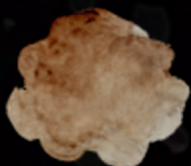
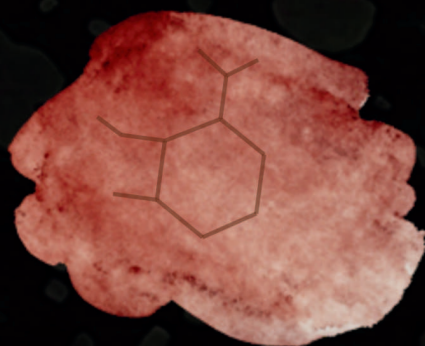
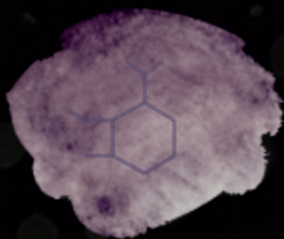


Soil Organic Carbon:
*Pools and Predictors in Nordic Agricultural
Soils, and Insights into SOM Fractionation
Methodology*



Anna-Reetta Salonen

Propositions

1. Soil carbon fractionation methods shape the carbon pools they measure.
(this thesis)
2. Links between soil carbon fractions and field-scale soil functions remain uncertain.
(this thesis)
3. Peatland conversion to arable land demonstrates the gap between scientific certainty and societal action.
4. Scientific frameworks spread globally before validated locally.
5. Achieving sustainability depends more on society than on science.
6. The most obvious sustainability solution is transitioning to plant-based food systems.

Propositions belonging to the thesis, entitled

**Soil Organic Carbon: Pools and Predictors in Nordic Agricultural Soils,
and Insights into SOM Fractionation Methodology**

Anna-Reetta Salonen

Wageningen, 19th of June 2026

Soil Organic Carbon:
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Methodology*

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Soil Organic Carbon:
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Methodology*

Anna-Reetta Salonen

Thesis

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Prof. Dr C Kroeze,

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Chapter 1

General Introduction

Anna-Reetta Salonen

1.1 Soil organic carbon

Soils provide the matrix that allows life to exist at the Earth's surface, a relationship so fundamental that it is reflected in the Latin language, as the word *humus* (soil) shares its root with *homo* (human) (Hillel 1992). The cultivation of soils began around 10 000 years ago (Purugganan & Fuller 2009), enabling sustained food production and underpinning the development of human societies. Today, soils sustain more than 95% of the global food production (FAO 2015). Soil organic matter (SOM), though constituting only a few percent of mineral soil by mass (Brady & Weil 2008), plays a central role in the life-giving properties of soils: nutrient cycling, soil structure, and water retention, all on which plant growth depends (Brady & Weil 2008; Schmidt et al. 2011).

In natural ecosystems, nutrient cycles are relatively closed: plant biomass returns through litterfall and root turnover, becomes soil organic matter, and through decomposition releases nutrients that support further plant growth. Carbon, by contrast, flows through the soil system. Plants assimilate it from the atmosphere through photosynthesis and it enters soil through root turnover, exudates, and litter. In the soil, this carbon is present as soil organic carbon (SOC) until decomposition returns it to the atmosphere (Janzen 2006; Schmidt et al. 2011). Human land use, including the conversion of natural ecosystems to agriculture, has disrupted these processes by removing biomass and reducing the return of carbon and nutrients to the soil (Lal 2004). In addition, food crops tend to have shallower root systems than natural vegetation, which additionally reduces carbon inputs compared to natural ecosystems (Jackson et al. 1996; Lal 2004). Furthermore, SOM decomposition can be accelerated by tillage that disrupts soil aggregates or by drainage which increases aeration, though effects vary with climate and soil conditions (Six et al. 2000; Powlson et al. 2014; Ogle et al. 2019).

The magnitude of soil carbon stocks is often expressed in comparison to the atmosphere and vegetation, highlighting soil as a major terrestrial carbon reservoir (e.g., Jobbágy & Jackson 2000; Ciais et al. 2013). Global estimates suggest that soils contain approximately 1500–2400 Pg SOC in the top metre, which is more than the atmospheric (~830–900 Pg) and terrestrial vegetation (~450–650 Pg) carbon pools combined (Jobbágy & Jackson 2000; Ciais et al. 2013; Batjes 1996; Friedlingstein et al. 2025). While this comparison highlights the importance of soils in the global carbon cycle, it obscures the

fact that SOC is unevenly distributed among land uses. Agricultural soils occupy approximately one third of the global land surface (~37–38%; FAO 2023), yet croplands and grazing lands together contain only a few hundred petagrams of SOC in the upper metre. Grassland and savanna biomes generally store approximately twice as much SOC per unit area as croplands, though grazing lands have also experienced substantial carbon losses (Jobbágy & Jackson 2000; Sanderman et al. 2017). In contrast, peatlands cover only about 3% of the global land area, yet store approximately 340 Pg SOC in the top metre alone (Leifeld & Menichetti 2018; Crézé et al. 2025). Forest soils, covering around 31% of the global land area, are estimated to store 1000–1500 Pg SOC in the top metre, potentially the largest share of global SOC stocks among terrestrial ecosystems (Jobbágy & Jackson 2000; Batjes 1996; FAO 2020; Sanderman et al. 2017). However, it should be noted that all global SOC stock estimates remain uncertain, as direct measurements are sparse and largely limited to surface soils, requiring extrapolation across depth and modelling to generalise the sparse measured data (Batjes 2016; Sanderman et al. 2017; FAO 2020).

The conversion of natural ecosystems to agriculture has led to substantial SOC losses since the onset of agriculture and these losses have accelerated with twentieth-century agricultural intensification (Sanderman et al. 2017). Consequently, agricultural soils receive much attention in the ongoing debate on climate change mitigation. However, complete recovery of the estimated agricultural SOC loss of 75 Pg C in the top metre since the beginning of cultivation (Sanderman et al. 2017) would offset less than a decade of current fossil fuel emissions, which now exceed 10 Pg C yr⁻¹ (Friedlingstein et al. 2025). The realistic recovery potential of SOC in agricultural soils is far smaller. Crucially, carbon sequestration in soils requires a net transfer of CO₂ from the atmosphere via plants (Don et al. 2024); the use of organic inputs such as manure or compost does not sequester carbon but rather redistributes SOC within the agricultural system. Estimates of the global soil carbon sequestration potential in croplands range from 0.4 to 1.85 Pg C yr⁻¹ (Smith et al. 2008; Zomer et al. 2017), but these include gains from redistributing existing carbon, such as manure additions, and assume achievable increases in soil carbon stocks without accounting for whether photosynthetic inputs are sufficient to deliver them. Under the constraints imposed by photosynthetic inputs and the need to

harvest biomass, Janzen et al. (2022) estimated the maximum net sequestration of cropland soils to be only 0.1-0.2 Pg C yr⁻¹. At these rates, recovering historical losses would require at least several centuries.

These considerations argue against framing SOC sequestration as a climate mitigation tool. However, maintaining or improving SOC levels remains essential for the ecosystem services that agricultural soils provide (Dominati et al. 2010): SOC fuels biological activity and releases nutrients for plant growth through decomposition, promotes aggregate formation, and improves soil structure, water infiltration, and resistance to erosion (Hoffland et al. 2020). Janzen (2006) articulated this tension as the 'carbon dilemma': the same decomposition that releases carbon to the atmosphere also releases nutrients essential for plant growth. The organic matter we might wish to store for climate benefits, we also wish to cycle to sustain soil biological activity and nutrient supply. The challenge therefore is not simply to maximize SOC storage, but to steward SOC in ways that maintain the soil properties on which agriculture depends, recognizing that a productive soil is one in which organic matter turns over (Janzen 2024). To approach this challenge, distinguishing SOM components with different turnover times and functions is essential. This has motivated efforts to separate SOM into operationally defined pools.

1.2 The POM/MAOM framework

SOM exists along a continuum that spans from fresh plant residues to compounds extensively transformed by microbial activity. Molecules on this continuum vary in their chemical composition and stability (Schmidt et al. 2011; Lavalley et al. 2020). To tackle this complexity, SOM is commonly divided into pools that are assumed to have different origins, states of decomposition, turnover times, and functional roles. A widely adopted framework distinguishes particulate organic matter (POM) from mineral-associated organic matter (MAOM) (Tiessen & Stewart 1983; Cambardella & Elliott 1992; Lavalley et al. 2020).

According to the framework, POM consists of fresh or partially decomposed plant residues that turn over on timescales of years to decades (Lavalley et al. 2020). POM can be further distinguished as free POM, which exists loosely in soil pores, and occluded POM, which is physically protected within soil aggregates. This distinction is not static:

free POM can become a nucleus around which aggregates form, slowing its decomposition by reducing access for microorganisms (Six et al. 2000). POM is less protected by mineral interactions than MAOM and, within the POM/MAOM framework, is expected to decompose more rapidly, though its persistence depends on environmental constraints such as temperature and oxygen availability, as well as physical protection within aggregates or at depth (Lavallee et al. 2020; Button et al. 2022). Furthermore, POM can include pyrogenic carbon such as charcoal, which persists far longer than the years to decades typically assumed (Lehmann et al. 2015; Lavallee et al. 2020). MAOM comprises organic compounds associated with mineral surfaces, particularly clays and metal oxides. It is considered to be enriched in microbially processed compounds, formed through microbial transformation or direct sorption of dissolved organic matter (Lavallee et al. 2020). These mineral associations are presumed to allow MAOM to persist from decades to millennia (Lavallee et al. 2020).

The POM and MAOM pools play distinct roles in the carbon dilemma (Janzen 2006): much of the organic matter we might wish to store for climate benefits also needs to cycle to sustain biological activity and nutrient supply. In this framing, POM represents the cycling pool, contributing to nutrient availability and microbial activity, while MAOM represents long-term carbon storage. However, the assumed correspondence between the pools and their functions is increasingly questioned. While MAOM typically stores the majority of SOC in agricultural soils (often around 80%; Cotrufo et al. 2022; Georgiou et al. 2022), association with minerals does not guarantee stability: the pool size can remain stable while individual molecules reside only for limited times (Jilling et al. 2018; Cotrufo & Lavallee 2022; Yu et al. 2022). Conversely, POM can persist longer than expected by its pool description (Witzgall et al. 2021; Heckman et al. 2022). The extent to which the operationally defined POM and MAOM pools reflect distinct functions and turnover time in soils remains unclear.

Earlier approaches characterized SOM through chemical extraction, yielding operationally defined humic fractions (humic acids, fulvic acids, humin) that are now often viewed as artifacts of the harsh extraction process rather than distinct compounds in the soil (Lehmann & Kleber 2015). Physical fractionation emerged as an alternative, separating SOM based on size or density rather than solubility (e.g., Tiessen & Stewart

1983; Cambardella & Elliott 1992; Golchin et al. 1994). In practice, POM and MAOM pools are now commonly assessed through physical methods, which yield operationally defined fractions assumed to represent them. However, any separation method imposes an abrupt cutoff on what is in reality a continuum (Schmidt et al. 2011) and methodological differences can make outcomes vary between methods.

Two main physical POM/MAOM fractionation approaches have emerged: separation by particle size and by density. Size fractionation separates dispersed soil at a cutoff, typically 50–53 μm (Poeplau et al. 2018; Cotrufo et al. 2019). Material larger than the cutoff is classified as POM, material smaller than the cutoff as MAOM. Density fractionation uses flotation in a heavy liquid, typically 1.6–1.8 g cm^{-3} (Cerli et al. 2012; Poeplau et al. 2018). POM, being lighter organic material, floats, while MAOM, associated with denser mineral particles, sinks. Depending on the protocol, density fractionation can also separate free from occluded POM, yielding three fractions. Both approaches produce fractions termed POM and MAOM, yet direct comparisons suggest they may isolate material with distinct isotopic and spectroscopic properties (Poeplau et al. 2018; Leuthold et al. 2024). This raises the question whether fractions defined by size correspond to those defined by density, and whether either SOM fractionation method adequately captures the functional distinction between labile and mineral-stabilised organic matter.

A possible approach to assess whether operationally defined fractions differ in composition is to combine POM/MAOM fractionation with thermal analyses (Poeplau et al. 2025; Schiedung et al. 2025). Rock-Eval thermal analysis characterizes organic matter based on the temperature at which it decomposes, providing information on thermal stability that relates to chemical composition (Disnar et al. 2003; Sebag et al. 2016). Applying such analysis to physically separated SOM fractions can test whether POM and MAOM exhibit the distinct chemical and thermal properties that their assumed functional roles would imply.

1.3 The carbon saturation concept

As MAOM should represent organic matter associated with soil mineral surfaces, the amount of mineral surfaces in a given soil theoretically sets an upper limit to MAOM

accumulation. This has led to the concept of MAOM saturation: the idea that soils have a finite capacity to stabilise organic carbon, determined by mineral surface area (Hassink 1997; Six et al. 2002; Stewart et al. 2007; Feng et al. 2013; Georgiou et al. 2022). MAOM saturation has implications for carbon sequestration, as soils approaching their saturation limit may have limited capacity for additional MAOM accumulation regardless of management (Moinet et al. 2023; Georgiou et al. 2025).

The amount of fine-sized soil minerals has traditionally been considered a predictor for SOC storage capacity. This is supported by the well-documented pattern that soils with higher clay content tend to store more organic carbon (Jobbágy & Jackson 2000; Kaiser & Guggenberger 2000; Arrouays et al. 2006). Hassink (1997) proposed that SOC storage capacity could be estimated from the proportion of clay and silt (<20 µm fraction). This was based on an empirical linear relation found in old grasslands and uncultivated soils, which were assumed to have reached their maximum carbon content under the given conditions (i.e., to be carbon saturated). However, Feng et al. (2013) argued that this approach could include potentially unsaturated soils, which would underestimate the maximum carbon stabilisation potential. They proposed boundary line analysis as an alternative. This method groups soils by their clay and silt content, identifies the soils with the highest carbon content within each group, and fits a regression through these upper values. The aim is to estimate the upper boundary of carbon storage capacity rather than the average across soils at varying degrees of saturation.

An alternative metric, the clay (or clay and silt) to SOC ratio, has also been used to assess the soil carbon saturation state (Dexter et al. 2008; Schjøning et al. 2012; Prout et al. 2021). This ratio reflects how much clay is available per unit of organic carbon to associate with: the lower the ratio, the more the mineral surfaces are occupied by SOC. Ratios below 10 have been proposed to indicate SOC saturation, while higher ratios suggest capacity for further SOC accumulation.

These texture-based approaches for estimating soil carbon saturation status assume that the content of fine soil particles (clay and silt) reflects the reactive mineral surface area available for MAOM binding (Sollins et al. 1996; Wiesmeier et al. 2019). However, textural clay (<2 µm) is defined solely by particle size and does not distinguish between different mineral types. In this thesis, 'clay' refers to this particle size fraction unless

otherwise specified. Through weathering, primary minerals inherited from the parent material (such as quartz, feldspars, and micas) are transformed into secondary minerals, including phyllosilicates (e.g. smectite, illite, vermiculite) and aluminum (Al) and iron (Fe) (hydr)oxides. Among these, 2:1 phyllosilicates in particular have high specific surface areas (for example, 700–800 m² g⁻¹ for smectite compared with <5 m² g⁻¹ for quartz; Brady & Weil 2008). This large reactive surface enables the adsorption and stabilisation of organic compounds through a range of mechanisms, including electrostatic interactions, ligand exchange, and cation bridging (Kleber et al. 2007). Some saturation frameworks account for mineralogical variation by developing separate estimates for soils dominated by 1:1 or 2:1 clay minerals (Feng et al. 2013; Georgiou et al. 2022; Six et al. 2024). However, the clay fraction can also include variable proportions of non-reactive primary minerals that are clay-sized but less reactive (Churchman 2010). This implies that clay content alone does not necessarily reflect reactive mineral surface area, particularly in soils where a substantial proportion of the clay fraction consists of primary minerals rather than reactive secondary minerals. Alongside clay, Al and Fe (hydr)oxides have received attention as predictors of MAOM storage capacity (Rasmussen et al. 2018; Wiesmeier et al. 2019). These secondary minerals, formed through weathering, provide reactive surfaces for organic matter binding, particularly in acidic soils where they carry positive surface charge (Kleber et al. 2007).

1.4 Subsoil as a carbon pool

Most soil carbon research focuses on topsoils (often 0–30 cm; Yost & Hartemink 2020), yet a substantial proportion of SOC stock lies below the topsoil: globally, over half of the carbon in the first metre is found below 30 cm depth (Sanderman et al. 2017). This SOC pool behaves differently from topsoil carbon. Radiocarbon dating shows subsoil carbon to be considerably older and slower to turn over (Rumpel & Kögel-Knabner 2011; Mathieu et al. 2015), suggesting greater potential for long-term storage than in the topsoil. The stability of subsoil SOC also reflects environmental conditions at depth: lower temperatures, reduced oxygen availability, limited fresh carbon inputs, and greater spatial separation between decomposers and SOC (Salomé et al. 2010; Button et al. 2022). SOC stabilisation mechanisms may also differ between topsoil and subsoil. While clay content often predicts SOC in surface horizons, this relationship can weaken at depth, where Al

and Fe oxides may play a larger role (Rumpel & Kögel-Knabner 2011; Rasmussen et al. 2018). At the same time, subsoils appear to be substantially further from MAOM saturation than surface soils (Georgiou et al. 2022). This suggests both unused capacity and potential for long-term carbon stabilisation at depth.

Carbon reaches the subsoil through several pathways. Dissolved organic carbon leaches downward with percolating water and can be stabilised at depth through interactions with Al and Fe oxides (Kaiser & Kalbitz 2012; Rumpel & Kögel-Knabner 2011). Organic matter can also move attached to clay particles during illuviation (the downward transport of fine particles to lower soil horizons; Torres-Sallan et al. 2017). Tillage mixes carbon throughout the plough layer and may displace some below it, while deep roots deposit carbon directly at depth through turnover and exudation (Rasse et al. 2005; Button et al. 2022). The relative importance of these pathways varies with soil type, climate, and management, and likely shapes the distribution of SOC between POM and MAOM at depth. Yet many SOC monitoring programmes sample only the topsoil (Heikkinen et al. 2013, Orgiazzi et al. 2018). This leaves subsoil carbon stocks and their distribution between POM and MAOM largely uncharacterised.

1.5 Nordic agricultural soils

As explained above, the capacity of soils to store organic carbon depends on several factors. The contents of textural clay (<2 µm) and SOC storage have long been connected, as fine particles provide surface area for organic matter binding (Dexter et al. 2008; Wiesmeier et al. 2019). However, the reactive properties of the clay-sized minerals matter alongside their quantity: the link between clay content and SOC storage rests on the assumption that clay-sized particles are mineralogically reactive. In young, glacially-derived soils such as found in Nordic environments, this assumption may not hold. Finnish agricultural soils e.g., are relatively young and formed from materials deposited during the Weichselian glaciation approximately 10 000 years ago (Brady & Weil 2008; Koljonen 1992). Glacial action has ground primary rock-forming minerals such as quartz and feldspars to clay size, but the relatively short time since deglaciation has limited the chemical weathering needed to transform them into reactive secondary minerals (Keskinen et al. 2022).

Furthermore, cold and humid conditions distinguish these soils from the temperate systems where saturation concepts were developed: low temperatures slow decomposition, allowing organic matter to accumulate even where mineral protection is limited (Jobbágy & Jackson 2000; Cotrufo & Lavelle 2022). The relatively high SOC levels in Nordic soils (Matschall et al. 2018; Heikkinen et al. 2021) may therefore reflect slow SOM turnover, mineral stabilisation, or both. Regardless of the underlying mechanism, these carbon stocks are changing: long-term monitoring has documented declining SOC in Finnish cropland soils over recent decades (Heikkinen et al. 2013), with recent evidence linking these losses to climate warming (Heikkinen et al. 2022). How SOC is stored and distributed between POM and MAOM in these soils, and whether saturation concepts developed in more weathered systems hold, remains largely unexplored.

1.6 Research objectives and outline of this thesis

This thesis examines soil organic carbon in Finnish agricultural soils, as well as POM/MAOM fractionation methods applied to diverse soils across a wide range of properties and origins. The objectives are to: (1) characterize carbon stocks and their distribution into fast-cycling (POM) and slow-cycling (MAOM) fractions, (2) assess which soil properties, including soil texture and Al and Fe oxide content, control MAOM storage and whether SOC saturation concepts apply to Finnish soils, (3) assess whether operationally defined POM and MAOM fractions represent distinct SOM pools that align with their assumed roles as fast- and slow-cycling carbon, and (4) examine whether chemical dispersion prior to POM/MAOM size fractionation is complete, and how incompleteness of dispersion affects the distribution of SOC into POM and MAOM pools.

In **Chapter 2**, I examine a 24-year field experiment located in South-Western Finland comparing organic and conventional arable cropping systems and an adjacent unmanaged meadow, where all above and belowground plant inputs return to the soil, as a reference site. I assess how these contrasting land uses affect the soil carbon stocks and their distribution into size-based POM and MAOM fractions with soil depth (to 70 cm), evaluate the relative importance of clay content compared to aluminum and iron oxides

in explaining carbon variation through the soil profile. I also assess the soil carbon saturation state using Hassink's Equation and clay to SOC ratio.

Chapter 3 broadens the scope to 93 fields across southern Finland, spanning a wide range of soil types and management histories. I evaluate which soil properties explain variation in topsoil SOC and its distribution into size-based POM and MAOM fractions, and assess the soil carbon saturation state using several commonly applied methods (Hassink's Equation, boundary line analysis and clay to SOC ratio).

Chapter 4 addresses an assumption underlying the POM/MAOM framework: do POM and MAOM fractions obtained by size-based and density-based fractionation represent distinct SOM pools as their assumed functional roles imply? I compare fractions isolated by both methods using Rock-Eval thermal analysis, assessing whether they differ in chemical and thermal properties as the conceptual POM/MAOM framework assumes.

Chapter 5 examines a critical methodological assumption in size-based fractionation: whether chemical dispersion of soil aggregates is complete prior to size separation, and how incomplete dispersion affects the distribution of SOC between POM and MAOM. Using 38 widely diverse soils, we assess how soil properties, particularly SOC content and Al and Fe oxide content, affect dispersion completeness, and quantify the resulting errors in SOC distribution between POM and MAOM.

Finally, **Chapter 6** brings together the findings from the preceding chapters in a broader discussion of what they mean for understanding and measuring fast- and slow-cycling carbon (POM/MAOM) pools, the assumptions underlying current fractionation approaches and SOC saturation concepts, and the particular challenges of Nordic soils.

Chapter 2

Assessing the effect of arable management practices on carbon storage and fractions after 24 years in boreal conditions of Finland

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Abstract

Soil organic matter (SOM) plays an important role in soil functions that are crucial for sustainable agriculture. Understanding how agricultural management and soil properties affect SOM in different soil depths would aid in maintaining and increasing SOM throughout the soil profile. We sampled a 24 year-old cultivation field experiment with organic and conventional cropping systems, and an adjacent unmanaged meadow to 70 cm soil depth to assess the total organic carbon (OC) stocks and the distribution of OC into mineral-associated (MAOM), particulate (POM) and dissolved (DOM) organic matter. We found that >83% of the soil OC was in the MAOM fraction, and that the distribution of OC across the MAOM, POM and DOM within a soil depth was not strongly affected by soil management. Largest OC stocks (169 t ha^{-1}) together with the largest plant root biomass was found in the unmanaged meadow, which highlights the potential of deep-rooting plants in sequestering OC into the soil. The OC saturation state of the soil was assessed based on the clay to OC ratio and Hassink's Equation (Hassink 1997). Hassink's Equation seemed to underestimate the MAOM accrual capacity of these soils and thus overestimated soil OC saturation state, whereas the clay to OC ratio indicated potential for OC accrual in all soil depths except for the meadow topsoil. These varying results suggest that the applied metric should be soil type and -depth specific. We also determined the contribution of clay content and aluminum and iron oxides in explaining the amount of total OC, MAOM-C and POM-C. In contrast to the aluminum and iron oxides, clay was not well correlated to any of the OC fractions below the 20 cm depth, suggesting that estimating the OC accrual potential of the deeper soil should not be based on the soil texture alone. Our results indicate that aluminum and iron oxides can play an important role in transporting and stabilizing of OC in the soil profile.

2.1 Introduction

Current agricultural management has led to a decline in global carbon (C) stocks in soil organic matter (SOM) in topsoils (0-30 cm) by 26%, and in the deeper soil profile (0-100 cm) by 16% (Sanderman et al. 2017), and a continuing annual decrease of arable soil organic carbon has been reported in Finland (Heikkinen et al. 2013). The SOM loss threatens the viability of agriculture, as it is essential in a multitude of soil functions such as; primary productivity, carbon sequestration and climate regulation, water purification and regulation, providing habitat for biodiversity, and in the recycling of nutrients (e.g., Schulte et al. 2014, Wiesmeier et al. 2019, Hoffland et al. 2020). The management-derived depletion of SOM also contributes to climate change by increasing atmospheric carbon dioxide (CO₂) concentrations (Paustian et al. 2016). To prevent further SOM reduction and to mitigate climate change, it is important to learn how soil properties and agricultural management affect the form of soil organic carbon (OC) and the size of OC stocks across different soil depths.

To determine the form of soil C, SOM can be physically divided into two fractions (Tiessen & Stewart 1983, Cambardella & Elliott 1992, Lavallee et al. 2020): to relatively stable mineral associated (MAOM), and to more labile particulate (POM) organic matter. MAOM is presumably mainly of microbial origin (Cotrufo et al. 2013, Kallenbach et al. 2016) but some portion of it derives from leached plant material (Angst et al. 2021, Yu et al. 2022), whereas POM consists of relatively intact plant material (Lavallee et al. 2020). MAOM is stabilised by its interactions with soil minerals while POM has only limited interaction with minerals, although soil aggregation or the soil physicochemical status may hinder its decomposition (Lavallee et al. 2020). In addition to MAOM and POM fractions, a minor part of the soil organic carbon can be extracted from the soil as dissolved organic matter (DOM) (Kalbitz et al. 2000, von Lützow et al. 2007).

In arable mineral soils, largest part of the OC stock is in the MAOM (Kögel-Knabner et al. 2008, Georgiou et al. 2022). Various aspects regulate the total amount of SOM and its distribution to the different OC forms (MAOM-C, POM-C and DOM-C). Soil properties, such as texture and amount of aluminum (Al) and iron (Fe) oxides control on their part the size of the soil OC stock in MAOM (von Lützow et al. 2007). The amount of clay (< 2 µm) sized particles has traditionally been considered as an adequate predictor for a ma

ximum capacity of the mineral-associated soil OC stock (Dexter et al. 2008) as the proportion of these particles gives a reference of mineral surface area available for MAOM binding (Wiesmeier et al. 2019). Hence, combining soil clay content with soil OC content (i.e., clay to OC ratio) could serve as a sensible metric for assessing OC deficit or OC accrual potential of a given soil (Dexter et al. 2008, Schjønning et al. 2012, Prout et al. 2021).

Soil properties, such as the contents of clay and Al and Fe oxides, derive primarily from the weathering of the soil parent material, in combination with environmental conditions, such as precipitation and temperature (Jenny 1941). The weathering capacity of parent material is therefore dependent on the regional climatic conditions. This context specificity in terms of climatic region is critically important when understanding the underlying mechanisms for carbon sequestration and may require that clay content alone is not considered as the most suitable predictor of MAOM-C storage capacity. Al and Fe oxides act efficiently as OC binding agents (Wiseman & Püttmann 2006, Wiesmeier et al. 2019, Mendez et al. 2020, Hall & Thompson 2022), and in humid cold climates with relatively low pH, such as the Boreal conditions in Finland (Metzger et al. 2012), their oxalate-extractable species have been shown to be better predictors for the soil OC than soil texture alone (Rasmussen et al. 2018, Wiesmeier et al. 2019, Fukumasu et al. 2021). In humid Boreal climate, periodic water saturated conditions may have two-way effects on the SOM dynamics: waterlogging is likely to hinder the decomposition of POM due to microbial inhibition (Lugato et al. 2021). On the other hand, water saturation may cause Fe to reduce, potentially releasing MAOM-C from mineral complexes (Huang & Hall 2017).

In addition to soil properties, agricultural management is an important regulator of OC storage capacity in arable soils (e.g., Tiefenbacher et al. 2021). Examples of the management practices that can be applied to aid increasing soil carbon storage are reduced tillage (Smith et al. 2008), cultivation of deep rooting plants (Thorup-Kristensen et al. 2020), and the use of organic fertilizers, such as manure (Maillard & Angers 2014). However, as the outcome of these procedures is highly context-dependent, it is challenging to make universally applicable generalizations on their effects on soil OC stocks. In addition, as most soil carbon studies only consider the top layer of ca. 30 cm of

arable soils (Yost & Hartemink 2020), there is less knowledge on the impact of these practices on the whole soil profile OC stock. For example, the effect that deep-rooting plants have on soil OC is not unambiguous. Deep-rooting grassland plants can allocate as much as 60% of photosynthesised OC belowground (Jackson et al. 2017) which could potentially increase soil OC stocks. However, root exudates may also have opposite effects: they may cause MAOM desorption from mineral surfaces (Keiluweit et al. 2015), as well as increase soil microbial activity which leads to MAOM-C mineralization (so called priming effect; Kuzyakov et al. 2000). Likewise, effects of tillage can be contrasting in different soil depths (Haddaway et al. 2016, Ogle et al. 2019, Cai et al. 2022). Disturbance caused by tillage may hasten SOM mineralization in the topsoil (through increased aeration and disturbance of aggregates, Grandy & Robertson 2007) but concurrently, may translocate SOM into the deeper soil depths which may guard it against decomposition by soil organisms (Button et al. 2022).

There are several potential stabilisation mechanisms for SOM below the plough layer. In general, lower temperatures and periodical anoxic conditions may hinder SOM mineralisation (Button et al. 2022). As in the topsoil, soil properties may promote the formation of organo-mineral association with clay-sized soil minerals and binding to Al and Fe oxides. There is estimated to be considerable potential for increasing subsoil OC storage, as globally it is approximated that only 21% of the subsoil (below 30 cm) MAOM storage capacity is currently occupied (Georgiou et al. 2022). As the mechanisms behind SOM stabilisation and its distribution to different fractions may vary with the soil depth, assessing predictors and potential metrics for the total amount of OC and stabilised OC (i.e., MAOM-C) in different soil depths could support the attempts to increase deep soil OC stocks for climate mitigation (Dynarski et al. 2020).

We explored the effects of organic and conventional crop rotations on soil OC stocks down to the depth of 70 cm, and on the distribution of OC into different SOM fractions (MAOM, POM and DOM) in a Finnish long-term field experiment (established in 1995). We hypothesized that the organic crop rotation system receiving organic fertilization (manure) would have higher carbon stock when compared to the conventional crop rotation receiving mineral fertilization only. The results from the cropping systems were compared to an adjacent permanent meadow that was assumed to have the highest OC

stock and to be closest to the OC saturation due to deep-rooting plants and high litter- and root-derived OC inputs. The unmanaged meadow was representing an undisturbed soil and maximum OC input without external SOM additions and expected to reveal the practical OC accrual potential of soil in relation to soil properties (i.e., clay content and Al- and Fe oxides). We anticipated the MAOM-C levels to be positively correlated with clay, Al and Fe oxides in the topsoil of all the studied soils. As OC levels are assumed to drop with increasing soil depth, below 30 cm depth MAOM-C was expected to correlate with Al and Fe oxides only as they are supposedly preferential binding sites for the OC.

2.2 Material and methods

2.2.1 Study site and soil sampling

Soil samples were taken in October 2019 from Yöni field experiment (Natural Resources Institute Finland) in Jokioinen, South-Western Finland (60°51'44.5"N 23°31'24.5"E). Parent material of the soil is glaciofluvial clay (Yli-Halla et al. 2009) and the field is classified as a Vertic Stagnosol (IUSS Working Group WRB, 2015; Yli-Halla et al. 2000). Mean annual precipitation is 600 mm and temperature 5 °C. Artificial drainage was installed in the field at the depth of 1 meter in 1989.

The field experiment was established in 1995 on a site that had been extensively managed grassland since the beginning of the 20th century. Cultivation in some parts of the field started in 1990 and between 1990 and 1995, the crop rotations differed slightly from the fixed crop rotation system which was established in 1995 in the whole experimental area (Table 2.1). In 1990, soil samples for soil fertility assessment (composite samples from c. 0-25 cm) were collected from the field. In the plots where unmanaged meadow, and organic and conventional crop rotations were later established OC contents (g kg^{-1} ; $\pm\text{SE}$) were: 52 ± 7.1 , 50 ± 3.0 , and 53 ± 2.2 , respectively. At the time of establishing the experiment (year 1995), part of the field was left to develop as a natural, unmanaged meadow. The unmanaged meadow closely resembles to a grassland by its vegetation but is not used as a pasture. No management procedures were performed for the unmanaged meadow, except for removal of trees if they appeared. The remainder of the field had two cultivation systems: 1) organically and 2) mineral fertilized cereal crop rotations in three randomized blocks. Plot size was approximately 150 x 20 m. Both systems had the

same cultivated plants in a five-year rotation since the start of the experiment (Table 2.1) and similar ploughing; during each five-year crop rotation, soil was ploughed three times to 20 cm depth. Equal amount of harvest residues was left in the organic and conventional systems. The conventional system was minerally fertilized according to the nitrogen and phosphorus fertilization limits of the prevailing Agri-Environmental Programme and herbicides (glyphosate and MCPA) were applied approximately six times per five-year crop rotation cycle. In the organic system, cattle manure was applied as the form of fertilizer and no herbicides were used. During the five-year crop rotation, the organic system received the amount of manure containing total N excreted by a 0.5 dairy cow ($50 \text{ kg ha}^{-1} \text{ y}^{-1}$). The amount was based on the sufficiency of achieved yield levels before 1995 to meet a fodder requirement of 0.5 dairy cows. The amount of phosphorus in the applied manure was determined by the nitrogen-adjusted manure application rate. From the start of the field experiment to the time of our soil sampling, average yearly nutrient amounts that the cropping systems received in the fertilization were: 26 kg ha^{-1} phosphorus and 95 kg ha^{-1} nitrogen for the conventional system, and 10 kg ha^{-1} phosphorus and 50 kg ha^{-1} nitrogen for the organic system. In the conventional system, all the fertilizer nitrogen was in a soluble form, and in the organic system 43% (22 kg y^{-1}). There were no OC inputs to the conventional system. As the OC content of the manure used as organic fertilizer was not analysed, the yearly OC inputs to the organic cropping system were estimated based on literature (Ylivainio et al. 2019, Saastamoinen et al. 2022) to be $0.62\text{-}0.73 \text{ t OC ha}^{-1}$, adding up to $14.9\text{-}17.4 \text{ t OC ha}^{-1}$ in total during the 24-year duration of the field experiment. Experimental plots have not been limed regularly. Some of the plots were limed 14 years prior to our sampling but judged by the low variation in measured pH (Table 2.2) across the managements and soil depths, the possible effects from liming have faded before our soil sampling.

In each of the cultivated plots, three one-meter-deep soil cores were collected (i.e., 9 soil cores per soil management) such that the distance from the outer edges of the plot were 10 and 6 meters, and the inter-sample distance was approximately 12 meters. Sampling was performed with the technique presented in Persson & Bergström (1991) and Uusitalo et al. (2012). In short, one-meter-long PVC – tubes (inner diameter 6.8 cm, outer wall of the tube 3 mm) were screwed into the soil and then pulled out. The adjacent permanent

meadow, which was not part of the complete randomized block design, was divided into three plots, sized approximately 50 x 20 m each, and sampled in the same way as the cultivated fields.

The PVC tubes holding the intact soil columns were stored at -20 °C until summer (May-July) 2020. Tubes were defrosted to +4°C and cut into 10 cm intervals while the soil remained in place inside the tube. After the cutting, the soil was pushed out from the 10 cm long tube sections. Part of the soil in each 10 cm soil section was air dried at +30 °C, and part stored at +4 °C.

Despite most of the one-meter long sampling cores penetrated the soil completely during soil sampling, all contained less than 100 cm soil. Soil depths that the PVC tubes contained were; conventional: min. 62 cm, max. 76 cm, and average 69 ($\pm$ SE 1.4) cm, organic: min. 64 cm, max 85 cm, and average 71 ($\pm$ SE 2.0) cm, and unmanaged meadow: min. 62 cm, max. 73 cm, and average 68 ($\pm$ SE 1.1) cm. There are several possible explanations for the cores not holding the full 100 cm of the soil. According to Persson & Bergström (1991), heavy clay soils that are near or in the water saturated state may stick to the edges of the tube and this may prevent the soil cutting motion of the edges and hence restrains the tube thoroughly penetrating the soil (Persson & Bergström 1991). We contemplate the most probable reason for cores having less than 100 cm soil to be that lowest part of the soil columns from inside of the sampling tube have dropped back to the drilling hole when the tube was pulled out from the soil. Also, soil may be somewhat condensed during the sampling. However, judged by the soil bulk densities (Figure 2.A1) that are comparable with values reported for heavy clay soils from the area of the field experiment (e.g., Yli-Halla et al. 2009), we estimate this to be only a minor explaining factor.

For most of the laboratory analyses (except for soil textural analyses, see details below), soil layers down to 40 cm depth were kept separately in 10 cm layers, and soil layers below 40 cm (i.e., layers 40-50 cm, 50-60 cm, and 60-70 cm) were mixed to form one pooled sample. The decision to pool the layers below 40 cm was based on soil OC measurements performed for homogenized, air-dried subsamples of each 10 cm soil interval as in the soil depths 40-70 cm of all the studied profiles, the total soil OC concentrations were comparable (data not shown).

2.2.2 Soil properties and root biomass

Soil pH and electric conductivity (Table 2.2) were measured in 1:2.5 (w:v) soil-deionized water-solution. Soil was mixed into the water and incubated overnight at room temperature. The next morning, electric conductivity was measured from the unmixed sample, after which the sample was mixed and pH was measured immediately thereafter. Al- and Fe oxalates were extracted with acid ammonium oxalate extraction according to Niskanen (1989; 0.05 M oxalate, pH 3.3) using 5 g of air-dried soil. Al and Fe were measured with inductively coupled plasma mass spectrometry (ICP–OES Thermo Scientific iCAP 6300 MFC DUO).

Roots were washed from a subsample (~100 g of soil) that was stored at +4 °C prior to the washing, using tap water and a sieve with a mesh size of 125 µm. Before weighing, the roots were dried at +60 °C.

Soil texture (size classes: clay < 2 µm; silt 2-20 µm; fine sand 20-200 µm; and coarse sand > 200 µm) was analyzed with the pipette method (Elonen 1971). Soils were pretreated with H₂O₂ for SOM removal, and then acidified with 2 M HCl and dispersed with 0.05 M Na₄P₂O₇. For texture analyses, air-dried soil was pooled together to create one sample per plot for each of the following soil depths: 0-20 cm, 20-40 cm and 40-70 cm (Table 2.3). The dominant clay mineral in the field is illite (X-Ray Diffractionation with Bruker D8 (Bruker Axs); minerals identified with Diffrac EVA 5.2 software. TOPAS 5.0 software for quantification WAXS measurements: 10–90°2θ; and SAXS measurements (for determining the d-values): 3–13°2θ)).

2.2.3 SOM fractionation and metrics to estimate soil OC saturation

Samples were fractionated by size to MAOM (< 53 µm) and POM (> 53 µm) according to Cotrufo et al. (2019) with the exception that after testing the protocol, we decided to use field moist soil instead of air-dried soil to assist complete dispersion of the studied heavy clay soil (air-drying fortifies the existing aggregate structure and possibly creates new aggregates, e.g., Kaiser et al. 2015). In short, soil samples (around 5 g by dry weight) were sieved (mesh size 2 mm) and dispersed by shaking 18 h at 120 rpm (Ika LabortechnikKS 501 digital) with glass beads and 5 g l⁻¹ sodium hexametaphosphate. After the dispersion, the suspension was rinsed on a sieve (mesh size 53 µm). The fraction that passed through the sieve was collected as MAOM, and the fraction that remained on

top of the sieve was collected as POM. Fractions were dried to constant weight in a forced-air oven (60 °C). The level of soil dispersion was checked by using two internal reference soils (also field-moist; clay- and coarse textured soil) with each set of dispersed soils to confirm reproducible weights of the MAOM and POM. Samples were analysed for OC (MAOM-C and POM-C) by dry combustion (LecoCHN 628, St. Joseph, Michigan, USA). OC recovery was on average 97 %, and ranged from 100 to 84%. All the analysed OC can be taken to represent OC due to the inherently low pH in Finnish soils as this prevents the formation of carbonate minerals (Nelson and Sommers 1996).

DOM was extracted from air-dried soil (5 g) that was shaken in deionized water in 1:10 (w:v) ratio for 1 h at 180 rpm, and then left overnight at room temperature. Next morning, the sample was shaken for 15 min at 180 rpm and centrifuged for 10 min at 1069 g after which the solution was decanted and filtered (0.2 µm, Nucle-pore® polycarbonate, Whatman International Ltd) using a water suction. Dissolved carbon (DOM-C) was measured from the filtered solution with a Shimadzu TOC-V CPH/CPN analyser (Kyoto, Japan). When calculating the proportion of the total OC in each of the SOM fractions, the OC present in DOM-C was subtracted from MAOM-C as when performing the MAOM/POM fractionation procedure, we consider DOM-C to end up in the MAOM fraction (i.e., to pass through the 53 µm sieve).

Soil texture was used to estimate the MAOM-C accrual capacity for each soil. We calculated the capacity of fine-sized particles (clay+silt, < 20 µm) to stabilise MAOM-C using the Hassink's (1997) Equation: $\text{MAOM-C (g kg}^{-1}\text{) at OC saturation} = 0.370 \times (\text{clay+silt}\%) + 4.09$. We also used the theory by Dexter et al. (2008) by which the soil OC saturation state can be estimated with clay to OC ratio. A ratio below 10 indicates that mineral surfaces are being saturated with OC, and a ratio above 10 suggests OC saturation deficit. These accrual capacity estimations were compared with measured MAOM-C to assess the potential for further MAOM-C accrual for the cultivated soils.

Laboratory analyses were performed for each soil depth layer (i.e., 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, and 40-70 cm) from each of the sampling tubes (leading to n=9 per depth layer and soil management). For the data analyses, the results of the laboratory analyses were averaged plotwise (leading to n=3). For variables of total C, the mass and OC content of SOM fractions, and Al and Fe oxides within a depth per plot, the coefficient

of variation (CV) was calculated from three plot replicates. Where within a plot variation exceeded 20%, samples were re-analysed to ascertain the high level of variation.

Table 2.1. Cultivated plants of the five-year crop rotation in the Yöni field. The rotation has been similar since 1995 excluding some exceptions*: in cases when the winter rye was not wintering well, oat was sown in the spring. In the organic plots, occasional years of bare fallow were utilized to assist in weed management.

Yöni crop rotation, since 1995	
Year 1	Spring barley (<i>Hordeum vulgare</i> L.) and perennial grass mixture of timothy (<i>Phleum pratense</i> L.), meadow fescue (<i>Festuca pratensis</i> (Huds.) P. Beauv.) and red clover (<i>Trifolium pratense</i> L.)
Year 2	A perennial grass mixture of timothy, meadow fescue and red clover
Year 3	A perennial grass mixture of timothy, meadow fescue and red clover, with winter rye (<i>Secale cereale</i> L.) seeds sown in autumn
Year 4	Winter rye
Year 5	A mixture of oat (<i>Avena sativa</i> L.) and pea (<i>Pisum sativum</i> L.)
*Exceptions in crop rotation: 2012, plot 10 (org.) was fallow; in 2013, plot 5 (conv.), and plot 6 (org.) cultivated with oat; in 2015, plot 13 (org.) was fallow; in 2016, plot 9 (org.), and 10 (conv.) cultivated with oat; in 2017, plot 10 (org.) was fallow.	

Table 2.2. Soil pH, electric conductivity (EC, 0.1mS/cm), and Al and Fe oxides (g kg⁻¹) in three management systems (conventional, organic and meadow) per soil depth ($\pm$ SE, n=3).

Soil depth	Management	pH	EC	Al-ox	Fe-ox
0-10 cm	Conventional	5.8 $\pm$ 0.1	44 $\pm$ 2.6	2.2 $\pm$ 0.1	5.4 $\pm$ 0.3
	Organic	6.1 $\pm$ 0.1	64 $\pm$ 5.0	2.1 $\pm$ 0.1	5.6 $\pm$ 0.2
	Meadow	5.7 $\pm$ 0.1	138 $\pm$ 4.3	3.2 $\pm$ 0.4	6.7 $\pm$ 0.4
10-20 cm	Conventional	6.0 $\pm$ 0.1	45 $\pm$ 2.7	2.2 $\pm$ 0.1	5.5 $\pm$ 0.3
	Organic	6.2 $\pm$ 0.1	55 $\pm$ 4.8	1.9 $\pm$ 0.1	5.7 $\pm$ 0.2
	Meadow	5.7 $\pm$ 0.1	58 $\pm$ 3.7	3.5 $\pm$ 0.3	7.7 $\pm$ 0.2
20-30 cm	Conventional	6.3 $\pm$ 0.1	42 $\pm$ 3.2	1.7 $\pm$ 0.1	3.6 $\pm$ 0.6
	Organic	6.4 $\pm$ 0.1	48 $\pm$ 7.3	1.8 $\pm$ 0.1	4.6 $\pm$ 0.5
	Meadow	5.8 $\pm$ 0.1	42 $\pm$ 5.9	2.9 $\pm$ 0.3	7.8 $\pm$ 0.0
30-40 cm	Conventional	6.8 $\pm$ 0.1	53 $\pm$ 0.6	1.7 $\pm$ 0.0	1.5 $\pm$ 0.2
	Organic	7.0 $\pm$ 0.1	37 $\pm$ 1.0	1.7 $\pm$ 0.0	1.7 $\pm$ 0.0
	Meadow	6.3 $\pm$ 0.2	40 $\pm$ 1.9	2.1 $\pm$ 0.2	6.3 $\pm$ 0.9
40-70 cm	Conventional	7.2 $\pm$ 0.1	49 $\pm$ 5.0	1.4 $\pm$ 0.0	1.4 $\pm$ 0.0
	Organic	7.4 $\pm$ 0.1	40 $\pm$ 2.5	1.4 $\pm$ 0.0	1.4 $\pm$ 0.0
	Meadow	6.9 $\pm$ 0.2	40 $\pm$ 2.0	1.5 $\pm$ 0.0	3.5 $\pm$ 1.0

Table 2.3. Soil texture, average across the field (n=3).

Soil depth	Management	Coarse Sand	Fine Sand	Silt	Clay
		> 200 μm	200–20 μm	2–20 μm	< 2 μm
g kg ⁻¹					
0-20 cm	Conventional	100	200	170	530
	Organic	110	200	160	530
	Meadow	24	170	126	680
20-40 cm	Conventional	40	190	140	640
	Organic	40	180	160	620
	Meadow	15	160	115	710
40-70 cm	Conventional	0	167	173	660
	Organic	0	186	154	660
	Meadow	0	180	140	680

2.2.4 Data analyses

All statistical analyses were performed with R (R Core Team 2022, version 4.1.3). Analyses were performed between conventional and organic cropping systems as the unmanaged meadow was not a part of the randomized block design and therefore not included in the statistical analyses. Since some of the data was not normally distributed and had unequal variances, we used the nonparametric Kruskal-Wallis test to determine possible differences in the forms of carbon (MAOM-C, POM-C and DOM-C), Clay to OC ratio, and C to N ratios of the bulk soil, MAOM and POM. We regarded p-values of 0.05 or less as statistically significant.

For comparing soil carbon stocks, we used the Fixed depth (FD) intervals and Equal soil mass (ESM) approach (e.g., Wendt & Hauser 2013, von Haden et al. 2020), and Kruskal-Wallis test for determining statistical differences in the OC stocks between the cropping systems. In the fixed depth approach, soil carbon stocks were calculated as follows: OC stock (t OC ha^{-1}) = OC concentration (g OC kg^{-1} soil) $\times$ soil bulk density (kg m^{-3}) $\times$ depth of a soil layer (cm) / 1000. We estimated soil OC stocks also with the ESM method as FD may be prone to errors caused by compaction during the sampling and the following segmenting the soil into FD layers (e.g., Wendt & Hauser 2013, von Haden et al. 2020).

The ESM method may also more accurately reflect the true state of the soil OC stocks when comparing differing managements as it disregards possible differences in the soil density that are derived from soil management and/or SOM content. ESM OC stock calculations were performed with the Excel spreadsheet from Wendt & Hauser (2013). For the Fixed depth OC stock calculations, we scaled all the sampling tubes to 70 cm soil depth (actual sampling depths were 62-85 cm, see Section 2.2.1). For the ESM OC stock calculations, we estimated the OC stocks to the soil mass of 7500 t ha⁻¹ (this soil mass is reached in the soil depth of 57.0 (±0.8) - 62.3 (0.8) cm; Table 2.A2) as all of the sampling tubes (with one exception) contained this soil mass when the area of the sampling tube was scaled to a hectare. One sampling tube from the unmanaged meadow contained 7390 t ha⁻¹ of soil and was extrapolated to represent the soil mass of 7500 t ha⁻¹. As we wanted to avoid further extrapolations, we decided to assess the soil OC stocks to the soil mass of 7500 t ha⁻¹ throughout the field.

As ESM and FD methods gave resembling results, and the soil bulk densities did not imply differing soil compaction in any of the soil depth layers between the management systems when sampling (Figure 2.A1), further detailed analyses (MAOM/POM/DOM fractionation, Al and Fe oxide extraction, root biomass analyses) were performed for the fixed depth soil layers (FD). Similarities of the soil bulk densities gave additional support for the reliability of the comparisons of the OC stocks and other studied parameters of the soil layers with both approaches, FD and ESM.

We applied Spearman rank correlation analyses to estimate the association between soil properties (clay content, and Al and Fe oxide contents) and total OC, MAOM-C, and POM-C. Soil profiles of conventional and organic cropping systems, and the adjacent unmanaged meadow were analysed in three depth segments: 0-20 cm, 20-30 cm and 30-70 cm. Analyses were performed within a depth segment for soil samples with all the managements combined. We selected these specific layers as we observed the highest variation in most of the studied variables in the soil layer of 20-30 cm, which we believe expresses that ploughing affects to some extent also the soil below 20 cm in the cropped systems, causing heterogeneity in this layer. The plough layer of 0-20 cm was assumed to be relatively homogenous due to the regular mixing effect from the ploughing, and the depth 30-70 cm was also assumed relatively homogenous as it is not disturbed by the

tillage operations. All the plots were created using R packages ‘ggplot2’, ‘ggpubr’ and ‘ggbreak’ (Wickham et al. 2016, Kassambara 2020, Xu et al. 2021).

2.3 Results

2.3.1 Soil carbon stock in the unmanaged meadow in relation to the cultivated soil

Total soil OC stock down to 70 cm depth in the unmanaged meadow was 169 t ha^{-1} ($\pm \text{SE } 6.94$) which was 1.5-fold compared to the conventional ($112 \text{ t ha}^{-1} \pm \text{SE } 3.32$), and 1.3-fold compared to the organic ($129 \text{ t ha}^{-1} \pm \text{SE } 3.88$) cropping system. The OC stocks of the unmanaged meadow were higher in all the individual (FD) soil layers (Figure 2.1A) than in the cropping systems. In all the soil management types, most of the OC, around 85%, was in the top 30 cm of the soil, where also most of the plant root biomass was located (Figure 2.1B). The unmanaged meadow had distinctly larger root biomass in the uppermost soil layers when compared to the cropping systems; in the soil depth of 0-10 cm, root biomass in the meadow was around 5-fold, in the layer 10-20 cm around 3-fold, and in the 20-30 cm soil depth more than double compared to the cropping systems. Within the cropping systems, root biomass tended to be larger in the organic than in the conventional system.

2.3.2 Soil carbon stocks in organically and conventionally managed cropping systems

We determined the total soil carbon stocks of the cropping systems to the equivalent soil mass of 7500 t ha^{-1} . In the whole of this soil mass, the OC stock was significantly higher in the organic ($125 \text{ t ha}^{-1} \pm \text{SE } 1.3$) than in the conventional cropping system ($107 \text{ t ha}^{-1} \pm \text{SE } 1.3$, p-value 0.04953). Further, we divided the soil mass into three layers: 0-1500 t ha^{-1} , 1500-3000 t ha^{-1} and 3000-7500 t ha^{-1} (see Table 2.A2 for the soil depths (cm) in which the Equal soil masses were reached). When comparing these individual layers, the 1500-3000 t ha^{-1} layer had significantly larger OC stocks in the organic ($48 \text{ t ha}^{-1} \pm \text{SE } 2.8$) than in the conventional ($35 \text{ t ha}^{-1} \pm \text{SE } 2.8$) cropping system (p-value 0.04953). The upper (0-1500 t ha^{-1}) and lower (3000-7500 t ha^{-1}) layers had resembling OC stocks in both of the cropping systems; OC stocks were in the upper layer 53 t ha^{-1} ($\pm \text{SE } 1.0$) in the conventional, and 57 t ha^{-1} ($\pm \text{SE } 1.3$) in the organic; and in the lower layer 19 t ha^{-1} ($\pm \text{SE } 1.3$) in the conventional, and 20 t ha^{-1} ($\pm \text{SE } 0.8$) in the organic system.

When considering the total OC stocks by depth (to 70 cm; Figure 2.1), rather than mass, we observed a significantly larger stock in the organic than in the conventional cropping system (p-value 0.04953). The total OC stocks were on average 17 t ha⁻¹ larger in organic than in the conventional cropping system, corresponding to a yearly difference of 0.7 t ha⁻¹ in OC stock. In the topmost soil layer of 0-10 cm, OC stocks were comparable (34 t ha⁻¹ conventional, and 35 t ha⁻¹ organic) but in the soil depths from 10 to 30 cm, the organic cropping system had larger OC stocks than the conventional system (layer 10-20 cm: organic 44 t ha⁻¹, conventional 41 t ha⁻¹; layer 20-30 cm: organic 31 t ha⁻¹, conventional 19 t ha⁻¹, p-value 0.04953). Below 30 cm, OC stocks were similar: in the layer 30-40 cm, the conventional system had 6 t OC ha⁻¹, and the organic system 7 t ha⁻¹. In the deepest studied soil layer of 40-70 cm, both of the cropping systems had a OC stock of 12 t OC ha⁻¹.

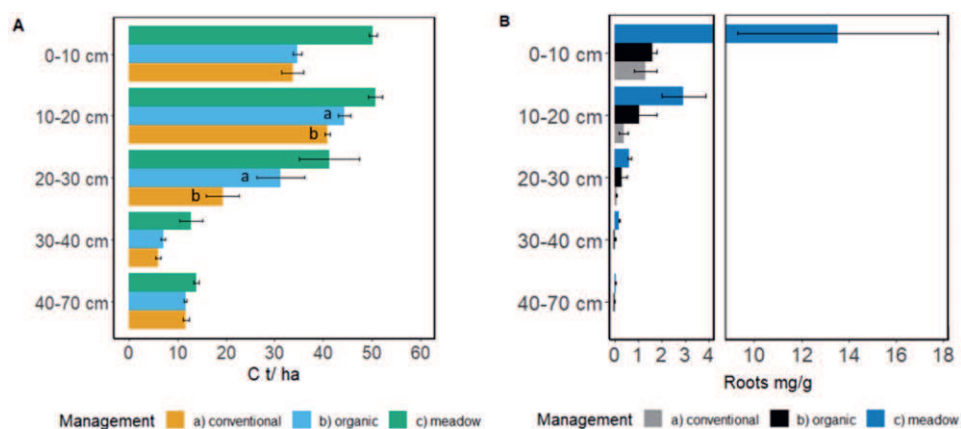


Figure 2.1. A) Distribution of soil organic carbon stocks (t ha⁻¹) in the cropping systems (conventional and organic) and unmanaged meadow ($\pm$ SE, n=3) in Fixed depth soil layers to 70 cm soil depth. Statistical analyses were performed only between the conventional and organic cropping systems, and letters a and b denote statistical differences within a soil layer (Kruskal-Wallis test, p-value 0.04953). B) Root mass (mg in g⁻¹ of soil) in the soil depth layers ($\pm$ SE, n=3). Roots were observed in all the soil depths of unmanaged meadow and organic cropping system, but in the conventional system, there were no measurable amounts of roots below the soil depth of 30 cm (neither from the root washing procedure nor in visual inspection). Note the break in the x-axis

Table 2.4. Average ($\pm$ SE, $n=3$) organic carbon concentrations of total soil, MAOM, POM and DOM, clay to OC-ratio, estimated MAOM-C accrual potential (g kg^{-1}) and the realized MAOM-C accrual (i.e., the measured OC content (%)) from the estimated accrual potential) according to Hassink's Equation, and C to N ratios of the bulk soil, MAOM and POM per soil depth and cropping system. The total soil C to N ratio was calculated from the MAOM and POM C and N content. Statistical analyses were performed only between the conventional and organic cropping systems (except for Hassink's Equation), and letters a and b denote statistical differences within a soil layer (Kruskal-Wallis test; all statistically significant tests had a p-value 0.04953)

Hassink's Equation											C to N ratio		
Soil depth	Management	Total OC g kg ⁻¹	soil g kg ⁻¹	MAOM-C g kg ⁻¹	POM-C g kg ⁻¹	DOM-C mg g ⁻¹	Clay to OC ratio	MAOM-C				Bulk soil	POM
								accrual potential (g kg ⁻¹)	Realized MAOM-C accrual (%)	MAOM	POM		
0-10 cm	Conventional	32.8 ± 1.2		28.6 ± 0.6	4.2 ± 0.8	0.33 ± 0.01	16 ± 1.1	29.6	97 ± 2.9	13 ± 0.7	12 ± 0.6	23 ± 5.8	
	Organic	35.5 ± 0.8		29.6 ± 1.2	5.9 ± 0.5	0.36 ± 0.07	15 ± 0.7	29.8	99 ± 5.7	12 ± 0.3	11 ± 0.4	19 ± 0.4	
	Meadow	70.0 ± 2.5		59.5 ± 1.7	10.5 ± 0.8	0.86 ± 0.03	10 ± 0.6	33.9	176 ± 7.7	14 ± 1.1	14 ± 1.3	18 ± 0.9	
10-20 cm	Conventional	32.0 ± 1.4		27.2 ± 1.1	4.8 ± 0.9	0.28 ± 0.01	17 ± 1.3	29.6	92 ± 3.8	13 ± 0.6	12 ± 0.6	21 ± 1.5	
	Organic	35.1 ± 0.9		29.5 ± 0.9	5.6 ± 0.5	0.31 ± 0.05	15 ± 0.7	29.8	99 ± 4.8	12 ± 0.3	11 ± 0.4	19 ± 0.4	
	Meadow	46.9 ± 1.8		42.3 ± 1.6	4.6 ± 0.2	0.51 ± 0.06	15 ± 1.0	33.9	125 ± 6.3	14 ± 0.2	14 ± 0.1	23 ± 3.9	
20-30 cm	Conventional	13.5 ± 2.3 ^a		11.7 ± 1.8 ^a	1.8 ± 0.7	0.12 ± 0.00 ^a	57 ± 13.6	32.9	36 ± 6.7	11 ± 1.1	11 ± 0.8	16 ± 3.1	

	Organic	22.4 ± 3.9 ^b	19.0 ± 3.5 ^b	3.4 ± 0.5	0.22 ± 0.03 ^b	40 ± 10.8	32.8	58 ± 12.1	12 ± 0.7	11 ± 0.7	19 ± 1.1
	Meadow	32.9 ± 5.2	30.4 ± 4.6	2.4 ± 0.7	0.33 ± 0.07	26 ± 6.5	34.4	89 ± 15.4	15 ± 0.4	15 ± 0.3	27 ± 6.5
30-40 cm	Conventional	4.1 ± 0.3	3.9 ± 0.3	0.2 ± 0.0	0.06 ± 0.00 ^a	158 ± 17.0	32.9	12 ± 0.7	7 ± 1.6	7 ± 1.6	8 ± 1.9
	Organic	4.9 ± 0.3	4.5 ± 0.2	0.3 ± 0.1	0.09 ± 0.01 ^b	131 ± 8.0	32.8	14 ± 1.0	6 ± 0.4	6 ± 0.4	11 ± 0.8
	Meadow	9.0 ± 1.6	8.5 ± 1.4	0.5 ± 0.1	0.11 ± 0.01	98 ± 15.7	34.4	25 ± 4.9	14 ± 2.8	14 ± 2.8	10 ± 3.0
	Conventional	2.6 ± 0.1	2.5 ± 0.1	0.1 ± 0.0	0.04 ± 0.00	254 ± 4.6 ^a	34.9	7 ± 0.1	8 ± 3.3	5 ± 0.4	7 ± 1.2
40-70 cm	Organic	2.7 ± 0.1	2.6 ± 0.1	0.1 ± 0.0	0.05 ± 0.00	246 ± 1.8 ^b	33.3	8 ± 0.1	5 ± 0.2	5 ± 0.2	7 ± 0.5
	Meadow	3.3 ± 0.2	3.0 ± 0.2	0.2 ± 0.0	0.06 ± 0.00	215 ± 6.0	34.4	9 ± 0.6	8 ± 0.4	7 ± 0.5	9 ± 3.2

2.3.3 Distribution of soil OC to MAOM and POM fractions

In all the studied FD soil depths, the majority of soil C, 83-97%, was in the MAOM fraction (Table 2.A1). The proportion of soil OC in the POM fraction was at its highest close to the soil surface (0-30 cm) and decreased with soil depth. The distribution of soil OC between SOM fractions did not differ statistically between organic and conventional cropping systems but there was a tendency of higher MAOM-C, POM-C, and DOM-C in the organically managed system (Table 2.4).

In general, there was a strong positive relationship between total soil OC content and the amount of OC in all the studied SOM fractions in the cropping systems (i.e., OC was comparably shared into MAOM-C, POM-C, and DOM-C with different OC levels; Table 2.4, Figure 2.2A and B, Table 2.A1). Total OC content of the soil samples varied between 2.6-70 g kg⁻¹ (Table 2.4), and the MAOM-C was linearly related to the total OC content (Figure 2.2A). Similarly, the highest POM contents were measured in soils with the highest OC contents (Figure 2.2B), but the variation in the OC content of POM was larger than in MAOM. In the soil depth of 20-30 cm, total OC and MAOM-C were larger in the organic than conventional cropping system (Table 2.4, p-value 0.04953). The amount of DOM-C was significantly correlated to total C, and larger in the organic than in the conventional cropping system in the soil depths of 20-30 cm and 30-40 cm (p-value 0.04953). Bulk soil C to N ratios were higher in the topsoil (0-30 cm) than deeper in the soil. C to N ratios tended to be lower in the MAOM than in the POM down to 30 cm depth but below that this difference narrowed down, and the values were closer to each other (Table 2.4).

2.3.4 Soil OC saturation state, and MAOM-C and POM-C along the soil profile in relation to soil properties

When the capacity of soil to accrue OC was estimated based on the clay to OC ratio (Table 2.4), the surface soil (0-10 cm) of the unmanaged meadow had on average a clay to OC ratio of 10, indicating that the mineral surfaces were close to or at the OC saturated state. However, already in the 10-20 cm soil layer, the ratio was 15, implying an OC saturation deficit. In the cropping systems, the clay to OC ratios in the plough layer (0-20 cm) varied between 15 to 17, also indicating an OC saturation deficit. Below the soil depth of 20 cm,

all the soils had a saturation deficiency as judged by clay to OC ratios; they were larger than 10 (26-254) for all the studied soils

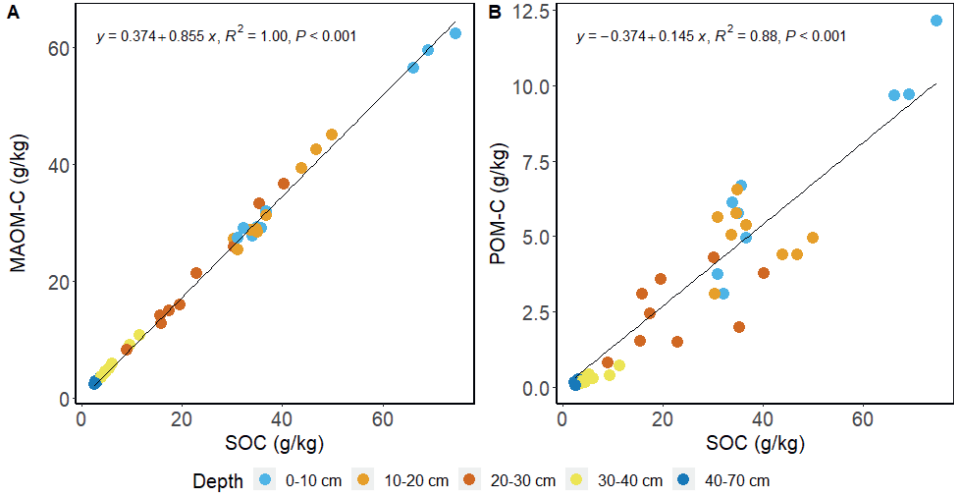


Figure 2.2. The soil organic OC content versus MAOM-C (A), POM-C (B; $n=9$ per soil depth layer). Graphs are based on the data from all soil depths in both cropping systems and the unmanaged meadow. Note differing y-axis scales.

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When estimating the saturation point of MAOM-C with the Hassink's Equation (Hassink 1997), the topsoil layers of the unmanaged meadow were above maximum saturation; soil layer 0-10 cm had reached 176%, and the layer 10-20 cm 125% of the estimated saturation

OC level. Also topsoil layers of the cropping systems were very close to or at the MAOM-C saturated state when estimated with the Hassink's Equation. In the ploughed layer, MAOM-C saturation was 97% and 99% in the conventionally managed fields and 92% and 99% in the organically managed fields in the 0-10 and 10-20 cm soil layer, respectively. However, in soil layers below 30 cm, all the soil management types had low MAOM-C and were estimated to have potential to accrue MAOM-C. It is noteworthy that despite both clay to OC ratio and Hassink's Equation showed that all topsoils were close or even above OC saturation, the OC to MAOM-C ratio (Figure 2.2A) did not show any indications of MAOM-C saturation, not even at the levels higher than 60 g kg^{-1} of total soil carbon.

In the topsoil (0-20 cm), Al-ox and clay, and Al-ox and Fe-ox were strongly correlated, whereas deeper in the soil (30-70 cm) the individual oxides were not correlated with each other but correlated with clay in different soil depths; Al-ox in 0-30 cm, and Fe-ox in 30-70 cm (Table 2.5). Spearman rank correlation analyses showed clear positive relationship between clay content and total OC and MAOM-C in the topsoil (0-20 cm, Figure 2.3). However, below 20 cm soil depth, clay content could not be connected to the total OC nor the MAOM-C or POM-C. Al-ox and Fe-ox were significantly associated with both total OC and MAOM-C in the most of studied soil layers (0-70 cm), but in the soil depth of 20-30 cm only Fe-ox correlated with total OC and MAOM-C whereas Al-ox was more strongly correlated with these below 30 cm depth. In the soil depths from 0 to 30 cm, Spearman correlation analyses did not link any of the soil properties to POM-C, but in the 30-70 cm, correlation between Al and Fe oxides and POM-C was found.

Table 2.5. Spearman rank correlation coefficients (ρ) for the soil clay content (%), aluminum (Al-ox, g kg⁻¹) and iron (Fe-ox, g kg⁻¹) oxides in different soil depths. Significant correlations bolded ($p < 0.05$).

0-20 cm				20-30 cm				30-70 cm			
	Clay	Al-ox	Fe-ox								
				Clay	Al-ox	Fe-ox		Clay	Al-ox	Fe-ox	
Clay	1			Clay	1		Clay	1			
Al-				Al-			Al-				
ox	0.56	1		ox	0.87	1	ox	-0.08	1		
Fe-				Fe-			Fe-				
ox	0.44	0.71	1	ox	0.42	0.57	ox	0.27	0.68	1	

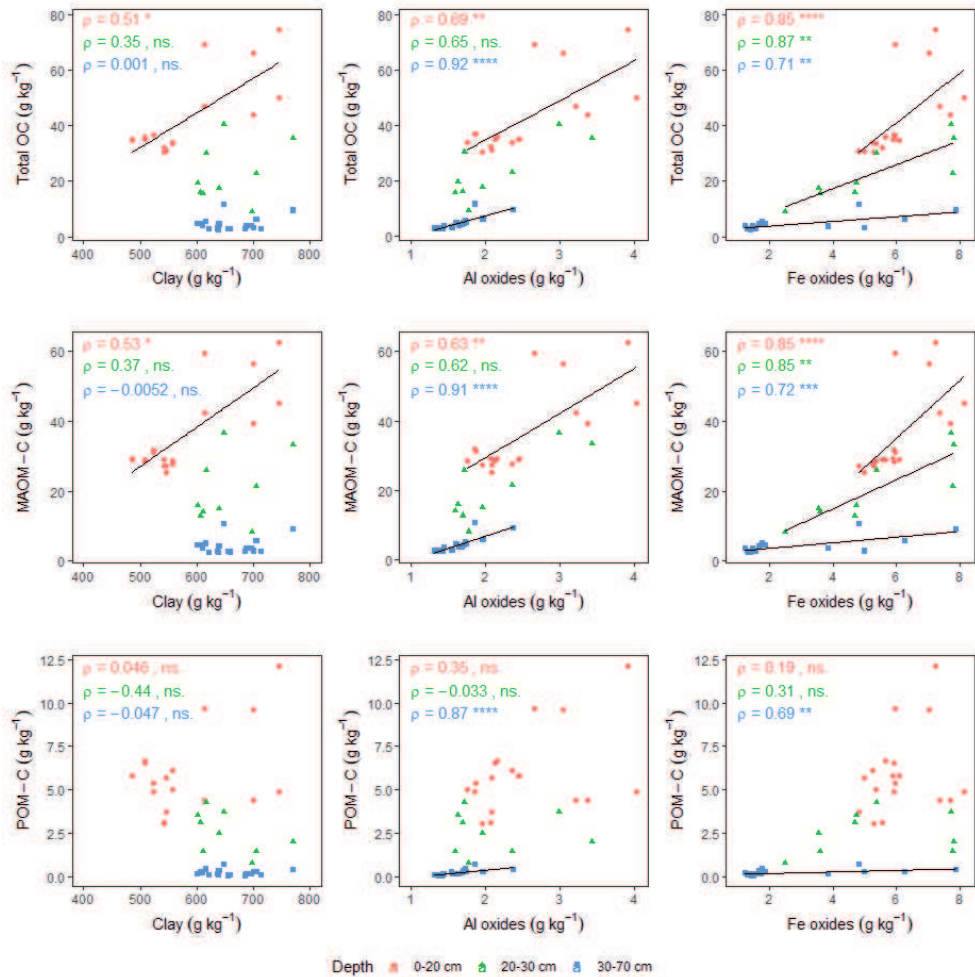


Figure 2.3. Spearman rank correlations between total soil OC, MAOM-C and POM-C, and the soil properties clay, Al- and Fe oxides (g kg^{-1}). Analysis was performed for all the soil samples from all the management systems (conventional and organic cropping systems and unmanaged meadow) in three depth intervals: 0-20 (contains depths 0-10 cm and 10-20 cm ($n=18$), 20-30 cm ($n=9$), and 30-70 cm (contains soil depths 30-40 cm and 40-70 cm, $n=18$). Significant p-values are marked as follows: $< 0.05=*$, $< 0.01=**$, $< 0.001=***$, and $0.0001=****$. In case of a significant correlation, regression line added to illustrate the relation between soil properties and OC fraction.

2.4 Discussion

2.4.1 OC stocks and plant roots across soil depths

The largest soil OC stock and plant root biomass was observed from the unmanaged meadow when compared to the conventional and organic cropping systems (Figure 2.1). The unmanaged meadow having the highest OC stocks despite not receiving additional nutrient- or OC inputs in the past 24 years, illustrates the high potential of undisturbed ecosystems with perennial plants to either accumulate new OC to the soil or both, accumulate new OC and maintain high OC stocks from the previous land use. The unmanaged meadow and the cropping systems had the same land use history as an extensively managed grassland before the start of the experiment (prior to 1995), dating back at least to the beginning of the 20th century (excluding some exceptions during 1989-1995; see Section 2.2.1). Prior to land clearing, the site of the field experiment was a forest (Gylden 1850). In general, the long-term effects of forest clearance depend on the subsequent land use: in northern latitudes, soil OC stocks tend to drop after conversion to an arable field (Guo & Gifford 2002, Heikkinen et al. 2013), but increase if the change is from forest to pasture (Guo & Gifford 2002; the unmanaged meadow studied in here may be accounted to resemble a pasture). Consistent with this, the OC stocks of the unmanaged meadow in our study were four times greater compared to the reported OC stocks of boreal forest soils around the same latitude in Scandinavia (including Finland; average of 41 t ha⁻¹ OC to one meter depth, Callesen et al. (2003)). However, as the remaining forests in Scandinavia are mainly growing on coarser soils with limited OC binding capacity and inherently more fertile, fine-textured soils have been cleared for agricultural production (Heikkinen et al. 2013), OC stock comparisons should be done with great caution and preferably taking soil texture into consideration. As the baseline OC stocks at the start of the field experiment were not measured, it is not possible to ascertain the origin of the high OC stocks of the unmanaged meadow. Nevertheless, there are indications of increase in the OC stocks as they were 15-53% larger in all the observed soil layers of the meadow than in the cultivated systems. We also found the unmanaged meadow to be closer to the OC saturation (see Section 2.4.5). Therefore, there are implications that perennial plants, with high OC input from plant litter and root exudates (e.g., Jackson et al. 2017, Bai &

Cotrufo 2022) combined with a lack of disturbances are a potentially effective means of sequestering new OC to the soil.

Focusing on the cropping systems, organically managed soil had higher OC stocks in the studied profile (0-70 cm; Figure 2.1A) than the conventional cropping system after 24 years of unchanged management. Before the start of the field experiment (in 1990), OC concentrations of the topsoil (c. 0-25 cm, see section 2.2.1) in the experimental field and in the adjacent, unmanaged meadow were uniform, and higher than the OC concentrations we measured from the cropping systems in 2019 (Table 2.4). This indicates that the change from extensively managed grassland to the crop rotations (in 1995) may have decreased soil OC in the cultivated soils whereas in the unmanaged meadow, lack of disturbance and abundant plant root biomass have enabled maintaining higher level of the soil OC stocks. Between the cultivated systems, OC levels have decreased more in the conventional than in the organic cropping system. Higher measured OC levels of the organic systems are in line with several other studies reporting that organic management practices can increase the size of the soil OC stocks (e.g., Leifeld & Fuhrer 2010, Kätterer et al. 2014, Tiefenbacher et al. 2021), although the amount of applied fertilizer-OC is an important determinant for the size of the OC stocks (Maillard & Angers 2014, Bolinder et al. 2020). As part of the manure can be decomposed relatively fast after application to the soil (Maillard & Angers 2014), the larger OC stocks in the organically fertilized soils are likely to derive from both the continuous OC additions from the applied manure and from the root inputs.

Although both studied cropping systems had similar crop rotation and soil management (see Section 2.2.1), roots were found growing deeper in the organic system (Figure 2.1B). The deeper rooting depth and larger root biomass in the organic system was not a consequence of any apparent physical limitations (such as a plough pan or higher bulk density) keeping the roots in the conventional system from growing deeper (Figure 2.A1). The differing root growth patterns in the two cropping systems may relate to a nutrient acquisition strategy of plants as the organic system received less phosphorus and nitrogen (both in total and in a soluble form) than the conventional system (see Section 2.2.1). However, it is noteworthy that organic cropping systems typically have a larger density

of weeds than conventional systems (Bårberi 2002). In this work, we did not have a possibility to distinguish which portion of the root biomass originated from the cropped plants and which from the weeds, but it is probable that the density of the weed roots was higher in the organic system. Nevertheless, roots are shown to be efficient in increasing soil OC levels in Nordic conditions as they have been shown to contribute to the OC accumulation as much as organic amendments in an organic system in Sweden (Menichetti et al. 2015). In addition to plant-root derived OC additions, ploughing (to 20 cm depth in both cropping systems) and leaching may contribute to the translocation of part of the organic fertilizer from the topsoil to deeper soil layers (Button et al. 2022), due to which roots may grow deeper to reach the nutrients mineralised from the organic fertilizer (Chapman et al. 2012).

While topsoil (0-15 cm) OC stocks in the cropping systems (54 t ha^{-1} in the conventional, and 56 t ha^{-1} organic management) were comparable to each other and to the national average for the Finnish mineral arable soil (54 t OC ha^{-1} , Heikkinen et al. (2013)), differences in total OC stocks between the cropping systems derived primarily from the organic system having a higher OC stocks in the soil depth of 10-30 cm (Figure 2.1A). In both the organic and conventional management systems, the soil is ploughed to a depth of 20 cm within 12-14 months from fertilizer application, resulting in inversion tillage being applied >10 times over a 24-year period from the start of the field experiment to our sampling. This may be an important reason for the significant difference in OC stock between the organic and conventional management systems at 20-30 cm soil depth. Boreal climatic conditions hinder the mineralisation of organic fertilizer, and this is particularly evident in the subsoil where lower oxygen levels and colder temperatures result in the longer-term preservation of OC (Ogle et al. 2019).

In the 30-40 cm soil depth, we probably observed effects of organic fertilising and/or root inputs as the OC stocks in this soil layer were 16% larger compared to the conventional managed fields. However, we cannot quantify which part of soil OC originated from organic fertilization, and which part was of plant origin. In the subsoil (40-70 cm), the differing fertilisation management had no effect on the OC stocks, suggesting that the rate of OC accumulation in the subsoil was indeed slow (Button et al. 2022). The rooting patterns of the arable crops may restrict subsoil OC accrual as the belowground OC

allocation can be less than 10% of the photosynthesized OC in the croplands (Jackson et al. 2017). The slow increase in subsoil carbon compared to higher soil depths (0-40 cm) is supported by Kätterer et al. (2014) who reported that during 13 years of field treatments in Sweden, organic amendments were responsible for as much as 27% of OC stock increase in the upper subsoil (25-40 cm), but with no observable effects on soil OC stocks below the 40 cm.

2.4.2 Distribution of soil OC to MAOM and POM fractions

The majority of soil OC was in the MAOM fraction (Table 2.A1). Throughout the soil profile, we found that only 3-17% of the OC was associated with the POM-fraction. As expected, the share of OC in the POM tended to be higher in the topsoil (10-17% of soil OC in POM in 0-20 cm) where the plant litter production and root exudation are more abundant than in deeper soil depths (3-15% of soil OC in POM in 30-70 cm). However, the proportion of soil OC in the POM fraction was lower than expected when compared to the findings of Sokol et al. (2022), who reported a global average of 35% of the total OC found in the POM fraction in mineral soils, and Guillaume et al. (2022) who reported 20% for temperate climate conditions, despite the land use. This can in part be explained by methodological observations. We utilised the fractionation method of Cotrufo et al. (2019) with the application of glass beads to assist dispersion due to the high clay content of the soils. The glass bead approach likely results in some crumbling of the larger POM fragments into finer pieces ($< 53 \mu\text{m}$). This fine-sized POM passes through the sieve to the MAOM-fraction leading to a somewhat overestimating the MAOM-C pool. On the other hand, despite the dispersion with glass beads, we observed some very stable aggregates ($> 53 \mu\text{m}$) in the POM fraction. While occluded POM inside these dispersion-resistant aggregates (often aggregates build surrounding POM; Besnard et al. 1996, Totsche et al. 2018) belongs in the POM-C pool, their mineral matrix will contain some MAOM-C, falsely determined as POM-C. Despite it being likely that some portion of OC from both SOM fractions was determined in the incorrect OC pool, we assume that this represents a relatively minor source of error as these errors would at least partly cancel each other out.

2.4.3 C to N ratios of the bulk soil and MAOM and POM fractions

C to N ratios of the total soil and MAOM were closely corresponding (Table 2.4; C in the C to N ratio can be accounted as OC). The C to N ratios of the topsoil were comparable to that found in cereal cropping systems on a clay soil in Finland (Soinne et al. 2021), but slightly higher than reported in Sweden (Börjesson et al 2018). The C to N ratio (C/N=5-15) for total soil and MAOM was lower than for POM (C/N=7-27) throughout the soil profile (0-70 cm). Our findings are comparable with the literature (Lavallee et al. 2020) as C to N ratios tend to be lower in MAOM (C/N=8-13) than in POM (C/N=10-40). In the subsoil (40-70 cm), the C to N ratio of POM (C/N=7-11) was reduced and resembled that of the MAOM (C/N=5-14), which is in line with Rumpel & Kögel-Knabner (2011) who also found C to N ratios to be higher in the topsoil than deeper in the soil. The lower C to N ratio of the POM fraction in the subsoil (below 30 cm) and its significant correlation with the Al and Fe oxides (Figure 2.3) may signal resembling quality of the operational SOM fractions MAOM and POM (Yu et al. 2022) deeper in the soil. The shift of POM C to N ratio with depth from a ratio similar to plant material in the topsoil to a ratio comparable to the microbial biomass (Miltner et al. 2012) in the subsoil implies that a larger relative share of the OC is microbially processed in the subsoil than in the topsoil (Button et al. 2022).

2.4.4 Assessing soil OC saturation state

We estimated the soil MAOM-C saturation state from the relationship between total soil OC and MAOM-C. A total OC content of 50 g kg⁻¹ is suggested to be a flex point soil OC concentration where new soil OC begins mainly to accrue to the POM-C fraction (Stewart et al. 2007, Cotrufo et al. 2019). In our data, the relationship between total soil OC and MAOM-C remained linear also above the flex point concentration (Figure 2.2A), indicating that in soils with high clay content, the MAOM-C accrual capacity may exceed the proposed maximum of 50 g kg⁻¹. Similarly, the MAOM-C content at the surface soil of the unmanaged meadow surpassed the estimate of its accrual capacity calculated according to Hassink's Equation (Hassink 1997; Table 2.4) as it was 125-176% in 0-20 cm, and the plough layers (0-20 cm) of the cropping systems were found to be near or at saturation (92-99% of the estimated accrual capacity with the Hassink's Equation). These findings indicate that assessing the MAOM-C accrual capacity based on just the soil OC

content (Cotrufo et al. 2019) or with approaches that just include the share of clay and silt fraction (e.g., Hassink 1997) will lead to an underestimation of the MAOM-C accrual capacity in heavy clay soils.

In addition to the larger specific surface area of clay-sized particles and therefore the larger mass-based sorption capacity compared to silt-sized particles, the capacity of clay soils to accrue MAOM may be enhanced due to the formation of small microaggregates ($< 20 \mu\text{m}$) in which the clay-sized particles and microbial derived organic materials act as building units (Totsche et al. 2018). Encrustation of clay-sized particles around an organic molecule can lead to the formation of very stable aggregates (Zimmermann & Horn, 2020) that are able to increase the OC protection capacity even after the full MAOM-C surface sorption potential is reached. We found very stable aggregates (or precipitates) after the MAOM-POM fractionation procedure (See Section 2.4.2), especially in the soils from the conventional and organic crop rotation systems. A reason for aggregates being more prevalent in the cropping systems may be the lower SOM content compared to the unmanaged meadow. In the meadow systems, high OC levels may lead to complexation of Al and Fe with SOM, which may disturb the further precipitation of these metals towards more crystalline forms and therefore reduce their aggregate-stabilizing effect in the unmanaged meadow (Hall & Thompson 2022). However, as the interactions with SOM and metals in oxide precipitation and dissolution processes and aggregate formation are very complex, more research to verify the origin of these very stable aggregates is needed.

We also used the clay to OC ratio for assessing the C- saturation state of the soil. Dexter et al. (2008) suggested that the maximum capacity of mineral soils to bind OC would be reached at a clay to OC ratio of 10 as 1 gram of OC can be at maximum complexed by 10 grams of clay. We found that the clay to OC ratio in the uppermost surface soil (0-10 cm) of the unmanaged meadow was 10 (Table 2.4), suggesting that based on this measure, the topsoil of the meadow has reached C saturation. The clay to OC ratios also suggested that at most soil depths the cropping systems were more carbon deficient than in the unmanaged meadow, except for the soil layer of 10-20 cm where comparable clay to OC ratios were found (ratios between 15-17). The similarity of ratios in this soil layer is probably caused by the ploughing of the cropping systems as this introduces organic

matter below the topmost surface soil layer (here, below 10 cm), leading to a relatively homogenous OC content throughout the plough layer (in this experimental field, 0-20 cm). At the same time, soil OC content in the unploughed meadow was found to be highest between 0-10 cm and decline in the 10-20 cm layer, leading to an abrupt increase in clay to OC ratio (i.e., the soil was further away from OC saturation) below the top layer of the soil.

Below the depth of 30 cm, we found high (from 26 to 254) clay to OC ratios in all the studied management systems, implying potential for further MAOM-C accrual. Also, this demonstrates that reaching the OC saturation below the top soil without active incorporation of external OC inputs (for example by ploughing) is likely unattainable. In the deepest studied soil depth (40-70 cm), the clay to OC ratio of the organically managed system was significantly lower than in the conventional system. At such deep soil depths all systems remained well away from the OC saturation as their clay to OC ratios were higher than 200. However, it may not be meaningful to estimate the MAOM-C accrual capacity of the deeper soil layers (below 40 cm) based on the same metrics as in the surface soil layers, since physical, biological and often also chemical properties differ from the surface soil (Salome et al. 2010, Liebmann et al. 2020) which may hamper the OC accrual in the deep soil.

2.4.5 Role of clay, and Al and Fe oxides in transporting and storing OC

In addition to management induced translocations, OC moves down in the profile via leaching as DOM-C (Kaiser & Kalbitz 2012, Button et al. 2022) and attached to mineral surfaces as MAOM-C with clay illuviation (Yli-Halla et al. 2009, Torres-Sallan et al. 2017). The larger DOM-C concentrations in the soil layers of 20-30 and 30-40 cm in the organic cropping system may indicate dynamic cycling of OC originating from the organic amendments and rhizodeposition. Downward movement of OC can be facilitated by large biopores and other cracks, such as old root channels and earthworm burrows, that form preferential flow paths in heavy clay soils as the one studied here (Shipitalo et al. 2004). Deeper in the soil, DOC may be stabilized again by interactions with Fe and Al oxides (Rumpel & Kögel-Knabner 2011). In addition, downward movement of OC can be initiated when organic acids (for example root exudates) enhance the dissolution of minerals and therefore increase the solubility of aluminum and iron (Bais et al. 2006).

Soluble complexes of Al and Fe with organic ligands can leach through the soil profile during which the physicochemical properties of the surrounding soil support their alternating precipitation and re-mobilisation cycles (i.e. podzolisation; Lundström et al. 2000). Therefore, the tight relationship between Al and Fe oxides, OC and MAOM-C along the soil profile may also indicate DOC-driven relocation of dissolved metals in which case the oxalate extractable Al and Fe would be responding to instead of driving the OC content as was suggested by Hall & Thompson (2022). Whichever the mechanism that translocates OC in the soil profile, the observed positive relationship between OC and DOC encourages the strive for reaching high OC content in the surface soil. The infiltration and percolation of water into the soil profile can support a continuous yet slow seeping of OC down into the soil profile, where it could potentially be stored for a long time.

2.5 Conclusions

After 24 years from the start of the field experiment, the organically managed system had larger OC stocks (17 t ha^{-1} in the 0-70 cm) compared to the conventionally managed cropping system (total stock size: 129 and 112 t ha^{-1} in the organic and conventional system, respectively). Specifically, the organically managed soil had higher stocks between 10-30 cm depth. The higher OC content of this soil layer points towards ploughing-induced translocation of the organic amendments and/or increased leaching of OC originating from surface applied organic fertilizer, and specifically for the organic system possibly increased OC inputs due to a larger root biomass compared to the conventional system. However, the highest total OC stock was found in the unmanaged meadow (169 t ha^{-1}) which was part of the same field, indicating that there is significant potential for increasing or maintaining soil OC stocks via OC sequestration by plants. In the unmanaged meadow, the MAOM-C content of the uppermost 10 cm of the soil was nearly 60 g kg^{-1} and did not show signs of saturation, which demonstrates a high potential of heavy clay soils (in here, $> 530 \text{ g kg}^{-1}$ of clay) for the OC accrual. In all of the studied management systems, the MAOM-C content of the surface soil was tightly related to the clay content, but deeper in the soil profile this relationship was not detected. Instead, in deeper soil layers both MAOM-C and POM-C were positively related to Al and Fe oxides, which indicates that oxides play a significant role in transporting and stabilizing OC in

soils. This suggests that when estimating the OC accrual potential of deeper soil layers (below 40 cm), estimations should not be based on soil texture alone. Further, the slow rate of OC inputs reaching deeper soil layers and/or biogeochemical constraints preventing OC stabilization may reduce the ability to increase subsoil OC stocks. However, at least some of the OC that enters the subsoil can potentially be retained for a long time.

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Conflict of interests

The authors declare no conflict of interest.

Appendix

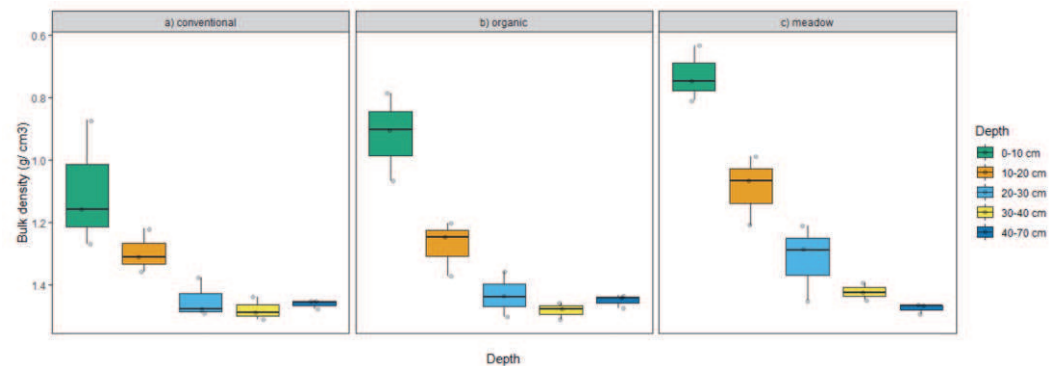


Figure 2.A1. Soil bulk densities ($n=3$). No statistical differences between conventional and organic management.

Table 2.A2. Average ($\pm SE$, $n=9$) soil depths in which the equal soil masses were fulfilled.

ESM t ha ⁻¹	Management	Soil depth (cm)
1500	Conventional	14.5 $\pm$ 0.7
	Organic	15.2 $\pm$ 0.6
	Meadow	18.8 $\pm$ 0.5
3000	Conventional	25.9 $\pm$ 0.7
	Organic	26.8 $\pm$ 0.9
	Meadow	31.2 $\pm$ 0.7
7500	Conventional	57.0 $\pm$ 0.8
	Organic	58.0 $\pm$ 1.2
	Meadow	62.3 $\pm$ 0.8

Table 2.A1. Proportions (%) of soil OC and sample mass in the MAOM and POM fractions. For all the studied soil layers, proportion of the total soil OC in the DOM was < 0.032% (not shown in the table). No statistical differences were observed between conventional and organic management.

Soil depth	Management	Proportion of the total soil OC (%) in the fraction		Proportion of the sample mass (%) in the fraction	
		MAOM	POM	MAOM	POM
0-10 cm	Conventional	87 ± 2.0	13 ± 9.5	84 ± 1.9	16 ± 1.9
	Organic	83 ± 1.7	17 ± 10.5	84 ± 0.3	17 ± 0.3
	Meadow	85 ± 0.6	15 ± 9.2	86 ± 1.5	14 ± 1.5
10-20 cm	Conventional	85 ± 2.5	15 ± 10.7	83 ± 1.0	17 ± 1.0
	Organic	84 ± 1.3	16 ± 8.4	84 ± 1.2	16 ± 1.2
	Meadow	90 ± 0.2	10 ± 5.6	88 ± 2.3	12 ± 2.3
20-30 cm	Conventional	87 ± 3.9	13 ± 12.1	86 ± 3.2	14 ± 3.2
	Organic	85 ± 1.8	15 ± 7.3	87 ± 0.8	14 ± 0.8
	Meadow	93 ± 1.0	7 ± 5.2	88 ± 3.0	12 ± 3.0
30-40 cm	Conventional	95 ± 0.6	5 ± 3.3	93 ± 2.6	7 ± 2.6
	Organic	94 ± 1.0	6 ± 3.9	95 ± 1.3	5 ± 1.3
	Meadow	95 ± 0.6	5 ± 2.4	93 ± 2.2	7 ± 2.2
40-70 cm	Conventional	96 ± 0.3	4 ± 1.7	96 ± 1.1	4 ± 1.1
	Organic	97 ± 0.4	3 ± 1.5	96 ± 1.5	4 ± 1.5
	Meadow	93 ± 1.5	7 ± 3.7	95 ± 0.9	5 ± 0.9

Chapter 3

Soil organic carbon fractions and storage potential in Finnish arable soils

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Abstract

Understanding the factors affecting the total amount and distribution of soil organic carbon (OC) across different functional carbon pools is important to better define future management of soil OC stocks. The interactions between soil management practices, local physicochemical soil properties, and climate are essential for determining the OC content of the soil. Nevertheless, how these factors affect the total amount of OC and its distribution across carbon pools, i.e., more labile particulate (POC) and more stable mineral-associated (MAOC) organic carbon, is only partly known. In this study we assessed topsoil (0-20 cm) samples from 93 arable farms in the southern half of Finland to determine the total amount of OC, and its distribution in MAOC and POC, along with relevant soil properties (amount of clay and silt, aluminium and iron oxides and pH), climate (precipitation and temperature), and fertilization (mineral versus organic). The fertilization did not affect the total soil carbon content (12-58 g OC kg⁻¹ soil). The share of OC in the MAOC fraction (on average 86% of total OC) was relatively stable across the large range of OC contents and clay contents (2-68%). We assessed the highest feasible MAOC of the soils with boundary line analyses and their OC saturation state with Hassink's equation (Hassink 1997). Only soils with the lowest clay content (<10% clay) were assumed to be carbon saturated, suggesting that most of the studied soils have a capacity to accrue more MAOC. Simple linear regression showed that clay, aluminium and iron oxides explained 9, 21 and 22% of the variation in MAOC, respectively. Multiple regression analyses including the amount of clay, clay+silt, aluminium and iron oxides, pH, type of fertilization, precipitation, and temperature as explanatory variables explained 33-53% of the variation in OC and MAOC. In all soils, aluminium oxides were important explanatory variable for MAOC, whereas Fe oxides were significant only in soils with higher clay content (>30%). In soils with a low clay content (<30%), pH had added value in explaining MAOC. Altogether, it seems that various climatic, edaphic and soil management-related factors are context-dependently controlling OC, and that soil textural information alone is not necessarily an adequate predictor to assess the MAOC saturation state of the soil.

3.1 Introduction

Soil organic matter (SOM) is recognized as key for a multitude of soil functions (Wiesmeier et al. 2019, Hoffland et al. 2020), one of which, climate regulation, a potential sink for atmospheric carbon dioxide (CO₂; Paustian et al. 2016) is receiving a lot of attention. Agricultural management has resulted in the depleted soil organic carbon (OC) globally (Paustian et al. 2016, Sanderman et al. 2017). There is now a strong focus on carbon farming practices which promote the incorporation of organic matter into soils as part of agricultural management to maintain or increase soil carbon stocks. However, to date, our mechanistic understanding of the soil processes relating to the soil OC dynamics and stabilization are still inadequate and results in variable outcomes in relation to carbon farming practices (Angst et al. 2023).

SOM is a complex mixture of organic molecules in different phases of decomposition (Schmidt et al. 2011) and hence present in the soil in a multitude of forms (Simpson & Simpson 2012). To determine the forms of SOM in the soil, it is often fractionated into mineral-associated (MAOM) and particulate (POM) organic matter (Tiessen & Stewart 1983, Cambardella & Elliott 1992, Lavalée et al. 2020). In mineral soils, the majority of OC is often present as mineral-associated organic carbon (MAOC; Kögel-Knabner et al. 2008, Georgiou et al. 2022) which is regarded as a relatively stable and long-lasting form of soil OC since its interactions with soil minerals guards it against further decomposition (Lavalée et al. 2020). In contrast, particulate organic carbon (POC) has only limited interaction with soil minerals and is hence considered more labile than MAOC, but soil aggregation or soil physicochemical status may still slow the rate of its decomposition (Lavalée et al. 2020). Around half of MAOC occurring in arable soils is presumed to be of microbial origin and half directly of plant origin (Angst et al. 2021), whereas POC comprises of relatively undecomposed plant material (Lavalée et al. 2020).

MAOC is considered important from a climate mitigation perspective, as it potentially decomposes slower than POC and can form a relatively stable OC storage (Lavalée et al. 2020). The more labile POC is used as an energy source for soil biota (Yu et al. 2022) which in turn also becomes a source for MAOC production (Angst et al. 2023). Approximately 20% of the OC content of an arable soil is considered to be POC (Guillaume et al. 2022, Mayer et al. 2022, Sokol et al. 2022), however, the POC pool is

considered to be dynamic which means its pool-size can readily change as the result of soil management interventions. One of the soil management practices to which total OC, POC and MAOC may respond on a relatively short timescale is the type of fertilization applied (Angst et al. 2023). The application of organic fertilizers introduces OC in the form of POC into the soil, but depending on climatic and edaphic factors, most of the OC may decompose relatively soon after application (Maillard & Angers 2014, Mayer et al. 2022), meaning that maintaining a certain level of soil OC stocks would require a continuous input of organic materials (Powlson & Neil 2021). Decomposition of the added organic materials results in respiration of some of the OC in the form of carbon dioxide (CO₂) and therefore a loss of OC from the soil. The rate of this loss is dependent on the temperature, moisture and quality of the organic materials (Powlson & Neil 2021, Berthelin et al. 2022). However, this process also results in assimilation of soil organic carbon into the microbial biomass, and this biologically processed OC contributes to a relative higher proportion of OC in the MAOC fraction (Angst et al. 2021).

The long-term stabilisation of OC in the form of MAOC is proposed to be strongly dependent on soil properties such as mineral particles and various metal oxides, and soil pH (Rasmussen et al. 2018, Wiesmeier et al. 2019). The amount of clay sized (<2 µm) and silt (<20 µm or <60 µm) particles has traditionally been considered as an adequate predictor of the soil OC storing capacity (Dexter et al. 2008, Feng et al. 2013, Wiesmeier et al. 2019) as the proportion of these particles provides a reference of mineral surface area available for MAOC binding. Furthermore, clay is a paramount factor in soil aggregation which may hinder further OC decomposition as aggregates often form around POC particles, making it physically inaccessible for decomposer organisms (Besnard et al. 1996, Totsche et al. 2018). However, despite the acknowledged importance of the clay-sized soil minerals for OC storage, it remains unclear what the limitations are for the maximum binding capacity for MAOC on these mineral surfaces, and some studies find no upper limit for the operationally defined MAOC (Matus 2021, Begill et al. 2023, Salonen et al. 2023, Urbanski et al. 2023).

Finnish soils are relatively young as they were formed by Weichselian glaciation around 10,000 years ago (Koljonen 1992). During the glaciation, soils and their clay and silt-size minerals were formed from rock by abrasion of the ice. The felsic parent material (Yli-

Halla & Mokma 2002) from which these soils are formed are strongly durable and resistant to in-situ weathering, leading to a relatively large proportion (more than 50%; Keskinen et al. 2022) of the minerals in the clay and silt-sized fractions of Finnish soils being only moderately weathered. This means that they have a relatively low surface activity in comparison to soils that are further weathered and hence containing a larger share of 1:1 or 2:1 type of clay minerals that are important in stabilising soil OC (Six et al. 2002, Georgiou et al. 2022, Six et al. 2023).

As soil mineralogy is spatially variable, so are the associated surface properties such as charge density and surface area (Mitchell & Soga 2005) and soils with corresponding clay+silt content may have differing types of mineral surfaces. Therefore, the surface activity and MAOC storage capacity may be different despite a similar clay+silt content (Feng et al. 2013, Georgiou et al. 2022). Different metrics applying the amount of specific sized soil minerals (i.e., clay and/or silt) or the type of the dominating clay mineral (i.e., 1:1 or 2:1) can be used to assess the OC saturation state of soil and to estimate OC accrual potential of soil (e.g., Hassink 1997, Dexter et al. 2008, Schjønning et al. 2012, Feng et al. 2013, Georgiou et al. 2022, Six et al. 2023). Estimating the soil OC saturation state can provide information on the OC sequestration potential of agricultural soils, which can be potentially useful from the climate mitigation perspective since soils can act as a carbon sink (Keenan & Williams 2018, Paustian et al. 2016).

In addition to clay and silt, oxides of aluminium (Al) and iron (Fe) can act efficiently as OC binding agents (Wiseman & Püttmann 2006, Wiesmeier et al. 2019, Mendez et al. 2020, Hall & Thompson 2022). Their oxalate-extractable species (Al oxides and Fe oxides) have been shown to be better predictors for soil OC than soil texture (i.e., clay or clay+silt content) in soils of humid cold climates with relatively low pH (Rasmussen et al. 2018, Wiesmeier et al. 2019, Fukumasu et al. 2021, Salonen et al. 2023). Such soils are typical of boreal conditions in Finland (Metzger et al. 2012), where relatively cold mean annual temperatures and prevalent moist conditions can hamper SOM decomposition (Wiesmeier et al. 2019), and prevalent moist conditions may lead to relatively low soil pH which may further retard microbial decomposition of soil carbon (Keiluweit et al. 2016, Slessarev et al. 2016).

Our objectives were: i) to assess the amount of OC, and its distribution across MAOC and POC carbon fractions in arable soils receiving organic or mineral fertilization; ii) to estimate the OC saturation state of the studied soils; iii) to assess which soil properties (clay, silt, Al and Fe oxides, and pH) explain the different proportions of the carbon fractions; and iv) to determine whether climatic factors, i.e. precipitation and temperature, control the total amount of OC and its distribution into MAOC and POC. We analysed topsoil (0-20 cm) samples from 93 farms with varying soil textures (clay content 2-68%) located across the southern half of Finland (60° N – 65° N) for the total amount of OC and its distribution between MAOC and POC. We used a boundary line analyses (Feng et al. 2013, Georgiou et al. 2022, Six et al. 2023) to assess the highest feasible MAOC values for these soils under the current agricultural management. Further, the MAOC saturation state of the soil was assessed with Hassink's equation (Hassink 1997). We hypothesized that: 1) there will be higher levels of total OC, MAOC and POC in organically fertilized soils due to higher inputs of organic matter; 2) as agricultural management depletes soil OC stocks, the studied soils will not be OC saturated; 3) Al- and Fe oxides will be better predictors of MAOC than clay content, as they act as the preferential binding sites; and that 4) soil OC concentration increases with increasing precipitation and decreases with increasing temperature.

3.2 Materials and methods

3.2.1 Soil sampling and basic soil characterisation

Soils samples were collected during years 2018 and 2019 right after establishing the Finnish Carbon Action project (Mattila et al. 2022) focusing on the effects of a range of soil management practices that are estimated to have the potential to increase soil OC levels. At the start, participating farmers selected a field within their farm for the project and reported its soil management history for the 5 years preceding the start and baseline soil sampling (i.e., for the years 2014-2018 or 2015-2019, depending on the year that the farm entered the project). Studied soils were in arable use and there were no permanent grasslands included. Most of the farms had cereals (n=78) and grasses (n=52) included in the five-year crop rotation before the soil sampling. Based on the soil management history from five years before the soil sampling, the studied farms were divided into two groups: farms applying mainly mineral fertilizers and farms applying mainly organic fertilization.

The quality and amounts of applied fertilizing (in both, farms that applied mineral and organic fertilizing) varied from farm to farm, considering that the applied maximum amounts of nutrients were in line with the national regulations. Individual rates of the amounts of nutrients applied with fertilizing were not available. The farms were located across the southern half of Finland (60° N – 65° N; Figure 4.1). Long-term mean annual precipitation (MAP) in the studied farms was 636 mm, with a median of 631 mm (range 548-727 mm year⁻¹). Long-term mean annual temperature (MAT) was 5.1 °C, with a median of 5.2 °C (range of 3.3-6.4 °C). MAP and MAT are 19 year averages before the soil sampling (years 1998-2017; Finnish Meteorology Institute. Data from the weather stations closest to each farm).

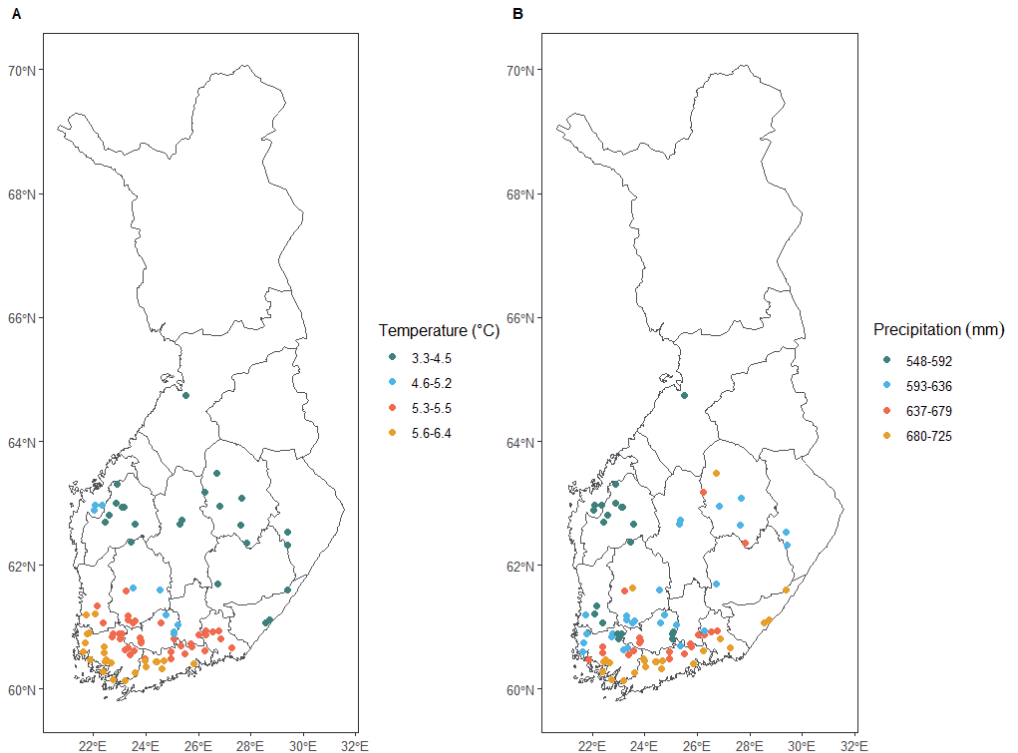


Figure 3.1. Locations of the studied farms in Finland and mean annual temperature (1A, MAT, °C) and precipitation (1B, MAP, mm). MAT and MAP are 19 year averages.

The samples analysed in this study were taken from three geo-referenced sampling points per field as part of the baseline sampling. Baseline sampling took place in autumn of the start year, after the last harvest, before the carbon farming practices were commenced. The topsoil (0-20 cm) was sampled by the participating farmers who collected 10 soil core ($\varnothing$ 14 mm) samples on the circumference of a 10 m radius circle. The 30 samples were bulked to form one composite sample which was sent in a plastic container by a courier service to the University of Helsinki (Helsinki, Finland), all samples arrived within three days of sampling. Soils were sieved (mesh size 10 mm) and part of the samples were stored in -20°C prior pending further analyses, and the other part was analysed for elemental C and N (LECO, Michigan, USA).

3.2.2 MAOM and POM fractionation, Al and Fe oxide extraction, and soil textural analyses

Soils were fractionated by size into MAOM ($<53\ \mu\text{m}$) and POM ($>53\ \mu\text{m}$) according to Cotrufo et al. (2019). In short, frozen soils (stored in -20°C prior the analyses) were melted in $+4^{\circ}\text{C}$, sieved (mesh size 2 mm) and air-dried. Then the soil samples were dispersed by shaking in $5\ \text{g l}^{-1}$ sodium hexametaphosphate and glass beads for 18 h at 120 rpm (Ika LabortechnikKS 501 digital). After the dispersion, the soil was rinsed on a sieve (mesh size $53\ \mu\text{m}$). The soil that passed through the sieve was collected as MAOM ($<53\ \mu\text{m}$), and the soil on top of the sieve was collected as POM ($>53\ \mu\text{m}$). Fractions were dried to a constant weight in a forced-air oven ($60\ ^{\circ}\text{C}$). To confirm reproducibility of the soil dispersion, two internal reference soils (clay- and coarse textured soil) were included in each set of fractionated soils. Samples were analysed for OC (MAOC and POC) by dry combustion (LecoCHN 628, St. Joseph, Michigan, USA). OC recovery was on average 97% and ranged from 100 to 84%. All the measured C was taken to represent OC as due to the inherently low pH in Finnish soils, carbonate minerals are absent (Nelson & Sommers 1996).

Poorly crystalline Al- and Fe-oxides were extracted with acid ammonium oxalate extraction according to Niskanen (1989; 0.05 M oxalate, pH 3.3). Prior to the analysis, soil was stored in -20°C , then defrosted to $+4^{\circ}\text{C}$, sieved (mesh size 2 mm) and air-dried. We used 5 g of air-dried soil in the extraction. Al and Fe were measured with inductively

coupled plasma mass spectrometry (ICP–OES Thermo Scientific iCAP 6300 MFC DUO).

Soil texture (size classes: clay <2 µm; fine silt 2–20 µm; coarse silt 20–60 µm; fine sand 60–200 µm; and coarse sand >200 µm) was analysed with the pipette method (Elonen 1971). Following the protocol, soils were pre-treated with H₂O₂ for SOM removal, and then acidified with 2 M HCl and dispersed with 0.05 M Na₄P₂O₇.

3.2.3 Estimations of soil OC saturation and OC accrual potential

We applied a modified boundary line analysis to estimate the maximum capacity for MAOC stabilization (as in Feng et al. 2013, Georgiou et al. 2022, Six et al. 2023). In the boundary line analyses, the soils having the highest MAOC contents were used in analysing a feasible maximum for OC accrual in the studied climate and under the current arable land management. For the analysis, data were sorted by mass proportions of clay+silt (<60µm) particles in bulk soil (g kg⁻¹ soil), and then separated into groups with intervals of 100 g of the clay+silt fraction kg⁻¹ soil. Next, the upper tenth percentile of the MAOC content of clay+silt particles in each group were identified, and then the upper tenth percentile of the MAOC contents of clay+silt particles and the corresponding mass proportions from each group were combined and used in a regression analysis where the intercept was forced through zero. We then used the slope from the boundary line analyses (Figure 3.2) to estimate the highest feasible MAOC content (g OC kg⁻¹ clay+silt) for the studied soils as follows (Table 3.2): Maximum MAOC = clay+silt content (%) * maximum MAOC for the studied soils (55 g C kg⁻¹ silt+clay). We also estimated the maximum MAOC for the studied soils assuming 2:1 or 1:1 clay mineral dominated (Six et al. 2023) as follows: Maximum MAOC= clay+silt content (%) * maximum MAOC for 1:1 (46 g C kg⁻¹ silt+clay) or 2:1 (82 g C kg⁻¹ silt+clay).

Soil texture was used to estimate the MAOC accrual capacity for each soil (Table 3.2). We calculated the capacity of fine-sized particles to stabilise MAOC using the Hassink's (1997) equation: MAOC (g kg⁻¹) at OC saturation = 4.09+0.370 × (clay+silt%). For clay and silt, we used two size cut-offs: clay+silt <20 µm, as in Hassink 1997, and clay+silt <60 µm. Larger clay+silt (<60 µm) was included as it is suggested that minerals up to this size have relevant specific surface area for MAOC binding (Six et al. 2023). These accrual capacity estimations were compared with measured MAOC to assess the potential

for further MAOC accrual of the soils. Further, we divided soils into saturated and non-saturated according to Hassink's equation (with clay+silt size cut-off $<20\ \mu\text{m}$) to test the effect of fertilizing into the distribution of soil OC into MAOC and POC (Table 3.3).

3.2.4 Data analyses

Out of the 105 farms participating in the Carbon Action project, soil samples from 97 were available for our analyses. Among these 97 farms, there were four soils that were classified as highly organic (OC $> 7\%$, OM 12-20%; Paasonen-Kivekäs et al. 2009) according to the Finnish soil classification system. As these highly organic soils had considerably higher OC content than the rest of the studied soils, we excluded them from the statistical analyses, leaving 93 soils for a part of the statistical analyses.

For three out of the remaining 93 farms, details on the fertilization history were not available (not reported by farmers) and hence these three farms were also excluded from the statistical analyses where fertilization (mineral vs organic) was assessed. The remaining 90 farms were divided into two groups based on their fertilization history (mineral, $n=46$, and organic, $n=44$). Two-way ANOVA with a Tukey HSD test (package 'stats') was conducted to evaluate statistical differences between OC and nitrogen (N) concentrations and OC to N ratios of total soil, MAOM and POM, OC contents of MAOM and POM, soil pH, and clay+silt content ($\%;$ $<60\ \mu\text{m}$) in mineral and organically fertilized soils grouped by clay content (0-100, 100-200, 200-300 g clay kg^{-1} soil etc). Division to the clay content groups was chosen as some studies have reported increasing OC content with increasing clay content (Hassink 1997, Kaiser & Guggenberger 2003, Arrouays et al. 2006).

Further, to assess whether fertilization (mineral vs organic) would yield different outcomes depending on the soil OC saturation state, we divided soils into two groups: MAOC-saturated and non-saturated. We then employed a t-test (using the 'rstatix' package; Kassambara, 2022) to evaluate differences in OC and N contents.

We performed a multiple linear regression to identify predictors for soil OC and MAOC. The selection of the best explanatory variables was performed on an AIC-driven model selection approach ('dredge' function from the package MuMIn (Barton 2018). As explanatory variables, we used textural class (clay $<2\ \mu\text{m}$ or clay+silt $<60\ \mu\text{m}$ (g kg^{-1} soil;

see details below), amount of Al- and Fe-oxides (g kg^{-1} soil), pH, cumulative annual rainfall (mm; yearly average from the 19 year time period 1998-2017), mean annual temperature ($^{\circ}\text{C}$; yearly average from 19 year time period 1998-2017), and fertilization (mineral vs organic fertilizers). We divided soils into two groups according to their clay content: 1) $<30\%$ of clay and 2) $>30\%$ of clay. The 30% cut-off was chosen as according to the Finnish soil classification system clay soil has $>30\%$ of clay (Paasonen-Kivekäs et al. 2009). The variance inflation factor for the covariates of the models was checked using the 'vif' function of the car package (Fox & Weisberg 2019) and this was found to be <2 , meaning that no co-linearity of the covariates of the best-fit models was observed. We inspected the distribution of the residuals of the final models visually to check that model assumptions were not violated.

All statistical analyses were performed with R (R Core Team 2023, version 4.3.1), and all the plots were created using R packages 'ggplot2' and 'ggpubr' (Wickham et al. 2016, Kassambara 2020). We regarded p-values of 0.05 or less as statistically significant.

3.3 Results

3.3.1 MAOC and POC across clay contents

Soil OC and N contents (g kg^{-1} soil) tended to increase with increasing clay content, with the exception of the clay content interval of 200-300 g kg^{-1} which had less OC than the previous clay intervals, and the clay interval 300-400 g kg^{-1} that had the highest measured OC content (Table 3.1). N contents were significantly higher in soils having $>400 \text{ g kg}^{-1}$ clay than in soils that contained less clay. OC content (C%) of the $<53 \mu\text{m}$ soil fraction (i.e., MAOM) was highest in the lowest clay content interval (0-100 g kg^{-1} , p-value <0.05). In contrast, the OC content (C%) of the $>53 \mu\text{m}$ soil fraction (i.e., POM) increased with increasing clay content.

The proportion of the total OC in the MAOM fraction varied between 83-89% (Supplementary Table 3.2). The total amount of MAOC expressed per g OC kg^{-1} total soil was lowest in the clay content interval of 200-300 g kg^{-1} and tended to be different in the soils with differing clay contents, whereas the total amount of POC expressed per g OC kg^{-1} total soil did not differ between the clay classes. The bulk soil C to N ratio in the different clay classes was 15 on average (Table 3.1), and a significantly higher ratio (C to

N ratio=18) was observed in the 0-100 g per kg clay content interval compared to the soils with a higher clay content (C to N ratio=12-14). Such a decrease in C to N ratio with increasing soil clay content was found also for MAOM (decrease from C to N ratio 18 to 12), but not for POM, where the C to N ratio increased from 18 to 23. No statistically significant differences were observed in soil pH between the different clay content intervals.

There were no statistically significant differences observed in the carbon and nitrogen associated parameters across the clay content intervals for either mineral or organically fertilized sites (Supplementary Table 3.1).

Table 3.1. Average ($\pm$ SE) organic carbon and nitrogen concentration (g kg^{-1} soil) of total soil, the C to N ratio of the total soil, MAOM ($<53 \mu\text{m}$ soil) and POM ($>53 \mu\text{m}$ soil), the OC concentration (g kg^{-1} soil) and contents (%) of MAOC and POC in the <53 and $>53 \mu\text{m}$ soil mass fraction, respectively, and soil pH in different clay classes by 100 g kg^{-1} intervals. Letters denote statistical differences (Tukey's HSD, significance level <0.05). Clay and silt content not tested for statistical differences. n = number of farms sampled (in total n=93). The horizontal line between the clay classes of 200-300 g kg^{-1} and 300-400 g kg^{-1} indicates the cut-off between low clay ($<30\%$) and high clay ($>30\%$) soils.

Clay (g kg^{-1})	n	Total OC (g kg^{-1})	Total N (g kg^{-1})	MAOM		POM		POM		pH
				Soil CN	C-% ($<53\mu\text{m}$ soil)	MAOC (g kg^{-1} soil)	MAOM CN	C-% ($>53\mu\text{m}$ soil)	POC (g kg^{-1} soil)	
0-100	14	24.3 $\pm$ 3.1 ^{ab}	1.90 $\pm$ 0.16 ^a	18 $\pm$ 0.9 ^a	7.1 $\pm$ 0.9 ^a	20.2 $\pm$ 2.7 ^{ab}	18 $\pm$ 1.0 ^a	0.7 $\pm$ 0.1 ^a	4.1 $\pm$ 0.6	6.43 $\pm$ 0.19
100-200	12	29.0 $\pm$ 3.6 ^{ab}	1.90 $\pm$ 0.16 ^a	15 $\pm$ 0.8 ^b	4.0 $\pm$ 0.6 ^b	25.3 $\pm$ 3.2 ^{ab}	14 $\pm$ 0.8 ^b	1.8 $\pm$ 0.4 ^a	3.7 $\pm$ 0.5	6.38 $\pm$ 0.15
200-300	12	22.6 $\pm$ 1.4 ^a	1.69 $\pm$ 0.09 ^a	13 $\pm$ 0.5 ^b	2.6 $\pm$ 0.3 ^b	18.7 $\pm$ 1.3 ^a	13 $\pm$ 0.4 ^b	2.5 $\pm$ 0.5 ^{ab}	3.9 $\pm$ 0.8	6.33 $\pm$ 0.12
300-400	23	33.9 $\pm$ 2.3 ^b	2.24 $\pm$ 0.11 ^{ab}	15 $\pm$ 0.4 ^b	3.4 $\pm$ 0.2 ^b	29.3 $\pm$ 2.1 ^b	14 $\pm$ 0.4 ^b	5.4 $\pm$ 0.9 ^b	4.6 $\pm$ 0.4	6.38 $\pm$ 0.09
400-500	8	31.1 $\pm$ 3.4 ^{ab}	2.35 $\pm$ 0.33 ^b	14 $\pm$ 0.6 ^b	3.3 $\pm$ 0.4 ^b	27.8 $\pm$ 3.3 ^{ab}	13 $\pm$ 0.6 ^b	3.5 $\pm$ 0.8 ^{ab}	3.4 $\pm$ 0.3	6.24 $\pm$ 0.13
500-600	12	30.1 $\pm$ 1.2 ^{ab}	2.20 $\pm$ 0.06 ^b	14 $\pm$ 0.4 ^b	3.0 $\pm$ 0.1 ^b	26.2 $\pm$ 1.2 ^{ab}	13 $\pm$ 0.4 ^b	3.9 $\pm$ 0.9 ^{ab}	3.9 $\pm$ 0.5	6.35 $\pm$ 0.10
600-700	12	32.6 $\pm$ 2.7 ^{ab}	2.45 $\pm$ 0.2 ^b	13 $\pm$ 0.4 ^b	3.1 $\pm$ 0.3 ^b	28.0 $\pm$ 2.4 ^{ab}	12 $\pm$ 0.3 ^b	5.8 $\pm$ 0.6 ^b	4.5 $\pm$ 0.7	6.40 $\pm$ 0.11

3.3.2 The OC saturation status of the soils under current agricultural management

With the boundary line analyses, the estimated feasible practical maximum MAOC in the studied climate and under the current agricultural management was 55 g MAOC kg⁻¹ clay+silt (Figure 3.2).

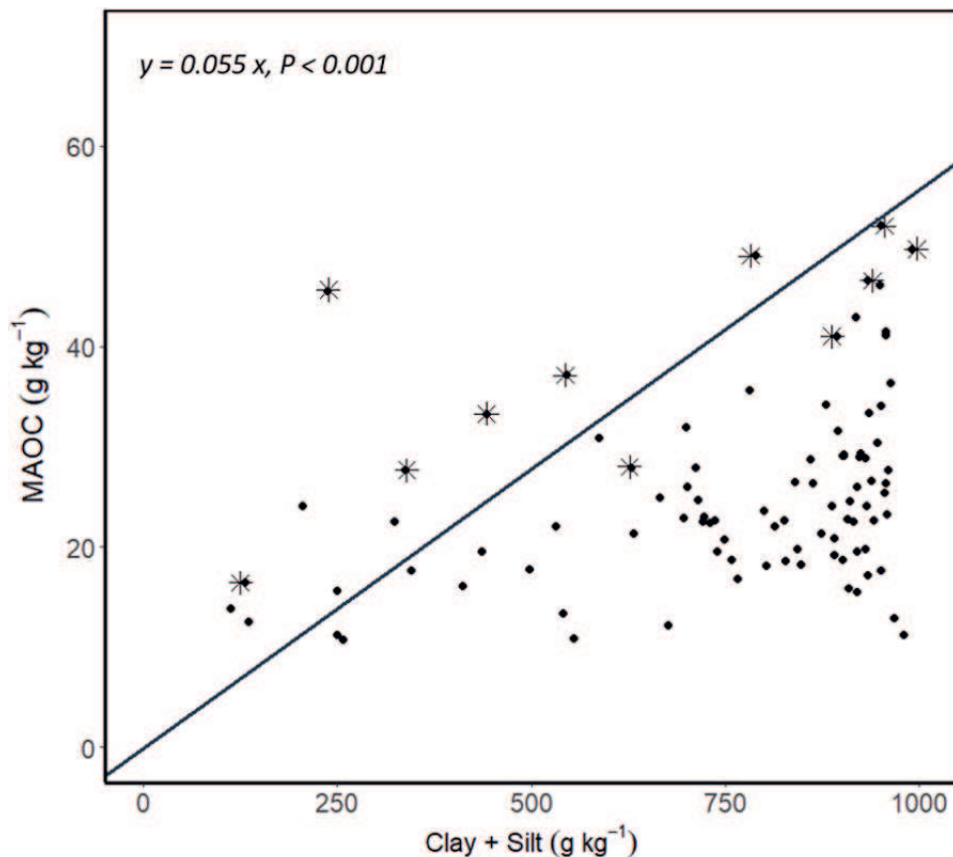


Figure 3.2. Prediction of the maximal MAOC content of clay+silt (<60 μ m). Regression is based on boundary line analyses with the data of the upper tenth percentile of MAOC contents included within clay+silt 100 g kg⁻¹ soil intervals (marked with asterisks).

The estimated feasible highest possible MAOC contents based on the boundary line analyses were higher than the estimates assuming 1:1 clay mineral dominated soils (46 g OC kg⁻¹ clay+silt; Six et al. 2023) and lower than when estimated for 2:1 clay mineral dominated soils (82 g OC kg⁻¹ clay+silt). Only the soils in the lowest clay content interval

(0-100 g kg⁻¹) reached their MAOC saturation according to the boundary line analyses as they contained 118% of the estimated feasible MAOC, whereas the other clay content intervals contained 45-66% of the estimated feasible MAOC.

To critically assess the results of the boundary line analysis, we applied Hassink's equation (Hassink 1997) for verification. When the MAOC saturation was estimated based on Hassink's equation (Hassink 1997) including a clay+silt fraction sized <20 µm, the MAOC accrual capacities were exceeded in the low-clay soils (0-100 and 100-200 g clay kg⁻¹ soil; Table 3.2). However, when including also the larger silt fraction (clay+silt <60 µm), only soils in the lowest clay content interval (0-100 g clay kg⁻¹ soil) were estimated to be carbon saturated, and soils with a clay content >100 g kg⁻¹ were found to be below their estimated saturation level. In the >200 g clay kg⁻¹ soils, the estimated maximum MAOC saturation capacity was not observed.

When we divided the soils into carbon saturated and non-saturated soils according to Hassink's equation (with clay+silt size cut-off <20 µm; Table 3), we observed no fertilization (mineral vs organic) related differences (t-test) in carbon and nitrogen associated soil parameters within the saturated or non-saturated soils.

Table 3.2. Clay (%; <2 μm) and clay+silt content (%; <20 μm and <60 μm) of the soils, estimated maximum MAOC (g OC kg⁻¹ soil) according to Hassink's equation (with clay+silt size cut-offs <20 μm and <60 μm) and maximum OC accrual estimates by boundary line analyses (g OC kg⁻¹ clay+silt <60 μm) for 1:1 and 2:1 clay mineral dominated soils, and estimated feasible MAOC (g MAOC kg⁻¹ clay+silt) in the studied soils under current agricultural management in different clay classes by 100 g kg⁻¹ intervals. Letters in MAOC from estimated maximum with Hassink's equation denote statistical differences (Tukey's HSD, significance level <0.05), n = number of farms sampled. Other variables were not tested for statistical differences. The horizontal line between the clay classes of 200-300 g kg⁻¹ and 300-400 g kg⁻¹ indicates the cut-off between low clay (<30%) and high clay (>30%) soils.

Hassink's Equation															Estimated maximum OC content with Boundary Line Analyses				
Clay (g kg ⁻¹)	n	Clay (<2 μm)			Clay + silt (<20 μm)			Clay + Silt (<60 μm)			Clay+silt <20 μm			Clay+silt <60 μm					
		%	%	%	%	%	%	%	%	%	%	%	%	%					
Total OC (g kg ⁻¹ soil)	MAOC (g kg ⁻¹ soil)	Estimated maximum (g OC kg ⁻¹ soil)	MAOC from estimated maximum (%)	Estimated maximum (g OC kg ⁻¹ soil)	MAOC from estimated maximum (%)	Estimated maximum (g OC kg ⁻¹ soil)	MAOC from estimated maximum (%)	Dominated clay mineral		Estimated feasible MAOC in own soils		Current MAOC from the estimated feasible (%)							
								1:1	2:1	1:1	2:1								
0-100	14	5 ± 0.6	15 ± 1.7	31 ± 4.5	24.3 ± 3.1 ^{ab}	20.2 ± 2.7 ^{ab}	9.5 ± 0.6	213 ± 27.4 ^a	15.6 ± 1.7	130 ± 20.4 ^a	14	26	17	118					
100-200	12	15 ± 0.8	43 ± 4.2	70 ± 5.4	29.0 ± 3.6 ^{ab}	25.3 ± 3.2 ^{ab}	20.1 ± 1.6	126 ± 21.6 ^b	30.2 ± 2.0	84 ± 12.9 ^b	32	59	39	66					
200-300	12	24 ± 0.6	64 ± 5.1	76 ± 5.2	22.6 ± 1.4 ^a	18.7 ± 1.3 ^a	27.6 ± 1.9	68 ± 8.2 ^b	32.1 ± 1.9	58 ± 6.7 ^b	35	64	42	45					
300-400	23	36 ± 0.5	75 ± 2.4	87 ± 2.2	33.9 ± 2.3 ^b	29.3 ± 2.1 ^b	32.0 ± 0.9	92 ± 5.8 ^b	36.2 ± 0.8	81 ± 4.9 ^b	40	73	48	61					
400-500	8	46 ± 1.0	77 ± 3.9	85 ± 3.5	31.1 ± 3.4 ^{ab}	27.8 ± 3.3 ^{ab}	32.4 ± 1.4	86 ± 10.4 ^b	35.5 ± 1.3	78 ± 9.1 ^b	39	71	47	59					
500-600	12	55 ± 0.8	80 ± 2.6	85 ± 2.8	30.1 ± 1.2 ^{ab}	26.2 ± 1.2 ^{ab}	33.6 ± 1.0	78 ± 3.6 ^b	35.7 ± 1.0	73 ± 2.8 ^b	39	71	47	56					
600-700	12	62 ± 0.6	85 ± 1.1	92 ± 0.9	32.6 ± 2.7 ^{ab}	28.0 ± 2.4 ^{ab}	35.7 ± 0.4	78 ± 7.2 ^b	38.0 ± 0.3	74 ± 6.2 ^b	42	77	51	55					

Table 3.3. Soil organic carbon properties and pH of soils divided according to the MAOC saturation level (saturated and non-saturated, respectively, according to Hassink's equation with clay+silt size cut-off <20 µm) and fertilizer application. Within saturated and non-saturated soils, no statistically significant differences were observed between the soils with mineral and organic fertilization (n=number of farms sampled (total number of farms = 90)).

MAOC saturation state of the soil	Fertilization	n	Total OC (g kg ⁻¹)	Total N (g kg ⁻¹)	Soil		MAOC (g kg ⁻¹ soil)		MAOM (<53 μm soil)		POC (g kg ⁻¹ soil)		POM (>53 μm soil)		pH	Clay %		Clay+silt %	Al-ox (g kg ⁻¹ soil)	Fe-ox (g kg ⁻¹ soil)	Mean Hassink Saturation (%)
					CN	C-%	C-%	CN	C-%	CN	C-%	CN	C-%								
Saturated	Mineral	16	32 ± 4	2.0 ± 0.2	16 ± 0.6	28 ± 3.2	4.9 ± 0.6	16 ± 0.6	3.7 ± 0.5	2.6 ± 0.8	18 ± 1.2	6.4 ± 0.1	25 ± 5	65 ± 7	1.9 ± 0.2	4.1 ± 0.6	145				
	Organic	19	35 ± 3	2.2 ± 0.2	17 ± 0.7	30 ± 2.9	6.1 ± 0.7	16 ± 0.8	4.4 ± 0.3	3.4 ± 1.1	21 ± 1.1	6.2 ± 0.1	22 ± 4	56 ± 7	2.5 ± 0.2	4.0 ± 0.6	179				
Non-saturated	Mineral	30	27 ± 1	2.0 ± 0.1	14 ± 0.2	23 ± 0.9	2.8 ± 0.1	13 ± 0.2	4.3 ± 0.4	3.7 ± 0.5	22 ± 0.7	6.5 ± 0.1	43 ± 3	84 ± 2	1.9 ± 0.1	4.7 ± 0.3	73				
	Organic	25	26 ± 1	1.9 ± 0.1	14 ± 0.3	22 ± 1.2	2.5 ± 0.1	13 ± 0.3	3.7 ± 0.4	4.3 ± 0.5	22 ± 0.8	6.4 ± 0.1	40 ± 3	88 ± 2	1.8 ± 0.1	4.5 ± 0.2	70				

3.3.3 Soil properties, climate and fertilization regime as explanatory variables of total OC and its distribution into MAOC and POC

When observing all the studied soils together (clay content 2-68%), clay content strongly correlated with the concentration of Fe oxides. While Al oxides did not significantly correlate with clay content, there was a significant correlation between Al and Fe oxides concentration (Table 3.4). Likewise, in the clay <30% soils, clay and Fe oxides correlated strongly, whereas in clay >30% soils, Fe oxides and clay did not correlate but Al-oxides and clay, as well as Fe oxides and Al oxides correlated.

Table 3.4. Pearson correlation coefficients (*r*) for the soil clay content (%), aluminum (Al-ox, g kg⁻¹) and iron (Fe-ox, g kg⁻¹) oxides in all studied soils, and in soils with clay <30 % or clay >30 %. Significant correlations bolded (*p* < 0.05).

All soils				Clay <30%				Clay >30%			
	Clay	Al-ox	Fe-ox		Clay	Al-ox	Fe-ox		Clay	Al-ox	Fe-ox
Clay	1			Clay	1			Clay	1		
Al-ox	0.16	1		Al-ox	-0.28	1		Al-ox	0.30	1	
Fe-ox	0.63	0.23	1	Fe-ox	0.69	0.016	1	Fe-ox	0.16	0.31	1

For total OC and MAOC, simple linear regression analyses for all soils together showed positive relationships with clay, Al and Fe (p-values <0.05; Figure 3.3). There was a significant positive relationship between soil clay content and total OC and MAOC (p=0.005 for both), which accounted for 9% of the variation in the carbon data. Al and Fe oxides accounted for more of the variation, describing for around 21-23% of the variation in total OC and MAOC. Neither clay, nor Al and Fe oxides explained variation in POC.

Multiple regression models explained 33-50% of the variation in total OC, and 36-53% of the variation in MAOC (Table 3.5). Yearly annual temperature (°C) was not chosen as an explanatory variable in any of the models, nor was the clay content (g kg⁻¹ soil), whereas clay+silt content (g kg⁻¹ soil) was a significant explanatory variable in the total OC and MAOC models (p-value <0.001; Table 5, Supplementary Table 3.3). Al oxide was the only explanatory parameter that contributed (p-value <0.05) to the explanation of variance in all models, whereas Fe oxides were not included in the models for soils with low (<30%) clay content for both total OC and MAOC. Instead, pH was important in explaining total OC and MAOC in the clay <30% soils. Generally, best-fit models explaining total OC and MAOC per clay content class (< or >30% clay) were very similar.

The only deviation between the models was that mean annual precipitation (MAP) was not a significant explanatory variable for MAOM in clay <30% soils, whereas it was significant in all the other models.

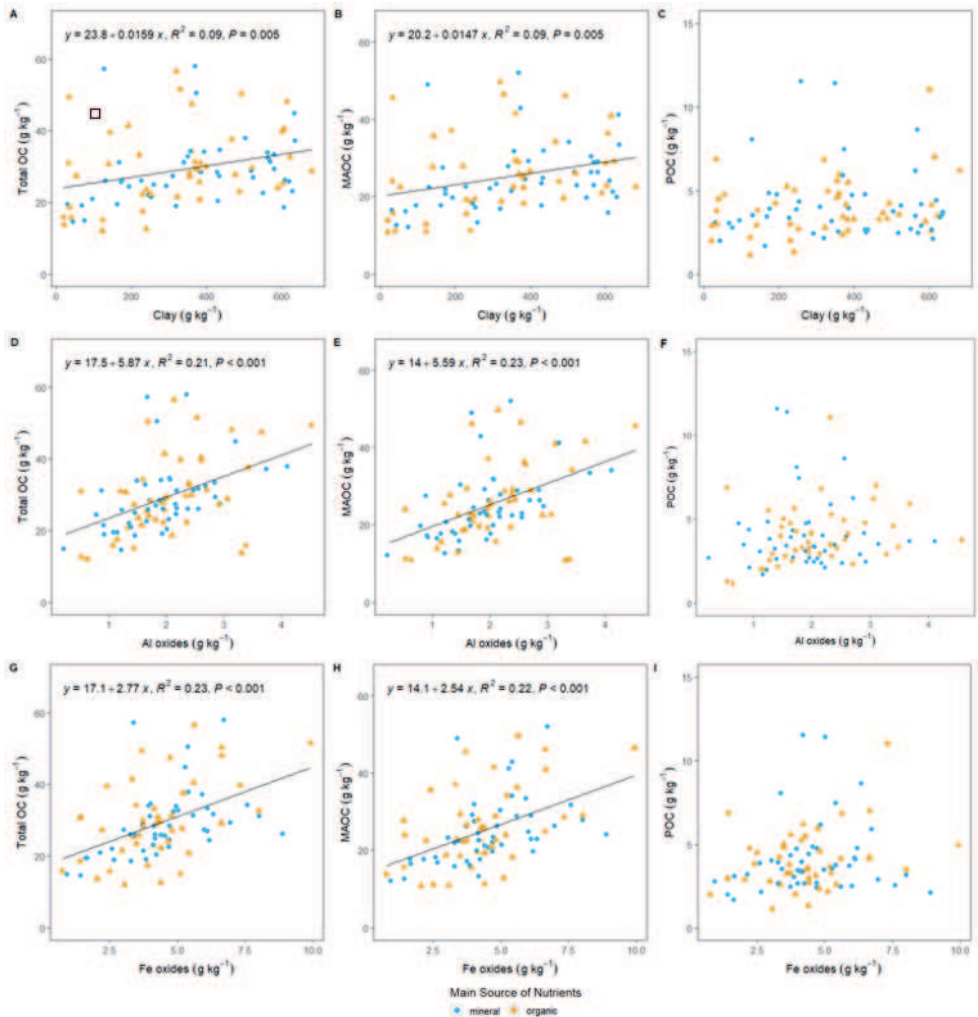


Figure 3.3. Simple linear regressions for total OC, MAOC and POC (g kg^{-1} soil) with clay, Al- and Fe oxides as explanatory variables in fields ($n=90$) with mineral (blue, $n=46$) or organic (orange, $n=44$) fertilization. In case of a significant relationship ($p < 0.05$), a regression line was added to illustrate the relationship.

Table 3.5. Best-fit regression models from AIC-driven model selection explaining total OC and MAOC. Models were tested for all soils together (n=90) and for soils with a clay content <30% (n=35) and >30% (n=55), separately. Explanatory variables were clay content (g kg⁻¹), clay plus silt content (g kg⁻¹), Al and Fe oxides (g kg⁻¹), cumulative long-term annual precipitation (MAP, mm/year) and yearly mean temperature (MAT; °C; 19 year average), pH, and fertilization (mineral or organic, coded 0 and 1, respectively). Significant (p <0.05) explanatory variables **bolded**. More model details in Supplementary Table 3.

	OC fraction *	Soils	Regression Model	Adj. R ²
1)		Clay <30 %	0.03 clay + 5.06 Al_{ox} - 0.86 pH - 0.10 MAP - 4.26 fertilization + 129.9	0.33
2)	Total OC	Clay >30 %	-0.02 clay + 6.56 Al_{ox} + 1.57 Fe_{ox} - 0.10 MAP + 83.2	0.50
3)		All soils	4.91 Al_{ox} + 1.91 Fe_{ox} - 3.91 pH - 0.08 MAP + 89.8	0.44
4)		All soils	0.008 clay & silt + 5.26 Al_{ox} + 1.26 Fe_{ox} - 3.78 pH - 0.09 MAP + 87.5	0.46
5)		Clay <30 %	3.56 Al_{ox} - 0.97 pH - 0.06 MAP - 3.94 fertilization + 116.4	0.36
6)	MAOM-C	Clay >30 %	-0.019 clay + 6.34 Al_{ox} + 1.39 Fe_{ox} - 0.09 MAP + 76.4	0.53
7)		All soils	4.65 Al_{ox} + 1.69 Fe_{ox} - 4.48 pH - 0.08 MAP + 86.8	0.47
8)		All soils	0.008 clay& silt + 5.01 Al_{ox} + 1.01 Fe_{ox} - 4.34 pH - 0.08 MAP + 84.4	0.49

*In the models 1-3 and 5-7, clay as an explanatory variable. In the models 4 and 8, clay + silt as an explanatory variable.

3.4 Discussion

3.4.1 OC, MAOC and POC in the soils

Contrary to our expectation, we did not find that the implementation of organic fertilization practices resulted in a higher total OC content of soils, when comparing to mineral fertilization (Supplementary Table 3.1). OC in minerally and organically fertilized soils was on average 29.9 and 28.8 g OC kg⁻¹ kg soil, respectively. This result differs from several long-term studies that show OC increase after changing from mineral to organic fertilization (Maillard & Angers 2014, Just et al. 2023).

There are several potential explanations for the similarity of soil OC contents in our soils that differed in fertilization regime. As plants are important in bringing OC to the soil via root exudates and crop residues (Jackson et al. 2017), it is possible that as long as there is sufficient amount of nutrients to maintain a specific level of primary productivity, the type of fertilization (mineral versus organic) is in some cases not a decisive factor for the soil OC storage (Gregorich et al. 1996, Ladha et al. 2011, Gocke et al. 2023). It is also possible that we did not detect higher OC stocks in organically fertilized soils due to relatively high baseline levels of OC in the Finnish arable soils (Heikkinen et al. 2013). In this case, the pre-existing OC pool may mask OC additions by organic fertilization, as it is difficult to discern small gradual changes in soil OC levels (e.g., Schrumpf et al. 2011). Furthermore, one possibility is that part of the OC from the organic fertilizers is located below the ploughed layer (i.e., below 20 cm soil depth we studied in here) as ploughing and leaching may translocate OC from the organic fertilizer into deeper soil layers (e.g., Kätterer et al. 2014, Salonen et al. 2023, Skadell et al. 2023).

Similarly, the type of fertilization had no effect on the OC contents of the MAOM and POM fractions (Supplementary Table 3.1). This is in line with what has been previously reported for a Finnish arable topsoil (0-20 cm) in field trials comparing mineral vs organic systems after 35 years (Mayer et al. 2022) and after 24 years (Salonen et al. 2023). This may further indicate that comparable MAOC and POC levels can be achieved with both organic and mineral fertilization, as long as in mineral farming systems there are enough nutrients to support the plant growth providing OC inputs to the soil.

Across the studied soils, the proportion of OC in MAOC was relatively constant, (83-89% of the total OC) and this result agrees with what has been determined before for a Finnish arable clay soil (83-87% of OC in MAOC; Salonen et al. 2023), for temperate arable soils (86%; Begill et al. 2023), and in a global meta-analysis (83%, Matus et al. 2021), indicating that soils of the agroecosystems across differing environments and climates are having corresponding proportions of the OC in the MAOC and POC fractions.

3.4.2 Testing OC saturation concepts in the studied soils

There was a strong positive linear relationship between total OC and MAOC across the range of OC contents (12 to 58 g total OC kg⁻¹ soil, Supplementary Figure 3.1). A similar relationship was found by Begill et al. (2023). In contrast, some studies have shown that after a specific OC content, the relationship between total OC and MAOC diminishes. For example, Cotrufo et al. (2019) observed this levelling off after a total OC content of 50 g kg⁻¹ in a set of European soils, which was not the case in the current study despite using a similar physical MAOM/POM fractionation method. Our study was restricted to a set of mineral arable soils from the Boreal climate zone with a very specific pedogenic history (i.e., formed by abrasion by ice, Koljonen et al. 1992). Therefore, our results possibly better reflect soil OC saturation status in the specific case of boreal agricultural soils.

We estimated the feasible maximum amount of MAOC under the prevailing agricultural management for our soils with boundary line analyses and found it to be 55 g MAOC kg⁻¹ clay+silt (Figure 3.2, Table 3.2). This estimate sits between the proposed maxima (Six et al. 2023) for 1:1 clay-mineral dominated soils (46 g MAOC kg⁻¹ clay+silt) and 2:1 clay-mineral dominated soils (82 g MAOC kg⁻¹ clay+silt). The result of the boundary line analyses is consistent with what could be expected based on background knowledge of the mineralogy of Finnish soils, since they are formed by ice abrasion and are relatively young (Koljonen et al. 1992) and non-weathered (Keskinen et al. 2022) and thus are assumed to have lower surface activity than 2:1 clay mineral dominated soils. According to the boundary line analyses, studied soils had 45-118% of the estimated feasible MAOC (g OC kg⁻¹ clay+silt) under the current arable land management, and only the lowest clay

content interval (0-100 g kg⁻¹) was above the estimated maximum (118% of the estimated feasible MAOC), meaning that the majority (85%) of the studied soils were not deemed to be saturated and could theoretically accumulate more MAOC. However, it is worth emphasising that we do not know whether the soils selected in this study and for the boundary line analyses are truly representative of a maximum achievable OC content with the current agricultural management, as we do not have time series measurements of the soils that confirm a steady state of OC in terms of a specific level of OC inputs. Nonetheless, since our dataset is relatively large and contains a diverse selection of farms from the southern half of Finland with different farming histories, we can assume that the results reveal a reasonable estimate of the maximum MAOC under the current agricultural management.

We also assessed soil carbon saturation with Hassink's equation (Hassink 1997; Table 3.2) with two particle size cut-offs for clay+silt: <20 µm (as in Hassink 1997) and <60 µm (as theoretically minerals up to this size have relevant specific surface area for MAOC binding; Six et al. 2023). With this approach, all soils with the clay content >200 g kg⁻¹ (72% of the studied soils) had OC saturation deficit, while soils with the lowest studied clay content (0-100 g kg⁻¹) were found to be above their theoretical maximum (i.e., >100%) MAOC saturation.

As some of the soils, with a clay content <100 g kg⁻¹, were found to be oversaturated with MAOC (i.e., having more than 100% of the maximum MAOC proposed by the boundary line analyses and Hassink's equation), it is possible that the used approaches are not well suited for the soils in question. These saturation estimation approaches oversimplify the binding capacity associated with MAOC as they only take into account soil texture, whereas there is a multitude of other factors (such as climate, vegetation, soil chemistry, Al and Fe oxides) affecting soil OC stabilisation soils (e.g., Six et al. 2002, Rasmussen et al. 2018, Guillaume et al. 2022, Soinne et al. 2024).

Furthermore, observed oversaturation could also indicate methodological error in the size fractionation of MAOM (<53µm soil) / POM (> 53µm soil). Similarities of the C to N ratios of MAOM and POM in the low clay soils (<100 g clay kg⁻¹ soil, C to N ratio=18, Table 3.1) may imply POM contamination, e.g., fine-sized POM passing through the sieve to the MAOM fraction during the MAOM/POM fractionation, which would

increase the OC content of the MAOM. Due to the relatively high clay content of most of our soils, we needed to use glass beads to assist full dispersion of the soils, and the glass bead approach may have led to some crumbling of the larger POM fragments into smaller, MAOM-sized fragments ($<53\ \mu\text{m}$).

Yet, it is possible that the observed similarity of the C to N ratios of the MAOM and POM in the $<100\ \text{g clay kg}^{-1}$ soil is not a sign of a contamination but instead indicates a chemical resemblance of MAOC and POC in the low clay soils. Some studies have found MAOM and POM to be chemically resembling (e.g., Yu et al. 2022, Chang et al. 2024), and Yu et al. (2022) reported C to N ratios corresponding to the ones in here in MAOM and POM fractionated with the same method (size cut-off $53\ \mu\text{m}$). Also, it has been shown that in coarse soils, POM-sized MAOM (i.e., mineral bound MAOC $>53\ \mu\text{m}$) can have higher C to N ratio than smaller sized MAOM ($<53\ \mu\text{m}$; Samson et al. 2020, Sokol et al. 2022). Hence, we cannot rule out the possibility that the resembling C to N ratios of the MAOM and POM in the studied low clay soils are a true finding.

3.4.3 Soil properties, climate parameters and OC, MAOC and POC

As expected, we found that Al and Fe oxides were more strongly correlated with OC and MAOC than clay (Figure 3.3). However, as simple linear regressions could explain only 9-23% of the variation in OC and its fractions, neither clay, Al and Fe were well suited as single predictors to explain OC and its fractions in the studied soils. As soil OC and clay content have previously reported to be connected (e.g., Kaiser & Guggenberger 2003, Arrouays et al. 2006, Wiesmeier et al. 2019), it was somewhat surprising that the soil clay content was not associated stronger with OC (clay explained only 9% of OC and MAOC variation; Figure 3.3). Some previous studies have also stated that clay content is not the only or even an important determinant of OC (Rasmussen et al. 2018, Prout et al. 2021, Salonen et al. 2023). One possible explanation for this in the current study relates to the relatively low primary productivity of soils with a high clay content. As very clayey soils in Finland tend to have lower yield levels than coarser soils, there is possibly a lower plant-derived OC input in the soil (Soinne et al. 2021) which could lead to lower SOM levels compared to soils with a higher primary productivity.

While Al and Fe oxides had a stronger correlation with OC and MAOC than the soil clay content, also they both remained poor single predictors for the amount of soil carbon.

However, soils estimated to be MAOC-saturated had relatively more Al and Fe oxides per gram of clay or clay+silt than non-saturated soils (Table 3), indicating a close relationship between oxides and MAOC (Rasmussen et al. 2018, Hall & Thompson 2022). In the multiple regression models (Table 3.5), Al oxides were particularly important as an explanatory variable in all the models, whereas Fe oxides were significant for OC and MAOC only in soils with high clay content (>30%). Soil pH correlated negatively with OC and MAOC in soils with low clay (<30%). This is consistent with previous research, as low soil pH has been found to correlate with slower OC decomposition (Keiluweit et al. 2016, Slessarev et al. 2016) and OC accumulation (Lugato et al. 2021).

Consistent with the simple linear regressions, clay content was not a significant predictor in any of the multiple regression models. However, clay+silt was a significant predictor of OC and MAOC when all the studied soils were included in the analysis. It is possible that this is a consequence of higher primary productivity and plant-derived OC inputs in coarser soils (Soinne et al. 2021), as more compacted soil structure of the more clayey soils may limit plant OC inputs.

In a global meta-analysis, Jobbágy & Jackson (2000) reported that soil total OC content increased with precipitation and decreased with temperature. Likewise, Hansen et al. (2024) found that MAP was connected to higher MAOC levels in a global scale. Opposite to these findings and our expectations, OC decreased with increasing precipitation, and clay and temperature had no relationship with OC or MAOC in our multiple regression analyses. It is possible that the range of mean temperature in our data (3.3-6.4°C) is not wide enough to make a noticeable difference in the soil OC content as for the large part of year, the studied soils are far from the optimal temperature for the microbial growth (20-25°C for Boreal agricultural soil; Pietikäinen et al. 2005) which halts OC decomposition and transformation processes.

Our finding that the mean annual precipitation (range 548-725 mm year⁻¹) was negatively correlated with OC and MAOC was unexpected as greater MAP has been previously connected to larger OC as prevalent moist condition can cause anoxic conditions which hinders SOM decomposition (Jobbágy & Jackson 2000, Wiesmeier et al. 2019). On the other hand, prevalent rain may restrain OC inputs into the soil as it may limit plant growth

and consequently curb OC inputs to the soil due to waterlogging and following anoxic conditions (Drew 1997).

All in all, the best-fit models explained 33-50% of the variation in total OC, and 36-53% of the variation in MAOC. The relatively low model fit could indicate that other factors explaining soil OC that were not included in this study (such as plant productivity, topography, long-term land use and fertilizing history) could affect OC/MAOC levels in our soils. In particular, data on soil productivity/ yield levels could improve the models, as most of our soils were found to have low carbon saturation, with the implication that mineral adsorption surfaces were not a limiting factor.

3.5 Conclusions

We fractionated soil from 93 arable fields with a physical MAOM (<53 μm) / POM (>53 μm) fractionation method. The total amount of OC (12-58 g kg^{-1} soil) was not affected by the type of fertilization (mineral vs organic) and the share of OC in MAOC ($86 \pm 3\%$) was found to be relatively stable and also not affected by fertilizer type. Largest total OC and MAOC contents were measured in high clay soils but considering the general assumption of the significant role of clay in defining the protective capacity for MAOC, we observed surprisingly high MAOC contents even in the low clay soils. Both applied soil OC saturation assessments, boundary line analysis and Hassink's equation, revealed that the lowest clay content soils were above their predicted maximum (i.e., >100%) MAOC saturation, and as expected, soils with higher clay content have further OC accrual potential. As the low clay soils seemed to be oversaturated with OC/MAOC, it is possible that the applied OC saturation estimation approaches misjudge the OC accrual capacity of these soils because they rely merely on soil textural information. Also, as clay was not an important predictor for soil OC in the multiple regression models, there are indications that in the studied, relatively moderately weathered soils, clay content is not a decisive factor for the amount of stabilised OC. In our soils, single soil properties (clay, Al and Fe oxides) were not well-suited as predictors for total OC or MAOC, but multiple regression models revealed that soil parameters (in particular Al and Fe oxides and pH) and weather parameters (MAP and MAT) combined could explain more of the variation in soil carbon. Multiple regression models at their best explained 53% of the variation of MAOC,

indicating that there are other context-dependent factors (such as long-term land use history) contributing to soil OC related processes that deserve attention in further studies.

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Conflicts of Interests

The authors have no conflicts of interest to declare

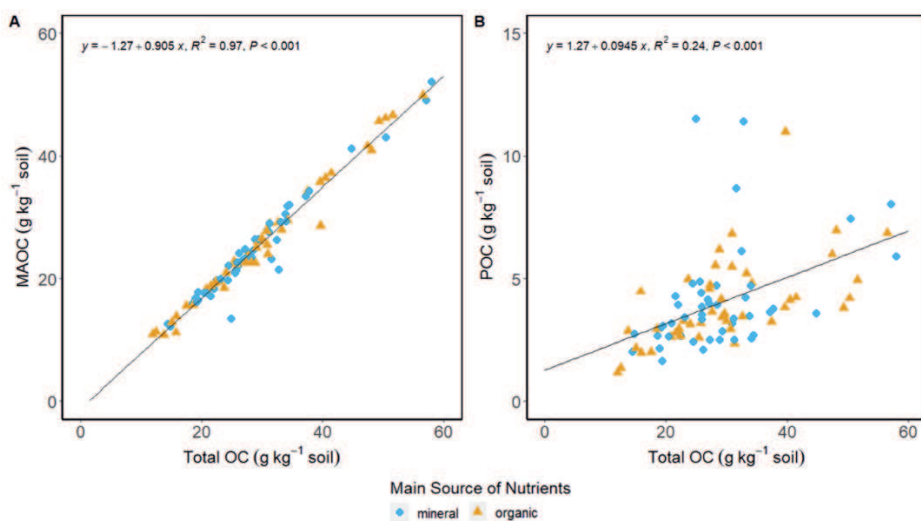
Appendix

Supplementary Table 3.1. Average ($\pm$ SE) organic carbon and nitrogen concentration (g kg^{-1} soil) of total soil, the C to N ratios of total soil, MAOM ($<53 \mu\text{m}$ soil) and POM ($>53 \mu\text{m}$ soil), the OC concentration (g kg^{-1} soil) and contents (%) of MAOC and POC in the $<53 \mu\text{m}$ and $>53 \mu\text{m}$ soil mass fraction, soil pH, and clay+silt content (%; $<60 \mu\text{m}$) in different clay classes by 100 g kg^{-1} intervals. No statistical differences between the soils with mineral and organic fertilization within a clay class (g kg^{-1}) were observed, n =number of farms sampled (in total $n=90$). Horizontal line between the clay classes of 200–300 g kg^{-1} and 300–400 g kg^{-1} indicates the cut-off between low clay ($<30\%$) and high clay ($>30\%$) soils.

Clay (g kg ⁻¹)	Fertilization	n	Total OC (g kg ⁻¹)	Total N (g kg ⁻¹)	MAOM		POM		pH	Clay + Silt %			
					Soil CN	MAOC (g kg ⁻¹ soil) C-%	MAOM (>53 μm soil) CN	POC (g kg ⁻¹ soil) C-%					
0-100	Mineral	5	17.8 ± 1.3	1.08 ± 0.10	17 ± 1.2	6.1 ± 1.7	15.0 ± 1.1	17 ± 1.3	0.4 ± 0.1	2.8 ± 0.2	16 ± 2.4	6.7 ± 0.2	34 ± 10.1
	Organic	7	24.5 ± 4.8	1.39 ± 0.25	18 ± 1.5	8.0 ± 1.5	20.5 ± 4.6	18 ± 1.8	0.6 ± 0.1	4.0 ± 0.6	18 ± 1.2	6.5 ± 0.2	23 ± 2.4
100-200	Mineral	7	30.0 ± 4.7	1.95 ± 0.19	15 ± 0.9	3.6 ± 0.6	25.7 ± 4.1	14 ± 0.9	2.0 ± 0.5	4.3 ± 0.7	19 ± 1.7	6.6 ± 0.1	75 ± 5.2
	Organic	5	27.7 ± 6.1	1.83 ± 0.29	14 ± 1.4	4.5 ± 1.3	24.9 ± 5.6	14 ± 1.4	1.6 ± 0.8	2.8 ± 0.6	20 ± 3.4	6.1 ± 0.3	64 ± 10.8
200-300	Mineral	5	23.8 ± 0.9	1.86 ± 0.14	13 ± 0.6	2.7 ± 0.3	18.7 ± 1.7	12 ± 0.4	2.5 ± 0.9	5.1 ± 1.6	19 ± 3.0	6.4 ± 0.3	73 ± 8.1
	Organic	6	21.9 ± 2.8	1.58 ± 0.12	14 ± 0.8	2.6 ± 0.6	18.7 ± 2.3	13 ± 0.8	2.7 ± 0.7	3.2 ± 0.7	22 ± 1.3	6.3 ± 0.1	78 ± 8.5
300-400	Mineral	9	35.0 ± 4.0	2.33 ± 0.20	15 ± 0.5	3.5 ± 0.4	30.0 ± 3.7	14 ± 0.5	4.9 ± 1.2	5.0 ± 1.0	21 ± 1.0	6.4 ± 0.2	85 ± 3.9
	Organic	14	33.1 ± 2.9	2.18 ± 0.13	15 ± 0.5	3.2 ± 0.3	28.8 ± 2.7	14 ± 0.5	5.8 ± 1.3	4.3 ± 0.4	23 ± 0.9	6.4 ± 0.1	88 ± 2.6
400-500	Mineral	4	27.6 ± 2.9	2.02 ± 0.20	14 ± 0.6	3.2 ± 0.5	24.5 ± 2.9	13 ± 0.6	2.3 ± 1.0	3.1 ± 0.6	22 ± 2.8	6.4 ± 0.2	79 ± 5.7
	Organic	4	34.6 ± 6.1	2.68 ± 0.62	13 ± 1.1	3.4 ± 0.6	31.0 ± 5.9	13 ± 1.1	4.6 ± 1.1	3.6 ± 0.2	23 ± 3.1	6.1 ± 0.2	91 ± 1.6
500-600	Mineral	10	30.8 ± 1.3	2.22 ± 0.07	14 ± 0.4	3.1 ± 0.1	26.7 ± 1.3	13 ± 0.4	4.1 ± 1.0	4.1 ± 0.6	23 ± 0.5	6.3 ± 0.1	86 ± 2.9
	Organic	2	26.8 ± 2.8	2.11 ± 0.17	13 ± 0.3	2.7 ± 0.5	23.5 ± 2.6	12 ± 0.4	3.0 ± 1.0	3.3 ± 0.2	20 ± 1.6	6.5 ± 0.4	80 ± 9.5
600-700	Mineral	6	29.3 ± 4.0	2.33 ± 0.29	13 ± 0.4	2.8 ± 0.4	26.0 ± 3.9	12 ± 0.4	5.0 ± 0.8	3.3 ± 0.3	23 ± 1.6	6.5 ± 0.1	92 ± 1.1
	Organic	6	35.9 ± 3.5	2.57 ± 0.30	14 ± 0.4	3.3 ± 0.4	30.1 ± 3.0	13 ± 0.4	6.5 ± 0.9	5.7 ± 1.3	23 ± 1.6	6.3 ± 0.2	91 ± 1.5

Supplementary Table 3.2. Distribution of OC and sample mineral mass between the MAOM (<53 μm) and POM (>53 μm) fraction in different soil clay content classes. n = number of farms sampled (n_{tot} = 93 farms). Average ($\pm\text{SE}$) are presented.

Clay (g kg^{-1})	n	Proportion in < 53 μm fraction (i.e., MAOM)	
		Total soil OC (%)	Mineral mass (%)
0-100	14	83 ± 1.7	32 ± 3.5
100-200	12	87 ± 1.3	69 ± 5.0
200-300	12	83 ± 3.1	76 ± 5.0
300-400	23	86 ± 0.7	87 ± 2.0
400-500	8	89 ± 1.2	86 ± 3.2
500-600	12	87 ± 1.5	87 ± 2.1
600-700	12	86 ± 1.7	92 ± 0.9



Supplementary Figure 3.1. The soil organic carbon (OC) content versus MAOC (g kg^{-1} soil; A) and POC (g kg^{-1} soil; B) in fields ($n=90$) with mineral (blue, $n=46$) or organic (orange, $n=44$) fertilizer as main source of nutrients. Note differing y-axis scales.

Supplementary Table 3.3. Best-fit regression models from AIC-driven model selection explaining total OC and MAOC. Models were tested for all soils together (n=90) and for soils with a clay content <30% (n=35) and >30% (n=55), separately. Explanatory variables were clay content (g kg⁻¹), clay plus silt content (g kg⁻¹), Al and Fe oxides (g kg⁻¹), cumulative long-term annual precipitation (MAP; mm/year) and yearly mean temperature (°C; 19 year average), pH, and fertilization (organic or mineral). When p-values are presented in the Table, the variable in question was included in the best fit model. Significant p-values of the coefficients are presented in bold.

			p-values of the coefficients				
OC fraction *	Soils	Adj. R ²	Clay / clay+silt	Al _{ox}	Fe _{ox}	pH	MAP Fertilization
1)	Clay <30 %	0.33	0.66	<0.05	<0.05	<0.05	0.17
2)	Clay >30 %	0.50	0.31	<0.001	<0.001	<0.001	
3)	All soils	0.44		<0.001	<0.001	0.052	<0.001
4)	All soils	0.46	<0.001	<0.001	<0.05	0.057	<0.001
5)	Clay <30 %	0.36		<0.001	<0.001	0.13	0.15
6)	Clay >30 %	0.53	0.30	<0.001	<0.001	<0.001	
7)	All soils	0.47		<0.001	<0.001	<0.05	<0.001
8)	All soils	0.49	<0.001	<0.001	<0.05	<0.05	<0.001

*In the models 1-3 and 5-7, clay as an explanatory variable. In the models 4 and 8, clay + silt as an explanatory variable.

Chapter 4

Density fractionation better isolates functionally distinct POM and MAOM fractions from soil organic carbon than size fractionation

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Abstract

Soil organic matter (SOM) is commonly fractionated into fast-cycling particulate organic matter (POM), and more stable mineral-associated organic matter (MAOM) using methods based on density or particle size. As the data originating from size- and density-based fractionation methods can be interpreted interchangeably, it is important to verify whether these methods isolate POM and MAOM pools that: 1) are distinct and match their conceptual definitions, and 2) contain similar proportions of the total soil organic carbon (SOC). To evaluate this, we fractionated POM and MAOM in topsoil (0-10 cm; immature SOM) and subsoil (30-50 cm; mature SOM) from three arable fields with differing clay (5-60%) and SOC contents (0.3-2.7%). We assessed size- and density-fractionated POM and MAOM and the corresponding bulk soil with Rock-Eval thermal analysis for chemical composition (HI and OI indices, O/C ratio) and thermal stability ($S3CO_2_T50$, $S4CO_2_T50$, R- and I-index). Size fractionation allocated more SOC to POM than density fractionation in most soils (1.4-3.6 fold in 5 out of 6 soils). Density-fractionated POM retained the chemical composition and thermal lability of weakly decomposed plant material, whereas size-fractionated POM more closely resembled MAOM, suggesting incomplete separation of the two pools by size-based method. Size-fractionated POM, in contrast, showed greater thermal stability than density fractionated POM, in some cases exceeding that of MAOM. Our results demonstrate that the choice of fractionation method critically affects SOM pool characterisation. From the fractionation methods analysed, density fractionation better isolates functionally distinct POM and MAOM fractions than size fractionation.

4.1 Introduction

Soil organic matter (SOM) is commonly fractionated into particulate (POM) and mineral-associated organic matter (MAOM), with an aim to separate soil organic carbon (SOC) into pools with distinct functionality, stability, and turnover rates (Tiessen and Stewart, 1983; Cambardella and Elliott, 1992; Lavalley et al. 2020). Methods to achieve the separation vary (von Lützow et al. 2007), and whether different fractionation methods isolate comparable POM and MAOM pools is not established (Poeplau et al. 2018; Leuthold et al. 2024), though recent work suggests it may be particularly problematic in coarse-textured soils (Georgiou et al. 2025; Poeplau et al. 2025).

According to the conceptual functional definition, POM consists mainly of relatively undecomposed plant residues that exist freely in the soil or within dynamic aggregates, making it physically more available for decomposition (Tiessen & Stewart 1983; Six et al. 2000; Lavalley et al. 2020). In contrast, MAOM derives from a range of organic inputs, including microbially processed plant material, dissolved organic matter, and microbial compounds such as extracellular polymeric substances and necromass (Cotrufo et al. 2013; Angst et al. 2021). These organic compounds associate with mineral surfaces through sorption, ligand exchange, and cation bridging (Kleber et al. 2021), and are considered more resistant to decomposition than POM (Cotrufo et al. 2022). However, before POM and MAOM can be connected to SOC dynamics in the field, it is important to verify that the fractions isolated in the laboratory match their conceptual pool descriptions.

Various physical fractionation protocols are used to separate POM and MAOM either at a particle size cutoff (commonly 20, 50, or 63 μm , with POM being the larger fraction) or a density cutoff (commonly 1.60–1.85 g cm^{-3} , with POM being the lighter fraction; e.g., Poeplau et al. 2018). Recent research on SOC has increasingly focused on POM and MAOM, with a consensus that MAOM stores the majority of agricultural soil organic carbon (often around 80%; Cotrufo et al. 2022; Georgiou et al. 2022; Begill et al. 2023; Soinne et al. 2024). However, while the majority of SOC of agricultural soils is typically found in MAOM, the exact distribution between POM and MAOM can vary depending on the fractionation method. Some studies report similar SOC proportions in POM and MAOM regardless of whether size- or density-based methods are utilised (e.g., Leuthold

et al. 2024), whereas others find a difference (e.g., Georgiou et al. 2025, Poeplau et al. 2025). Also, the comparability of size- and density-based SOM fractionation methods may be dependent on the soil type. In high-sand and high-SOC soils, size-based fractionation allocates a comparatively larger proportion of SOC to MAOM than in high-clay soils, possibly because fine POM passes through the sieve and is counted as MAOM (Poeplau et al. 2025).

While MAOM accounts for the largest share of SOC, POM plays a critical role in agroecosystems by supporting soil life and nutrient cycling (Hoffland et al. 2020; Witzgall et al. 2021). As the more labile and management-sensitive carbon pool (Angst et al. 2023), POM can serve as an indicator of SOM responses to agricultural practices (Bongiorno et al. 2019; van Rijssel et al. 2025). Methodological inconsistencies in POM measurement could therefore lead to misinterpretation of its functional role in soils.

Such inconsistencies may arise because size- and density-based fractionation methods, despite their widespread use, do not necessarily separate POM and MAOM into fractions that truly reflect their conceptual functional definitions, i.e., POM as a more labile, decomposer-accessible pool and MAOM as a more stable, long-term storage pool (Delahaie et al. 2024; Leuthold et al. 2024; Schiedung et al. 2025). As SOM exists along a continuum from POM to MAOM, any fractionation method imposes an operational cut-off that most likely results in some degree of overlap between the POM and MAOM pools (Six et al. 2024; Wasner et al. 2024; Poeplau et al. 2025), and both fractionation approaches can cause cross-contamination between pools. In size fractionation, MAOM can be misallocated to POM through incomplete aggregate dispersion or the presence of coarse mineral-associated carbon (Breure et al. 2026), while conversely, fine POM passing through the sieve can be misallocated to MAOM (Poeplau et al. 2025). These challenges may be amplified in clay-rich soils where organo-mineral associations are more abundant and stronger (Mikutta et al. 2006; Kleber et al. 2007). Evaluating whether different fractionation methods actually isolate chemically distinct SOM pools is therefore essential for their functional interpretation. Density fractionation is not without its own biases, as incomplete dispersion or inappropriate density cutoffs can also lead to misallocation between fractions (Cerli et al. 2012). For this, Rock-Eval thermal analysis offers one approach, providing both thermal properties and chemical data that can indicate

the degree of organic matter processing (Disnar et al. 2003; Delahaie et al. 2024; Poeplau et al. 2025). Immature, fresh SOM is higher in hydrogen (H) and as organic matter becomes more mature and processed, it accumulates oxygen (O)-rich carboxyl and phenolic groups (Lehmann & Kleber 2015; Sebag et al. 2016), which facilitate binding to mineral surfaces through ligand exchange and cation bridging (Kleber et al. 2007).

These fractionation challenges have been studied primarily in topsoils, yet subsoils store a substantial proportion of total SOC stocks and are increasingly a research focus (Fulton-Smith & Cotrufo 2019; Anthony et al. 2022; Salonen et al. 2023). Subsoil SOM is generally more microbially processed and exists in a cooler, more oxygen-limited decomposition environment (Rumpel & Kögel-Knabner 2011; Button et al. 2022). How well size- and density-based fractionation methods separate POM and MAOM under these conditions is unclear.

This paper addresses three knowledge gaps: 1) whether the chemical and thermal characteristics of POM and MAOM depend on the fractionation method used; 2) whether the fractionation method influences how much SOC is allocated to POM and MAOM; and 3) whether method performance is consistent across topsoil and subsoil. We analysed size- and density-fractionated POM and MAOM and the corresponding bulk soil using Rock-Eval thermal analysis (Disnar et al. 2003; Sebag et al. 2016). We hypothesised that POM would have chemical characteristics typical for immature, weakly decomposed SOM, with a higher hydrogen but a lower oxygen content. Moreover, we expected that POM would be thermally less stable than MAOM, reflecting weaker mineral protection. We expected method-derived differences to be more pronounced in topsoil, where fresher SOM inputs create a stronger contrast between POM and MAOM.

4.2 Materials and methods

4.2.1 Fractionated soils

We used topsoil (0–10 cm) and subsoil (30–50 cm) samples from three fields located in Germany, Switzerland, and Finland (Table 4.1), with four replicate samples per depth per field. The soils were selected to represent typical agricultural mineral soils with contrasting clay and silt contents. All fields had been under conventional ploughing (≥ 20 cm) for at least 19 years at the time of sampling, resulting in relatively homogeneous

topsoil layers and a less disturbed subsoil below the plough layer. We included both topsoil with higher plant-derived organic inputs and subsoil with lower organic inputs, so that a wide range of thermal and chemical characteristics for the POM and MAOM fractions could be expected. None of the soils contained carbonates.

Table 4.1. Soils collected from long term experiment studies that were utilised in this study; the year when the long-term experiment was established, the sampled soil layer (topsoil=0-10 cm, subsoil=30-50 cm), soil texture, SOC content, and pH.

Country	Established	Soil layer	Clay <2 μ m (%)	Silt 2-50 μ m (%)	Sand >50 μ m (%)	SOC (g OC kg ⁻¹ soil)	pH
Germany	1996	Topsoil	5	20	73	8.5 $\pm$ 0.9	7.1 $\pm$ 0
		Subsoil	5	22	73	2.5 $\pm$ 0.6	7.3 $\pm$ 0
Switzerland	2010	Topsoil	19	69	6	17.2 $\pm$ 1.0	6.3 $\pm$ 0.2
		Subsoil	20	71	5	6.3 $\pm$ 0.7	6.8 $\pm$ 0.2
Finland	2005	Topsoil	60	19	12	27.0 $\pm$ 2.4	5.6 $\pm$ 0.1
		Subsoil	45	10	38	9.3 $\pm$ 3.2	6.1 $\pm$ 0.2

4.2.2 POM/MAOM fractionation methods

Before applying the POM/MAOM fractionation methods, soils were sieved ($\emptyset$ 2 mm) and oven-dried at 40 °C overnight.

Size fractionation (yielding two fractions, POM_{Size} and MAOM_{Size}) was performed as described in Koorneef et al. (2023) (based on the protocols of Baldock et al. 2013; Sanderman et al. 2013). First, 10 g of dried soil was dispersed in 40 mL of 5 g l⁻¹ sodium hexametaphosphate (SHMP) by shaking 18 h at 180 rpm and then wet-sieved over 50 μ m in an automated wet sieving system (FRITSCH). The fraction that remained on top of the sieve was collected as POM_{Size} (i.e., all material sized >50 μ m), and the fraction that passed through the sieve was collected as MAOM_{Size} (i.e., all material sized <50 μ m). The fractions were dried at 105 °C to the constant weight.

For density fractionation (yielding two fractions, POM_{Density} and MAOM_{Density}), we followed a modified version of the Combined Size-Density 1 method described by Leuthold et al. (2024), using an SPT density of 1.60 g cm⁻³ (Cerli et al. 2012) and a higher centrifugation speed (12 000 G) during heavy fraction rinsing to ensure complete sedimentation of clay particles than was described in the Leuthold et al. (2024) paper. First, 25 mL of sodium polytungstate (SPT) with a density of 1.60 g cm⁻³ was added to 8 g of dried soil, along with twelve ~4 mm glass beads. The samples were shaken at 112

rpm for 18 h on a reciprocal shaker to fully disperse soil aggregates. After dispersion, the soil was centrifuged at 2,325 relative centrifugal force (RCF) for 20 min. The supernatant and the material floating on top of it ($\text{POM}_{\text{Density}}$, density $<1.60 \text{ g cm}^{-3}$) was aspirated with a vacuum filtration device, thoroughly washed with deionized water, and dried to a constant weight at 70°C . To remove SPT from the heavy fraction ($\text{MAOM}_{\text{Density}}$), the remaining soil was re-suspended in deionized water and centrifuged at 12 000 G for 25 min. The supernatant was decanted, and this process was repeated two more times. The sample was then dried at 70°C to a constant weight.

For both size and density fractionation, sample mass recoveries between 95-105% were considered acceptable.

4.2.3 SOC thermal analysis with Rock-Eval

All the studied SOM fractions ($\text{POM}_{\text{Density}}$, $\text{MAOM}_{\text{Density}}$, POM_{Size} , $\text{MAOM}_{\text{Size}}$ and bulk soils) were analysed with Rock-Eval® 7S (RE, Vinci technology, France) thermal analysis. Depending on the SOC content of the sample, either 60 mg (topsoils) or 90 mg (subsoils) of ball-milled (for 1 min with frequency 18.0 s^{-1}) samples were used.

All samples were run on the “soil cycle” program of the Rock-Eval. Briefly, samples were subjected sequentially to a pyrolysis and oxidation cycle. The pyrolysis cycle was under pure N_2 , with a 3 min isotherm at 200°C followed by heating at $30^\circ\text{C min}^{-1}$ to 650°C . The subsequent oxidation cycle was under CO_2 free air, and a 1 min isotherm at 300°C followed by heating at $20^\circ\text{C min}^{-1}$ to 850°C which was kept for 5 min. In both cycles, oxygenated compounds (CO and CO_2) were detected with infrared cells. In addition, during the pyrolysis cycle, emitted hydrocarbons were detected with a flame ionization detector. Thus, Rock-Eval analysis measures hydrocarbons (HC), CO , and CO_2 released during pyrolysis and oxidation phases. Parameters were derived from these signals using the R package RockEvalR (Van Hoef 2025). The temperature threshold separating organic from inorganic CO_2 , by default fixed at 400°C , was adjusted to the minimum CO_2 flux between 450 and 600°C to better represent the temperature-dependent mineralisation of soil organic matter.

The hydrogen index (HI, mg HC/g TOC) was calculated as the amount of hydrocarbons emitted divided by TOC and is a proxy for atomic H:C ratio of the SOM (Espitalié et al.

1977). In this paper the oxygen index (OI, mg O/g TOC) is based on the O emitted as both CO and CO₂ during pyrolysis and is a proxy for the atomic O:C ratio of the SOM. According to Disnar et al. (2003), higher HI values are indicative of less decomposed and more labile organic matter, while higher OI values are indicative for more oxidized and decomposed material. Higher OI is thought to reflect a higher functionality in terms of binding towards soil minerals (Sebag et al. 2016).

The thermal stability of the samples was assessed using four indices (Table 4.2). The I-index reflects the immature SOM fraction and is calculated from the amount of organic matter pyrolysed in the 200–340, 340–400, and 400–460°C temperature zones (Sebag et al. 2016). The R-index reflects the refractory SOM fraction and is calculated from organic matter pyrolysed in the 400–460°C and 460–500°C zones. The S3CO₂ T₅₀ represents the temperature at which 50% of total CO₂ has evolved during pyrolysis, and the S4CO₂ T₅₀ represents the same for the oxidation phase (Cécillon et al. 2021). The S4CO₂ T₅₀ has been shown to be an important predictor of centennially stable SOM, often attributed to MAOM (Cécillon et al. 2021). These indices were selected because they capture complementary aspects of thermal stability: the I- and R-indices describe the distribution of pyrolysed organic matter across defined temperature zones, while the T₅₀ parameters integrate the full pyrolysis and oxidation signals. In addition to these thermal indices, the molar O/C ratio of pyrolysed SOM was calculated for the same temperature zones used for the I- and R-index calculations, to explore how chemical composition may vary across thermal stability zones (Sebag et al. 2016). Two individual samples were excluded from analyses due to low SOC recoveries, resulting in n=3 on German topsoil (suboptimal SOC recovery in size fractionation), and Swiss subsoil (suboptimal SOC recovery in density fractionation).

Table 4.2. The Rock-Eval indices and how they reflect potential SOM stability. Negative (–) indicates that a higher value for the index stands for lower SOC stability, and positive (+) indicates that a higher value for the index indicates a higher SOC stability. Adapted from Koornneef et al. (2023).

Index	Description	Unit	Higher value associated with
HI	The hydrogen index. Proxy for atomic hydrogen to carbon (H:C) ratio of SOM. Higher values indicate less decomposed, more labile organic matter (Disnar et al. 2003)	mg CH g ⁻¹ C	POM
OI	The oxygen index. Proxy for atomic oxygen to carbon (O:C) ratio of SOM. Higher values reflect more oxidized and processed material, typically considered more stable due to increased reactivity towards soil minerals (Disnar et al. 2003)	mg O g ⁻¹ C	MAOM
I- index	Reflects thermally labile, less decomposed SOC (refers to immature SOC; Sebag et al. 2016)	Unitless	POM
R- index	Reflects thermally stable, more decomposed SOC (refers to refractory SOC; Sebag et al. 2016)	Unitless	MAOM

*Rock-Eval indices used here as indicators of chemical and thermal properties (Disnar et al. 2003; Sebag et al. 2016) associated with the conceptual definitions of POM and MAOM (e.g., Lavallee et al. 2020).

4.2.4 Statistical analysis

All statistical analyses were performed with R (R Core Team, 2024, version 4.4.1). Data analysis and visualisation were performed using R packages: 'tidyverse' (Wickham et al. 2019), 'ggplot2' (Wickham 2016), 'patchwork' (Pedersen 2025), 'cowplot' (Wilke 2024), 'ggrepel' (Slowikowski 2024), and 'vegan' (Oksanen et al. 2025). We also used the package RockEvalR (Van Hoef 2025).

To evaluate whether the size- and density-based fractionation methods differed in how SOC was distributed between the POM and MAOM fractions, we applied the non-parametric Wilcoxon rank-sum test due to non-normal distribution of the data. We also utilised the Wilcoxon test to compare individual Rock-Eval parameters (HI, OI, I-index, R-index, O/C ratio and S3CO₂_T50 & S4CO₂_T50) between corresponding fractions from each method (i.e., POM_{Density} vs. POM_{Size}, and MAOM_{Density} vs. MAOM_{Size}). Additionally, each individual fraction was compared to the bulk soil with Wilcoxon test to assess if fractionation successfully isolated distinct SOM pools by their chemical composition and thermal stability.

Principal component analysis (PCA) was performed on six Rock-Eval thermal parameters (HI, OI, R, I, S3CO₂_T50, S4CO₂_T50) using the 'rda' function in the 'vegan' package (Oksanen et al. 2007). Variables were standardized (mean = 0, SD = 1) prior to analysis (correlation-based PCA) to ensure equal weighting across parameters measured in different units. Separate PCAs were conducted for topsoil and subsoil.

4.3 Results

4.3.1 Density fractionation better separated POM from MAOM

In most cases, POM_{Density} separated chemically and thermally from the other fractions and bulk soil in the HI-OI and I-R diagrams (Figure 4.1), representing more hydrogen-rich, less oxidized organic matter (high HI, low OI) and lower thermal stability (high I-index, low R-index). This distinction was particularly evident in topsoils, and most pronounced in Swiss and Finnish topsoils and Finnish subsoil, while the German soil showed less differentiation.

In contrast, POM_{Size} clustered more closely with MAOM fractions and bulk soil, showing chemical and thermal characteristics atypical for relatively unaltered particulate organic matter. In some cases (Finnish soils, Swiss subsoil), POM_{Density} was notably more similar to MAOM_{Density} and MAOM_{Size} than to POM_{Size}, indicating that size- and density-based fractionation methods fail to isolate POM with comparable properties.

Statistical comparisons of individual parameters (Figure 4.2) confirmed the observed patterns. In topsoils, POM_{Density} differed significantly from POM_{Size} across all utilised Rock Eval indices (Wilcoxon, $p = 0.029$; except I- and R-index in Finland, where no statistical difference was observed). In subsoils, differentiation between POM fractions was less pronounced with no significant differences in most cases (except HI, OI and R in Finland).

In most soils, MAOM fractions differed significantly between the two fractionation methods and from bulk soil in terms of the thermal stability indices (I-index, R-index, $p < 0.05$), but not in the chemical composition indices (HI, OI; Figure 4.1). In the German topsoil sample no statistical difference was observed, while in Finnish soils MAOM fractions differed between the two fractionation methods but not from bulk soil. Despite MAOM comprising the majority of SOC, bulk soil SOM exhibited similar yet consistently higher thermal stability (higher R-index, lower I-index, $p < 0.05$) than the isolated MAOM fractions (Figure 4.2).

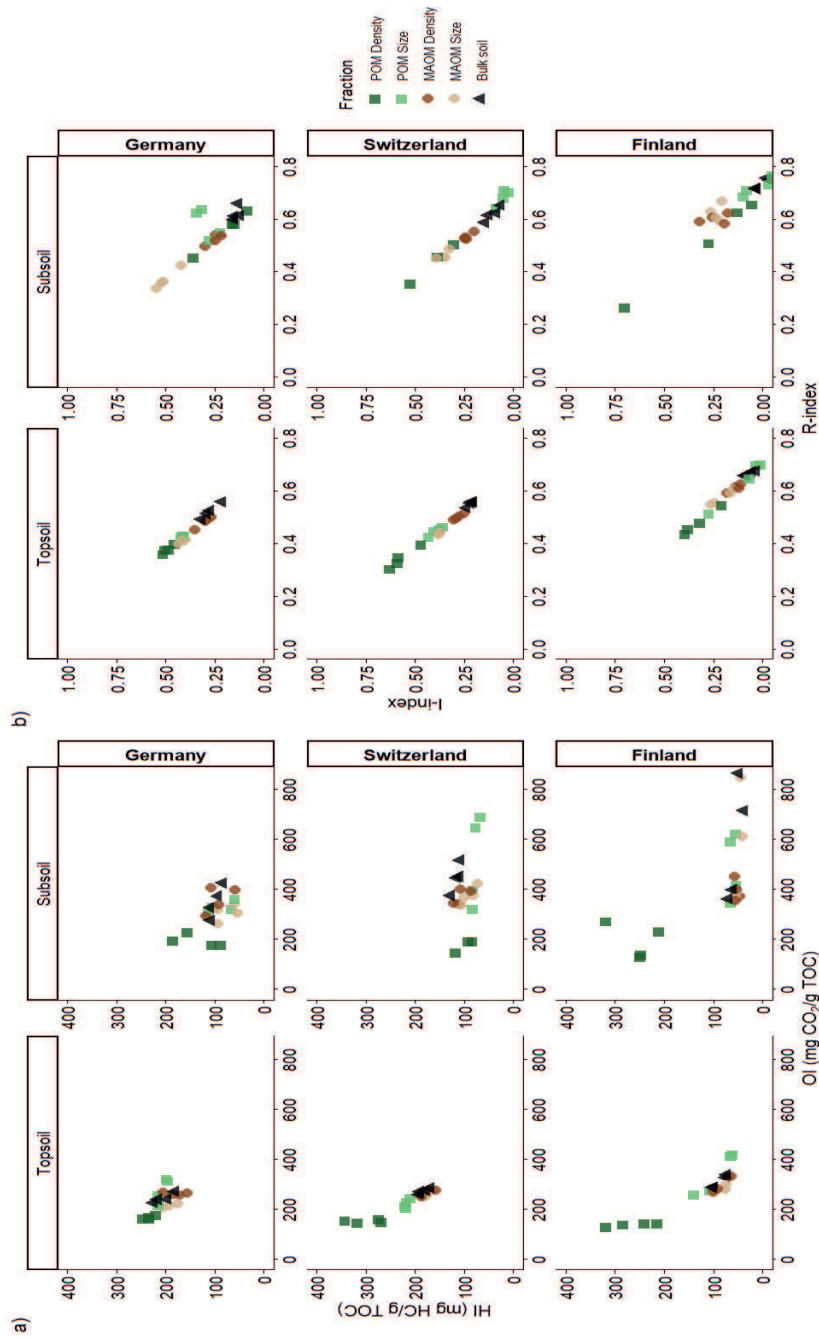


Figure 4.1. Hydrogen Index (HI, mg HC g⁻¹ TOC) vs Oxygen Index (OI, mg O₂ g⁻¹ TOC) (a) and I-index vs R-index (b) for POM and MAOM fractionated by density and size-based methods and the corresponding bulk soil.

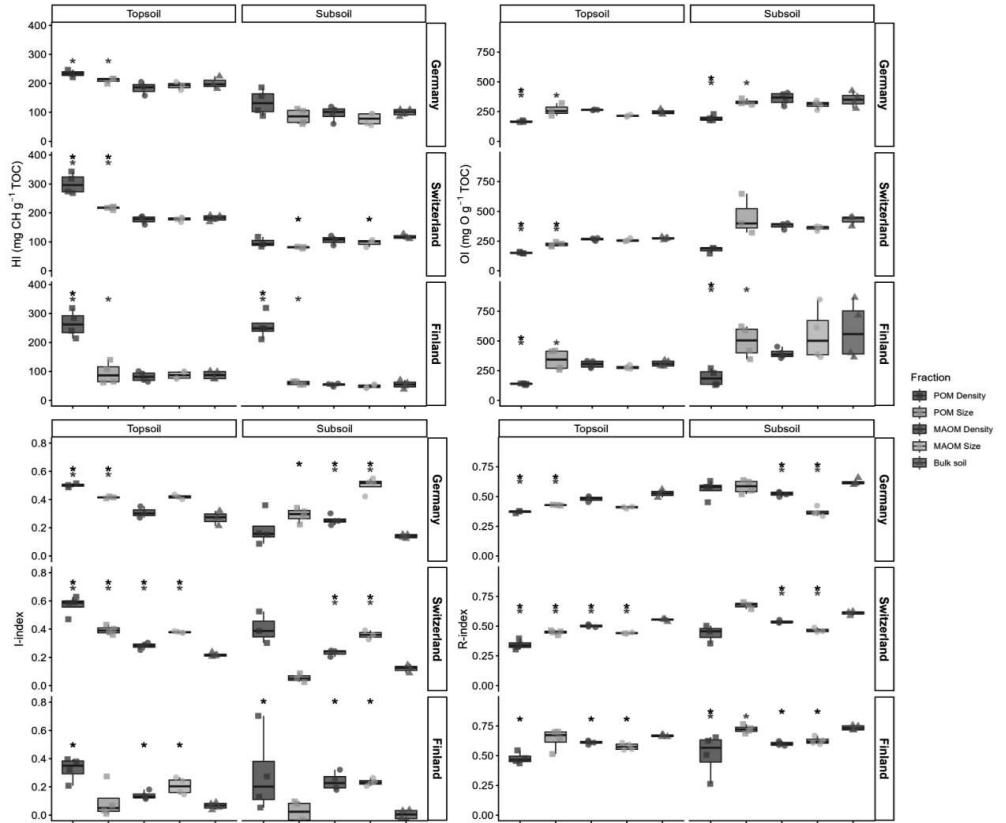


Figure 4.2. Hydrogen Index (HI, mg HC g⁻¹ TOC), Oxygen Index (OI, mg O g⁻¹ TOC), I-index, and R-index for POM and MAOM fractionated by density and size based methods and the corresponding bulk soil. Statistical tests were performed separately for each country and soil layer. Asterisks mark significant differences: green between POMDensity and POMSize, brown between MAOMDensity and MAOMSize, and the black with the POM/MAOM fraction in question and the bulk soil (i.e., black asterisk shown on top of the fraction that is significantly different from the bulksoil). Wilcoxon test, significant $p < 0.05$.

S3CO₂ T₅₀ and S4CO₂ T₅₀ showed variable patterns across fractions and depths (Figure 4.S1). These parameters were included in the PCA to capture the full thermal signature of each fraction. A principal component analysis of Rock-Eval parameters (HI, OI, I-index, R-index, S3CO₂ T₅₀, and S4CO₂ T₅₀) revealed distinct clustering patterns between fractionation methods and soil depths (Figure 4.3). In topsoil, PC1 and PC2 together explained 84% of the variance (67.3% and 16.7%, respectively), while in subsoil the PCs explained 70% (45.1% and 24.7%), indicating greater organic matter heterogeneity at depth.

In topsoil, POM_{Density} formed a distinct cluster characterised by higher HI and I-index, and lower OI and R-index values, indicating less thermally stable, less processed organic matter with lower functional group density. POM_{Size}, in contrast, showed substantial overlap with bulk soil and MAOM. MAOM_{Density} clustered closely with bulk soil across both PC1 and PC2, whereas MAOM_{Size} was positioned distinctly from both. This closer positioning of MAOM_{Density} to bulk soil was also reflected in individual Rock-Eval parameters (Figure 4.2).

In subsoil, fraction separation was less pronounced. POM_{Density} no longer formed a clearly distinct cluster, and confidence ellipses showed greater overlap between all fractions and bulk soil. MAOM_{Density} remained similar to bulk soil along PC2 but diverged along PC1, while MAOM_{Size} remained distinct from both bulk soil and MAOM_{Density}. Across both depths, Finnish samples exhibited notably higher variance than German and Swiss samples across all fraction types.

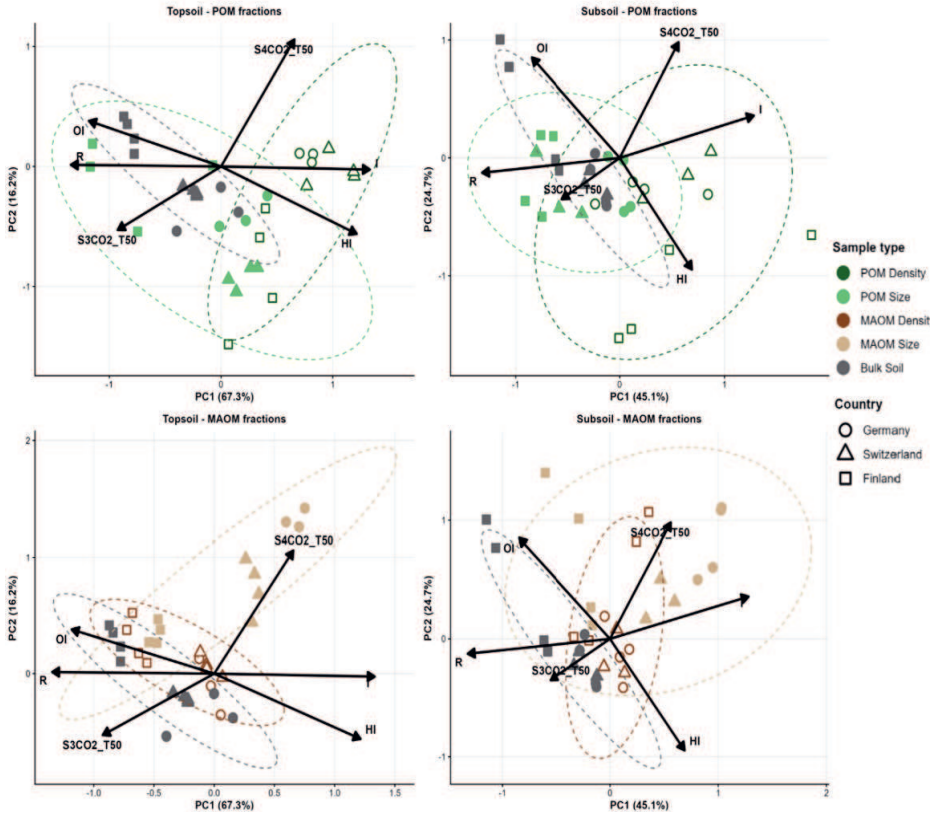


Figure 4.3. Principal component analysis (PCA) of Rock-Eval thermal parameters (HI, OI, R, I, S3CO₂_T50, S4CO₂_T50) for soil fractionated by density and size fractionation methods. Separate PCAs were performed for topsoil (left) and subsoil (right). Upper panels POM fractions with bulk soil; lower panels MAOM fractions alongside bulk soil. Dashed ellipses show 95% confidence intervals.

To further study the observed compositional differences between the density separated POM and MAOM, the pyrolyzed organic oxygen and carbon were integrated over distinct temperature intervals concurrent with the intervals used for I and R index calculation (Sebag et al. 2016). As shown in Figure 4.4, the O/C ratio of pyrolyzed POM_{Density} differed significantly (Wilcoxon tests, $p < 0.05$) from the bulk soil and the other SOC fractions for all temperature zones. No significant difference (Wilcoxon tests, $p > 0.05$) was found between other fractions or the bulk soil for any considered temperature interval.

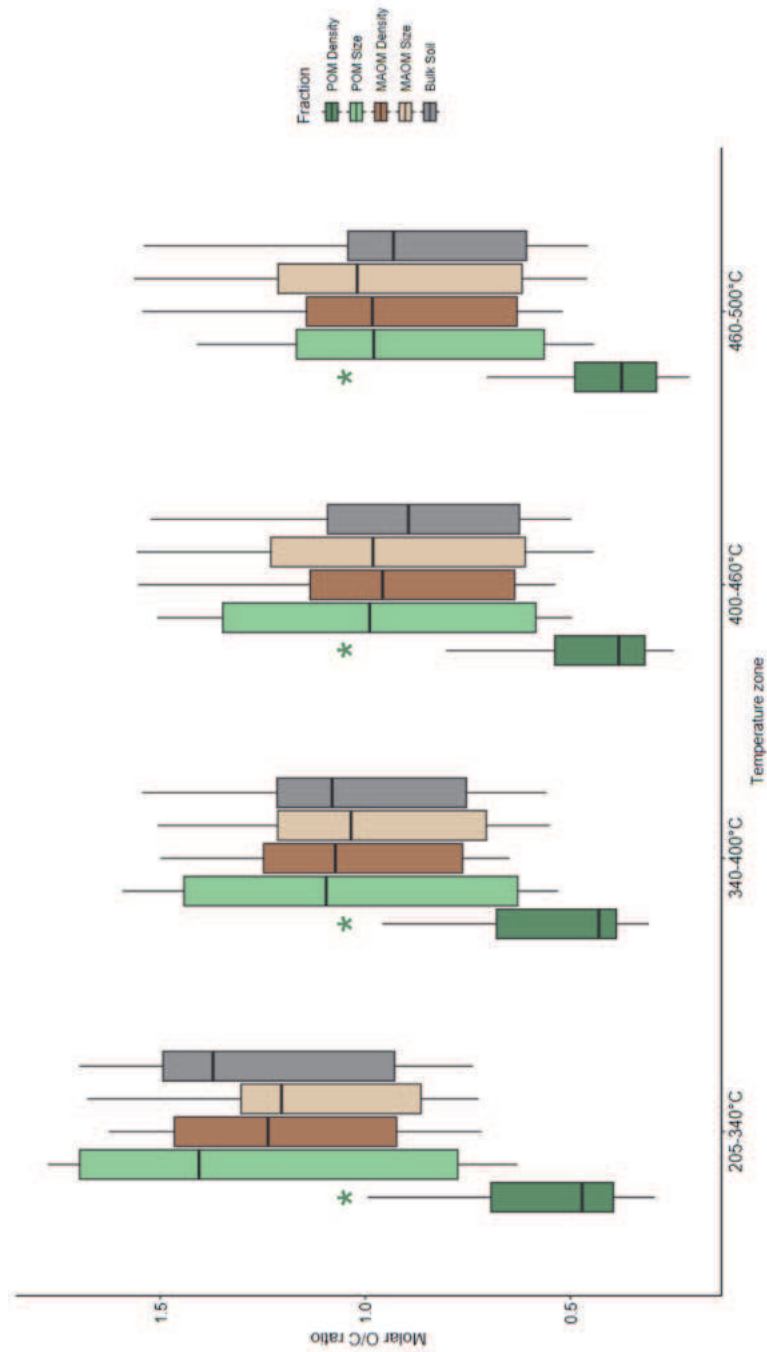


Figure 4.4. Molar oxygen to carbon ratio (O/C) of pyrolysed organic matter from top- and subsoils (all samples pooled together for the analysis, $n=23$) over distinct temperature zones. Temperature zones are concurrent with the intervals used for I - and R -index calculation (Sebag et al. 2016). The asterisks indicate significant difference from the other fractions or bulk soil (Wilcoxon test, $p < 0.05$).

4.3.2 Distribution of SOC into POM and MAOM across size and density-based fractionation methods

Total SOC in POM + MAOM (g OC kg^{-1} bulk soil) did not differ significantly from bulk SOC for either fractionation method (SOC recoveries 79–95%; Wilcoxon tests within country and depth, $p > 0.05$; Table 4.3).

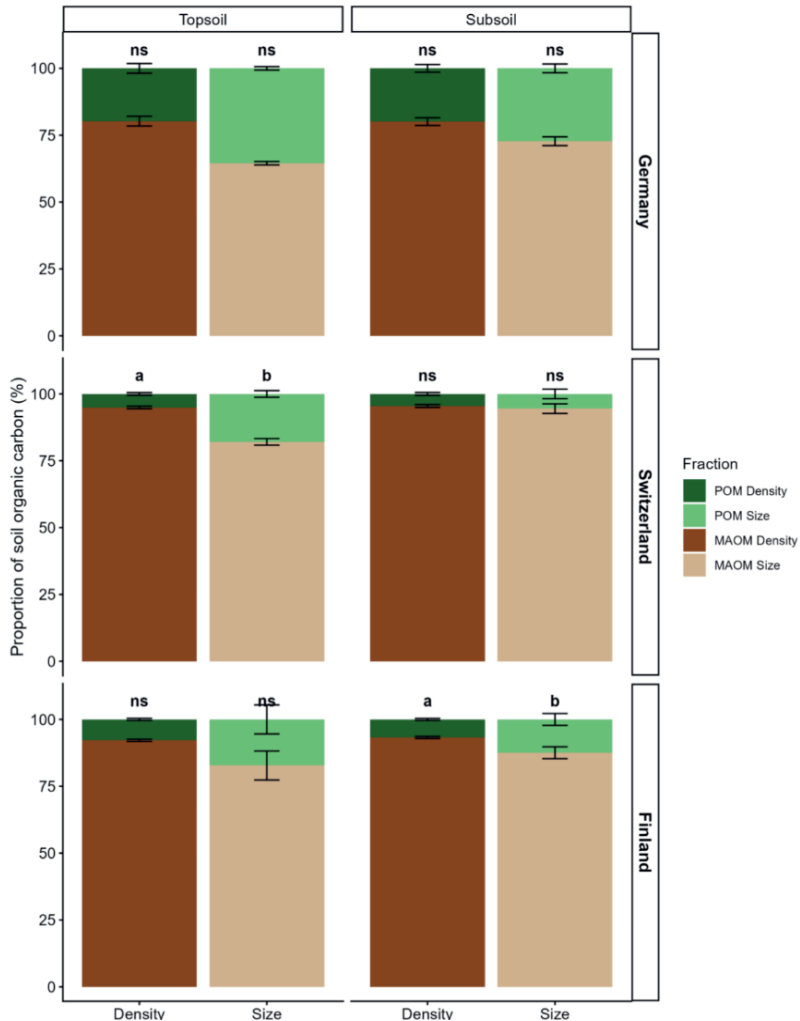


Figure 4.5. Distribution of soil organic carbon in fractionated pools. Proportional distribution of SOC between POM and MAOM fractions (POM-SOC + MAOM-SOC = 100%). Statistical tests were performed to compare the share of SOC in POM separately for each country and the soil layer (Wilcoxon tests, $p > 0.05$). Values are mean $\pm$ SE, $n=4$ (except for German topsoil and Swiss subsoil $n=3$).

Table 4.3. Soil organic carbon distribution and recovery by fractionation method. Total SOC content of POM and MAOM fractions (g C kg^{-1} POM+MAOM), SOC recovery in POM+MAOM by fractionation method (% of bulk soil OC), and bulk soil SOC content (g C kg^{-1} soil). Values are mean $\pm$ SE, $n=4$ (except for German topsoil and Swiss subsoil $n=3$). No significant differences (Wilcoxon tests, $p > 0.05$).

Country	Soil layer	<u>Density fractionation</u>				<u>Size fractionation</u>				<u>Bulk soil</u>	
		SOC in POM (g OC kg^{-1} bulk soil)	SOC in MAOM (g OC kg^{-1} bulk soil)	Total SOC (g OC kg^{-1} bulk soil)	SOC recovery (% from bulk soil OC)	SOC in POM (g OC kg^{-1} bulk soil)	SOC in MAOM (g OC kg^{-1} bulk soil)	Total SOC (g OC kg^{-1} bulk soil)	SOC recovery (% from bulk soil OC)	SOC (g OC kg^{-1} soil)	SOC (g OC kg^{-1} soil)
Germany	Topsoil	1.5 $\pm$ 0.1	5.9 $\pm$ 0.3	7.4 $\pm$ 0.2	88 $\pm$ 6	2.4 $\pm$ 0.2	4.4 $\pm$ 0.3	6.8 $\pm$ 0.5	81 $\pm$ 4	8.5 $\pm$ 0.9	
	Subsoil	0.4 $\pm$ 0.1	1.7 $\pm$ 0.4	2.1 $\pm$ 0.5	82 $\pm$ 4	0.6 $\pm$ 0.2	1.5 $\pm$ 0.4	2.0 $\pm$ 0.5	79 $\pm$ 5	2.5 $\pm$ 0.6	
Switzerland	Topsoil	0.8 $\pm$ 0.1	14.8 $\pm$ 0.9	15.6 $\pm$ 0.9	91 $\pm$ 1	2.8 $\pm$ 0.1	13.2 $\pm$ 1.1	16.0 $\pm$ 1.1	93 $\pm$ 2	17.2 $\pm$ 1.0	
	Subsoil	0.3 $\pm$ 0.0	5.5 $\pm$ 0.7	5.8 $\pm$ 0.7	92 $\pm$ 2	0.3 $\pm$ 0.1	5.4 $\pm$ 0.6	5.7 $\pm$ 0.6	91 $\pm$ 1	6.3 $\pm$ 0.7	
Finland	Topsoil	1.9 $\pm$ 0.1	23.1 $\pm$ 2.4	25.0 $\pm$ 2.5	92 $\pm$ 1	4.2 $\pm$ 1.4	20.5 $\pm$ 1.9	24.7 $\pm$ 1.6	92 $\pm$ 5	27.0 $\pm$ 2.4	
	Subsoil	0.5 $\pm$ 0.2	7.6 $\pm$ 2.8	8.1 $\pm$ 3.0	84 $\pm$ 4	1.3 $\pm$ 0.6	7.6 $\pm$ 2.6	8.9 $\pm$ 3.1	95 $\pm$ 2	9.3 $\pm$ 3.2	

4.4 Discussion

The aim of this study was to determine whether two commonly used SOM fractionation approaches, size- and density-based methods, isolate similar POM and MAOM fractions with matching thermal stability and chemical composition, and whether they allocate similar proportions of SOC into POM and MAOM pools.

Rock-Eval thermal analysis showed that POM_{Density} was chemically (HI, OI, O/C ratio) and thermally (I-index, R-index) (Figures 1-4) distinct from other fractions (POM_{Size}, MAOM_{Density} and MAOM_{Size}), and bulk soil SOM. In addition, the POM_{Density} fraction consistently revealed characteristics representative of less decomposed, thermally labile SOM. In contrast, POM_{Size} displayed characteristics closer to that of MAOM fractions and bulk soil, strongly indicating that size fractionation was unsuccessful in isolating a distinct POM pool. Additionally, size fractionation allocated 1.4–3.6 times more SOC to POM than density fractionation in five out of six soils (Table 4.3, Figure 4.5), further indicating that these methods capture fundamentally different organic matter pools. MAOM fractions exhibited chemical similarity between size and density fractionation methods (HI, OI) but differed significantly in thermal stability (I-index, R-index; Figure 4.2). Overall, these findings show that the choice of fractionation method affects the characterisation of both POM and MAOM.

4.4.1 Density fractionation isolates conceptually expected POM

Rock-Eval thermal analysis revealed that POM_{Density} and POM_{Size} differed fundamentally in both chemical composition (HI, OI, O/C ratio) and thermal stability (I-index, R-index) (Figures 1, 2 & 4). HI-OI diagrams (Figure 4.1) illustrated chemical composition gradients from hydrogen-rich to oxidized organic matter (Disnar et al. 2003), showing that POM_{Density} was less decomposed and oxidized compared to POM_{Size}, both MAOM fractions, and bulk soil. Thermal maturity indices (I-index and R-index; Sebag et al. 2016) confirmed this pattern, with POM_{Density} being more thermally labile than all other fractions and bulk soil. The PCA reinforced this distinction, showing POM_{Density} clustering separately from POM_{Size}, and bulk soil, with higher HI and I-index values characteristic of less processed organic matter (Figure 4.3). POM_{Density} remained distinct from POM_{Size} across both depths based on individual Rock-Eval parameters (Figure 4.2), though subsoil

fractions showed some convergence toward higher thermal stability. Subsoils also exhibited wider OI range, which may reflect preferential retention of more processed, O-enriched SOM on unsaturated mineral surfaces at depth, where OM loading is lower (Kleber et al. 2007).

For a fractionation method to successfully isolate POM, the resulting fraction should be distinct from both bulk soil and MAOM. POM_{Size} did not meet this criterion and was chemically similar to MAOM fractions (elevated OI, comparable HI and O/C ratios, Figures 2 & 4). The PCA confirmed this pattern: POM_{Size} overlapped with bulk soil rather than clustering with POM_{Density}, particularly in topsoil (Figure 4.3). In subsoil, all fractions showed greater overlap, though POM_{Density} still separated from POM_{Size} and bulk soil. The similarity between POM_{Size} and MAOM fractions aligns with Yu et al. (2022), who found that size-fractionated POM and MAOM often showed overlapping elemental compositions. Similarly, the results of Delahaie et al. (2024), indicated that size-fractionated MAOM includes thermally labile material inconsistent with conceptual definition of MAOM. Together, these findings suggest that POM_{Density} represents a functionally labile carbon pool matching conceptual expectations, while POM_{Size} does not. Treating POM_{Size} as equivalent to POM_{Density} in modeling or monitoring frameworks may therefore lead to misinterpretation of SOC vulnerability for decomposition.

4.4.2 MAOM thermal stability is method-dependent despite the chemical similarity

The proportions of SOC in MAOM_{Density} were remarkably consistent between topsoil and subsoil within each country (Germany: 80-80%; Switzerland: 95-95%; Finland: 92-93%, Table 4.S1), despite 3-4 fold decreases in total SOC with depth (Table 4.1). Even in Finnish soils, where clay content varied substantially between depths (60% topsoil, 45% subsoil), the proportional distribution remained stable. Proportion of SOC in MAOM_{Size} differed between soil types, being lower in sandy German soils (sand 73%; 65% of the SOC) than in clay-rich Swiss and Finnish soils (sand 5- 38%, 92-95% of the SOC). MAOM_{Density} showed greater consistency across depths and sites than MAOM_{Size}. Whether this reflects robustness of the method or lower sensitivity to real depth-related differences in MAOM composition remains an open question.

These MAOM proportions align with previously reported values (~80% of total SOC; Cotrufo et al. 2022; Georgiou et al. 2022; Begill et al. 2023; Soenne et al. 2024). While

MAOM fractions showed similar chemical composition (HI, OI) to bulk soil and to each other, they exhibited lower thermal stability than bulk soil (I and R-indices, Figure 4.2), with MAOM_{Size} tending to be less stable than MAOM_{Density}. This suggests that fractionation may alter organo-mineral associations without altering SOM chemistry, and that the soil dispersion method could affect the degree of this disruption.

Size fractionation uses chemical dispersion (SHMP) aimed at disintegrating soil aggregates through increased negative surface charge and repulsion between particles (Andreola et al. 2006), while density fractionation uses gentler physical dispersion (glass beads). However, SHMP may disrupt organo-mineral bonds beyond aggregate dispersion alone: Keskinen et al. (2024) demonstrated that SHMP decreased SOC recovery compared to water dispersion, indicating dissolution of SOM.

MAOM_{Size} showed higher I-index values than MAOM_{Density} or bulk soil (statistically significant in German and Swiss subsoils and Swiss topsoil; Figure 4.2), indicating lower thermal stability consistent with more aggressive disruption of organo-mineral associations. Furthermore, MAOM_{Size} showed soil-dependent variation in the PCA: German and Swiss MAOM_{Size} separated from bulk soil toward higher I-index and HI, while Finnish MAOM_{Size} clustered with bulk soil (Figure 4.3). In contrast, MAOM_{Density} consistently clustered with bulk soil, regardless of soil origin or depth, supporting density fractionation as more suitable for characterising MAOM quality (Leuthold et al. 2024).

4.4.3 Functionally distinct POM and MAOM

The significantly different O/C ratios observed between POM_{Density} and other studied samples (POM_{Size}, MAOM_{Density}, MAOM_{Size}, and bulk soil; Figure 4.4) indicate that density-separated POM represents a distinct, less oxidized SOM pool compared with all other fractions across all studied soils. Notably, POM_{Size} showed O/C ratios similar to MAOM_{Density}, MAOM_{Size}, and bulk soil, suggesting that size fractionation does not effectively separate POM from MAOM. These differences persisted across the entire 205–500°C temperature range, covering both labile biopolymers and more thermally stable compounds (Sebag et al. 2016), demonstrating that the chemical distinction between POM_{Density} and other fractions is not limited to a specific pyrolysis temperature.

The consistently lower O/C ratios of POM_{Density} strongly support the view that, as SOM matures, it either becomes mineral associated due to its increased functionality in terms of binding properties, or is mineralized (Guggenberger & Kaiser 2003). This aligns with conceptual models describing SOM decomposition as a continuous process, transforming larger plant and animal residues into smaller molecules enriched in oxygen-containing functional groups (Lehmann & Kleber 2015). This enrichment in charged functional groups, such as carboxylic and phenolic groups, increases the reactivity of SOM towards mineral surfaces, facilitating mineral protection against further decomposition (Kleber et al. 2007; Avneri-Katz et al. 2017). The tendency for lower HI values of POM_{Density} in subsoils compared with topsoils in Germany and Switzerland (Figure 4.2) may indicate the presence of recalcitrant POM that persists during decomposition (Disnar et al. 2003). However, methodological factors such as incomplete aggregate dispersion leading to MAOM contaminating POM cannot be excluded (Breure et al. 2026). Regardless of the mechanism, these findings highlight the importance of method selection when studying SOM.

4.4.4 Distribution of SOC across POM and MAOM

Using a single-pool density fractionation approach (i.e., extracting only one POM fraction instead of the more typical two-POM pools approach; e.g. Cerli et al. 2012), we found comparable SOC recoveries between density and size fractionation methods (79-95% for size, 82-92% for density; Table 4.3), indicating that the observed chemical differences between fractions are not driven by differential OC losses during separation. This contrasts with previous reports of better recoveries in size fractionation (Poeplau et al. 2018) but is consistent with Leuthold et al. (2024) who found similar recoveries across methods. Our results indicate that methodological losses were comparable between the two fractionation approaches, and that differences in SOC allocation to POM reflect a fractionation bias rather than, for example, incomplete recovery. Also, a single-pool approach (i.e., fractionating SOC only in a single POM pool) likely minimizes losses compared to multi-step methods (such as typical free and occluded POM, e.g. Cerli et al. 2012), as additional processing steps inherently increase the risk of SOC losses.

In most soils (five out of six soils), size fractionation tended to allocate a larger proportion (1.4–3.6 fold) of SOC to POM, than density fractionation. This is supported by studies

from Keskinen et al. (2024) and Gregorich et al. (2006). The larger allocation of SOC in POM in size fractionation can be explained by methodological biases. First, underdispersion of aggregates during size fractionation could result in some share of MAOM (i.e., SOC attached to the minerals of incompletely dispersed aggregates) being recovered in the POM fraction; Breure et al. (2026) found that underdispersion can overestimate the proportion of SOC in POM by as much as 235% (but on average 35%). Additionally, coarse mineral-bound organic carbon, i.e., SOC associated with sand-sized mineral particles ($>50\ \mu\text{m}$), is recovered in POM_{Size} pool, increasing its measured SOC content. Both fractionation methods could theoretically allocate some mineral-associated material to the POM fraction: in size fractionation, coarse minerals ($>50\ \mu\text{m}$) with attached SOC (i.e., MAOM) are allocated to the POM pool, while in density fractionation, minerals associated with organic matter (that have low density) may float.

However, our Rock-Eval results show that POM_{Density} consistently displayed characteristics of fresh, thermally labile organic matter, while POM_{Size} resembled MAOM and bulk soil. This indicates that in practice, density fractionation more effectively isolates a chemically and thermally distinct POM pool, even if the separation is never absolute (as there is always a continuum between fractions; Six et al. 2024). In sandy soils with high SOC content, size fractionation can overestimate MAOM due to fine POM contamination of the $<53\ \mu\text{m}$ fraction (Poeplau et al. 2025). Comparing these two biases, under-dispersion affects soils across all texture classes (but particularly clay-rich soils that form stronger aggregates; Totsche et al. 2018), while fine POM contamination is more pronounced in sandy soils with high organic carbon content (Poeplau et al. 2025). Our results, with larger POM_{Size} allocations and MAOM-resembling thermal characteristics of the POM_{Size} are consistent with underdispersion as a systematic bias in size fractionation. Fine POM ($<53\ \mu\text{m}$) passing into MAOM_{Size} represents a complementary bias operating in the opposite direction, but the net effect suggests under-dispersion dominates.

Our findings demonstrate that density fractionation isolates chemically more distinct POM and MAOM pools compared to size fractionation, as shown by Rock-Eval parameters HI-OI and I-R index patterns (Figures 1 and 2) and O/C ratios (Figure 4.4).

This aligns with Koorneef et al. (2023), who showed that thermal characterisation adds value to physical fractionation for assessing SOC quality.

4.5 Conclusions

We found that size- and density-based SOM fractionation methods do not isolate corresponding POM and MAOM fractions. POM_{Density} was chemically and thermally distinct from bulk soil and POM_{Size}, MAOM_{Density} and MAOM_{Size}, with characteristics indicating less processed, labile organic matter consistent with conceptual pool definitions. POM_{Size}, however, overlapped with MAOM and bulk soil, indicating contamination with mineral-associated material. The consistently lower O/C ratios of POM_{Density} may offer a diagnostic criterion for evaluating whether fractionation methods successfully isolate functionally distinct POM. Single-pool density fractionation combined with Rock-Eval thermal analysis hence seems to offer a robust approach for assessing SOM dynamics, though future research should evaluate whether this combination can detect soil management-induced changes in POM and MAOM quality. Moreover, our findings need to be validated across broader soil types beyond the six soils (7–60% clay) from three countries studied. Our findings highlight that POM and MAOM are method-dependent operational fractions rather than universal functional pools. More descriptive terminology, such as coarse and fine for size-fractionated pools and light and heavy for density-fractionated pools, would better reflect their operational nature. At minimum, we recommend that studies explicitly report fractionation methods alongside fraction names (e.g., POM_{Density}, POM_{Size} etc).

Acknowledgments

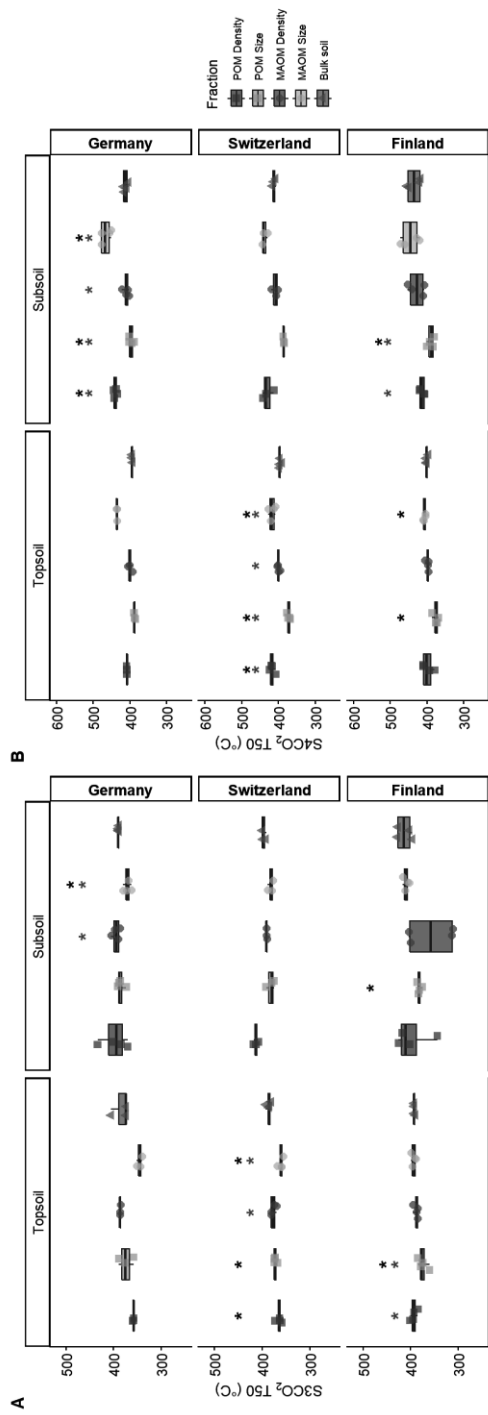
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collected from three Long-Term Experimental sites, for which we would like to thank LUKE, FiBl and ZALF for supporting sampling and providing basic site data.

Supplementary Material

Table 4.S1. Distribution of soil organic carbon in fractionated pools. Proportional distribution of SOC between POM and MAOM fractions (POM-SOC + MAOM-SOC = 100%). Mean $\pm$ SE.

Country	Soil layer	Fraction	n	Proportion (%) of OC in the fraction	
				<u>Density</u>	<u>Size</u>
Germany	Topsoil	<u>POM</u>	3	20 $\pm$ 1.8	35 $\pm$ 0.6
		<u>MAOM</u>	3	80 $\pm$ 1.8	65 $\pm$ 0.6
	Subsoil	<u>POM</u>	4	20 $\pm$ 1.4	27 $\pm$ 1.6
		<u>MAOM</u>	4	80 $\pm$ 1.4	73 $\pm$ 1.6
Switzerland	Topsoil	<u>POM</u>	4	5 $\pm$ 0.5 ^a	18 $\pm$ 1.2 ^b
		<u>MAOM</u>	4	95 $\pm$ 0.5 ^a	82 $\pm$ 1.2 ^b
	Subsoil	<u>POM</u>	3	5 $\pm$ 0.5	5 $\pm$ 1.8
		<u>MAOM</u>	3	95 $\pm$ 0.5	95 $\pm$ 1.8
Finland	Topsoil	<u>POM</u>	4	8 $\pm$ 0.4	17 $\pm$ 5.4
		<u>MAOM</u>	4	92 $\pm$ 0.4	83 $\pm$ 5.4
	Subsoil	<u>POM</u>	4	7 $\pm$ 0.4 ^a	12 $\pm$ 2.2 ^b
		<u>MAOM</u>	4	93 $\pm$ 0.4 ^a	88 $\pm$ 2.2 ^b



Chapter 5

Glued forever: incomplete dispersion hampers POM-MAOM fractionation in soils with high SOC and metal (hydr)oxide content

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Abstract

Fractionation of soil organic carbon (SOC) into particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) has contributed to our understanding of soil carbon cycling. Size fractionation following soil dispersion with sodium hexametaphosphate (SHMP) is a common method to separate POC ($>50\ \mu\text{m}$) from MAOC ($<50\ \mu\text{m}$). Complete dispersion of aggregates is essential to avoid overestimation of the POC and underestimation of the MAOC pools. We investigated how soil properties, particularly the contents of SOC, nanocrystalline aluminium (Al) and iron (Fe) (hydr)oxides, and exchangeable cations, affect soil dispersion with $5\ \text{g L}^{-1}$ SHMP using a set of 38 widely diverse soils. Completeness of dispersion was evaluated by assessing the content of fine particles ($<50\ \mu\text{m}$) present in the coarse fraction ($>50\ \mu\text{m}$) after size fractionation, using laser diffraction analysis. Multiple linear regression revealed that dispersion was less complete with increasing contents of SOC and Al and Fe (hydr)oxides, and also depended on soil texture. On average, incomplete dispersion led to $3.6\ \text{g MAOC kg}^{-1}$ soil (range: $0 - 42\ \text{g kg}^{-1}$) erroneously ending up in the coarse fraction, corresponding with a 33.4% (range $0 - 217\%$) overestimation of POC and a 7.8% (range: $0 - 53\%$) underestimation of MAOC. The residual moisture content of $40\ ^\circ\text{C}$ dried soils emerged as a cost-efficient and effective indicator for the risk of incomplete dispersion, except in soils high in exchangeable Na and/or Mg. Checking and reporting completeness of dispersion after size fractionation should be common practice when studying soil carbon pools. This study provides methodological insights that can guide improvements in SOC fractionation accuracy. Future refinements should focus on optimizing dispersion techniques while carefully balancing the risk of POM fragmentation.

5.1 Introduction

The potential of agricultural soils to capture atmospheric carbon and retain soil organic carbon (SOC) is widely acknowledged (Chenu et al. 2019; Palm et al. 2014; Van de Broek et al. 2019). Implementing sustainable land management practices that increase SOC could enhance multiple soil functions, such as climate regulation, nutrient cycling and water purification (Chenu et al. 2019; Creamer et al. 2022; Hoffland et al. 2020; Koorneef et al. 2024). Differentiating SOC pools with contrasting turnover rates has greatly advanced our understanding and modelling of soil carbon cycling and sequestration (Lavallee et al. 2020). SOC pools with different turnover rates have been operationally categorised into particulate organic matter (POM) and mineral associated organic matter (MAOM) (Cambardella and Elliott, 1993; Cotrufo et al. 2019; Lavallee et al. 2020; Poeplau et al. 2018). The residence time of POM is assumed to be in the order of years to decades, whilst MAOM could persist in soil for decades to millennia (Cotrufo and Lavallee, 2022; Lavallee et al. 2020; Spielvogel et al. 2008). POM is generally assumed to have a relatively short turnover time, because it is present in the soil in free form (free POM) or in aggregates that are easily disturbed (occluded POM), providing limited protection against decomposition. In contrast, MAOM is assumed to have a longer turnover time, due to the protection provided by organo-mineral association and occlusion in micropores (Arachchige et al. 2018; Mikutta et al. 2006; Totsche et al. 2018).

To evaluate management effects on SOC cycling, accurate separation of POM and MAOM is required: an overestimation of MAOM leads to an overestimation of the soil carbon sequestration potential and vice versa. The organic carbon in the POM (POC) and MAOM (MAOC) pools are typically assessed by size or density fractionation of soils (Lavallee et al. 2020; Leuthold et al. 2024; Poeplau et al. 2018). The commonly used high-throughput size fractionation method results in a coarse fraction (typically $>50\text{ }\mu\text{m}$) that theoretically primarily contains POC and a fine fraction (often $<50\text{ }\mu\text{m}$) that theoretically primarily contains MAOC, besides fine-sized POC (Lavallee et al. 2020). To effectively separate POC from MAOC, it is crucial to break down aggregates and disperse the soil (Lavallee et al. 2020). However, commonly applied methods for soil dispersion do not always result in separation into primary soil particles, since highly stable aggregates of organic and inorganic particles may persist (Lavallee et al. 2020; Pulleman et al. 2005; Totsche et al. 2018). Dispersion of aggregates by either chemical (e.g. pH adjustment, addition of a dispersion

agent) or physical (e.g. sonication, shaking with glass beads) processes is a balancing act. If a dispersion method is too aggressive, significant breakdown and alterations of the coarse fraction could occur, resulting in an overestimation of MAOC (Six et al. 2024). However, if the dispersion method is too gentle, this could lead to incomplete dispersion of stable aggregates and an underestimation of MAOC (Lavalley et al. 2020).

Soils are commonly dispersed with 5 g L⁻¹ sodium hexametaphosphate (SHMP), which has been recognized as an effective soil aggregate dispersant for a long time (Baldock et al. 2013; Cotrufo et al. 2019; Poeplau et al. 2018; Sanderman et al. 2013; Tyner, 1938; Wintermyer and Kinter, 1955). The cyclic polyphosphate binds to soil mineral surfaces through formation of surface complexes, thereby causing a strong desorption of SOC (Andreola et al. 2006; Han et al. 2016; Hinsinger, 2001; Kura et al. 1974). SHMP also strongly complexes polyvalent cations resulting in desorption of calcium (Ca) and magnesium (Mg) from soil organic matter and mineral surfaces (Kura et al. 1974). Moreover, the excess of sodium (Na) will replace Ca and Mg at negatively charged binding sites in the soil that collectively make up the cation exchange capacity (CEC), resulting in soil deflocculation and increased electrostatic repulsion between charged soil particles, leading to their dispersion (Andreola et al. 2006; Kura et al. 1974).

Certain cementing agents (e.g. organic matter, metal(hydr)oxides, polyvalent cations) can act as a glue between soil particles (Arachchige et al. 2018; Eusterhues et al. 2005; Kirsten et al. 2021; Rowley et al. 2021; Totsche et al. 2018). These agents can inhibit aggregate dispersion by SHMP and, consequently, may hinder a reliable size-based fractionation of SOC into POC and MAOC. The relevant cementing agents are soil specific. For example, under neutral to alkaline conditions, cementation could partly be mediated by calcium carbonates and Ca²⁺, which promote flocculation and aggregation of negatively charged clay and organic surfaces (Muneer and Oades, 1989; Oades, 1984; Rowley et al. 2018; Totsche et al. 2018). In acidic soils, aggregate stability is typically driven by reactivity of clay minerals (Lehtinen et al. 2014; Six et al. 2002) and metal (hydr)oxides, which can co-precipitate with organic matter, forming strong organo-mineral associations (Kaiser and Guggenberger, 2000; Kleber et al. 2005; Mikutta et al. 2006). Dispersion might also be dependent on the dominant clay mineralogy, particularly in relation to the size of the CEC and the swelling behaviour of clay minerals (Denef et al. 2002; Six et al. 2002). Finally, the silt fraction is considered relevant

for stabilising carbon (Hassink, 1997; Six et al. 2024) and likely also contributes to aggregate stability.

Dispersion with SHMP is generally assumed to be complete after 17 hours, irrespective of soil type (Cotrufo et al. 2019; Sanderman et al. 2013). However, the validity of this assumption in relation to the abundance of cementing agents remains to be tested. The main objective of this study was to establish whether and to what extent the aforementioned cementing agents affect soil dispersion by SHMP. In this context, a complete dispersion is defined as full separation of a soil into primary soil particles. To this end, size fractionation at 50 μm following dispersion with SHMP was performed for 38 soils varying in origin, texture, SOC, pH and other soil properties. Underdispersion was evaluated based on the content of fine particles ($<50\text{ }\mu\text{m}$) present in the coarse fraction after size fractionation. We hypothesize that underdispersion will increase with SOC, nanocrystalline metal (hydr)oxide, exchangeable Ca and Mg, IC, clay and silt contents, as these soil properties contribute to aggregate stability. The residual moisture content of soils dried at 40 °C has been proposed as an indicator for the specific surface area of soils (Beare et al. 2014; Emde et al. 2022; Kirschbaum et al. 2020; Parfitt et al. 2001), which is related to the stability of water-stable microaggregates (Totsche et al. 2018). As microaggregate stability is expected to hamper soil dispersion, the direct relationship between the moisture content of 40 °C dried soil and level of underdispersion is also explored.

5.2 Materials and methods

5.2.1 Definitions

In this manuscript, the terms ‘fine fraction’ and ‘MAOC’ (mineral associated organic carbon) are used to describe the $<50\text{ }\mu\text{m}$ fraction of the bulk soil after size fractionation and its respective organic carbon content. Likewise, ‘coarse fraction’ and ‘POC’ (particulate organic carbon) are used to describe the $>50\text{ }\mu\text{m}$ fraction of the bulk soil after size fractionation and its respective organic carbon content. MAOC and POC are operationally defined (based on size fractionation, not corrected for incomplete dispersion), and differ from the conceptually defined MAOC and POC pools according to Lavalley et al. (2020).

5.2.2 Soil selection

A set of 38 soil samples was selected, aiming to cover a wide range of soil properties which could affect soil dispersion. The set covers a variety of soil classes (IUSS Working Group WRB, 2022) as well as climatic zones, soil horizons and dominant clay mineralogies (Table 5.S1). Of this set, 24 soils (soils 10-33) are part of the International Soil Reference and Information Centre (ISRIC) soil reference collection. The remainder was selected from soils available in the Soil Biology and Soil Chemistry Groups of Wageningen University & Research.

5.2.3 Soil analyses

All soil analyses were performed on homogenised soils, sieved over 2 mm and dried at 40 °C for 48 hours. Soil pH was measured in 0.01 M CaCl₂ after shaking for 2 hours at a 1:10 soil:solution ratio (w/v) (Van Erp, 2002). The residual moisture content of 40 °C dried soil was determined by oven drying the soil at 105 °C for 24 hours. The presence of inorganic carbon (IC) was assessed by visual inspection of CO₂ development after adding droplets of 12M HCl to the soil samples. When relevant, the IC content was determined, following Koorneef et al. (2023). Briefly, organic matter was removed by loss on ignition (LOI) for 3 hours at 550 °C. Both bulk soil and ash samples were ball-milled to a fine powder before analysis of total carbon (C) using a LECO Truspec CHN Analyser (NEN-EN 15936, 2012). Soil IC content was corrected for weight loss during LOI, after which SOC content was derived by subtracting IC from total C content.

Nanocrystalline Al and Fe (hydr)oxide contents were determined by shaking 1.5 g of soil with 30 mL of 0.2 M ammonium oxalate (pH = 3.0) for 2 hours in the dark at 180 rpm on a horizontal shaker (NEN 5776, 2014; Schwertmann, 1964). For soils with pH >7 and containing IC, the soil to solution ratio was adjusted to 1:100 (w/v) for maintaining the pH of the extraction solution. After centrifugation, Al and Fe concentrations in the supernatant were measured by inductively coupled plasma - optical emission spectrometry (ICP-OES; Thermo iCAP 6500 DV). The sum of the oxalate-extractable Al and Fe (AlFe) was used in further analyses. The actual Cation Exchange Capacity (CEC) and exchangeable Ca, Mg and Na contents were determined using a 0.1 M BaCl₂ extraction (NEN 5738, 1996) and measured by ICP-OES. Clay (<2 µm), silt (2-50 µm) and sand (50-2000 µm) content of bulk soils were determined with the sieve and pipette methods (Van Reeuwijk, 2002), except for

soil 6-9 (Table 5.1) for which laser diffraction analysis was used. The texture of soil 30 could not be analysed because of its high iron content leading to flocculation after peptization.

5.2.4 Size fractionation of SOC

Size fractionation of SOC was based on Sanderman et al. (2013) and Baldock et al. (2013). A quantity of 10 g of soil was dispersed in 40 mL of 5 g L⁻¹ SHMP solution, while shaking horizontally at 180 rpm for 17 hours. Soils were then sieved over 50 µm, using an automated wet sieving apparatus (FRITSCH ANALYSETTE 3 PRO), shaking with a 1 mm amplitude for three minutes, with ±500 mL of demi water, applied from above through a rotating system. Soil were manually rinsed with an additional 100-200 mL of demi water to ensure that all <50 µm particles were washed through the sieve. The water was collected in a glass beaker. The soil remaining on the sieve was transferred into a second glass beaker, using a wash bottle. The fine (<50 µm) and coarse (>50 µm) fractions were dried at 105 °C for 48 hours. Each soil sample was fractionated in triplicate, but more replicates were included if needed to obtain sufficient material for C analysis and Laser Diffraction Analysis (LDA). One replicate had a weight recovery of 63% and was discarded. The remainder of the 167 replicates had weight recoveries ranging between 94 and 105%. Replicates were combined for further analysis.

Carbon in the bulk soil and combined coarse and fine fractions was measured using the LECO truspec CHN analyser (NEN-EN 15936, 2012) after ball-milling the samples to a fine powder. In soils containing IC, SOC was determined as described previously. The SOC recoveries were calculated as a quality check:

$$\text{SOC recovery (\%)} = \frac{\text{OC}_{\text{fine}} * \text{DW}_{\text{fine}} + \text{OC}_{\text{coarse}} * \text{DW}_{\text{coarse}}}{(\text{DW}_{\text{fine}} + \text{DW}_{\text{coarse}}) * \text{SOC}} * 100 \text{ (Equation 1)}$$

in which SOC, OC_{fine} and OC_{coarse} are the organic carbon contents (%) in the bulk soil, the fine fraction and the coarse fraction, respectively, and DW_{fine} and DW_{coarse} the dry weights (g) of the fine and the coarse fraction, respectively. DW_{fine} and OC_{fine} were corrected for the presence of 0.2 g SHMP. SOC recoveries ranged between 80 and 135%, but were between 90 and 110% for 30 out of 38 samples.

5.2.5 Laser Diffraction Analysis (LDA)

Completeness of dispersion was assessed by measuring the content of fine mineral particles (<50 µm) in the composite coarse fraction (>50 µm) and bulk soil, using Laser Diffraction

Analysis (LDA; Malvern MasterSizer 2000). For each sample, 2 g of material was used. Prior to LDA, carbonates were removed by adding droplets of 12M HCl. Subsequently, organic matter was removed by repeated (3 times; 4 times for soils with a SOC content >7%) addition of 15 mL 30% (w/w) H₂O₂ solution in a hot water bath (80°C). Floating organic material was carefully removed with forceps. Potential artifacts, such as partial dissolution of sensitive clay minerals or local aggregation due to changes in pH and ionic strength, were minimized by controlled, stepwise addition of H₂O₂ and thorough rinsing. It was assumed that removal of organic matter was equally effective for all soils, leaving only mineral particles. After digestion, the suspensions were washed with demi water until the solutions reached an electrical conductivity of <1000 $\mu\text{S m}^{-1}$. Finally, the solutions were decanted and samples were stored in demi water until further analysis (Supplementary Information 1).

5.2.6 Underdispersion

The output of LDA is a particle size distribution, based on volumes. We assume that particle density does not vary with particle size. Consequently, volume fractions are equivalent to mass fractions. Using LDA results, we quantified underdispersion in three ways (Underdispersion 1-3, Equations 2-4; Figure 5.1). The first measure of underdispersion (Underdispersion 1; Und1) represents the percentage of fine particles in the coarse fraction:

$$\text{Underdispersion 1 (\%)} = \text{Fine} - \text{in} - \text{coarse (\%)} \quad (\text{Equation 2})$$

Complete dispersion results in 0% fine particles in the coarse fraction. The results of Underdispersion 1 were used to calculate the weights of the fine and coarse fractions if dispersion would have been complete. The corrected POC and MAOC contents were then calculated, assuming that the <50 μm particles in the coarse fraction had the same C content as the <50 μm particles in the fine fraction.

The second measure of underdispersion (Underdispersion 2; Und2) quantifies the extent to which the fine particle content in the coarse fraction is lower relative to the bulk soil:

$$\text{Underdispersion 2 (\%)} = \frac{\text{Fine} - \text{in} - \text{coarse (\%)}}{\text{Fine} - \text{in} - \text{bulk (\%)}} * 100 \quad (\text{Equation 3})$$

in which fine-in-bulk is the percentage of fine particles (<50 μm) in the bulk soil sample.

The third measure for underdispersion (Underdispersion 3; Und3) corresponds with the percentage of fine particles originally present in the bulk soil sample that ended up in the coarse fraction:

$$\text{Underdispersion 3 (\%)} = \frac{\text{Fine} - \text{in} - \text{coarse (\%)} * \text{DWcoarse}}{\text{Fine} - \text{in} - \text{bulk (\%)} * \text{DWbulk}} * 100 \text{ (Equation 4)}$$

in which DWcoarse is the weight (g) of the coarse fraction after fractionation and DWbulk the weight (g) of the bulk soil sample before fractionation, corrected for the residual moisture content. Equal densities were assumed for the bulk soil and the coarse fraction. In the selected soils, the weight of the coarse fraction ranged between 8% and 95% of the bulk soil sample before fractionation. For soils with a large coarse fraction, a limited fine particle content in the coarse fractions may still account for a substantial part of the fine particles in the bulk soil. Underdispersion 3 can demonstrate this.

We did not know a priori which of the underdispersion measures would relate best to the errors in carbon estimates. Hence, to assess this, the differences between measured and corrected POC and MAOC were related to all three measures of underdispersion.

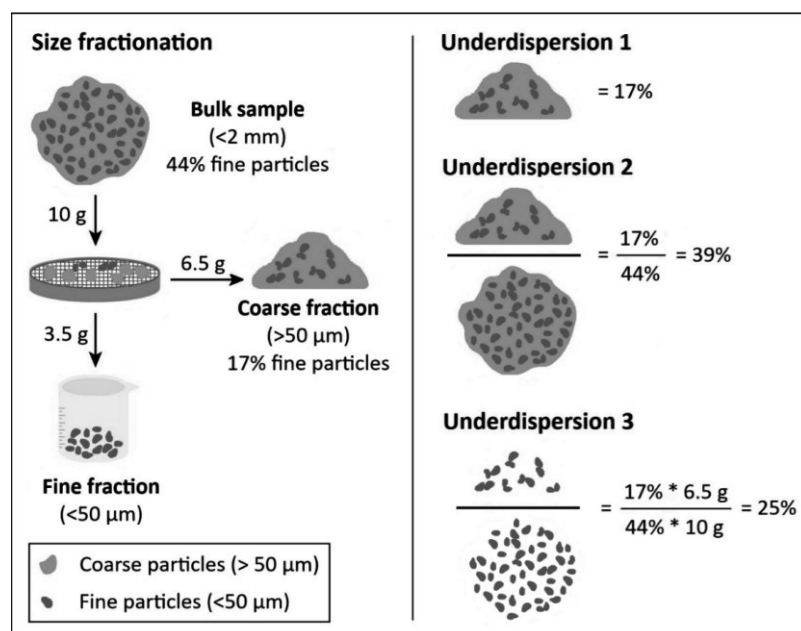


Figure 5.1: Schematic overview of the size fractionation procedure (left) and the quantification of underdispersion (right). In this example, the coarse fraction constitutes 65% of the bulk soil and contains 17% fine particles, compared to 44% of fine particles in the bulk soil.

5.2.7 Statistical analyses

Data analysis was done using R software (R core team, 2024) and Rstudio (R Studio Team, 2023). Plots were made using the ggplot function from the ggplot2 package, version 3.5.0 (Wickham, 2016). To explore direct relations among soil parameters and underdispersion, Spearman correlations were assessed. Furthermore, regression models were fitted to relate underdispersion to multiple soil parameters, using the generic lm function. To meet model assumptions and reduce leverage of highly influential points, Underdispersion 1, 2 and 3 were square root transformed. Underdispersion 1 was predicted based on pH, nanocrystalline AlFe, SOC, IC and exchangeable Na, Ca and Mg, as well as clay and silt content. Clay and silt together constitute the fine (<50 μm) fraction of the soil, which was also included in the model. Fine-in-bulk, used to calculate Underdispersion 2 and 3, equals the fine fraction when soils are completely dispersed. To prevent auto-correlation, clay, silt and the fine fraction were not included in the models describing Underdispersion 2 and 3.

Selection of the soil parameters explaining variation in underdispersion, was done based on the lowest Akaike's Information Criterion (AIC) value (Webster and McBratney, 1989) using the dredge function from the MuMIn package (Barton, 2020). Models were considered to have a similar performance if AIC values differed less than 2. The variables retained in the models were checked for multicollinearity, using the vif function from the car package, version 3.1-3 (Fox and Weisberg, 2019). All vif values were below 3 (Zuur et al. 2010), indicating no multicollinearity of variables. Normality of model residuals was inspected visually using histograms and tested with the Shapiro-Wilk test, using the function shapiro.test from the stats package, version 4.3.3 (R core team, 2024). Residuals were plotted against fitted values and explanatory variables to test for heterogeneity of variance and independence, respectively. Of the final models, the contribution of each explanatory variable to R^2 was evaluated using function calc.relimp from the relaimpo package, version 2.2–7 (Groemping, 2006).

5.3 Results

5.3.1 Soil properties and underdispersion

The selected soils varied widely in pH (2.9 - 7.9), clay content (1 - 86%), SOC content (0.2 - 12%), and AlFe oxide content (20 - 1549 mmol kg^{-1} ; Table 5.1). Across soils, levels of underdispersion varied: Underdispersion 1 (Und1) ranged between 0 and 67%,

Underdispersion 2 (Und2) between 0 and 84% and Underdispersion 3 (Und3) between 0 and 54%. Und1 and Und2 were strongly ($\rho = 0.96$) correlated, whereas the relation between Und2 and Und3 was largely determined by the size of the coarse fraction (Supplementary Information 3). Und1 was correlated with all measured soil properties, except pH (Table 5.2). Correlations between soil properties and Und2 were generally weaker than for Und1, and even weaker for Und3, except for SOC. Generally, underdispersion was positively related with SOC (Spearman's $\rho = 0.55 - 0.67$), AlFe oxides ($\rho = 0.50 - 0.73$), CEC ($\rho = 0.36 - 0.67$), silt ($\rho = 0.52 - 0.60$) and the residual moisture content ($\rho = 0.48 - 0.85$).

Und1 and Und2 levels above 25% were found for eight soils; four of these soils (Andosols) also had Und3 levels above 25% (Table 5.1). These soils had SOC contents above 2% and AlFe contents above 250 mmol kg⁻¹ and relatively high levels of exchangeable Mg and Ca (Figure S2). Nine soils had Und1, Und2 and Und3 levels below 2%. These soils were associated with relatively low levels of AlFe, OC and exchangeable Mg and Ca. However, the dataset also included soils with a high SOC or AlFe content that showed low levels of underdispersion (e.g. soil 7), as well as soils with a low exchangeable Ca and Mg content that exhibited high levels of underdispersion (e.g. soil 25).

The relative overestimation of POC (top row), the relative underestimation of MAOC (middle row) and the MAOC (g kg⁻¹ soil) erroneously ending up in the coarse fraction (bottom row) were plotted against Underdispersion 1, 2 and 3 (Figure 5.2). Incomplete dispersion had a larger relative effect on POC than on MAOC estimations. The POC overestimations ranged between 0 and 217% (average of 33.4%) and MAOC underestimations ranged between 0 and 53% (average of 7.8%). For 26 out of 38 soils, the POC was overestimated by more than 10% compared to when the soil would be fully dispersed, whereas the MAOC was underestimated by more than 10% in only 6 out of 38 cases. Across all 38 soils, the absolute amount of MAOC ending up in the coarse fraction ranged between 0 and 42 g kg⁻¹ soil (average of 3.6 g kg⁻¹ soil). This average was however strongly influenced by soils 27, 29, 30 (all Andosols), which had more than 20 g MAOC kg⁻¹ soil erroneously ending up in the coarse fraction. Excluding these soils showed that underdispersion led to an average of 1.0 g MAOC kg⁻¹ soil ending up in the coarse fraction, corresponding to 2.9% of the total bulk soil organic carbon.

Und1 and Und3 correlated equally strong with the POC overestimation ($\rho = 0.85$; Figure 5.2, top row), whereas Und2 had a slightly stronger correlation ($\rho = 0.92$). Und3 correlated best

with the underestimation of MAOC, with a Spearman's ρ of 0.98, compared to $\rho < 0.84$ for Und1 and Und2 (Figure 5.2, middle row). The MAOC in the coarse fraction correlated most strongly with Und3 ($\rho = 0.95$; Figure 5.2, bottom row).

Table 5.1.: Soil properties and underdispersion (Und1, 2 and 3). RMC = residual moisture content.

No	Soil class	pH	SOC	IC	Ca	Mg	Na	CEC	AlFe	Sand	Silt	Clay	RMC	Und1	Und2	Und3
		%		cmol+ kg ⁻¹		mmol kg ⁻¹		%		%		%				
1	Fluvisol	6.3	2.2	0	24.8	5.3	0.35	30	109	10	41	49	2.2	26.7	31.7	2.9
2	Anthrosol/ Arenosol	5.0	4.2	0	7.7	0.6	0.10	7.5	54	85	14	2	0.8	2.8	15.0	12.5
3	Fluvisol	7.6	1.0	1.0	17.0	1.0	0.10	17	76	34	41	25	1.3	5.2	10.3	4.0
4	Fluvisol	7.5	2.0	1.1	25.5	1.5	0.10	24	132	16	52	33	2.1	16.9	26.2	4.5
5	Anthrosol/ Arenosol	4.7	2.0	0	4.7	0.5	0.10	3.8	62	88	11	1	0.5	0.0	0.0	0.0
6	Regosol	7.4	1.1	1.9	22.0	5.3	0.27	24	118	22	34	44	2.3	6.4	10.0	2.7
7	Podzol	2.9	5.4	0	0.4	0.1	0.10	5.6	34	78	16	6	0.7	0.7	6.5	5.6
8	Podzol	3.7	1.7	0	0.4	0.1	0.10	4.3	175	88	8	4	0.8	0.0	0.0	0.0

No	Soil class	pH	SOC	IC	Ca	Mg	Na	CEC	AlFe	Sand	Silt	Clay	RMC	Und1	Und2	Und3
mmol										%						
			%	%	cmol+ kg ⁻¹			%								
9	Podzol	3.2	1.3	0	0.4	0.1	0.10	3.7	26	85	11	5	0.3	0.4	5.2	4.7
10	Humic Nitisol	5.2	2.5	<0.1	7.9	1.5	0.10	14	119	14	20	66	3.2	23.6	33.5	4.1
11	Humic Nitisol	5.7	0.7	<0.1	6.2	2.6	0.10	12	129	10	12	77	3.5	24.3	51.1	2.6
12	Pellic Vertisol	7.2	2.1	0	53.4	26.6	0.73	>40	174	8	7	85	8.8	13.2	14.5	1.0
13	Pellic Vertisol	7.9	1.6	0.3	45.1	28.5	8.99	>40	166	8	6	86	8.2	7.1	7.9	0.5
14	Fluvisol	7.7	0.4	1.6	34.4	1.0	0.25	8.6	66	61	22	17	0.9	4.4	8.4	3.2
15	Orthic Luvisol	5.9	0.4	0	10.6	1.0	0.10	11.3	71	59	17	24	1.3	13.1	16.7	1.4
16	Orthic Luvisol	6.0	0.2	0	8.5	1.0	0.10	9.8	59	65	15	21	1.2	16.7	23.2	2.2
17	Chromic Luvisol	7.4	1.7	1.1	18.8	2.6	0.10	17.6	99	48	18	34	1.6	4.9	7.4	2.7
18	Chromic Luvisol	7.1	0.4	<0.1	13.7	2.1	0.14	12.2	121	42	17	41	1.8	3.6	5.5	1.7
19	Ferric Acrisol	5.6	0.7	0	1.8	1.2	0.10	4.1	36	66	18	15	0.7	0.5	1.5	0.8

No	Soil class	pH	SOC	IC	Ca	Mg	Na	CEC	AlFe	Sand	Silt	Clay	RMC	Und1	Und2	Und3
		%			cmol+ kg ⁻¹			mmol kg ⁻¹			%			%		
20	Ferric Acrisol	4.4	0.5	0	1.3	1.0	0.10	5.4	48	56	19	26	0.5	0.4	1.0	0.5
21	Ferric Acrisol	4.0	0.5	0	0.4	0.1	0.10	1.9	63	67	1	32	0.5	0.2	2.1	1.4
22	Ferric Acrisol	4.2	0.3	0	0.4	0.1	0.10	1.6	42	64	1	35	0.5	0.0	0.0	0.0
23	Rhodic Ferralsol	5.1	0.6	0	0.4	0.2	0.10	0.5	20	83	2	15	0.4	0.0	0.0	0.0
24	Rhodic Ferralsol	5.0	0.2	0	0.4	1.4	0.10	3.4	25	62	2	36	0.6	0.0	0.0	0.0
25	Humic Ferralsol	4.1	2.0	<0.1	0.4	0.1	0.10	3.1	122	21	21	58	1.5	32.8	34.1	8.1
26	Humic Ferralsol	5.2	0.8	<0.1	0.4	0.1	0.10	2.7	126	28	19	54	1.3	1.1	1.4	0.5
27	Mollic Andosol	5.8	12.4	0.3	82.5	4.9	0.13	>40	417	43	39	19	3.6	59.7	83.5	53.7
28	Mollic Andosol	6.8	3.1	<0.1	15.6	3.0	0.14	24	1549	40	44	16	3.9	31.3	52.3	26.6
29	Humic Andosol	4.1	10.1	0	0.4	0.1	0.14	12	1056	32	39	29	4.2	61.2	83.9	53.9
30	Humic Andosol	4.7	5.3	<0.1	0.4	0.1	0.10	11	1272	NA	NA	NA	4.5	66.7	82.6	52.6

No	Soil class	pH	SOC	IC	Ca	Mg	Na	CEC	AlFe	Sand	Silt	Clay	RMC	Und1	Und2	Und3
			%		cmol+ kg ⁻¹			mmol		%		%				
			%		kg ⁻¹			%		%		%				
31	Xanthic Ferralsol	5.2	1.2	0	2.2	0.4	0.10	2.8	22	87	1	12	0.4	0.5	7.5	6.3
32	Xanthic Ferralsol	4.3	0.2	0	0.4	0.1	0.10	1.2	24	72	1	27	0.3	0.4	2.9	2.0
33	Podzol	4.3	5.2	0	0.4	0.1	0.10	3.5	780	77	16	7	4.0	17.3	43.1	27.8
34	Arenosol	4.8	1.9	0	6.8	1.4	0.10	8.7	147	62	25	13	0.8	3.5	18.1	13.1
35	Arenosol	5.6	2.5	0	5.1	1.3	0.10	5.9	74	91	8	2	0.6	0.0	0.0	0.0
36	Gleysol	4.7	5.6	0	12.4	7.3	0.35	22	254	17	13	70	2.7	35.2	47.7	11.4
37	Gleysol	4.7	4.0	0	8.8	8.0	0.41	21	257	17	13	71	2.3	26.7	34.6	5.8
38	Anthrosol/ Fluvisol	6.7	5.8	<0.1	29.8	4.9	0.33	35	138	37	25	38	1.9	12.9	16.3	5.9

Table 5.2: Spearman correlation coefficients (ρ) among underdispersion and soil parameters. Bold values are significant ($p < 0.05$). RMC = residual moisture content.

	Und1	Und2	Und3	pH	SOC	IC	Ca	Mg	Na	CEC	AlFe	Sand	Silt	Clay
pH	0.25	0.15	-0.07	1										
SOC	0.55	0.58	0.67	-0.13	1									
IC	0.35	0.25	0.18	0.65	0.06	1								
Ca	0.37	0.32	0.12	0.83	0.12	0.52	1							
Mg	0.37	0.30	0.04	0.67	0.19	0.33	0.82	1						
Na	0.35	0.26	0.11	0.29	0.19	0.18	0.56	0.58	1					
CEC	0.69	0.64	0.46	0.59	0.49	0.51	0.79	0.78	0.46	1				
AlFe	0.73	0.69	0.50	0.15	0.62	0.33	0.29	0.32	0.39	0.61	1			
Sand	-0.72	-0.58	-0.21	-0.47	-0.19	-0.50	-0.54	-0.67	-0.53	-0.62	-0.56	1		
Silt	0.60	0.55	0.52	0.39	0.37	0.55	0.40	0.29	0.08	0.71	0.47	-0.46	1	
Clay	0.48	0.32	-0.03	0.31	-0.05	0.37	0.35	0.55	0.54	0.32	0.36	-0.89	0.09	1
RMC	0.85	0.77	0.48	0.40	0.55	0.45	0.45	0.51	0.45	0.73	0.86	-0.77	0.49	0.56

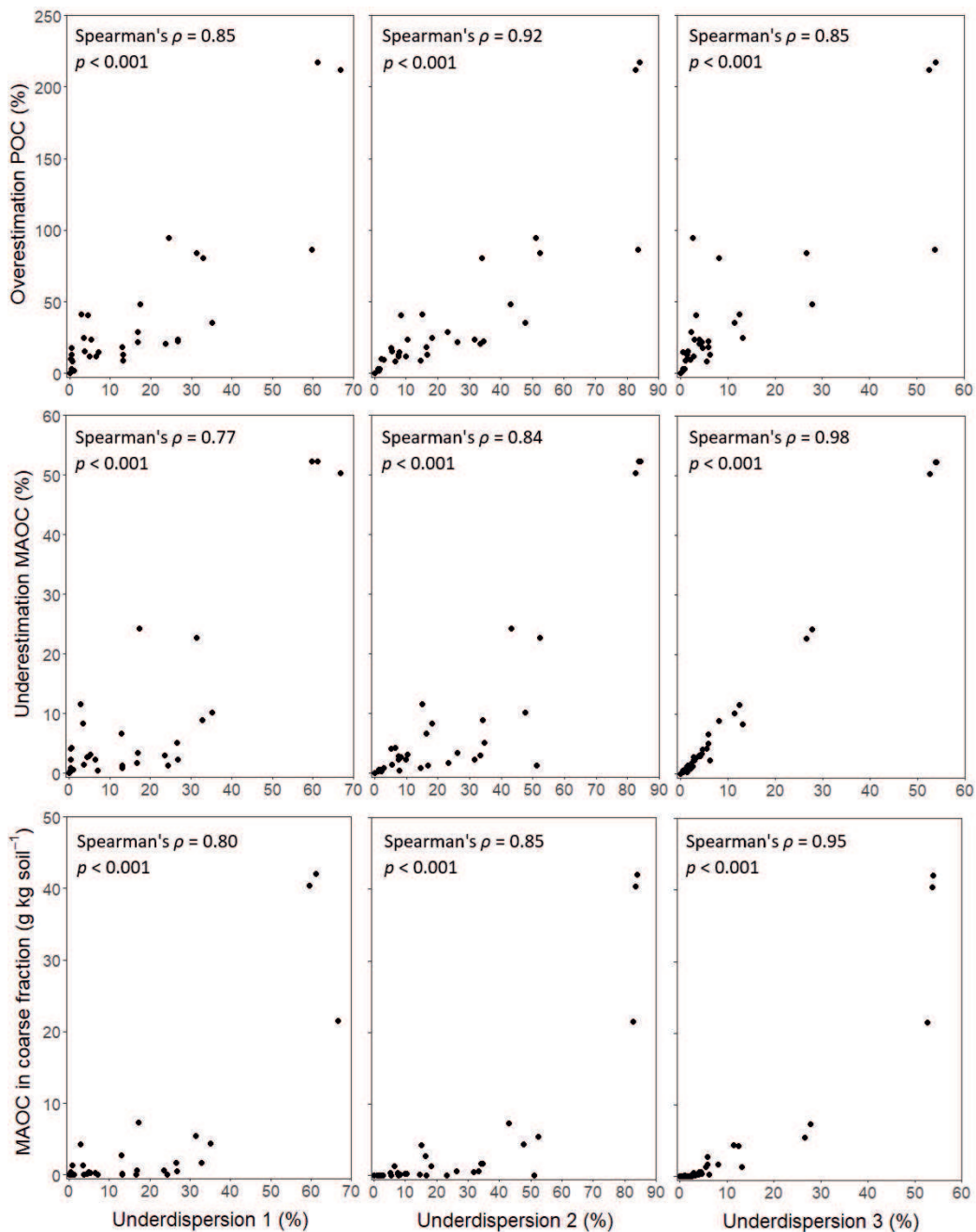


Figure 5.2: Relative overestimation of POC (top), relative underestimation of MAOC (middle) and absolute amounts of MAOC found in the coarse fraction (bottom) as a function of Underdispersion 1 (left), 2 (middle) and 3 (right).

5.3.2 Linear regression models

Several models described variation in Und1 ($n = 5$), Und2 ($n = 7$) and Und3 ($n = 10$) equally well (i.e. AIC values differed less than 2; Table S2); the models with the lowest AIC values are presented in Table 5.3. The soil parameters explained more variation in Und1 ($R^2 = 0.89$) and Und3 ($R^2 = 0.81$) than in Und2 ($R^2 = 0.54$; Figure 5.3). All models (including those not presented) retained SOC and AlFe as significant predictors. The SOC and AlFe content contributed positively to explaining underdispersion and had a comparable contribution to R^2 . For Und1, the fine fraction was also retained in all models. Exchangeable Mg was retained in four out of five models describing Und1 and six out of ten models describing Und3, whereas pH was retained in 5 out of 7 models describing Und2. Both Mg and pH contributed little to R^2 ($<3\%$), however. Removing these predictors had minimal impact on R^2 (reduced models; Table 5.3) and residuals were still normally distributed.

Table 5.3: Linear regression models describing Underdispersion 1, 2 and 3, fitted with all soil parameters (All) and performance of these models when omitting variables describing minimal variation (Reduced). Percentages below the predictors indicate relative contribution to R^2 .

Model	Predictors	R^2
Underdispersion 1 – All	SOC + AlFe + Fine fraction – Mg 36% 30% 32% 2%	0.89
Underdispersion 1 – Reduced	SOC + AlFe + Fine fraction 36% 31% 33%	0.88
Underdispersion 2 – All	SOC + AlFe + pH 51% 45% 3%	0.57
Underdispersion 2 – Reduced	SOC + AlFe 52% 48%	0.54
Underdispersion 3 – All	SOC + AlFe – Mg 53% 45% 2%	0.81
Underdispersion 3 – Reduced	SOC + AlFe 54% 46%	0.79

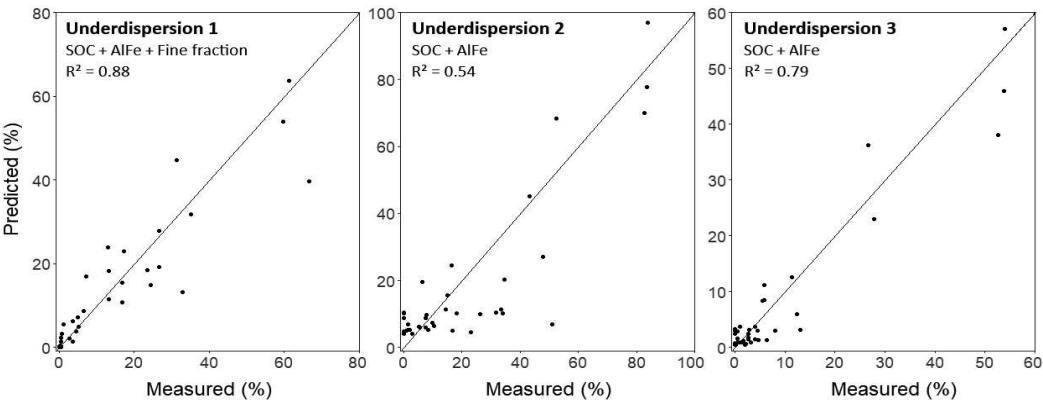


Figure 5.3: Predicted versus measured Und1 (left), Und2 (middle) and Und3 (right) and the 1:1 lines. Predicted values are based on the reduced models (Table 5.3) and were squared for back-transformation to the original scale.

5.3.3 Residual moisture content

The residual moisture content (RMC) of 40 °C dried soils ranged between 0.3 and 4.5%, with the exception of two heavy clay soils from Kenya with an RMC above 8% (soil 12 and 13; Table 5.1). The RMC was positively correlated with all measures of underdispersion (Table 5.2; Figure 5.4). The strongest correlations were found between RMC and Und1 ($\rho > 0.85$) and Und2 ($\rho > 0.77$). When excluding soils 12 and 13, the relations seemed linear for Und1 and Und2. Und3 was less strongly correlated with RMC ($\rho = 0.48$) and the relation seemed curvilinear.

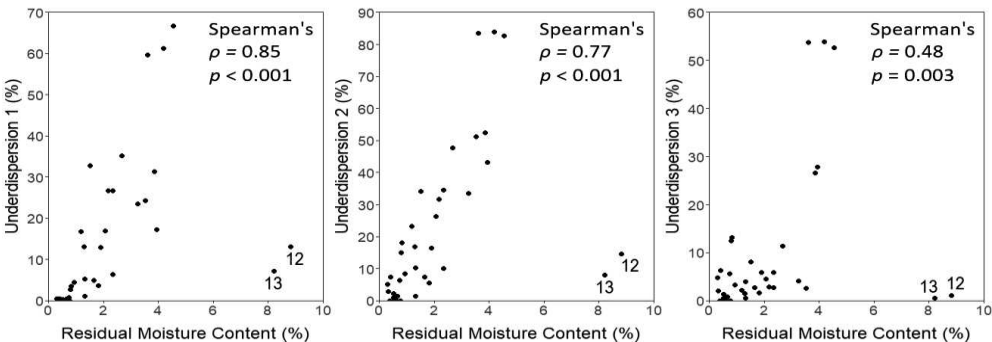


Figure 5.4: Underdispersion 1, 2 and 3 plotted against the residual moisture content. Numbers refer to the soils in Table 5.1 and are included for discussion purposes.

5.4 Discussion

POM-MAOM size fractionation following incomplete dispersion, leads to an overestimation of the fast-cycling POC pool and underestimation of the slow-cycling MAOC pool. Although the effect of underdispersion is likely partially compensated by fine POC (sized $<50\mu\text{m}$) ending up in the MAOC pool (Leuthold et al. 2024; Poeplau et al. 2025), this can have large implications for studies on soil carbon cycling. The aim of this study was therefore to explore how cementing agents that may inhibit dispersion, reflected by selected soil properties, affect soil dispersion during overnight shaking in a 5 g L^{-1} SHMP solution. Three distinct measures were used for quantifying underdispersion (Und1, Und2 and Und3). In general, SHMP was less effective in adequately separating the fine and the coarse fraction with increasing AlFe (hydr)oxide and SOC contents, irrespective of how underdispersion was quantified. Highest levels of underdispersion were found in three Andosols with relatively high nanocrystalline AlFe (hydr)oxide and SOC contents, yet some soils dispersed well despite also having high contents of AlFe (hydr)oxides and/or SOC. Soil texture also affected the amount of fine particles present in the coarse fraction (Und1). Aforementioned factors are known to influence aggregate stability (Bronick and Lal, 2005; Lehtinen et al. 2014) and were therefore expected to affect underdispersion. With the exception of the Andosols, underdispersion had a limited impact on the amounts of MAOC ending up in the coarse fraction (average of $1.0\text{ g MAOC kg}^{-1}$ soil and 2.9% of SOC). This furthermore will partially be compensated by POC ending up in the fine fraction (Poeplau et al. 2025).

5.4.1 Quantifying underdispersion

In this study, underdispersion was quantified in three ways. Und1 represented the percentage of fine particles in the coarse fraction, Und2 the decrease in fine particle content in the coarse fraction relative to the bulk soil sample, and Und3 the percentage of fine particles originally present in the bulk soil sample ending up in the coarse fraction. Complete dispersion corresponds with 0% fine particles in the coarse fraction, consequently with Und1, Und2 and Und3 equalling 0% . In principle, Und1 should be 0% . When Und1 $>0\%$, Und2 and Und3 might relate better to the errors in carbon estimates, as they are adjusted for textural differences.

The overestimation of POC was more strongly affected by underdispersion than the underestimation of MAOC. This is due to the fact that the fine fraction generally contained more C than the coarse fraction (average of 18.9 versus 5.7 g kg^{-1} soil; not presented). The

POC overestimation was best described by Und2, whereas the MAOC underestimation was best described by Und3. The latter can be explained mathematically (Supplementary Information 3). The absolute MAOC content in the coarse fraction, was also best described by Und3, although not linearly, as it is a function of both the amount of fine particles ending up in the coarse fraction as well as the C content of the fine fraction.

Based on these results, Und3 seems to be most suitable when the MAOC pool is most relevant, e.g. when studying soil C saturation (Cotrufo et al. 2022; Six et al. 2024) and Und2 when the POC pool is most relevant. Und2 was however poorly predicted based on soil properties; Und1 or Und3 thus might have more predictive power to assess the consequences of underdispersion also in studies that focus on POC. Irrespective of the measure of underdispersion selected, SOC and AlFe were retained as the most relevant variables in all models.

5.4.2 SOC

As hypothesised, underdispersion increased with SOC contents and high levels of underdispersion were associated with SOC contents above 2%. This is in line with Pulleman et al. (2005), who found incomplete dispersion in a (calcareous) soil containing 3.9% SOC, but not in soils <1.5% SOC. Soil organic carbon represents a heterogeneous pool of cementing agents and positively correlates with soil aggregate stability because it forms organo-mineral and organo-metal complexes (Al-Kaisi et al. 2014; Lehtinen et al. 2014; Totsche et al. 2018). Large quantities of carbon compounds, reflected by a high SOC content, may thus contribute to underdispersion, if SHMP cannot effectively dissociate aforementioned complexes. However, several soils had relatively high SOC contents, yet showed relatively limited underdispersion. Although SOC is an important factor explaining underdispersion, it cannot solely estimate the risk of underdispersion with SHMP, as metal (hydr)oxides were also relevant.

5.4.3 Nano-crystalline Al and Fe (hydr)oxides

The positive correlations between oxalate-extractable AlFe and underdispersion, as well as its significant contribution in the linear regression models, confirm our hypothesis that dispersion of soil aggregates decreases with increasing levels of nanocrystalline Al and Fe (hydr)oxides. Complete dispersion into primary soil particles seems impossible for soils rich

in Al and Fe (hydr)oxides, even when chemical dispersion is combined with sonication (Regelink et al. 2013).

The abundance of metal (hydr)oxides might cause underdispersion through two mechanisms. First, high contents of metal (hydr)oxides are generally associated with adsorption of SOC and high aggregate stability (Beare et al. 2014; Eusterhues et al. 2005; Lehtinen et al. 2014; Totsche et al. 2018). This implies that AlFe (hydr)oxides could inhibit dispersion through the formation of highly stable organo-mineral aggregates. Secondly, Al and Fe hydr(oxides) might interfere with the utilised dispersion agent (Han et al. 2016). Soil dispersion by SHMP is mainly attributed to binding of its phosphate groups to polyvalent cations, causing dispersion of clay-sized particles (Andreola et al. 2004; Han et al. 2016). Nanocrystalline metal (hydr)oxides typically drive P adsorption in soils (Antoniadis et al. 2016). High levels of nanocrystalline metal (hydr)oxides could therefore bind the P groups from SHMP, possibly reducing the effectiveness of dispersion. This mechanism might be especially relevant for soils with low P loading (Mendez et al. 2022).

5.4.4 Fine fraction

Clay and silt contents were not retained as significant predictors for Und1, but the fine fraction (i.e. the sum of the two) was. This is most likely caused by parsimony: one predictor is preferred over two separate predictors. When omitting the fine fraction, the clay and silt contents were indeed both retained as significant parameters (not presented).

The significance of the fine fraction can be explained both mechanistically and mathematically. Clay and silt particles are considered relevant for stabilising soil carbon and therefore likely contribute to aggregate stability (Dimoyiannis, 2012; Hassink, 1997; Salonen et al. 2024; Totsche et al. 2018), which is supported by the significant positive correlations of clay and silt content with Und1 in this study. This suggests that the silt fraction plays a significant additional role in describing underdispersion compared to using clay content alone. However, as Und1 represents the percentage of fine particles in the coarse fraction, a soil with a large fine fraction (i.e. a small coarse fraction) is also likely to have relatively more fine particles ending up in the coarse fraction, compared to a soil with a small fine fraction.

5.4.5 Cations

Of the three cations measured (Mg, Ca and Na), exchangeable Mg was the only cation retained as significant predictor in the models describing Und 1 and 3. Exchangeable Mg is generally considered to improve aggregate stability (Dimoyiannis, 2012; Lehtinen et al. 2014). In this study, exchangeable Mg was only weakly correlated with Und1 and contributed minimally to describing variation in Und1 and Und3. The significance of exchangeable Mg in the models is likely caused by soils 12 and 13, which showed relatively low underdispersion, despite having the highest levels of exchangeable Mg (Table 5.1; Figure 5.S1). Removing these soils from the dataset indeed made exchangeable Mg redundant as a predictor, whereas the models remained similar in terms of other predictors and R^2 (not presented).

Soils 12 and 13 are Kenyan Vertisols with a high clay content (>66%) and smectite as the dominant clay mineralogy (Table 5.S1). As both a high clay content and a clay mineralogy dominated by smectite increase aggregate stability (Dimoyiannis, 2012; Lehtinen et al. 2014), high levels of underdispersion would be expected for these soils, in contrast to our observations. However, soils 12 and 13 also had the highest exchangeable Na contents in the dataset (8.99 and 0.73 $\text{cmol}^+ \text{kg}^{-1}$ versus $<0.41 \text{ cmol}^+ \text{kg}^{-1}$ for all other selected soils; Table 5.1). As exchangeable Na facilitates clay dispersion (Chorom et al. 1994; Rengasamy et al. 2016; Zhang et al. 2024), this might have had a stronger effect on underdispersion in soils 12 and 13 than the exchangeable Mg content. Six et al. (2002) also found that Vertisols with a high exchangeable Na content dispersed easily, despite high exchangeable Mg, clay and smectite contents. Besides soils 12 and 13, the variation in exchangeable Na content was very limited (most soils had an exchangeable Na content below the detection limit of $0.10 \text{ cmol}^+ \text{kg}^{-1}$), so that Na appeared to be redundant as predictor for underdispersion in our study. Future studies are therefore recommended to include soils with more diversity in exchangeable Na content.

Calcium (both in exchangeable form and in carbonates) improves aggregate stability (Huang et al. 2019; Pulleman et al. 2005; Rowley et al. 2018; Virto et al. 2011) and is thus expected to contribute to underdispersion. Although the soils with the lowest levels of underdispersion tended to have the lowest exchangeable Ca content in the dataset (Figure 5.S1), exchangeable Ca content only correlated weakly with Und1 and was not retained as a significant variable in the regression models. These findings imply that Ca has limited relevance in describing

underdispersion compared to other soil properties (i.e. SOC and AlFe). As 31 out of 38 samples had an IC content below 0.1% (Table 5.1), we recommend future studies to include soils covering a larger variety in carbonate contents to assess its potential effect on underdispersion.

5.4.6 Residual moisture content

Underdispersion increased with an increasing residual moisture content (RMC) of soils dried at 40°C. The RMC likely relates to underdispersion, as it presumably reflects the specific surface area of soils (Emde et al. 2022; Kirschbaum et al. 2020; Parfitt et al. 2001), which is related to aggregate stability (Totsche et al. 2018). The RMC correlated well with oxalate-extractable AlFe and clay content (Table 5.2; Figure S2). The surface area of soils is to a substantial degree determined by the amounts of metal (hydr)oxides and clay particles present (Hiemstra et al. 2010; Mendez et al. 2022), which might explain the strong correlations.

Soils 12 and 13 had the highest RMC values, but low levels of underdispersion (section 5.4.5). The high RMC of soils 12 and 13 could be related to a combination of high clay (Figure S2) and high exchangeable Mg and Ca contents (Table 5.1; Figure 5.S1). Water molecules are retained in the primary hydration shells around these cations, even after drying at 105 °C (Guo et al. 2019; Kuligiewicz and Derkowski, 2017; Lehtinen et al. 2014). Despite its limitations in some soils, the RMC is determined as part of the size fractionation protocol and is thus a cost-effective indicator for potential underdispersion.

5.4.7 Limitations of the study

Although the soils used in this study covered a variety of soil classes, climatic zones, soil horizons and several dominant clay mineralogies, some soil properties had limited or skewed distributions. Furthermore, the number of soils included in this study (n=38) was relatively limited. To validate our findings, we therefore recommend future studies to include soils with more even and representative distributions in soil properties such as mineralogy, carbonates, CEC and exchangeable cations, but in particular SOC and Al and Fe (hydr)oxides.

Across laboratories, variations of the POM-MAOM fractionation protocol are in use (Poeplau et al. 2018). The protocol used in this study, differed in several aspects from other approaches. First, the coarse and fine fractions were separated using a 50 µm sieve, based on Sanderman et al. (2013) and in line with the USDA cutoff between sand and silt particles. However, the fractions are also commonly separated using 53 µm (e.g. Cotrufo et al. 2022;

Poeplau et al. 2018). As MAOC will mostly bind to the clay and fine silt (<20 µm) particles (Hassink, 1997; Salonen et al. 2024), we expect that a size cutoff of either 50 or 53 µm will have a limited effect on the findings of this study. Second, the fractions were dried at 105°C after separation, whereas they are commonly dried at 60°C (Cotrufo et al. 2019; Leuthold et al. 2024; Poeplau et al. 2018). Compared to drying at 60°C, drying at 105°C strongly reduces drying time and also removes residual moisture. Despite some potential loss of carbon due to thermal decomposition or volatilisation, differences in measured carbon between drying at 60 or 105°C are likely negligible (Dettmann et al. 2021; Even et al. 2025). Third, adding glass beads during shaking with SHMP to facilitate physical breakdown of aggregates or adding a sonication step are commonly employed to enhance dispersion (Jilling et al. 2020; Poeplau et al. 2018; Pulleman et al. 2005; Salonen et al. 2024; Supplementary Information 4). It is unclear to what extent these measures would have affected the findings of this study. Furthermore, it is unknown whether these measures only improve dispersion or also break up coarse POC that erroneously end up in the fine fraction (Leuthold et al. 2023; Oorts et al. 2005; Pulleman et al. 2005; Six et al. 2024). We therefore recommend further research to explore measures to improve soil dispersion without fragmenting POC.

Laser Diffraction Analysis (LDA) was used to assess the presence of fine particles in the bulk soil and coarse fraction samples. This method is known for underestimating volumes of clay particles and overestimating volumes of silt particles compared to the sieve and pipette method (Eshel et al. 2004; Svensson et al. 2022). The extent to which the choice of this methodology has affected underdispersion calculations, is highly dependent on the particle size distribution of the bulk soil, as well as the degree of underdispersion. Nevertheless, sand (and thus silt + clay) estimates appear to be relatively consistent across the two methods (Eshel et al. 2004), suggesting that, although LDA may have introduced some bias in the measured amounts of fine particles in the bulk soil and coarse fractions, it does not substantially alter the broader interpretation of our results.

5.5 Conclusions and recommendations

This study aimed to investigate the effect of soil properties on the effectiveness of soil dispersion during POM-MAOM size fractionation. The standard method of dispersion in 5 g L⁻¹ SHMP worked well for the majority of the studied soils, but was less effective in breaking down soil aggregates with increasing oxalate extractable metal hydroxide and SOC contents

and increasing size of the fine fraction ($<50\ \mu\text{m}$). Underdispersion was poorly predicted in soils with a high exchangeable Mg and/or Na content, underscoring the need for soil-specific considerations in assessing the risk for underdispersion. To enhance the accuracy of size fractionation, future refinements should focus on optimizing dispersion techniques while carefully balancing the risk of POM fragmentation. For example, fractionation of field-moist soils could be considered, as oven drying can lead to the formation of highly stable pseudo-aggregates not present under natural conditions (Kjaergaard et al. 2004; Salonen et al. 2023), especially in clay-rich soils that hardly experience drying in the field (Denef et al. 2001; Kaiser et al. 2015; Kjaergaard et al. 2004; Salonen et al. 2023; Totsche et al. 2018).

Checking and reporting completeness of dispersion after size fractionation should be common practice when studying soil carbon pools. The residual moisture content of soils dried at $40\ ^\circ\text{C}$ emerged as a valuable and cost-effective indicator for underdispersion, especially in combination with SOC content and the size of the fine fraction. As all these parameters are inherently obtained during the size fractionation procedure, they could be used in future studies to assess the risk of the underdispersion and potentially adjust the size fractionation protocol. Alternatively, underdispersion can be assessed visually under the microscope (Pulleman et al. 2005; Six et al. 2024). The particles of the dried coarse fraction should look clean, glassy and/or transparent and without visible aggregates (i.e. clusters held together by (in)organic cementing agents) if dispersion is complete.

Besides the implications for estimating the different soil carbon pools, the findings of this study could be relevant for texture determinations based on the sieve and pipette method, which also utilises $5\ \text{g L}^{-1}$ SHMP for dispersing soils (Van Reeuwijk, 2002). Such texture determinations could overestimate the sand and underestimate the silt and clay fractions with increasing levels of underdispersion.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper. Co-author Walter Schenkeveld is an Associate Editor for *Geoderma*, but was not involved in the editorial review or the decision to publish this article.

Data availability

Data are available at www.zenodo.org/records/16408680

Chapter 6

General Discussion

Anna-Reetta Salonen

6.1. Overview

Soil organic carbon (SOC) lies at the heart of processes that sustain agricultural production. It fuels soil biological activity by feeding decomposers that release nutrients for crops, binds mineral particles into aggregates that give soil its structure, regulates water infiltration and aeration, and retains nutrients against leaching (Hoffland et al. 2020). As the impacts of climate change have become more tangible, soils increasingly receive attention for contributing to climate mitigation by storing additional carbon (Minasny et al. 2017). However, a fundamental tension underlies this dual role of SOC. For carbon to accumulate in soil, it must resist microbial decomposition, whereas SOC must be decomposed to sustain soil biological activity and nutrient cycling (Janzen 2006).

Understanding how SOC is distributed among pools that differ in turnover time and stabilisation mechanisms is critical for reconciling carbon persistence with soil functioning. A widely used framework distinguishes particulate organic matter (POM), characterised by relatively rapid turnover, from mineral-associated organic matter (MAOM), which is considered to be stabilised through interactions with mineral surfaces and to persist over longer timescales (Lavalley et al. 2020). Building on this distinction, SOC saturation concepts propose that the accumulation of MAOM is constrained by the availability of reactive mineral surfaces, implying upper limits to long-term carbon storage (Hassink 1997; Six et al. 2002).

In practice, clay and silt contents of the soil are often used as a proxy for the reactive surface area to estimate MAOM saturation. However, the SOC saturation concept has been developed mostly in older, more weathered soils under temperate climates. Finnish agricultural soils are relatively young and glacially derived, with limited reactive mineral surfaces, and subject to cooler, more humid conditions that affect SOC cycling. Hence, their capacity to store SOC remains poorly understood, particularly below the plough layer where current monitoring efforts do not reach (Heikkinen et al. 2013; Luke 2018). Furthermore, clay and Al and Fe (hydr)oxides may predict SOC distribution and saturation in these soils, yet their relative importance has not been established (Rasmussen et al. 2018). Lastly, the concepts of SOC saturation rest on simplified assumptions. Physical fractionation methods used to separate POM from MAOM are

operational, and thus impose discrete boundaries (i.e. arbitrary cutoffs between POM and MAOM) on what in reality is a continuum of organic compounds at different stages of decomposition (Lehmann & Kleber 2015). Whether operationally defined POM and MAOM fractions consistently represent distinct carbon pools remains an open question, also across contrasting soil types, land uses and soil depths.

The main objectives of this thesis are (1) to assess whether SOC saturation concepts apply to Finnish (sub)soils and (2) to evaluate whether the properties of operationally defined POM and MAOM fractions align with the theoretical framework.

To this end, I assessed the applicability of MAOM saturation concepts using data from a 24-year field experiment in South-Western Finland (Chapter 2) and from 93 farms spanning the main agricultural regions of Finland (Chapter 3), and characterised the controls on SOC storage and the distribution of carbon between POM and MAOM fractions in these soils. To evaluate whether operationally defined POM and MAOM fractions represent functionally distinct carbon pools, I compared size- and density-based fractionation methods using Rock-Eval thermal analysis to test whether they isolate material with distinct chemical and thermal properties (Chapter 4). Finally, we tested a key assumption of size fractionation, that chemical dispersion fully breaks down soil aggregates, and assessed how incomplete dispersion affects the distribution of SOC into POM and MAOM fractions (Chapter 5).

6.2 Carbon stocks and their controls in Finnish agricultural soils

Long-term national monitoring shows that Finnish mineral arable soils have been losing carbon: mean SOC content in the top 0–15 cm decreased from approximately 38.9 g kg⁻¹ in the late 1980s to about 35.3 g kg⁻¹ by 2018 (Heikkinen et al. 2013; Luke 2018), and soils keep losing SOC (Heikkinen et al. 2022). The SOC concentrations measured in this thesis are broadly consistent with these values, though slightly lower: cropping systems in the 24-year field experiment contained 32.0–35.5 g C kg⁻¹ in the top 0–20 cm (Chapter 2), and topsoil concentrations across the 93 farms averaged 29.5 g kg⁻¹ (Chapter 3), though direct comparison is limited by differences in sampling depth.

MAOM constituted the majority of SOC across all studied soils, accounting for over 83% of total carbon in the field experiment (Chapter 2) and 86% in the farm topsoils (Chapter 3), consistent with the general pattern that MAOM stores the majority of agricultural SOC (Cotrufo & Lavalée 2022; Georgiou et al. 2022; Begill et al. 2023; Soinne et al. 2024). While this proportion was remarkably stable across soils with widely varying total OC and clay contents, clay content was a poor predictor of MAOM variation across the 93 farms (Chapter 3). These patterns raise questions about what controls MAOM accumulation in these soils, but also about how the operational separation of POM and MAOM affects the interpretation of their distribution (Chapter 4).

Across both Chapters 2 and 3, clay content was a poor predictor of MAOM. In the field experiment, clay content was correlated with MAOM only in the topsoil (Chapter 2) and accounted for only 9% of the variation in MAOM across the 93 farms (Chapter 3). While Al and Fe oxides performed better (21–22% each), they too remained poor single predictors. Regression models combining clay, Al and Fe oxides, pH and climate variables explained at most 53% of the variation in MAOM, indicating that current predictors, even in combination, leave substantial variation unexplained in the studied soils. Similar results have been reported from Swedish agricultural soils, where Chilipamushi et al. (2025) found that oxalate-extractable Al was the strongest single predictor of SOC whereas clay content showed no significant correlation. Likewise, Fukumasu et al. (2021) identified Al oxides alongside carbon inputs as major determinants of topsoil OC in Nordic and Canadian agricultural soils under humid climates. Rasmussen et al. (2018) demonstrated that the relative importance of SOM stabilisation mechanisms scales with climate and acidity: in water-limited, alkaline soils, exchangeable calcium was the strongest predictor, whereas with increasing moisture and acidity, Al and Fe oxides emerged as better predictors. Finnish agricultural soils, with their humid climate and moderately acidic to near-neutral pH, align with this gradient.

These studies reinforce two points that emerge also from the data presented in this thesis. First, clay content is a poor predictor of SOC storage capacity in young, glacially formed soils where the clay fraction is mineralogically distinct from the weathered soils on which texture-based saturation models were developed (Hassink 1997; Feng et al. 2013). As clay is defined by particle size ($<2\ \mu\text{m}$), not mineralogy, the fraction can include both

reactive secondary minerals with high specific surface area, as well as unreactive primary minerals that are clay-sized (Churchman 2010). In most soils, chemical weathering progressively transforms primary minerals into secondary phases, including phyllosilicates and Al and Fe oxides, that provide reactive surfaces for SOM binding (Strawn et al. 2019). Finnish soils, however, are relatively young and moderately weathered, and a substantial proportion of their clay-sized fraction consists of primary minerals such as quartz and feldspars rather than more reactive secondary phyllosilicates. Keskinen et al. (2022), analysing 120 soils from the Finnish national monitoring network, found that a substantial portion of the textural clay fraction consisted of finely ground primary minerals rather than clay minerals, a feature common to glacially derived soils where ice sheets reduced bedrock to clay-sized particles without the chemical weathering needed to form reactive secondary minerals (Locat et al. 1984). The specific surface area of primary minerals is orders of magnitude lower than that of secondary clay minerals (e.g. $<5 \text{ m}^2 \text{ g}^{-1}$ for quartz vs. $700\text{--}800 \text{ m}^2 \text{ g}^{-1}$ for smectite; Brady & Weil 2008). This means that clay content alone in Nordic soils is not a good proxy for the reactive surface area.

Second, even the strongest available predictors, including Al and Fe oxides, leave a large proportion of MAOM variation unexplained. This suggests that additional factors such as SOC input levels and land use history contribute substantially to explaining variation in MAOM contents in these soils. The limited predictive value of Al and Fe oxides can be explained by the finding that most of the studied soils appeared undersaturated relative to their estimated MAOM storage capacity (Chapter 3). This implies that MAOM content in these soils is constrained less by the availability of reactive surfaces than by the supply and processing of organic inputs. Moreover, even the relationship between Al and Fe oxides and MAOM may not reflect a simple stabilisation mechanism as the direction of causality between Al and Fe oxides and organic carbon is itself uncertain. Hall & Thompson (2022) showed that across chemically diverse North American soils, the Al pool most strongly associated with SOC likely consisted of organo-Al complexes rather than discrete oxide or aluminosilicate phases. This raises the possibility that organic inputs drive extractable Al concentrations through organic acid-mediated mineral weathering (Strawn et al. 2019) rather than Al phases controlling SOC storage. If this

applies to the Finnish soils studied in this thesis, where similarly positive correlations between oxalate-extractable Al and SOC were observed (Chapter 3), these relationships may partly reflect the history of organic matter inputs rather than the stabilisation capacity of the soil mineral matrix.

6.3 Soil carbon saturation

SOC saturation theory proposes that the accumulation of SOC is constrained by the availability of reactive mineral surfaces (Hassink 1997; Six et al. 2002; Six et al. 2024). The underlying logic is straightforward: more mineral surface area provides more binding sites for organic matter, and this capacity is finite. Clay or clay+silt content are commonly used as proxies for SOC storage capacity. However, this thesis challenges the transferability of texture-based estimates of SOC saturation to young, moderately weathered soils. When the clay-sized fraction contains a substantial proportion of less reactive primary minerals, as is common in Finnish soils (Keskinen et al. 2022), clay content by default overestimates reactive surface area, and saturation estimates derived from soil texture will do the same.

Soil carbon saturation concepts were therefore evaluated in both the field experiment (Chapter 2) and the farm survey (Chapter 3), with partly contrasting results depending on the metric applied. In the long-term field experiment, Hassink's equation suggested that the meadow topsoil exceeded its predicted MAOM capacity (125–176% in 0–20 cm) and that the cropping systems were near saturation (92–99%). The clay to OC ratio told a different story: only the meadow surface soil (0–10 cm) appeared saturated, while all other depths and management types indicated a substantial storage deficit. However, the meadow had a higher clay+silt content (806 g kg⁻¹) than the cropping systems (~695 g kg⁻¹), driven primarily by higher clay content. Since texture-based saturation metrics estimate MAOM capacity from fine particle content, and fine particle content does not fully reflect reactive surface area in these soils, it is unclear whether the apparent oversaturation of the meadow reflects genuine SOC accumulation beyond mineral capacity or a mismatch between the metric and the soil's actual stabilisation potential.

Across the 93 farms (Chapter 3), the pattern of divergence among SOC saturation metrics persisted. Hassink's equation suggested that many low-clay soils exceeded their predicted

MAOM storage capacity, whereas the boundary line analysis indicated that most soils with clay content above 100 g kg^{-1} had substantial remaining capacity for MAOM accrual. Hassink (1997) concluded that clay mineralogy did not affect the relationship between fine particle content and MAOM capacity. However, in the calibration dataset of Hassink (1997), soils with low-reactivity minerals (dominated by quartz and kaolinite) were predominantly coarse-textured, meaning that unreactive mineralogy coincided with low clay+silt content and had little influence on the regression. Finnish soils break this pattern in two ways. In high-clay soils, the clay fraction can be dominated by less reactive primary minerals, causing Hassink's equation to overestimate MAOM binding capacity. Here, the high clay content implies reactive surface area that is not actually present, so these soils appear falsely undersaturated. Conversely, in low-clay soils where MAOM storage is driven more by oxide content than clay, the equation underestimates binding capacity, and soils appear oversaturated despite additional binding capacity provided by reactive mineral phases.

The boundary line analysis identified a maximum of approximately $55 \text{ g MAOC kg}^{-1}$ clay+silt across the 93 farms. However, this estimate reflects the highest values observed under current arable management, not the theoretical maximum these soils could store. As all studied soils were under cultivation, and Finnish cropland soils have been losing carbon over recent decades (Heikkinen et al. 2022), the true MAOM storage capacity of these soils may be higher than the boundary line suggests. Indeed, the unmanaged meadow at the field experiment site (Chapter 2), where all plant inputs return to the soil, stored approximately $70 \text{ g MAOC kg}^{-1}$ clay+silt in the topsoil. This exceeds the boundary line maximum derived from the arable farms studied in Chapter 3. Whether the reactive mineral surfaces in the studied soils are genuinely approaching saturation, or simply receiving insufficient organic inputs under current management, cannot be resolved from these data alone.

In global-scale clay mineralogy maps (Ito & Wagai 2017), Finnish soils are classified as illite/mica-dominated, placing them in the 2:1 category for which Six et al. (2024) estimated a maximum of 82 g C kg^{-1} clay+silt. However, such maps identify only the dominant clay mineral group at broad spatial resolution, not the proportion of the clay-

sized fraction that actually consists of reactive clay minerals. At the field experiment site (Chapter 2), for example, illite is the dominant clay mineral, but clay minerals constitute only about half of the clay-sized fraction. The remainder consists of primary minerals with specific surface areas orders of magnitude lower than those of clay minerals (Keskinen et al. 2022; Section 6.2 of this thesis). That the boundary line estimate falls well below the 2:1 estimate of 82 g C kg^{-1} clay+silt is consistent with this: the reactive clay minerals in these soils are 2:1 type, but their contribution is diluted by a large proportion of unreactive primary minerals in the clay-sized fraction.

In response to our finding that soils of the long-term field experiment appeared largely below MAOM saturation (Chapter 2), Six et al. (2024) pointed out that the 2:1 clay mineralogy of these soils predicts a higher MAOM capacity of 82 g C kg^{-1} clay+silt, well above the boundary line maximum of $55 \text{ g MAOC kg}^{-1}$ clay+silt derived from the arable farms studied for this thesis (Chapter 3). Against this mineralogy-adjusted estimate, the highest value observed in this thesis, approximately $70 \text{ g MAOC kg}^{-1}$ clay+silt in the meadow topsoil (Chapter 2), would nevertheless still appear somewhat undersaturated. This is a valid point, but it does not resolve the underlying problem. The mineralogy-adjusted estimate of Six et al. (2024) corrects for mineral type by assigning a higher capacity to 2:1 clays, consistent with the illite-dominated mineralogy in the field experiment. However, the estimate still assumes that the clay-sized fraction consists primarily of reactive clay minerals. In Finnish soils, where clay minerals constitute only about half the clay-sized fraction (Keskinen et al. 2022), even the mineralogy-adjusted estimate probably still overstates the reactive surface area available for MAOM stabilisation. This illustrates a point Six et al. (2024) themselves raise: that better mineralogical data are needed to apply saturation frameworks reliably, particularly in soils where texture is a poor proxy for reactive mineral content.

If a mineralogical limit to MAOM accumulation exists, it may in young, glacially-derived Nordic soils be better captured by oxide content than by texture alone. Chilipamushi et al. (2025) found that SOC content reached a plateau at Al oxide concentrations of approximately 3.5 g kg^{-1} in Swedish agricultural soils, suggesting a possible upper limit determined entirely by oxide content. Whether such a plateau applies to Finnish soils

could not be assessed, as the highest Al oxide concentration measured across both studies was 3.5 g kg⁻¹ (Chapter 2).

Beyond mineralogy, additional factors complicate the assumption that mineral surface abundance directly translates into SOC storage. Nanoscale imaging has shown that organo-mineral associations are distributed patchily, with only a minority of mineral surfaces covered by organic matter (Vogel et al. 2014; Vidal et al. 2021). Estimates suggest that fewer than one-fifth of mineral surfaces carry organic coatings even in soils rich in MAOM (Cotrufo & Lavalley 2022). Where organic matter does accumulate, it tends to form multilayered structures in which organic molecules bind to previously attached organic matter rather than directly to mineral surfaces (Kleber et al. 2007; Vogel et al. 2014; Schweizer et al. 2021; Begill et al. 2023), with carbon in outer layers likely being far more accessible to decomposition than carbon directly bound to reactive surfaces. Given this, apparent oversaturation (i.e., MAOM contents exceeding the maximum predicted by a saturation equation), likely reflects constraints of organo-mineral contact or decomposition dynamics rather than soils having exceeded their mineral binding capacity. The patchy, multilayered nature of organo-mineral associations (Vogel et al. 2014; Begill et al. 2023) also has implications for MAOM stability. Although MAOM dominates SOC stocks, individual molecules within this pool may cycle on relatively short timescales, resulting in possibly stable pool sizes despite rapid molecular turnover (Jilling et al. 2018; Yu et al. 2022). At the same time, POM can persist longer than expected under certain conditions (Witzgall et al. 2021; Heckman et al. 2022). These observations challenge the notion that MAOM accumulation alone represents a reliable pathway to long-term carbon sequestration.

Taken together, these findings suggest that SOC saturation concepts, as currently operationalised, have limited applicability. The problems documented here for Finnish soils are not unique to glacially derived soils, but point to fundamental limitations in equating texture with carbon storage potential. For any soil, understanding carbon storage capacity requires moving beyond texture towards characterising the reactive mineral surfaces, organic matter inputs, and decomposition dynamics that govern carbon stabilisation and turnover.

6.4 Subsoil carbon

Most soil carbon research focuses on the topsoil (often, 0–30 cm) yet a substantial proportion of total SOC is stored below that (Jobbágy & Jackson 2000; Rumpel & Kögel-Knabner 2011). Carbon in subsoils is generally older, turns over more slowly, and is governed by different controls than carbon in topsoil, including reduced organic inputs, lower oxygen availability, and lower biological activity (Rumpel & Kögel-Knabner 2011; Mathieu et al. 2015; Button et al. 2022). Understanding what controls subsoil carbon is therefore important for assessing whether deeper soil layers could meaningfully contribute to long-term carbon storage under agricultural management.

In all management types in the long-term field experiment (Chapter 2), around 85% of the SOC was concentrated in the top 30 cm, where also most plant root biomass was found. To 70 cm depth, total carbon stocks were highest in the unmanaged meadow (169 Mg ha⁻¹), followed by the organic (129 Mg ha⁻¹) and conventional (112 Mg ha⁻¹) cropping systems, values broadly comparable to the 83–413 Mg C ha⁻¹ (mean ~163 Mg C ha⁻¹) reported for 30 farms across southern Finland (Wang et al. 2026). The MAOM pool dominated SOC throughout the soil profiles, but the distribution of SOC over the POM and MAOM pools changed with depth: POM comprised 13–17% of SOC in the top 10 cm but only 3–7% below 40 cm. The C:N ratio of size-fractionated POM in the topsoil (16–27) converged toward MAOM values in the subsoil (7–11), consistent with increasing microbial processing of POM with depth (Miltner et al. 2012; Yu et al. 2022).

The predictors of carbon storage also changed with depth. Clay content was positively correlated with total SOC and MAOM in the topsoil (0–20 cm), but this relationship disappeared in deeper soil layers (Chapter 2). Instead, oxalate-extractable Al and Fe were the dominant correlates of MAOM throughout the soil profile, with Fe oxides particularly prominent in the 20–30 cm transition layer and both oxides significant below 30 cm. The meadow maintained notably higher Fe oxide concentrations throughout the profile, a pattern that may reflect enhanced mineral weathering driven by root exudates and organic acids in the carbon-rich meadow soil, or co-transport of iron with dissolved organic carbon from surface layers (Kaiser & Kalbitz 2012; Keiluweit et al. 2015). This depth-dependent shift from partly clay-associated to oxide-dominated correlates of SOC is consistent with the pattern reported by Rasmussen et al. (2018) for the topsoil, where

oxalate-extractable Al was a stronger correlate of SOC than clay content in acidic, humid soils.

Nonetheless, oxides correlated with MAOM in the subsoil. There are several possible explanations for this. With a clay content of approximately 650 g kg^{-1} , the subsoil is likely subject to periodic waterlogging and redox fluctuations, under which the cycling of Fe between reduced and oxidised states can generate short-range-order Fe phases that co-precipitate with dissolved organic matter upon re-oxidation (Chen et al. 2020; Button et al. 2022). Kronberg et al. (2024) demonstrated that such redox cycling proceeds even under boreal temperatures in Finnish clay soils with stagnic properties. However, a positive correlation between oxalate extractable oxides and SOC does not in itself demonstrate a stabilisation mechanism. Oxides and organic matter may co-accumulate without a direct stabilisation relationship: for example, the higher oxalate-extractable Fe throughout the meadow profile could reflect enhanced mineral weathering by root exudates and organic acids in soil, rather than Fe driving MAOM retention (Hall & Thompson 2022). From correlational data alone, whether oxides are stabilising organic matter or organic matter is mobilising oxides cannot be distinguished.

Global estimates suggest that only 21% of subsoil MAOM capacity is currently occupied (Georgiou et al. 2022). In the soils studied here, subsoil clay to SOC ratios of 158–254 at 30–70 cm (Chapter 2) far exceeded the clay to SOC ratio of 10 above which soils are generally considered SOC-depleted (Prout et al. 2021), and Hassink-derived MAOM filled only 7–25% of estimated capacity, indicating large theoretical SOC deficits. If this theoretical capacity were readily fillable, subsoil SOC stocks should respond to sustained differences in root inputs. The unmanaged meadow maintained substantially higher root mass throughout the profile (Chapter 2), yet below 40 cm, SOC stocks appeared similar across all three management systems despite 24 years of contrasting inputs.

The one exception was the 20–30 cm transition layer, where the organic system had higher SOC than the conventional system. The organic system also tended to have more root biomass than the conventional system, though the difference was not statistically significant. This, together with the repeated incorporation of organic amendments into the plough layer (targeted to 20 cm depth), may explain the higher SOC observed in this

transition zone. Some proportion of the applied manure may also have reached below the plough layer through preferential flow paths or biological mixing. Under boreal conditions, lower oxygen availability and colder temperatures could have favoured some part of the SOC from the manure to preserve (Ogle et al. 2019; Button et al. 2022).

Deeper in the soil profile, no management-induced differences in SOC were observed. The unmanaged meadow had roughly double the SOC concentration at 30–40 cm (9.0 vs 4.1–4.9 g kg⁻¹ in the conventional and organic cropping systems), but in the deepest studied soil depth of 40–70 cm the differences had largely disappeared (3.3 vs 2.6–2.7 g kg⁻¹). Whether this reflects an ongoing process of root-derived carbon inputs slowly reaching the subsoil, or preservation of legacy carbon from the previous land use before the experiment, cannot be determined without baseline data. Also, as the meadow was not part of the randomised block design, these differences remain descriptive. These results suggest that subsoil carbon accumulation is constrained not by the capacity of the soil to store additional carbon, but by how little carbon reaches these depths and how slowly it accumulates there, even under perennial vegetation (Janzen et al. 2022).

6.5 What type of SOC do POM and MAOM represent?

Soil organic matter is heterogeneous in origin, chemical composition and persistence (Schmidt et al. 2011). The POM/MAOM framework distinguishes two fractions: particulate organic matter (POM), consisting of partially decomposed plant and root fragments not bound to mineral surfaces, and mineral-associated organic matter (MAOM), consisting of organic compounds associated with mineral surfaces, typically more microbially processed and enriched in nitrogen than POM (Lavalley et al. 2020). In the laboratory, these pools are often separated either by size (often at 53 µm; Cotrufo et al. 2019) or by density (light fraction floating in heavy liquid typically at 1.6–1.8 g cm⁻³; Cerli et al. 2012; Poeplau et al. 2018). This conceptual distinction is assumed to divide SOM into functionally differing pools with different stabilisation mechanisms and different turnover times: POM is expected to cycle on years to decades, while MAOM is expected to persist for decades to centuries, protected by its association with reactive mineral surfaces (Lavalley et al. 2020; Cotrufo et al. 2019).

In Chapters 2 and 3, I used the size fractionation method to quantify carbon in POM and MAOM in Finnish soils. Cotrufo et al. (2019) recommended the size-based approach for separating SOM into two meaningful fractions with different characteristics and dynamics. The method is relatively straightforward: chemically dispersed soil is rinsed over a 53 μm sieve to separate the fractions. As Chapters 2 and 3 required processing of around 300 soil samples, this practicality was important given that alternative SOM fractionation methods, including density fractionation, are considerably more laborious (Poeplau et al. 2018). However, the method assumes that the chemical dispersion fully breaks soil aggregates, so that all material remaining on the sieve is true POM. Observations during size fractionation, including visibly persisting aggregates after dispersion, suggested that this assumption was not always met, motivating further investigation into whether size and density fractionation methods isolate POM and MAOM matching their conceptual pool descriptions (Chapter 4), and how reliably size fractionation achieves complete soil dispersion (Chapter 5).

Rock-Eval thermal analysis, originally developed for petroleum exploration (Espitalié et al. 1977) and increasingly applied to characterise soil organic matter quality (Disnar et al. 2003; Sebag et al. 2016; Delahaie et al. 2024; Koorneef et al. 2023), offered a way to assess whether the two methods produce POM/MAOM consistent with their conceptual pool definitions. (Chapter 4). Density-fractionated POM was consistently more hydrogen-rich and less oxidised than MAOM across all soils and depths, implying lower degree of decomposition and consistent with its expected plant material-like characteristics (Sebag et al. 2016; Koorneef et al. 2023). In contrast, size-fractionated POM had thermal and chemical properties overlapping with MAOM (both size- and density fractionated), particularly in topsoil, suggesting that size-fractionated POM includes mineral-associated SOC. Taken together, in the studied set of soils, density fractionation isolates fractions that are thermally and chemically distinct and consistent with the conceptual definitions of POM and MAOM, while size fractionation produces fractions that overlap in the properties that should distinguish them. This was the case even though the density fractionation method used here (Leuthold et al. 2024) is a single-POM-pool separation that does not distinguish free from occluded POM, as several other methods do (Cerli et al. 2012; Poeplau et al. 2018). As size fractionation also yields only

one POM pool, this single-pool density method was appropriate for comparison, and in practice is fast and readily applicable to large sample sets. However, a single-POM-pool separation cannot distinguish free POM, which is assumed to respond to management and inputs, from occluded POM protected within aggregates, which may cycle on different timescales (Poeplau et al. 2018). For some research questions, further separation of POM into free and occluded fractions may be necessary.

Chapter 5 quantified the dispersion error directly. Two composite samples from the field experiment of Chapter 2 were among the soils tested (soils 36 and 37 in Chapter 5). Incomplete dispersion led to an overestimation of SOC in POM by 22.5 and 35.6% in these soils, while SOC in MAOM was underestimated by 5.1 and 9.9%. Because POM constitutes only a small fraction of total SOC in these mineral soils, even a modest transfer of undispersed MAOM material inflates the POM pool proportionally far more than it depletes the MAOM pool. However, the size fractionation protocol used in Chapter 2 differed from that in Chapter 5: Chapter 2 used field-moist soils and employed glass beads to assist dispersion, whereas Chapter 5 tested air-dried soils without glass beads. As both soil moisture state and mechanical movement from glass beads affect soil dispersion efficiency, the errors reported in Chapter 5 cannot be applied as a correction to Chapter 2, and the possible misallocation of SOC between POM and MAOM pools because of under-dispersion in the field experiment remains unknown.

Taken together, Chapters 4 and 5 suggest that in Chapters 2 and 3 of this thesis, size-fractionated POM likely contained some mineral-associated material due to incomplete soil dispersion, while glass bead fragmentation may have transferred true POM into the MAOM fraction. As these errors work in opposite directions, and without a way to quantify their magnitude, the true distribution of SOC between POM and MAOM in Chapters 2 and 3 remains uncertain.

These findings have broader implications. If size fractionation does not reliably separate functionally distinct pools, then studies reporting POM responses to management and meta-analyses combining size- and density-fractionated data require careful interpretation. Before POM and MAOM pools can be incorporated into SOC models, the methods used to quantify them need to produce consistent and comparable results. The parallel with humic substances is worth noting: humic acid, fulvic acid, and humin were

treated for decades as distinct pools with distinct properties, but now they are largely seen as artefacts of the extraction procedure itself (Lehmann & Kleber 2015). The POM/MAOM framework, while based in physical rather than chemical separation, faces analogous risks if operational fractions do not correspond to functionally meaningful SOM pools. Whether any POM/MAOM fractionation method can achieve this across the full diversity of soils remains an open question. Density fractionation, as characterised by Rock-Eval thermal analysis in Chapter 4, appears to isolate more distinct pools. However, density-fractionated POM is largely free of minerals, while size-fractionated POM is ground and homogenised with the mineral matrix during ball milling, and these differing matrices may themselves influence Rock-Eval thermal signals in ways that are not yet fully understood (Kanari et al. 2021). Comparisons of thermal properties between fractions with very different mineral content should therefore be interpreted with caution. Rock-Eval thermal analysis applied to bulk soil bypasses these fractionation-related uncertainties by characterising SOC composition without physical separation (Delahaie et al. 2024; Schiedung et al. 2025). However, thermal stability is not equivalent to biological stability, and the relationship between Rock-Eval SOM pools and SOC turnover under field conditions remains to be established.

The implications of incomplete dispersion extend beyond carbon pool estimation, as standard texture analysis by the sieve and pipette method relies on the same chemical dispersion with sodium hexametaphosphate. This implies that soils prone to under-dispersion during fractionation are also prone to underestimation of their clay content in routine texture analysis. As an accurately measured clay content already overestimates the reactive mineral surface area in Finnish soils (Sections 1.5 and 6.2 of this thesis; Keskinen et al. 2022), this further complicates texture-based assessments of SOC storage capacity in these soils.

6.6 Carbon for soils

In the preceding sections, I discussed the controls on SOC storage in Finnish agricultural soils, application of SOC saturation concepts, and whether operational SOC fractionation methods isolate functionally distinct faster-cycling (POM) and slower-cycling (MAOM) SOC pools. However, even perfectly working laboratory methods and analysis tools still

only provide a snapshot of the distribution of SOC among pools at a single point in time. Understanding changes in soil organic carbon over time requires temporal SOC data and flux measurements (Moinet et al. 2023). Systematic reviews of SOC simulation models suggest this temporal data is largely missing. For example, Le Noë et al. (2023) found that only 23% of predictive SOC models had been validated with independent data, with the majority only validated against spatial variation in carbon stocks across sites, rather than temporally validated with time-series data measured at the same sites. This problem extends beyond temporal validation: Garsia et al. (2023) found that 71% of the SOC models had not been validated at all, and when validated, only covered a narrow range of land use types, soil conditions, and climatic contexts. Yet these same models are increasingly being relied upon to underpin carbon credit schemes and verify sequestration claims (Garsia et al. 2023; Saifuddin et al. 2024). This highlights that soil carbon policy risks being heavily reliant on assumptions rather than evidence.

The lack of temporal data also applies to observational studies like those in this thesis. The SOC stocks and their distribution across POM and MAOM fractions and soil depths were measured in the long-term field experiment to 70 cm depth (Chapter 2) and across topsoils of 93 farms (Chapter 3). Although they provide a characterisation of current conditions, these single-timepoint measurements cannot reveal whether soils are gaining or losing carbon, how quickly SOC fractions turn over, or how stocks will respond to changing climate or management (Moinet et al. 2023). Without repeated measurements over time and estimates of carbon inputs and outputs, the distributions of SOC, POM, and MAOM described here cannot be interpreted as stable or changing or for example, responding to soil management.

This has large implications for the extent to which soil carbon research is able to support climate policy. The potential scale of soil carbon sequestration in agricultural mineral soils is modest relative to the anthropogenic carbon emissions. Janzen et al. (2022) estimated that maximum net sequestration from global cropland soils under best management would reach approximately 0.1 to 0.2 Pg carbon per year, less than 2% of current yearly fossil fuel carbon emissions (approximately 10 Pg C yr⁻¹; Friedlingstein et al. 2025). For Finland specifically, Soinne et al. (2024) estimated that mineral agricultural topsoils potentially can accrue 0.005-0.01 Pg SOC in MAOM in total, equivalent to less

than one year of national greenhouse gas emissions. Meanwhile, Finnish peatlands store an estimated 5.1 Pg SOC (Turunen & Valpola 2020) and forest mineral soils a further 1.0 Pg (Kauppi et al. 1997). At the same time, organic soils account for only about 10% of Finnish agricultural field area, yet dominate agriculture-related carbon dioxide and nitrous oxide emissions at the national scale (Regina et al. 2018). This highlights that protecting existing carbon stocks, particularly by halting further conversion of peatlands to agriculture and reducing emissions from soils already cultivated, would deliver far greater climate benefits than pursuing small gains in mineral soil carbon sequestration. For these agricultural mineral soils, halting the currently observed decline in SOC would already constitute a meaningful climate change mitigation measure. National soil monitoring data show that climate change enhances SOC losses from Finnish mineral agricultural soils, but diversifying crop rotations with perennial grasses can partly counteract this trend (Heikkinen et al. 2022). At the national scale in Finland, recent shifts toward more diverse and perennial-dominated rotations were found to mitigate SOC losses from mineral soils (Kostensalo et al. 2024). While large-scale carbon sequestration in these soils may be unrealistic, mitigating ongoing SOC losses through improved management is a possible way for soils to contribute to climate mitigation. Beyond its possible role in carbon accounting, maintaining SOC in agricultural mineral soils remains essential for agricultural sustainability.

However, how SOC is maintained matters for climate mitigation. The long-term field experiment studied in this thesis (Chapter 2; Kanari et al. 2025) provides an example. The organic cropping system had higher SOC stocks than the conventional system, and the difference corresponded closely to the cumulative carbon input from manure over 24 years of contrasting management. The organic system also tended to have more root biomass, which may have contributed additional sequestered SOC. If the higher measured carbon stock originates even in part from manure, it would represent a redistribution of carbon within the agricultural system rather than a net removal from the atmosphere (Powlson et al. 2011; Don et al. 2024). While some of this carbon may become stabilised as MAOM over time, attributing such increases to SOC sequestration without accounting for carbon origin may misrepresent net atmospheric carbon removal. This could lead to an overestimation of the climate benefits of agricultural management practices that

increase SOC stocks through organic inputs rather than through enhanced photosynthesis, as soil carbon sequestration requires a net transfer of CO₂ from the atmosphere via photosynthesis (Don et al. 2024). Furthermore, the livestock systems that produce manure are themselves responsible for substantial greenhouse gas emissions (Gerber et al. 2013; Xu et al. 2021), and the carbon returned to soil represents only a small fraction of their total greenhouse gas cost. Applying organic residues to soil contributes to nutrient cycling and organic matter maintenance, but does not in itself constitute soil carbon sequestration. From a climate mitigation perspective, maintaining existing SOC stocks and increasing them through enhanced photosynthetic carbon inputs would be a notable co-benefit of agriculture.

This thesis revealed that widely applied SOC frameworks did not perform consistently even in Finnish soils, despite the fact that Finland is part of the prosperous and better-studied global north. If these frameworks struggle here, also their application in soils of global south deserves critical scrutiny. The empirical basis for our understanding of SOC dynamics remains disproportionately derived from temperate agroecosystems, while regions where SOC conservation is critical for food security and climate adaptation remain underrepresented in long-term research (Powers et al. 2011; Garsia et al. 2023).

6.7 On the operational nature of soil carbon measurements

The journey of a soil sample from field to laboratory measurement involves multiple steps, each of which can introduce changes to the material being analysed. For example, the common practice of drying soil prior to analysis can modify soil aggregate structure as well as organo–mineral associations, altering the soil from the state in which it existed in the field. Then during SOM fractionation, aggregates are deliberately disrupted, and chemical dispersants used to achieve this may further modify organic matter in ways that are difficult to quantify. Consequently, what is measured in the laboratory is not an intact reflection of field conditions, but always to some extent an operational product shaped by the methodology used to generate the data.

In this thesis, I utilised operational laboratory approaches to study soil organic carbon, including physical fractionation into POM and MAOM, and oxalate extractable aluminium and iron as proxies for reactive mineral surfaces in the soil. However, these

approaches may either alter the soil they seek to characterise or rely on simplifying assumptions about the soil.

The operational nature of laboratory-derived data is easy to overlook, yet becomes critical when the operational fractions are interpreted as functionally meaningful pools in the field, as is the case with the POM/MAOM distinction. While the reasoning behind separation into these categories is theoretically well founded, it remains uncertain whether specific fractionation procedures isolate the pools described by the theory. In this thesis, size- and density-based fractionation approaches produced chemically differing POM and MAOM fractions from the same soils. This illustrates how methodological choices can shape conclusions about soil carbon dynamics.

In this thesis, I also applied commonly used texture-based carbon saturation concepts. These yielded conflicting results when applied to the soils studied in this thesis, whose mineralogical properties differed from those on which the metrics were originally developed. This highlights that empirically derived frameworks and concepts require validation under differing contexts, as they do not automatically transfer across soils.

None of this diminishes the value of operational and empirical approaches. Our understanding of complex soil systems progresses by testing where simplified tools and frameworks hold and where they need adjustment. Recognising the limits of applicability is equally important, and in that sense, this thesis contributes by clarifying where some widely used frameworks (POM/MAOM) and concepts (texture-based carbon saturation) can be applied in Northern agricultural soils such as the ones studied in here.

References

- Al-Kaisi, M.M., Douelle, A., Kwaw-Mensah, D., 2014. Soil microaggregate and macroaggregate decay over time and soil carbon change as influenced by different tillage systems. *J. Soil Water Conserv.* 69, 574-580. <https://doi.org/10.2489/jswc.69.6.574>
- Andreola, F., Castellini, E., Ferreira, J.M.F., Olhero, S., Romagnoli, M., 2006. Effect of sodium hexametaphosphate and ageing on the rheological behaviour of kaolin dispersions. *Appl. Clay Sci.* 31, 56-64. <https://doi.org/10.1016/j.clay.2005.08.004>
- Andreola, F., Castellini, E., Manfredini, T., Romagnoli, M., 2004. The role of sodium hexametaphosphate in the dissolution process of kaolinite and kaolin. *J. Eur. Ceram. Soc.* 24, 2113-2124. [https://doi.org/10.1016/S0955-2219\(03\)00366-2](https://doi.org/10.1016/S0955-2219(03)00366-2)
- Angst, G., Mueller, K. E., Castellano, M. J., Vogel, C., Wiesmeier, M., & Mueller, C. W. (2023). Unlocking complex soil systems as carbon sinks: multi-pool management as the key. *Nature Communications*, 14(1), 2967. <https://doi.org/10.1038/s41467-023-38700-5>
- Angst, G., Mueller, K. E., Nierop, K. G., & Simpson, M. J. (2021). Plant-or microbial-derived? A review on the molecular composition of stabilized soil organic matter. *Soil Biology and Biochemistry*, 156, 108189. <https://doi.org/10.1016/j.soilbio.2021.108189>
- Antoniadis, V., Koliniati, R., Efstratiou, E., Golia, E., Petropoulos, S., 2016. Effect of soils with varying degree of weathering and pH values on phosphorus sorption. *Catena* 139, 214-219. <https://doi.org/10.1016/j.catena.2016.01.008>
- Arachchige, P.S.P., Hettiarachchi, G.M., Rice, C.W., Dynes, J.J., Maurmann, L., Wang, J., Karunakaran, C., Kilcoyne, A.L.D., Attanayake, C.P., Amado, T.J.C., Fiorin, J.E., 2018. Sub-micron level investigation reveals the inaccessibility of stabilized carbon in soil microaggregates. *Sci. Rep.* 8, 1-13. <https://doi.org/10.1038/s41598-018-34981-9>
- Arrouays, D., Saby, N., Walter, C., Lemercier, B., & Schvartz, C. (2006). Relationships between particle-size distribution and organic carbon in French arable topsoils. *Soil Use and Management*, 22(1), 48-51. <https://doi.org/10.1111/j.1475-2743.2006.00020.x>
- Avneri-Katz, S., Young, R. B., McKenna, A. M., Chen, H., Corilo, Y. E., Polubesova, T., ... & Chefetz, B. (2017). Adsorptive fractionation of dissolved organic matter (DOM) by

- mineral soil: Macroscale approach and molecular insight. *Organic Geochemistry*, 103, 113-124. <https://doi.org/10.1016/j.orggeochem.2016.11.004>
- Bai, Y., & Cotrufo, M. F. (2022). Grassland soil carbon sequestration: Current understanding, challenges, and solutions. *Science*, 377(6606), 603-608. <https://doi.org/10.1126/science.abo2380>
- Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual review of plant biology*, 57(1), 233-266. <https://doi.org/10.1146/annurev.arplant.57.032905.105159>
- Baldock, J.A., Sanderman, J., MacDonald, L.M., Puccini, A., Hawke, B., Szarvas, S., McGowan, J., 2013. Quantifying the allocation of soil organic carbon to biologically significant fractions. *Soil Res.* 51, 561-576. <https://doi.org/10.1071/SR12374>
- Bàrberi, P. (2002). Weed management in organic agriculture: are we addressing the right issues?. *Weed research*, 42(3), 177-193. <https://doi.org/10.1046/j.1365-3180.2002.00277.x>
- Bartoń, K. (2023). MuMIn: Multi-Model Inference. R package version 1.47.5, <<https://CRAN.R-project.org/package=MuMIn>>.
- Batjes, N.H. (1996). Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science*, 47, 151-163. <https://doi.org/10.1111/j.1365-2389.1996.tb01386.x>
- Beare, M.H., McNeill, S.J., Curtin, D., Parfitt, R.L., Jones, H.S., Dodd, M.B., Sharp, J., 2014. Estimating the organic carbon stabilisation capacity and saturation deficit of soils: A New Zealand case study. *Biogeochemistry* 120, 71-87. <https://doi.org/10.1007/s10533-014-9982-1>
- Begill, N., Don, A., & Poeplau, C. (2023). No detectable upper limit of mineral-associated organic carbon in temperate agricultural soils. *Global Change Biology*. <https://doi.org/10.1111/gcb.16804>
- Berthelin, J., Laba, M., Lemaire, G., Powlson, D., Tessier, D., Wander, M., & Baveye, P. C. (2022). Soil carbon sequestration for climate change mitigation: Mineralization

kinetics of organic inputs as an overlooked limitation. *European Journal of Soil Science*, 73(1), e13221. <https://doi.org/10.1111/ejss.13221>

Besnard, E., Chenu, C., Balesdent, J., Puget, P., & Arrouays, D. (1996). Fate of particulate organic matter in soil aggregates during cultivation. *European Journal of Soil Science*, 47(4), 495-503. <https://doi.org/10.1111/j.1365-2389.1996.tb01849.x>

Bolinder, M. A., Crotty, F., Elsen, A., Frac, M., Kismányoky, T., Lipiec, J., ... & Kätterer, T. (2020). The effect of crop residues, cover crops, manures and nitrogen fertilization on soil organic carbon changes in agroecosystems: A synthesis of reviews. *Mitigation and Adaptation Strategies for Global Change*, 25, 929-952. <https://doi.org/10.1007/s11027-020-09916-3>

Bongiorno, G., Bünemann, E. K., Oguejiofor, C. U., Meier, J., Gort, G., Comans, R., ... & de Goede, R. (2019). Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. *Ecological Indicators*, 99, 38-50. <https://doi.org/10.1016/j.ecolind.2018.12.008>

Börjesson, G., Bolinder, M. A., Kirchmann, H., & Kätterer, T. (2018). Organic carbon stocks in topsoil and subsoil in long-term ley and cereal monoculture rotations. *Biology and Fertility of Soils*, 54(4), 549-558. <https://doi.org/10.1007/s00374-018-1281-x>

Brady, N.C., & Weil, R.R. (2008). *The Nature and Properties of Soils* (14th ed.). Pearson.

Breure, M. S., van der Sluijs, A. J., Koorneef, G. J., Salonen, A. R., Pulleman, M. M., Hagens, M., ... & Di Lonardo, D. P. (2026). Glued forever: incomplete dispersion hampers POM-MAOM fractionation in soils with high SOC and metal (hydr) oxide content. *Geoderma*, 467, 117704. <https://doi.org/10.1016/j.geoderma.2026.117704>

Bronick, C.J., Lal, R., 2005. Soil structure and management: A review. *Geoderma* 124, 3-22. <https://doi.org/10.1016/j.geoderma.2004.03.005>

Button, E. S., Pett-Ridge, J., Murphy, D. V., Kuzyakov, Y., Chadwick, D. R., & Jones, D. L. (2022). Deep-C storage: Biological, chemical and physical strategies to enhance carbon stocks in agricultural subsoils. *Soil Biology and Biochemistry*, 108697. <https://doi.org/10.1016/j.soilbio.2022.108697>

- Cai, A., Han, T., Ren, T., Sanderman, J., Rui, Y., Wang, B., ... & Xu, M. (2022). Declines in soil carbon storage under no tillage can be alleviated in the long run. *Geoderma*, 425, 116028. <https://doi.org/10.1016/j.geoderma.2022.116028>
- Callesen, I., Liski, J., Raulund-Rasmussen, K., Olsson, M. T., Tau-Strand, L., Vesterdal, L., & Westman, C. J. (2003). Soil carbon stores in Nordic well-drained forest soils-Relationships with climate and texture class. *Global change biology*, 9(3), 358-370. <https://doi.org/10.1046/j.1365-2486.2003.00587.x>
- Cambardella, C.A., Elliott, E.T., 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.* 56, 777-783. <https://doi.org/10.2136/sssaj1992.03615995005600030017x>
- Cambardella, C.A., Elliott, E.T., 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. *Soil Sci. Soc. Am. J.* 57, 1071-1076. <https://doi.org/10.2136/sssaj1993.03615995005700040032x>
- Cécillon, L., Baudin, F., Chenu, C., Christensen, B. T., Franko, U., Houot, S., ... & Barré, P. (2021). Partitioning soil organic carbon into its centennially stable and active fractions with machine-learning models based on Rock-Eval® thermal analysis (PARTY SOC v2.0 and PARTY SOC v2.0 EU). *Geoscientific Model Development*, 14(6), 3879-3898. <https://doi.org/10.5194/gmd-14-3879-2021>
- Cerli, C., Celi, L., Kalbitz, K., Guggenberger, G., & Kaiser, K. (2012). Separation of light and heavy organic matter fractions in soil-Testing for proper density cut-off and dispersion level. *Geoderma*, 170, 403-416. <https://doi.org/10.1016/j.geoderma.2011.10.009>
- Chang, Y., Sokol, N. W., van Groenigen, K. J., Bradford, M. A., Ji, D., Crowther, T. W., ... & Ding, F. (2024). A stoichiometric approach to estimate sources of mineral-associated soil organic matter. *Global Change Biology*, 30(1), e17092. <https://doi.org/10.1111/gcb.17092>
- Chapman, N., Miller, A. J., Lindsey, K., & Whalley, W. R. (2012). Roots, water, and nutrient acquisition: let's get physical. *Trends in plant science*, 17(12), 701-710. <https://doi.org/10.1016/j.tplants.2012.08.001>

References

- Chen, C., Hall, S. J., Coward, E., & Thompson, A. (2020). Iron-mediated organic matter decomposition in humid soils can counteract protection. *Nature Communications*, 11(1), 2255. <https://doi.org/10.1038/s41467-020-16071-5>
- Chenu, C., Angers, D.A., Barré, P., Derrien, D., Arrouays, D., Balesdent, J., 2019. Increasing organic stocks in agricultural soils: Knowledge gaps and potential innovations. *Soil Tillage Res.* 188, 41-52. <https://doi.org/10.1016/j.still.2018.04.011>
- Chilipamushi, M., Von Brömssen, C., Colombi, T., Kätterer, T., & Larsbo, M. (2025). Oxalate-extractable aluminium is a key predictor of organic carbon content in Swedish agricultural topsoils. *Geoderma Regional*, e01038. <https://doi.org/10.1016/j.geodrs.2025.e01038>
- Chorom, M., Rengasamy, P., Murray, R.S., 1994. Clay dispersion as influenced by pH and net particle charge of sodic soils. *Aust. J. Soil Res.* 32, 1243-1252. <https://doi.org/10.1071/SR9941243>
- Churchman, G. J. (2010). Is the geological concept of clay minerals appropriate for soil science? A literature-based and philosophical analysis. *Physics and Chemistry of the Earth, Parts A/B/C*, 35(15-18), 927-940. <https://doi.org/10.1016/j.pce.2010.05.009>
- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., ... & Thornton, P. (2014). Carbon and other biogeochemical cycles. In *Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 465-570). Cambridge University Press. <https://doi.org/10.1017/CBO9781107415324.015>
- Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter?. *Global change biology*, 19(4), 988-995. <https://doi.org/10.1111/gcb.12113>
- Cotrufo, M.F., Haddix, M.L., Kroeger, M.E., Stewart, C.E., 2022. The role of plant input physical-chemical properties, and microbial and soil chemical diversity on the formation

- of particulate and mineral-associated organic matter. *Soil Biol. Biochem.* 168, 108648. <https://doi.org/10.1016/j.soilbio.2022.108648>
- Cotrufo, M.F., Lavalley, J.M., 2022. Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration, 1st ed, *Advances in Agronomy*. Elsevier Inc. <https://doi.org/10.1016/bs.agron.2021.11.002>
- Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. *Nat. Geosci.* 12, 989-994. <https://doi.org/10.1038/s41561-019-0484-6>
- Creamer, R.E., Barel, J.M., Bongiorno, G., Zwetsloot, M.J., 2022. The life of soils: Integrating the who and how of multifunctionality. *Soil Biol. Biochem.* 166, 108561. <https://doi.org/10.1016/j.soilbio.2022.108561>
- Crézé, C., Saatchi, S., Kwon, N., Yang, Y., & Li, S. (2025). High-resolution global map (100 m) of soil organic carbon reveals critical ecosystems for carbon storage. *Earth System Science Data Discussions*, 2025, 1-46. <https://doi.org/10.5194/essd-2025-294>
- Delahaie, A. A., Cécillon, L., Stojanova, M., Abiven, S., Arbelet, P., Arrouays, D., ... & Barré, P. (2024). Investigating the complementarity of thermal and physical soil organic carbon fractions. *Soil*, 10(2), 795-812. <https://doi.org/10.5194/soil-10-795-2024>
- Denef, K., Six, J., Merckx, R., Paustian, K., 2002. Short-term effects of biological and physical forces on aggregate formation in soils with different clay mineralogy. *Plant Soil* 246, 185-200. <https://doi.org/10.1023/A:1020668013524>
- Denef, K., Six, J., Paustian, K., Merckx, R., 2001. Importance of macroaggregate dynamics in controlling soil carbon stabilization: Short-term effects of physical disturbance induced by dry-wet cycles. *Soil Biol. Biochem.* 33, 2145-2153. [https://doi.org/10.1016/S0038-0717\(01\)00153-5](https://doi.org/10.1016/S0038-0717(01)00153-5)
- Dettmann, U., Kraft, N.N., Rech, R., Heidkamp, A., Tiemeyer, B., 2021. Analysis of peat soil organic carbon, total nitrogen, soil water content and basal respiration: Is there a 'best' drying temperature? *Geoderma* 403. <https://doi.org/10.1016/j.geoderma.2021.115231>

References

- Dexter, A. R., Richard, G., Arrouays, D., Czyz, E. A., Jolivet, C., & Duval, O. (2008). Complexed organic matter controls soil physical properties. *Geoderma*, 144(3-4), 620-627. <https://doi.org/10.1016/j.geoderma.2008.01.022>
- Dimoyiannis, D., 2012. Wet aggregate stability as affected by excess carbonate and other soil properties. *L. Degrad. Dev.* 23, 450-455. <https://doi.org/10.1002/ldr.1085>
- Disnar, J. R., Guillet, B., Kéravis, D., Di-Giovanni, C., & Sebag, D. (2003). Soil organic matter (SOM) characterization by Rock-Eval pyrolysis: scope and limitations. *Organic geochemistry*, 34(3), 327-343. [https://doi.org/10.1016/S0146-6380\(02\)00239-5](https://doi.org/10.1016/S0146-6380(02)00239-5)
- Dominati, E., Patterson, M., & Mackay, A. (2010). A framework for classifying and quantifying the natural capital and ecosystem services of soils. *Ecological Economics*, 69(9), 1858-1868. <https://doi.org/10.1016/j.ecolecon.2010.05.002>
- Don, A., Seidel, F., Leifeld, J., Kätterer, T., Martin, M., Pellerin, S., ... & Chenu, C. (2024). Carbon sequestration in soils and climate change mitigation - Definitions and pitfalls. *Global Change Biology*, 30(1), e16983. <https://doi.org/10.1111/gcb.16983>
- Drew, M. C. (1997). Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annual review of plant biology*, 48(1), 223-250. <https://doi.org/10.1146/annurev.arplant.48.1.223>
- Dynarski, K. A., Bossio, D. A., & Scow, K. M. (2020). Dynamic stability of soil carbon: reassessing the "permanence" of soil carbon sequestration. *Frontiers in Environmental Science*, 8, 514701. <https://doi.org/10.3389/fenvs.2020.514701>
- Elonen, P. 1971. Particle-size analysis of soils. *Acta Agr.Fenn.* 122: 1-122.
- Emde, D., Hannam, K.D., Midwood, A.J., Jones, M.D., 2022. Estimating mineral-associated organic carbon deficits in soils of the Okanagan valley: a regional study with broader implications. *Front. Soil Sci.* 2, 1-17. <https://doi.org/10.3389/fsoil.2022.812249>
- Eshel, G., Levy, G.J., Mingelgrin, U., Singer, M.J., 2004. Critical evaluation of the use of laser diffraction for particle size distribution analysis. *Soil Sci. Soc. Am. J.* 68, 736-743. <https://doi.org/10.2136/sssaj2004.7360>

- Espitalié, J., Laporte, J. L., Madec, M., Marquis, F., Leplat, P., Paulet, J., & Boutefeu, A. (1977). Méthode rapide de caractérisation des roches mères, de leur potentiel pétrolier et de leur degré d'évolution. *Revue de l'Institut français du Pétrole*, 32(1), 23-42. <https://doi.org/10.2516/ogst:1977002>
- Eusterhues, K., Rumpel, C., Kögel-Knabner, I., 2005. Organo-mineral associations in sandy acid forest soils: Importance of specific surface area, iron oxides and micropores. *Eur. J. Soil Sci.* 56, 753-763. <https://doi.org/10.1111/j.1365-2389.2005.00710.x>
- Even, R.J., Machmuller, M.B., Lavallee, J.M., Zelikova, T.J., Cotrufo, M.F., 2025. Large errors in soil carbon measurements attributed to inconsistent sample processing. *SOIL* 11, 17-34. <https://doi.org/10.5194/soil-11-17-2025>
- FAO. (2015). Status of the World's Soil Resources. Food and Agriculture Organization of the United Nations.
- FAO. (2020). Global Forest Resources Assessment 2020: Main Report. Rome. <https://doi.org/10.4060/ca9825en>
- FAO. (2023). Land Use. In: FAOSTAT. Rome. <http://www.fao.org/faostat/en/#data/RL>
- Feng, W., Plante, A. F., & Six, J. (2013). Improving estimates of maximal organic carbon stabilization by fine soil particles. *Biogeochemistry*, 112, 81-93. <https://doi.org/10.1007/s10533-011-9679-7>
- Fox J., & Weisberg, S. (2019). *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks CA.
- Friedlingstein, P., O'Sullivan, M., Jones, M. W., Andrew, R. M., Bakker, D. C. E., Hauck, J., ... & Zeng, J. (2025). Global carbon budget 2025. *Earth System Science Data Discussions*, 2025, 1–139. <https://doi.org/10.5194/essd-2025-659>
- Fukumasu, J., Poeplau, C., Coucheney, E., Jarvis, N., Klöffel, T., Koestel, J., Kätterer, T., Nimblad Svensson, D., Wetterlind, J., & Larsbo, M. (2021). Oxalate-extractable aluminum alongside carbon inputs may be a major determinant for organic carbon content in agricultural topsoils in humid continental climate. *Geoderma*, 402, 115345. <https://doi.org/10.1016/j.geoderma.2021.115345>

References

- Fulton-Smith, S., & Cotrufo, M. F. (2019). Pathways of soil organic matter formation from above and belowground inputs in a *Sorghum bicolor* bioenergy crop. *GCB Bioenergy*, 11(8), 971-987. <https://doi.org/10.1111/gcbb.12598>
- Garsia, A., Moinet, A., Vazquez, C., Creamer, R. E., & Moinet, G. Y. (2023). The challenge of selecting an appropriate soil organic carbon simulation model: A comprehensive global review and validation assessment. *Global Change Biology*, 29(20), 5760-5774. <https://doi.org/10.1111/gcb.16896>
- Georgiou, K., Angers, D., Champiny, R. E., Cotrufo, M. F., Craig, M. E., Doetterl, S., ... & Wieder, W. R. (2025). Soil carbon saturation: what do we really know?. *Global Change Biology*, 31(5), e70197. <https://doi.org/10.1111/gcb.70197>
- Georgiou, K., Jackson, R. B., Vindušková, O., Abramoff, R. Z., Ahlström, A., Feng, W., ... & Torn, M. S. (2022). Global stocks and capacity of mineral-associated soil organic carbon. *Nature communications*, 13(1), 1-12. <https://doi.org/10.1038/s41467-022-31540-9>
- Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A. and Tempio, G. (2013). Tackling climate change through livestock: A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome.
- Gocke, M. I., Guigue, J., Bauke, S. L., Barkusky, D., Baumecker, M., Berns, A. E., ... & Amelung, W. (2023). Interactive effects of agricultural management on soil organic carbon accrual: A synthesis of long-term field experiments in Germany. *Geoderma*, 438, 116616. <https://doi.org/10.1016/j.geoderma.2023.116616>
- Golchin, A., Oades, J. M., Skjemstad, J. O., & Clarke, P. (1994). Study of free and occluded particulate organic matter in soils by solid state ¹³C CP/MAS NMR spectroscopy and scanning electron microscopy. *Australian Journal of Soil Research*, 32(2), 285-309. <https://doi.org/10.1071/SR9940285>
- Grandy, A. S., & Robertson, G. P. (2007). Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. *Ecosystems*, 10(1), 59-74. <https://doi.org/10.1007/s10021-006-9010-y>

- Gregorich, E. G., Beare, M. H., McKim, U. F., & Skjemstad, J. O. (2006). Chemical and biological characteristics of physically uncomplexed organic matter. *Soil Science Society of America Journal*, 70(3), 975-985. <https://doi.org/10.2136/sssaj2005.0116>
- Gregorich, E. G., Liang, B. C., Ellert, B. H., & Drury, C. F. (1996). Fertilization effects on soil organic matter turnover and corn residue C storage. *Soil Science Society of America Journal*, 60(2), 472-476.
<https://doi.org/10.2136/sssaj1996.03615995006000020019x>
- Groemping, U., 2006. Relative Importance for Linear Regression in R: The Package relaimpo. *J. Stat. Softw.* 17, 1-27. <https://doi.org/10.18637/jss.v017.i01>
- Guggenberger, G., & Kaiser, K. (2003). Dissolved organic matter in soil: challenging the paradigm of sorptive preservation. *Geoderma*, 113(3-4), 293-310. [https://doi.org/10.1016/S0016-7061\(02\)00366-X](https://doi.org/10.1016/S0016-7061(02)00366-X)
- Guillaume, T., Makowski, D., Libohova, Z., Bragazza, L., Sallaku, F., & Sinaj, S. (2022). Soil organic carbon saturation in cropland-grassland systems: Storage potential and soil quality. *Geoderma*, 406, 115529. <https://doi.org/10.1016/j.geoderma.2021.115529>
- Guo, L. B., & Gifford, R. M. (2002). Soil carbon stocks and land use change: a meta analysis. *Global change biology*, 8(4), 345-360. <https://doi.org/10.1046/j.1354-1013.2002.00486.x>
- Guo, L., Gu, W., Peng, C., Wang, W., Jie Li, Y., Zong, T., Tang, Y., Wu, Z., Lin, Q., Ge, M., Zhang, G., Hu, M., Bi, X., Wang, X., Tang, M., 2019. A comprehensive study of hygroscopic properties of calcium-and magnesium-containing salts: Implication for hygroscopicity of mineral dust and sea salt aerosols. *Atmos. Chem. Phys.* 19, 2115-2133. <https://doi.org/10.5194/acp-19-2115-2019>
- Gylden, C. W. (1850). Karta öfver Finland utvisande skogstillgångarna i landets särskilda delar år 1850. <https://helda.helsinki.fi/handle/1975/162>
- Haddaway, N. R., Hedlund, K., Jackson, L. E., Kätterer, T., Lugato, E., Thomsen, I. K., ... & Isberg, P. E. (2016). How does tillage intensity affect soil organic carbon? A systematic review protocol. <https://doi.org/10.1186/s13750-016-0052-0>

References

- Hall, S. J., & Thompson, A. (2022). What do relationships between extractable metals and soil organic carbon concentrations mean?. *Soil Science Society of America Journal*, 86(2), 195-208. <https://doi.org/10.1002/saj2.20343>
- Han, Y., Liu, W., Zhou, J., Chen, J., 2016. Interactions between kaolinite Al-OH surface and sodium hexametaphosphate. *Appl. Surf. Sci.* 387, 759-765. <https://doi.org/10.1016/j.apsusc.2016.07.002>
- Hansen, P. M., Even, R., King, A. E., Lavallee, J., Schipanski, M., & Cotrufo, M. F. (2024). Distinct, direct and climate-mediated environmental controls on global particulate and mineral-associated organic carbon storage. *Global Change Biology*, 30(1), e17080. <https://doi.org/10.1111/gcb.17080>
- Hassink, J. (1997). The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant and Soil*, 191, 77-87. <https://doi.org/10.1023/A:1004213929699>
- Heckman, K., Hicks Pries, C. E., Lawrence, C. R., Rasmussen, C., Crow, S. E., Hoyt, A. M., ... & Wagai, R. (2022). Beyond bulk: Density fractions explain heterogeneity in global soil carbon abundance and persistence. *Global Change Biology*, 28(3), 1178-1196. <https://doi.org/10.1111/gcb.16023>
- Heikkinen, J., Keskinen, R., Kostensalo, J., & Nuutinen, V. (2022). Climate change induces carbon loss of arable mineral soils in boreal conditions. *Global Change Biology*, 28(12), 3960-3973. <https://doi.org/10.1111/gcb.16164>
- Heikkinen, J., Keskinen, R., Regina, K., Honkanen, H. and Nuutinen, V. (2021). Estimation of carbon stocks in boreal cropland soils - methodological considerations. *European Journal of Soil Science*, 72(2), 934-945. <https://doi.org/10.1111/ejss.13033>
- Heikkinen, J., Ketoja, E., Nuutinen, V., & Regina, K. (2013). Declining trend of carbon in Finnish cropland soils in 1974-2009. *Global change biology*, 19(5), 1456-1469. <https://doi.org/10.1111/gcb.12137>
- Hiemstra, T., Antelo, J., Rahnemaie, R., Riemsdijk, W.H. va., 2010. Nanoparticles in natural systems I: The effective reactive surface area of the natural oxide fraction in field

- samples. *Geochim. Cosmochim. Acta* 74, 41-58.
<https://doi.org/10.1016/j.gca.2009.10.018>
- Hillel, D. (1992). *Out of the Earth: Civilization and the Life of the Soil*. Univ of California Press. <https://doi.org/10.1097/00010694-199108000-00014>
- Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237, 173-195.
<https://doi.org/10.1023/A:1013351617532>
- Hoffland, E., Kuyper, T.W., Comans, R.N.J., Creamer, R.E., 2020. Eco-functionality of organic matter in soils. *Plant Soil* 455, 1-22. <https://doi.org/10.1007/s11104-020-04651-9>
- Huang, W., & Hall, S. J. (2017). Elevated moisture stimulates carbon loss from mineral soils by releasing protected organic matter. *Nature communications*, 8(1), 1774.
<https://doi.org/10.1038/s41467-017-01998-z>
- Huang, X., Jia, Z., Guo, J., Li, T., Sun, D., Meng, H., Yu, G., He, X., Ran, W., Zhang, S., Hong, J., Shen, Q., 2019. Ten-year long-term organic fertilization enhances carbon sequestration and calcium-mediated stabilization of aggregate-associated organic carbon in a reclaimed Cambisol. *Geoderma* 355, 113880.
<https://doi.org/10.1016/j.geoderma.2019.113880>
- Ito, A., & Wagai, R. (2017). Global distribution of clay-size minerals on land surface for biogeochemical and climatological studies. *Scientific Data*, 4(1), 1-11.
<https://doi.org/10.1038/sdata.2017.103>
- IUSS Working Group WRB, 2015 IUSS Working Group WRB World Reference Base for Soil Resources. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps, Update 2015. World Soil Resources Report No. 106 FAO, Rome (2015).
- IUSS Working Group WRB, 2022. World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps, 4th ed. International Union of Soil Sciences (IUSS), Vienna, Austria.

References

- Jackson, R. B., Canadell, J., Ehleringer, J. R., Mooney, H. A., Sala, O. E., & Schulze, E. D. (1996). A global analysis of root distributions for terrestrial biomes. *Oecologia*, 108(3), 389-411. <https://doi.org/10.1007/BF00333714>
- Jackson, R. B., Lajtha, K., Crow, S. E., Hugelius, G., Kramer, M. G., & Piñeiro, G. (2017). The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. *Annual review of ecology, evolution, and systematics*, 48, 419-445. <https://doi.org/10.1146/annurev-ecolsys-112414-054234>
- Jaeger, B. (2017). Package 'r2glmm'. R Found Stat Comput Vienna available CRAN R-project org/package= R2glmm.
- Janzen, H. H. (2024). RUSSELL REVIEW. Soil carbon stewardship: Thinking in circles. *European Journal of Soil Science*, 75(5), e13536. <https://doi.org/10.1111/ejss.13536>
- Janzen, H.H. (2006). The soil carbon dilemma: Shall we hoard it or use it? *Soil Biology and Biochemistry*, 38, 419-424. <https://doi.org/10.1016/j.soilbio.2005.10.008>
- Janzen, H.H., van Groenigen, K.J., Powlson, D.S., Schwinghamer, T., & van Groenigen, J.W. (2022). Photosynthetic limits on carbon sequestration in croplands. *Geoderma*, 416, 115810. <https://doi.org/10.1016/j.geoderma.2022.115810>
- Jenny, H. (1941) *Factors of Soil Formation: A System of Quantitative Pedology*. Dover Publications, New York, 281 p.
- Jilling, A., Kane, D., Williams, A., Yannarell, A.C., Davis, A., Jordan, N.R., Koide, R.T., Mortensen, D.A., Smith, R.G., Snapp, S.S., Spokas, K.A., Stuart Grandy, A., 2020. Rapid and distinct responses of particulate and mineral-associated organic nitrogen to conservation tillage and cover crops. *Geoderma* 359, 114001. <https://doi.org/10.1016/j.geoderma.2019.114001>
- Jilling, A., Keiluweit, M., Contosta, A. R., Frey, S., Schimel, J., Schneck, J., ... & Grandy, A. S. (2018). Minerals in the rhizosphere: overlooked mediators of soil nitrogen availability to plants and microbes. *Biogeochemistry*, 139(2), 103-122. <https://doi.org/10.1007/s10533-018-0459-5>

- Jobbágy, E. G., & Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological applications*, 10(2), 423-436. [https://doi.org/10.1890/1051-0761\(2000\)010\[0423:TVDOSO\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2)
- Just, C., Armbruster, M., Barkusky, D., Baumecker, M., Diepolder, M., Döring, T. F., ... & Wiesmeier, M. (2023). Soil organic carbon sequestration in agricultural long-term field experiments as derived from particulate and mineral-associated organic matter. *Geoderma*, 434, 116472. <https://doi.org/10.1016/j.geoderma.2023.116472>
- Kaiser, K., & Guggenberger, G. (2003). Mineral surfaces and soil organic matter. *European Journal of Soil Science*, 54(2), 219-236. <https://doi.org/10.1046/j.1365-2389.2003.00544.x>
- Kaiser, K., & Kalbitz, K. (2012). Cycling downwards - dissolved organic matter in soils. *Soil Biology and Biochemistry*, 52, 29-32. <https://doi.org/10.1016/j.soilbio.2012.04.002>
- Kaiser, K., Guggenberger, G., 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. *Org. Geochem.* 31, 711-725. [https://doi.org/10.1016/S0146-6380\(00\)00046-2](https://doi.org/10.1016/S0146-6380(00)00046-2)
- Kaiser, M., Kleber, M., Berhe, A.A., 2015. How air-drying and rewetting modify soil organic matter characteristics: An assessment to improve data interpretation and inference. *Soil Biol. Biochem.* 80, 324-340. <https://doi.org/10.1016/j.soilbio.2014.10.018>
- Kalbitz, K., Solinger, S., Park, J. H., Michalzik, B., & Matzner, E. (2000). Controls on the dynamics of dissolved organic matter in soils: a review. *Soil science*, 165(4), 277-304. <https://doi.org/10.1097/00010694-200004000-00001>
- Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature communications*, 7(1), 1-10. <https://doi.org/10.1038/ncomms13630>
- Kanari, E., Barré, P., Baudin, F., Berthelot, A., Bouton, N., Gosselin, F., ... & Cécillon, L. (2021). Predicting Rock-Eval® thermal analysis parameters of a soil layer based on samples from its sublayers; an experimental study on forest soils. *Organic Geochemistry*, 160, 104289. <https://doi.org/10.1016/j.orggeochem.2021.104289>

References

- Kanari, E., Karhu, K., Salonen, A. R., Lemola, R., Soinne, H., Barré, P., ... & Heinonsalo, J. (2025). Sensitivity of soil organic carbon stabilization indicators to 24 years of land-use change across soil depth. *Geoderma*, 463, 117573. <https://doi.org/10.1016/j.geoderma.2025.117573>
- Kassambara, A. (2020). Ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0.999. <https://rpkgs.datanovia.com/ggpubr/>
- Kassambara, A. (2022). Rstatix: pipe-friendly framework for basic statistical tests. 2021.
- Kätterer, T., Börjesson, G., & Kirchmann, H. (2014). Changes in organic carbon in topsoil and subsoil and microbial community composition caused by repeated additions of organic amendments and N fertilisation in a long-term field experiment in Sweden. *Agriculture, Ecosystems & Environment*, 189, 110-118. <https://doi.org/10.1016/j.agee.2014.03.025>
- Kauppi, P. E., Posch, M., Hänninen, P., Henttonen, H. M., Ihalainen, A., Lappalainen, E., ... & Tamminen, P. (1997). Carbon reservoirs in peatlands and forests in the boreal regions of Finland. *Silva Fennica*, 31, 13-25. <https://doi.org/10.14214/sf.a8507>
- Keenan, T. F., & Williams, C. A. (2018). The terrestrial carbon sink. *Annual Review of Environment and Resources*, 43, 219-243. <https://doi.org/10.1146/annurev-environ-102017-030204>
- Keiluweit, M., Bougoure, J. J., Nico, P. S., Pett-Ridge, J., Weber, P. K., & Kleber, M. (2015). Mineral protection of soil carbon counteracted by root exudates. *Nature Climate Change*, 5(6), 588-595. <https://doi.org/10.1038/nclimate2580>
- Keiluweit, M., Nico, P. S., Kleber, M., & Fendorf, S. (2016). Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils?. *Biogeochemistry*, 127, 157-171. <https://doi.org/10.1007/s10533-015-0180-6>
- Keskinen, R., Hillier, S., Liski, E., Nuutinen, V., Nyambura, M., & Tiljander, M. (2022). Mineral composition and its relations to readily available element concentrations in cultivated soils of Finland. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science*, 72(1), 751-760. <https://doi.org/10.1080/09064710.2022.2075790>

- Keskinen, R., Nikama, J., Kostensalo, J., Rätty, M., Rasa, K., & Soinne, H. (2024). Methodological choices in size and density fractionation of soil carbon reserves-A case study on wood fiber sludge amended soils. *Heliyon*, 10(2). <https://doi.org/10.1016/j.heliyon.2024.e24450>
- Kirschbaum, M.U.F., Giltrap, D.L., McNally, S.R., Liáng, L.L., Hedley, C.B., Moinet, G.Y.K., Blaschek, M., Beare, M.H., Theng, B.K.G., Hunt, J.E., Whitehead, D., 2020. Estimating the mineral surface area of soils by measured water adsorption. Adjusting for the confounding effect of water adsorption by soil organic carbon. *Eur. J. Soil Sci.* 71, 382-391. <https://doi.org/10.1111/ejss.12892>
- Kirsten, M., Mikutta, R., Vogel, C., Thompson, A., Mueller, C.W., Kimaro, D.N., Bergsma, H.L.T., Feger, K.H., Kalbitz, K., 2021. Iron oxides and aluminous clays selectively control soil carbon storage and stability in the humid tropics. *Sci. Rep.* 11, 1-12. <https://doi.org/10.1038/s41598-021-84777-7>
- Kjaergaard, C., de Jonge, L.W., Moldrup, P., Schjønning, P., 2004. Water-dispersible colloids: effects of measurement method, clay content, initial soil matric potential, and wetting rate. *Vadose Zo. J.* 3, 403-412. <https://doi.org/10.2136/vzj2004.0403>
- Kleber, M., Bourg, I. C., Coward, E. K., Hansel, C. M., Myneni, S. C., & Nunan, N. (2021). Dynamic interactions at the mineral-organic matter interface. *Nature Reviews Earth & Environment*, 2(6), 402-421. <https://doi.org/10.1038/s43017-021-00162-y>
- Kleber, M., Mikutta, R., Torn, M.S., Jahn, R., Kleber, M., Mikutta, R., Torn, M.S., Jahn, R., 2005. Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. *Eur. J. Soil Sci.* 56, 717-725. <https://doi.org/10.1111/j.1365-2389.2005.00706.x>
- Kleber, M., Sollins, P., & Sutton, R. (2007). A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry*, 85(1), 9-24. <https://doi.org/10.1007/s10533-007-9103-5>
- Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., & von Lützow, M. (2008). An integrative approach of organic matter stabilization in

temperate soils: Linking chemistry, physics, and biology. *Journal of Plant Nutrition and Soil Science*, 171(1), 5-13. <https://doi.org/10.1002/jpln.200700215>

Koljonen, T. (Ed.) (1992). *The geochemical atlas of Finland part 2: till*. Espoo: Geological Survey of Finland; 218 p.

Koorneef, G. J., de Goede, R. G., Pulleman, M. M., van Leeuwen, A. G., Barré, P., Baudin, F., & Comans, R. N. (2023). Quantifying organic carbon in particulate and mineral-associated fractions of calcareous soils-A method comparison. *Geoderma*, 436, 116558. <https://doi.org/10.1016/j.geoderma.2023.116558>

Koorneef, G.J., Pulleman, M.M., Comans, R.N., van Rijssel, S.Q., Barré, P., Baudin, F., de Goede, R.G., (2024). Assessing soil functioning: What is the added value of soil organic carbon quality measurements alongside total organic carbon content? *Soil Biol. Biochem.* 196. <https://doi.org/10.1016/j.soilbio.2024.109507>

Kostensalo, J., Hyväluoma, J., Jauhiainen, L., Keskinen, R., Nuutinen, V., Peltonen-Sainio, P., & Heikkinen, J. (2024). Diversification of crop rotations and soil carbon balance: impact assessment based on national-scale monitoring data. *Carbon Management*, 15(1), 2298373. <https://doi.org/10.1080/17583004.2023.2298373>

Kronberg, R., Kanerva, S., Koskinen, M., Polvinen, T., Heinonsalo, J., & Pihlatie, M. (2024). Controlled soil monolith experiment for studying the effects of waterlogging on redox processes. *Geoderma*, 452, 117110. <https://doi.org/10.1016/j.geoderma.2024.117110>

Kuligiewicz, A., Derkowski, A., 2017. Tightly bound water in smectites. *Am. Mineral.* 102, 1073-1090. <https://doi.org/10.2138/am-2017-5918>

Kura, G., Ohashi, S., Kura, S., 1974. Complex formation of cyclic phosphate anions with bivalent cations. *J. Inorg. Nucl. Chem.* 36, 1605-1609. [https://doi.org/10.1016/0022-1902\(74\)80631-7](https://doi.org/10.1016/0022-1902(74)80631-7)

Kuzyakov, Y., Friedel, J. K., & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry*, 32(11-12), 1485-1498. [https://doi.org/10.1016/S0038-0717\(00\)00084-5](https://doi.org/10.1016/S0038-0717(00)00084-5)

- Ladha, J. K., Reddy, C. K., Padre, A. T., & van Kessel, C. (2011). Role of nitrogen fertilization in sustaining organic matter in cultivated soils. *Journal of Environmental Quality*, 40(6), 1756-1766. <https://doi.org/10.2134/jeq2011.0064>
- Lal, R. (2004). Soil carbon sequestration impacts on global climate change and food security. *Science*, 304, 1623-1627. <https://doi.org/10.1126/science.1097396>
- Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Glob. Chang. Biol.* 26, 261-273. <https://doi.org/10.1111/gcb.14859>
- Le Noë, J., Manzoni, S., Abramoff, R., Bölscher, T., Bruni, E., Cardinael, R., ... & Guenet, B. (2023). Soil organic carbon models need independent time-series validation for reliable prediction. *Communications Earth & Environment*, 4(1), 158. <https://doi.org/10.1038/s43247-023-00830-5>
- Lehmann, J., & Kleber, M. (2015). The contentious nature of soil organic matter. *Nature*, 528(7580), 60-68. <https://doi.org/10.1038/nature16069>
- Lehmann, J., Abiven, S., Kleber, M., Pan, G., Singh, B. P., Sohi, S. P., & Zimmerman, A. R. (2015). Persistence of biochar in soil. In *Biochar for environmental management* (pp. 235-282). Routledge.
- Lehtinen, T., Lair, G.J., Mentler, A., Gísladóttir, G., Ragnarsdóttir, K.V., Blum, W.E.H., 2014. Soil aggregate stability in different soil orders quantified by low dispersive ultrasonic energy levels. *Soil Sci. Soc. Am. J.* 78, 713-723. <https://doi.org/10.2136/sssaj2013.02.0073>
- Leifeld, J., & Fuhrer, J. (2010). Organic farming and soil carbon sequestration: what do we really know about the benefits?. *Ambio*, 39(8), 585-599. <https://doi.org/10.1007/s13280-010-0082-8>
- Leifeld, J., & Menichetti, L. (2018). The underappreciated potential of peatlands in global climate change mitigation strategies. *Nature Communications*, 9(1), 1071. <https://doi.org/10.1038/s41467-018-03406-6>

References

- Leuthold, S., Lavalley, J.M., Haddix, M., Cotrufo, M.F., 2024. Contrasting properties of soil organic matter fractions isolated by different physical separation methodologies. *Geoderma* 445, 116870. <https://doi.org/10.1016/j.geoderma.2024.116870>
- Leuthold, S.J., Haddix, M.L., Lavalley, J.M., Cotrufo, M.F., 2023. Physical fractionation techniques, in: *Encyclopedia of Soils in the Environment*. pp. 68-80. <https://doi.org/10.1016/B978-0-12-822974-3.00067-7>
- Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature microbiology*, 2(8), 1-6. <https://doi.org/10.1038/nmicrobiol.2017.105>
- Liebmann, P., Wordell-Dietrich, P., Kalbitz, K., Mikutta, R., Kalks, F., Don, A., ... & Guggenberger, G. (2020). Relevance of aboveground litter for soil organic matter formation-a soil profile perspective. *Biogeosciences*, 17(12), 3099-3113. <https://doi.org/10.5194/bg-17-3099-2020>
- Locat, J., Lefebvre, G. & Ballivy, G. (1984). Mineralogy, chemistry, and physical properties interrelationships of some sensitive clays from eastern Canada. *Canadian Geotechnical Journal*, 21, 530-540. <https://doi.org/10.1139/t84-055>
- Lugato, E., Lavalley, J. M., Haddix, M. L., Panagos, P., & Cotrufo, M. F. (2021). Different climate sensitivity of particulate and mineral-associated soil organic matter. *Nature Geoscience*, 14(5), 295-300. <https://doi.org/10.1038/s41561-021-00744-x>
- Lundström, U. S., van Breemen, N., & Bain, D. (2000). The podzolization process. A review. *Geoderma*, 94(2-4), 91-107. [https://doi.org/10.1016/S0016-7061\(99\)00036-1](https://doi.org/10.1016/S0016-7061(99)00036-1)
- Maillard, É., & Angers, D. A. (2014). Animal manure application and soil organic carbon stocks: A meta-analysis. *Global Change Biology*, 20(2), 666-679. <https://doi.org/10.1111/gcb.12438>
- Mathieu, J. A., Hatté, C., Balesdent, J., & Parent, É. (2015). Deep soil carbon dynamics are driven more by soil type than by climate: a worldwide meta-analysis of radiocarbon profiles. *Global Change Biology*, 21(11), 4278-4292. <https://doi.org/10.1111/gcb.13012>
- Matschullat, J., Reimann, C., Birke, M., dos Santos Carvalho, D., Albanese, S., Anderson, M., ... & Demetriades, A. (2018). GEMAS: CNS concentrations and C/N ratios in

- European agricultural soil. *Science of the Total Environment*, 627, 975-984.
<https://doi.org/10.1016/j.scitotenv.2018.01.214>
- Mattila, T. J., Hagelberg, E., Söderlund, S., & Joona, J. (2022). How farmers approach soil carbon sequestration? Lessons learned from 105 carbon-farming plans. *Soil and Tillage Research*, 215, 105204. <https://doi.org/10.1016/j.still.2021.105204>
- Matus, F. J. (2021). Fine silt and clay content is the main factor defining maximal C and N accumulations in soils: a meta-analysis. *Scientific Reports*, 11(1), 6438. <https://doi.org/10.1038/s41598-021-84821-6>
- Mayer, M., Krause, H. M., Fliessbach, A., Mäder, P., & Steffens, M. (2022). Fertilizer quality and labile soil organic matter fractions are vital for organic carbon sequestration in temperate arable soils within a long-term trial in Switzerland. *Geoderma*, 426, 116080. <https://doi.org/10.1016/j.geoderma.2022.116080>
- Mendez, J. C., Hiemstra, T., & Koopmans, G. F. (2020). Assessing the reactive surface area of soils and the association of soil organic carbon with natural oxide nanoparticles using ferrihydrite as proxy. *Environmental science & technology*, 54(19), 11990-12000. <https://doi.org/10.1021/acs.est.0c02163>
- Mendez, J.C., Van Eynde, E., Hiemstra, T., Comans, R.N.J., 2022. Surface reactivity of the natural metal (hydr)oxides in weathered tropical soils. *Geoderma* 406, 115517. <https://doi.org/10.1016/j.geoderma.2021.115517>
- Menichetti, L., Ekblad, A., & Kätterer, T. (2015). Contribution of roots and amendments to soil carbon accumulation within the soil profile in a long-term field experiment in Sweden. *Agriculture, Ecosystems & Environment*, 200, 79-87. <https://doi.org/10.1016/j.agee.2014.11.003>
- Metzger, M. J., Shkaruba, A. D., Jongman, R. H. G., & Bunce, R. G. H. (2012). Descriptions of the European environmental zones and strata (No. 2281). *Alterra*.
- Mikutta, R., Kleber, M., Torn, M.S., Jahn, R., 2006. Stabilization of soil organic matter: Association with minerals or chemical recalcitrance? *Biogeochemistry* 77, 25-56. <https://doi.org/10.1007/s10533-005-0712-6>

- Miltner, A., Bombach, P., Schmidt-Brücken, B., & Kästner, M. (2012). SOM genesis: microbial biomass as a significant source. *Biogeochemistry*, 111(1), 41-55. <https://doi.org/10.1007/s10533-011-9658-z>
- Minasny, B., Malone, B. P., McBratney, A. B., Angers, D. A., Arrouays, D., Chambers, A., ... & Winowiecki, L. (2017). Soil carbon 4 per mille. *Geoderma*, 292, 59-86. <https://doi.org/10.1016/j.geoderma.2017.01.002>
- Mitchell, J. K., & Soga, K. (2005). *Fundamentals of soil behavior* (Vol. 3, p. USA). New York: John Wiley & Sons.
- Moinet, G.Y.K., et al. (2023). Carbon for soils, not soils for carbon. *Global Change Biology*, 29, 2384-2398. <https://doi.org/10.1111/gcb.16570>
- Muneer, M., Oades, J.M., 1989. The role of Ca-organic interactions in soil aggregate stability II. Field studies with ^{14}C -labeled straw, CaCO_3 and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$. *Soil Res.* 27, 401-409. <https://doi.org/10.1071/SR9890401>
- Natural Resources Institute Finland (Luke). (2018). Volume of carbon in agricultural land (Valse national soil monitoring, 1987-2018). <https://www.luke.fi/en/statistics/indicators/finlands-cap-impact-indicators/volume-of-carbon-in-agricultural-land>. Visited 4.2.2026.
- Nelson, D.W. & Sommers, L.E. (1996). Total carbon, organic carbon and organic matter. In: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T. & Sumner, M.E. (eds.). *Methods of Soil Analysis: Part 3 Chemical Methods*, 5.3. Number 5 in the Soil Science Society of America Book Series. Madison, Wisconsin, USA: Soil Science Society of America, Inc., American Society of Agronomy, Inc. p. 961-1010.
- NEN 5738, 1996. Bodem - Bepaling van de actuele kationen-uitwisselingscapaciteit en uitwisselbare basische kationen in grond.
- NEN 5776, 2014. Bodem - Bepaling van ijzer, aluminium en fosfor in een ammoniumoxalaat-oxaalzurextract voor het vaststellen van de fosfaatverzadiging.
- NEN-EN 15936, 2012. Slib, behandeld biologisch afval, bodem en afval - Bepaling van de totale organische koolstof (TOC) door droge verbranding.

- Niskanen, R. (1989). Extractable aluminium, iron and manganese in mineral soils: III Comparison of extraction methods. *Agricultural and Food Science*, 61(2), 89-97. <https://doi.org/10.23986/afsci.72357>
- Oades, J.M., 1984. Soil organic matter and structural stability: mechanisms and implications for management. *Plant Soil* 76, 319-337. https://doi.org/10.1007/978-94-009-6101-2_28
- Ogle, S. M., Alsaker, C., Baldock, J., Bernoux, M., Breidt, F. J., McConkey, B., ... & Vazquez-Amabile, G. G. (2019). Climate and soil characteristics determine where no-till management can store carbon in soils and mitigate greenhouse gas emissions. *Scientific reports*, 9(1), 11665. <https://doi.org/10.1038/s41598-019-47861-7>
- Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., et al. (2025). *vegan: Community Ecology Package*. R package version 2.7-2.
- Oorts, K., Vanlauwe, B., Recous, S., Merckx, R., 2005. Redistribution of particulate organic matter during ultrasonic dispersion of highly weathered soils. *Eur. J. Soil Sci.* 56, 77-91. <https://doi.org/10.1111/j.1351-0754.2004.00654.x>
- Paasonen-Kivekäs, M., Peltomaa, R., Vakkilainen, P., & Äijö, H. (2009). Maan vesi- ja ravinnetalous. Ojitus, kastelu ja ympäristö. Salaojajyhdistys ry. Gummerus Kirjapaino Oy, Jyväskylä.
- Palm, C., Blanco-Canqui, H., DeClerck, F., Gatere, L., Grace, P., 2014. Conservation agriculture and ecosystem services: An overview. *Agric. Ecosyst. Environ.* 187, 87-105. <https://doi.org/10.1016/j.agee.2013.10.010>
- Parfitt, R.L., Whitton, A.J.S., Theng, B.K.G., 2001. Surface reactivity of A horizons towards polar compounds estimated from water adsorption and water content. *Aust. J. Soil Res.* 39, 1105-1110. <https://doi.org/10.1071/SR00059>
- Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., & Smith, P. (2016). Climate-smart soils. *Nature*, 532(7597), 49-57. <https://doi.org/10.1038/nature17174>
- Pedersen, T. (2025). *_patchwork: The Composer of Plots_*. R package version 1.3.2.

References

- Persson, L., & Bergström, L. (1991). Drilling method for collection of undisturbed soil monoliths. *Soil Science Society of America Journal*, 55(1), 285-287. <https://doi.org/10.2136/sssaj1991.03615995005500010050x>
- Pietikäinen, J., Pettersson, M., & Bååth, E. (2005). Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS microbiology ecology*, 52(1), 49-58. <https://doi.org/10.1016/j.femsec.2004.10.002>
- Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., Cotrufo, M.F., Derrien, D., Gioacchini, P., Grand, S., Gregorich, E., Griepentrog, M., Gunina, A., Haddix, M., Kuzyakov, Y., Kühnel, A., Macdonald, L.M., Soong, J., Trigalet, S., Vermeire, M.L., Rovira, P., van Wesemael, B., Wiesmeier, M., Yeasmin, S., Yevdokimov, I., Nieder, R., 2018. Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils - A comprehensive method comparison. *Soil Biol. Biochem.* 125, 10-26. <https://doi.org/10.1016/j.soilbio.2018.06.025>
- Poeplau, C., Skovgaard, M., Vos, C., 2025. How biased is size fractionation derived mineral-associated organic carbon? *Geoderma* 463, 117584. <https://doi.org/10.1016/j.geoderma.2025.117584>
- Powers, J.S., Corre, M.D., Twine, T.E. & Veldkamp, E. (2011). Geographic bias of field observations of soil carbon stocks with tropical land-use changes precludes spatial extrapolation. *Proceedings of the National Academy of Sciences*, 108(15), 6318-6322. <https://doi.org/10.1073/pnas.1016774108>
- Powlson, D. S., & Neal, A. L. (2021). Influence of organic matter on soil properties: by how much can organic carbon be increased in arable soils and can changes be measured?. In *Proceedings of the International Fertiliser Society* (Vol. 862, pp. 1-32). International Fertiliser Society (IFS).
- Powlson, D. S., Stirling, C. M., Jat, M. L., Gerard, B. G., Palm, C. A., Sanchez, P. A., & Cassman, K. G. (2014). Limited potential of no-till agriculture for climate change mitigation. *Nature Climate Change*, 4(8), 678-683. <https://doi.org/10.1038/nclimate2292>
- Powlson, D. S., Whitmore, A. P., & Goulding, K. W. (2011). Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false.

- European journal of soil science, 62(1), 42-55. <https://doi.org/10.1111/j.1365-2389.2010.01342.x>
- Prout, J. M., Shepherd, K. D., McGrath, S. P., Kirk, G. J., & Haeefe, S. M. (2021). What is a good level of soil organic matter? An index based on organic carbon to clay ratio. *European Journal of Soil Science*, 72(6), 2493-2503. <https://doi.org/10.1111/ejss.13012>
- Pulleman, M.M., Six, J., Van Breemen, N., Jongmans, A.G., 2005. Soil organic matter distribution and microaggregate characteristics as affected by agricultural management and earthworm activity. *Eur. J. Soil Sci.* 56, 453-467. <https://doi.org/10.1111/j.1365-2389.2004.00696.x>
- Purugganan, M. D., & Fuller, D. Q. (2009). The nature of selection during plant domestication. *Nature*, 457(7231), 843-848. <https://doi.org/10.1038/nature07895>
- R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
- R Core Team (2023). *_R: A Language and Environment for Statistical Computing_*. R Foundation for Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>>
- R core team, 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org>.
- R Studio Team, 2023. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL <http://www.rstudio.com/>
- Rasmussen, C., Heckman, K., Wieder, W. R., Keiluweit, M., Lawrence, C. R., Berhe, A. A., ... & Wagai, R. (2018). Beyond clay: towards an improved set of variables for predicting soil organic matter content. *Biogeochemistry*, 137, 297-306. <https://doi.org/10.1007/s10533-018-0424-3>
- Rasse, D. P., Rumpel, C., & Dignac, M. F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and soil*, 269(1), 341-356. <https://doi.org/10.1007/s11104-004-0907-y>
- Regelink, I. C., Weng, L., Koopmans, G. F., & Van Riemsdijk, W. H. (2013). Asymmetric flow field-flow fractionation as a new approach to analyse iron-(hydr) oxide nanoparticles

References

in soil extracts. *Geoderma*, 202, 134-141.
<https://doi.org/10.1016/j.geoderma.2013.03.015>

Regina, K., Heikkinen, J., & Maljanen, M. (2018). Greenhouse gas fluxes of agricultural soils in Finland. In *Greenhouse Gas Emissions: Challenges, Technologies and Solutions* (pp. 7-22). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-3272-2_2

Rengasamy, P., Tavakkoli, E., McDonald, G.K., 2016. Exchangeable cations and clay dispersion: net dispersive charge, a new concept for dispersive soil. *Eur. J. Soil Sci.* 67, 659-665. <https://doi.org/10.1111/ejss.12369>

Rowley, M.C., Grand, S., Spangenberg, J.E., Verrecchia, E.P., 2021. Evidence linking calcium to increased organo-mineral association in soils. *Biogeochemistry* 153, 223-241. <https://doi.org/10.1007/s10533-021-00779-7>

Rowley, M.C., Grand, S., Verrecchia, É.P., 2018. Calcium-mediated stabilisation of soil organic carbon. *Biogeochemistry* 137, 27-49. <https://doi.org/10.1007/s10533-017-0410-1>

Rumpel, C., & Kögel-Knabner, I. (2011). Deep soil organic matter - a key but poorly understood component of terrestrial C cycle. *Plant and Soil*, 338(1), 143-158. <https://doi.org/10.1007/s11104-010-0391-5>

Saastamoinen, M., Manni, K. & Hellstedt, M. 2022. Kuivikemateriaalien ominaisuuksien vertailu. Julkaisussa: Manni, K. (toim.). Turvetta korvaavat uusiutuvat kuivikemateriaalit. *Luonnonvara- ja biotalouden tutkimus* 9/2022. Luonnonvarakeskus. Helsinki. S. 11-35.

Saifuddin, M., Abramoff, R. Z., Foster, E. J., & McClelland, S. C. (2024). Soil carbon offset markets are not a just climate solution. *Frontiers in Ecology and the Environment*, 22(7). <https://doi.org/10.1002/fee.2781>

Salome, C., Nunan, N., Pouteau, V., Lerch, T. Z., & Chenu, C. (2010). Carbon dynamics in topsoil and in subsoil may be controlled by different regulatory mechanisms. *Global Change Biology*, 16(1), 416-426. <https://doi.org/10.1111/j.1365-2486.2009.01884.x>

- Salonen, A.R., de Goede, R., Creamer, R., Heinonsalo, J., Soinne, H., 2024. Soil organic carbon fractions and storage potential in Finnish arable soils. *Eur. J. Soil Sci.* 75, 1-17. <https://doi.org/10.1111/ejss.13527>
- Salonen, A.R., Soinne, H., Creamer, R., Lemola, R., Ruoho, N., Uhlgren, O., de Goede, R., Heinonsalo, J., 2023. Assessing the effect of arable management practices on carbon storage and fractions after 24 years in boreal conditions of Finland. *Geoderma Reg.* 34. <https://doi.org/10.1016/j.geodrs.2023.e00678>
- Samson, M. É., Chantigny, M. H., Vanasse, A., Menasseri-Aubry, S., & Angers, D. A. (2020). Coarse mineral-associated organic matter is a pivotal fraction for SOM formation and is sensitive to the quality of organic inputs. *Soil Biology and Biochemistry*, 149, 107935. <https://doi.org/10.1016/j.soilbio.2020.107935>
- Sanderman, J., Creamer, C., Baisden, W. T., Farrell, M., & Fallon, S. (2017). Greater soil carbon stocks and faster turnover rates with increasing agricultural productivity. *Soil*, 3(1), 1-16. <https://doi.org/10.5194/soil-3-1-2017>
- Sanderman, J., Fillery, I.R.P., Jongepier, R., Massalsky, A., Roper, M.M., MacDonald, L.M., Maddern, T., Murphy, D. V, Baldock, J.A., 2013. Carbon sequestration under subtropical perennial pastures II: Carbon dynamics. *Soil Res.* 51, 771-780. <https://doi.org/10.1071/SR12351>
- Sanderman, J., Hengl, T., & Fiske, G. J. (2017). Soil carbon debt of 12,000 years of human land use. *Proceedings of the National Academy of Sciences*, 114(36), 9575-9580. <https://doi.org/10.1073/pnas.1706103114>
- Schiedung, M., Barre, P., & Peoplau, C. (2025). Separating fast from slow cycling soil organic carbon-A multi-method comparison on land use change sites. *Geoderma*, 453, 117154. <https://doi.org/10.1016/j.geoderma.2024.117154>
- Schjønning, P., de Jonge, L. W., Munkholm, L. J., Moldrup, P., Christensen, B. T., & Olesen, J. E. (2012). Clay dispersibility and soil friability-testing the soil clay-to-carbon saturation concept. *Vadose Zone Journal*, 11(1). <https://doi.org/10.2136/vzj2011.0067>.

References

- Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., ... & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, 478(7367), 49-56. <https://doi.org/10.1038/nature10386>
- Schrumpf, M., Schulze, E. D., Kaiser, K., & Schumacher, J. (2011). How accurately can soil organic carbon stocks and stock changes be quantified by soil inventories?. *Biogeosciences*, 8(5), 1193-1212. <https://doi.org/10.5194/bg-8-1193-2011>
- Schulte, R. P., Creamer, R. E., Donnellan, T., Farrelly, N., Fealy, R., O'Donoghue, C., & O'hallachain, D. (2014). Functional land management: A framework for managing soil-based ecosystem services for the sustainable intensification of agriculture. *Environmental Science & Policy*, 38, 45-58. <https://doi.org/10.1016/j.envsci.2013.10.002>
- Schweizer, S. A., Mueller, C. W., Höschen, C., Ivanov, P., & Kögel-Knabner, I. (2021). The role of clay content and mineral surface area for soil organic carbon storage in an arable toposequence. *Biogeochemistry*, 156(3), 401-420. <https://doi.org/10.1007/s10533-021-00850-3>
- Schwertmann, U., 1964. Differenzierung der eisenoxide des bodens durch extraktion mit ammoniumoxalat-lösung. *Zeitschrift für Pflanzenernährung, Düngung, Bodenkd.* 105, 194-202. <https://doi.org/10.1002/jpln.3591050303>
- Sebag, D., Verrecchia, E. P., Cécillon, L., Adatte, T., Albrecht, R., Aubert, M., ... & Trombino, L. (2016). Dynamics of soil organic matter based on new Rock-Eval indices. *Geoderma*, 284, 185-203. <https://doi.org/10.1016/j.geoderma.2016.08.025>
- Shipitalo, M. J., Nuutinen, V., & Butt, K. R. (2004). Interaction of earthworm burrows and cracks in a clayey, subsurface-drained, soil. *Applied Soil Ecology*, 26(3), 209-217. <https://doi.org/10.1016/j.apsoil.2004.01.004>
- Simpson, M. J., & Simpson, A. J. (2012). The chemical ecology of soil organic matter molecular constituents. *Journal of chemical ecology*, 38, 768-784. <https://doi.org/10.1007/s10886-012-0122-x>
- Six, J., Conant, R. T., Paul, E. A., & Paustian, K. (2002). Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and soil*, 241, 155-176. <https://doi.org/10.1023/A:1016125726789>

- Six, J., Doetterl, S., Laub, M., Müller, C. R., & Van de Broek, M. (2024). The six rights of how and when to test for soil C saturation. *Soil*, 10(1), 275-279. <https://doi.org/10.5194/soil-10-275-2024>
- Six, J., Doetterl, S., Laub, M., Müller, C. R., and Van de Broek, M.: The six rights of how and when to test for soil C saturation, *EGUsphere* [preprint], <https://doi.org/10.5194/egusphere-2023-2221>, 2023.
- Six, J., Elliott, E. T., & Paustian, K. (2000). Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry*, 32(14), 2099-2103. [https://doi.org/10.1016/S0038-0717\(00\)00179-6](https://doi.org/10.1016/S0038-0717(00)00179-6)
- Six, J., Feller, C., Denef, K., Ogle, S., de Moraes Sa, J., Albrecht, A., 2002. Soil organic matter, biota and aggregation in temperate and tropical soils - Effects of no-tillage. *Agronomie* 22, 755-775. <https://doi.org/10.1051/agro:2002043>
- Skadell, L. E., Schneider, F., Gocke, M. I., Guigue, J., Amelung, W., Bauke, S. L., ... & Don, A. (2023). Twenty percent of agricultural management effects on organic carbon stocks occur in subsoils-Results of ten long-term experiments. *Agriculture, Ecosystems & Environment*, 356, 108619. <https://doi.org/10.1016/j.agee.2023.108619>
- Slessarev, E. W., Lin, Y., Bingham, N. L., Johnson, J. E., Dai, Y., Schimel, J. P., & Chadwick, O. A. (2016). Water balance creates a threshold in soil pH at the global scale. *Nature*, 540(7634), 567-569. <https://doi.org/10.1038/nature20139>
- Slowikowski K (2024). `_ggrepel`: Automatically Position Non-Overlapping Text Labels with 'ggplot2'. R package version 0.9.6.
- Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., ... & Smith, J. (2008). Greenhouse gas mitigation in agriculture. *Philosophical transactions of the royal Society B: Biological Sciences*, 363(1492), 789-813. <https://doi.org/10.1098/rstb.2007.2184>
- Soinne, H., Hyyrynen, M., Jokubė, M., Keskinen, R., Hyvāluoma, J., Pihlainen, S., ... & Heikkinen, J. (2024). High organic carbon content constricts the potential for stable organic carbon accrual in mineral agricultural soils in Finland. *Journal of Environmental Management*, 352, 119945. <https://doi.org/10.1016/j.jenvman.2023.119945>

References

- Soinne, H., Keskinen, R., Rätty, M., Kanerva, S., Turtola, E., Kaseva, J., ... & Salo, T. (2021). Soil organic carbon and clay content as deciding factors for net nitrogen mineralization and cereal yields in boreal mineral soils. *European Journal of Soil Science*, 72(4), 1497-1512. <https://doi.org/10.1111/ejss.13003>
- Sokol, N. W., Whalen, E. D., Jilling, A., Kallenbach, C., Pett-Ridge, J., & Georgiou, K. (2022). Global distribution, formation and fate of mineral-associated soil organic matter under a changing climate: A trait-based perspective. *Functional Ecology*. <https://doi.org/10.1111/1365-2435.14040>
- Sollins, P., Homann, P., & Caldwell, B. A. (1996). Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma*, 74(1-2), 65-105. [https://doi.org/10.1016/S0016-7061\(96\)00036-5](https://doi.org/10.1016/S0016-7061(96)00036-5)
- Spielvogel, S., Prietzel, J., Kögel-Knabner, I., 2008. Soil organic matter stabilization in acidic forest soils is preferential and soil type-specific. *Eur. J. Soil Sci.* 59, 674-692. <https://doi.org/10.1111/j.1365-2389.2008.01030.x>
- Stewart, C. E., Paustian, K., Conant, R. T., Plante, A. F., & Six, J. (2007). Soil carbon saturation: concept, evidence and evaluation. *Biogeochemistry*, 86(1), 19-31. <https://doi.org/10.1007/s10533-007-9140-0>
- Strawn, D. G., Bohn, H. L., & O'Connor, G. A. (2019). *Soil chemistry*. John Wiley & Sons.
- Svensson, D.N., Messing, I., Barron, J., 2022. An investigation in laser diffraction soil particle size distribution analysis to obtain compatible results with sieve and pipette method. *Soil Tillage Res.* 223, 105450. <https://doi.org/10.1016/j.still.2022.105450>
- Thorup-Kristensen, K., Halberg, N., Nicolaisen, M., Olesen, J. E., Crews, T. E., Hinsinger, P., ... & Dresbøll, D. B. (2020). Digging deeper for agricultural resources, the value of deep rooting. *Trends in Plant Science*, 25(4), 406-417. <https://doi.org/10.1016/j.tplants.2019.12.007>
- Tiefenbacher, A., Sandén, T., Haslmayr, H. P., Miloczki, J., Wenzel, W., & Spiegel, H. (2021). Optimizing carbon sequestration in croplands: a synthesis. *Agronomy*, 11(5), 882. <https://doi.org/10.3390/agronomy11050882>

- Tiessen, H. J. W. B., & Stewart, J. W. B. (1983). Particle-size fractions and their use in studies of soil organic matter: II. Cultivation effects on organic matter composition in size fractions. *Soil Science Society of America Journal*, 47(3), 509-514. <https://doi.org/10.2136/sssaj1983.03615995004700030023x>
- Torres-Sallan, G., Schulte, R. P., Lanigan, G. J., Byrne, K. A., Reidy, B., Simó, I., ... & Creamer, R. E. (2017). Clay illuviation provides a long-term sink for C sequestration in subsoils. *Scientific Reports*, 7(1), 1-7. <https://doi.org/10.1038/srep45635>
- Totsche, K.U., Amelung, W., Gerzabek, M.H., Guggenberger, G., Klumpp, E., Knief, C., Lehdorff, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., Kögel-Knabner, I., 2018. Microaggregates in soils. *J. Plant Nutr. Soil Sci.* 181, 104-136. <https://doi.org/10.1002/jpln.201600451>
- Turunen, J. & Valpola, S. (2020). The influence of anthropogenic land use on Finnish peatland area and carbon stores 1950-2015. *Mires and Peat*, 26, Article 26. <https://doi.org/10.19189/MAP.2019.GDC.STA.1870>
- Tyner, E.H., 1938. The use of sodium metaphosphate for dispersion of soils for mechanical analysis. <https://doi.org/10.2136/sssaj1940.036159950004000C0020x>
- Urbanski, L., Kalbitz, K., Rethemeyer, J., Schad, P., & Kögel-Knabner, I. (2023). Unexpected high alkyl carbon contents in organic matter-rich sandy agricultural soils of Northwest Central Europe. *Geoderma*, 439, 116695. <https://doi.org/10.1016/j.geoderma.2023.116695>
- Uusitalo, R., Ylivainio, K., Hyväluoma, J., Rasa, K., Kaseva, J., Nylund, P., ... & Turtola, E. (2012). The effects of gypsum on the transfer of phosphorus and other nutrients through clay soil monoliths. *Agricultural and Food Science*, 21(3), 260-278. <https://doi.org/10.23986/afsci.4855>
- Van de Broek, M., Henriksen, C.B., Ghaley, B.B., Lugato, E., Kuzmanovski, V., Trajanov, A., Debeljak, M., Sandén, T., Spiegel, H., Decock, C., Creamer, R., Six, J., 2019. Assessing the climate regulation potential of agricultural soils using a decision support tool adapted to stakeholders' needs and possibilities. *Front. Environ. Sci.* 7, 1-17. <https://doi.org/10.3389/fenvs.2019.00131>

References

- Van Erp, P., 2002. The potential of multi-nutrient soil extraction with CaCl₂ in nutrient management. Wageningen University & Research.
- Van Hoef M., (2025). RockEvalR: Read and Manage Rock-Eval data files. R package version 0.0.1.0000, commit.65c3e689f155dd7b83a3891acc4a6773fcb06171, <<https://github.com/maartenvanhoef/RockEvalR>>.
- Van Reeuwijk, L.P., 2002. Procedures for soil analysis, 6th ed. International Soil Reference and Information Centre, Wageningen, The Netherlands.
- van Rijssel, S. Q., Koorneef, G. J., Veen, G. F., Pulleman, M. M., de Goede, R. G., Comans, R. N., ... & Mason-Jones, K. (2025). Conventional and organic farms with more intensive management have lower soil functionality. *Science*, 388(6745), 410-415. <https://doi.org/10.1126/science.adr0211>
- Vidal, A., Klöffel, T., Guigue, J., Angst, G., Steffens, M., Hoeschen, C., & Mueller, C. W. (2021). Visualizing the transfer of organic matter from decaying plant residues to soil mineral surfaces controlled by microorganisms. *Soil Biology and Biochemistry*, 160, 108347. <https://doi.org/10.1016/j.soilbio.2021.108347>
- Virto, I., Gartzia-Bengoetxea, N., Fernández-Ugalde, O., 2011. Role of organic matter and carbonates in soil aggregation estimated using laser diffractometry. *Pedosphere* 21, 566-572. [https://doi.org/10.1016/S1002-0160\(11\)60158-6](https://doi.org/10.1016/S1002-0160(11)60158-6)
- Vogel, C., Mueller, C. W., Höschen, C., Buegger, F., Heister, K., Schulz, S., ... & Kögel-Knabner, I. (2014). Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. *Nature Communications*, 5(1), 2947. <https://doi.org/10.1038/ncomms3947>
- Von Haden, A. C., Yang, W. H., & DeLucia, E. H. (2020). Soils' dirty little secret: Depth-based comparisons can be inadequate for quantifying changes in soil organic carbon and other mineral soil properties. *Global change biology*, 26(7), 3759-3770. <https://doi.org/10.1111/gcb.15124>
- Von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., & Marschner, B. (2007). SOM fractionation methods: relevance to

- functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry*, 39(9), 2183-2207. <https://doi.org/10.1016/j.soilbio.2007.03.007>
- Wang, S., Uhlgren, O., Salonen, A. R., Kanerva, S., Simojoki, A., Mattila, T., & Heinonsalo, J. (2026). Stocks and stoichiometry of carbon, nitrogen, and phosphorus in agricultural mineral soil profiles with views on the carbon sequestration. *Geoderma Regional*, e01058. <https://doi.org/10.1016/j.geodrs.2026.e01058>
- Wasner, D., Abramoff, R., Griepentrog, M., Venegas, E. Z., Boeckx, P., & Doetterl, S. (2024). The role of climate, mineralogy and stable aggregates for soil organic carbon dynamics along a geoclimatic gradient. *Global Biogeochemical Cycles*, 38(7), e2023GB007934. <https://doi.org/10.1029/2023GB007934>
- Webster, R., McBratney, A.B., 1989. On the Akaike Information Criterion for choosing models for variograms of soil properties. *J. Soil Sci.* 40, 493-496. <https://doi.org/10.1111/j.1365-2389.1989.tb01291.x>
- Wendt, J. W., & Hauser, S. (2013). An equivalent soil mass procedure for monitoring soil organic carbon in multiple soil layers. *European Journal of Soil Science*, 64(1), 58-65. <https://doi.org/10.1111/ejss.12002>
- Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemond G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019). "Welcome to the tidyverse." *Journal of Open Source Software*, 4(43), 1686. <https://doi.org/10.21105/joss.01686>
- Wickham, H., Chang, W., & Wickham, M. H. (2016). Package 'ggplot2'. Create elegant data visualisations using the grammar of graphics. Version, 2(1), 1-189.
- Wiesmeier, M., Urbanski, L., Hobbey, E., Lang, B., von Lützw, M., Marin-Spiotta, E., ... & Kögel-Knabner, I. (2019). Soil organic carbon storage as a key function of soils-A review of drivers and indicators at various scales. *Geoderma*, 333, 149-162. <https://doi.org/10.1016/j.geoderma.2018.07.026>
- Wilke, C (2024). *_cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'_*. R package version 1.1.3.

References

Wintermyer, A.M., Kinter, E.B., 1955. Dispersing agents for particle-size analysis of soils.

Wiseman, C. L. S., & Püttmann, W. (2006). Interactions between mineral phases in the preservation of soil organic matter. *Geoderma*, 134(1-2), 109-118. <https://doi.org/10.1016/j.geoderma.2005.09.001>

Witzgall, K., Vidal, A., Schubert, D. I., Höschen, C., Schweizer, S. A., Buegger, F., ... & Mueller, C. W. (2021). Particulate organic matter as a functional soil component for persistent soil organic carbon. *Nature communications*, 12(1), 4115. <https://doi.org/10.1038/s41467-021-24192-8>

Xu, S., Chen, M., Feng, T., Zhan, L., Zhou, L., & Yu, G. (2021). Use ggbreak to effectively utilize plotting space to deal with large datasets and outliers. *Frontiers in Genetics*, 2122. <https://doi.org/10.3389/fgene.2021.774846>

Xu, X., Sharma, P., Shu, S., Lin, T. S., Ciais, P., Tubiello, F. N., ... & Jain, A. K. (2021). Global greenhouse gas emissions from animal-based foods are twice those of plant-based foods. *Nature Food*, 2(9), 724-732. <https://doi.org/10.1038/s43016-021-00358-x>

Yli-Halla, M. and Mokma, D.L., (1999). Classification of soils of Finland according to Soil Taxonomy. *Soil Survey Horizons*, 40(2), pp.59-69. <https://doi.org/10.2136/sh1999.2.0059>

Yli-Halla, M., & Mokma, D. L. (2002). Problems encountered when classifying the soils of Finland. *Geoderma*, 107(3-4), 267-277. [https://doi.org/10.1016/S0016-7061\(01\)00152-9](https://doi.org/10.1016/S0016-7061(01)00152-9)

Yli-Halla, M., Mokma, D. L., & Alakukku, L. (2009). Evidence for the formation of Luvisols/Alfisols as a response to coupled pedogenic and anthropogenic influences in a clay soil in Finland. *Agricultural and food science*, 18(3-4), 388-401. <https://doi.org/10.23986/afsci.5965>

Yli-Halla, M., Mokma, D. L., Peltovuori, T., Sippola, J. (2000). Agricultural soil profiles in Finland and their classification. Publications of Agricultural Research Centre of Finland. Serie A 78. Agricultural Research Centre of Finland, Jokioinen.

- Ylivainio, K., Jermakka, J., Wikberg, H. and Turtola, E., 2019. Lämpökemiallisen käsittelyn vaikutus jätevesilietefosforin lannoitusarvoon: Jätevesien fosfori kiertoon lannoitteeksi (Precover) -hankkeen loppuraportti (in Finnish). Luonnonvara- ja biotalouden tutkimus 3/2019. 1Natural Resources Institute (Luke). Helsinki. 67 s.
- Yost, J. L., & Hartemink, A. E. (2020). How deep is the soil studied-an analysis of four soil science journals. *Plant and soil*, 452, 5-18. <https://doi.org/10.1007/s11104-020-04550-z>
- Yu, W., Huang, W., Weintraub-Leff, S. R., & Hall, S. J. (2022). Where and why do particulate organic matter (POM) and mineral-associated organic matter (MAOM) differ among diverse soils?. *Soil Biology and Biochemistry*, 172, 108756. <https://doi.org/10.1016/j.soilbio.2022.108756>
- Zhang, X., Liu, Z., Han, Y., 2024. Progress towards the identification and improvement of dispersive soils: A review. *Eur. J. Soil Sci.* 75, 1-27. <https://doi.org/10.1111/ejss.70002>
- Zimmermann, I. and Horn, R., 2020. Impact of sample pretreatment on the results of texture analysis in different soils. *Geoderma*, 371, p.114379. <https://doi.org/10.1016/j.geoderma.2020.114379>
- Zomer, R. J., Bossio, D. A., Sommer, R., & Verchot, L. V. (2017). Global sequestration potential of increased organic carbon in cropland soils. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-15794-8>
- Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems *Methods Ecol. Evol.* 1, 3-14. <https://doi.org/10.1111/j.2041-210x.2009.00001.x>

SUMMARY

Soil organic carbon (SOC) sustains many of the processes on which agriculture depends, including nutrient cycling, soil structure formation, and water regulation. It also represents one of the largest actively cycling carbon pools on Earth. Understanding how SOC is stored in agricultural soil and what determines its persistence is therefore important both for maintaining crop productivity and for assessing whether soils can contribute to climate change mitigation.

A widely adopted framework divides soil organic matter (SOM) into two pools with contrasting properties. Particulate organic matter (POM) consists mainly of relatively fresh plant residues with a residence time from years to decades. Mineral-associated organic matter (MAOM) comprises organic compounds associated with soil mineral surfaces and is assumed to persist for decades to centuries. Despite growing interest in this framework, SOC stocks and their distribution into POM and MAOM remain poorly characterised in northern agricultural soils, where short growing seasons and cold winters create conditions for SOC cycling that differ fundamentally from the systems where most research has been conducted. Moreover, most knowledge of what controls MAOM storage, including the concept of soil carbon saturation and fractionation protocols, was derived in older, more weathered soils. Finnish agricultural soils, one of the main focuses of this thesis, are relatively young, having been formed around 10 000 years ago from material deposited by glaciers. Where chemical weathering in older soils has transformed primary minerals into more reactive secondary minerals with larger surface areas, Finnish soils have not had sufficient time to undergo this transformation. Instead, the glacial action physically ground primary minerals into clay-sized minerals, with a substantial proportion of the clay fraction remaining composed of these primary minerals with a limited reactive surface area. This raises the question of whether the concepts and methods developed elsewhere are also applicable to these soils.

In this thesis, I examined SOC in Finnish agricultural soils with three main objectives: (1) to characterise carbon stocks and their distribution into POM and MAOM across soil depths and management systems, (2) to assess which soil properties control MAOM storage and whether SOC saturation concepts apply, and (3) to evaluate whether the

Summary

fractionation methods commonly used to separate POM and MAOM produce fractions that are consistent with the conceptual definitions of these pools. The first two objectives were addressed using Finnish soils (Chapters 2 and 3), while the methodological evaluation (Chapters 4 and 5) drew on a wider set of soils to test assumptions that are relevant across soil types.

To characterise SOC stocks across the soil profile, I studied soils to 70 cm depth in a 24-year field experiment in South-West Finland (Chapter 2). The experiment compared organic and conventional cropping systems with similar crop rotations but differing fertilisation, alongside an unmanaged meadow as reference. MAOM constituted the largest carbon pool throughout, accounting for 83% (topsoil) to 97% (subsoil) of total SOC. Aluminium and iron oxides were positively associated with MAOM across the soil profile, whereas clay content correlated with MAOM only in the topsoil (0–20 cm). While the topsoils of the cropping systems remained below their predicted MAOM saturation capacity, the meadow topsoil exceeded it, suggesting that texture-based models underestimate the carbon storage potential of these soils. In the 10–30 cm layer, the organic system stored significantly more SOC than the conventional system, possibly due to differences in organic matter input. Subsoil SOC stocks below 40 cm were comparable across all three systems despite very different management histories and, in the case of the meadow, higher root biomass at all depths.

Broadening the scope to 93 farms across southern Finland (Chapter 3), I found that MAOM accounted for on average 86% of total SOC in topsoils (0–20 cm), a proportion that remained stable across clay contents from 2% to 68%. Clay content explained only 9% of the variation in MAOM, whereas aluminium and iron oxides were better predictors (21–22% each) but still left most variation unexplained. SOC saturation was assessed with Hassink's equation, clay to SOC ratios, and boundary line analyses, and yielded contradictory results: some soils appeared oversaturated by one metric and undersaturated by another.

Chapters 4 and 5 address whether the two widely used approaches to separate POM and MAOM produce fractions consistent with their conceptual definitions. I compared size-based fractionation (53 μm cut-off) with density-based (1.6 g cm^{-3} cut-off) fractionation using Rock-Eval thermal analysis. Density fractionation isolated POM and MAOM with

clearly different thermal signatures, consistent with the conceptual framework. Size-fractionated POM, by contrast, had properties resembling MAOM rather than plant material, suggesting it contained substantial mineral-associated carbon (Chapter 4). Chapter 5 examined a likely contributing factor: incomplete dispersion of soil aggregates during size fractionation. Across 38 soils, chemical dispersion frequently failed to fully break down aggregates, leaving MAOM in the POM fraction. On average, this led to a 33% overestimation of SOC in POM and an 8% underestimation of SOC in MAOM, with the degree of under-dispersion increasing with SOC content and aluminium and iron oxide concentrations.

Dividing SOC into POM and MAOM may help to better understand SOC dynamics, but this requires fractionation methods that produce distinct pools in line with the conceptual framework. Based on this thesis, density fractionation seems a better tool than size fractionation for better understanding SOC cycling in future studies, despite operational challenges. This thesis furthermore illustrates that models and methods developed in older, more weathered soils do not necessarily transfer to soils with different mineralogy and weathering history. SOC saturation concepts based on soil texture did not perform consistently in the Finnish soils studied here, and local mineralogy may be more relevant than texture for understanding what controls MAOM storage. This highlights that conceptual frameworks, models and methods require validation across soils with contrasting properties before they can be applied as universally valid.

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About the Author

Anna-Reetta Salonen grew up in Espoo, Finland, by the sea, close to forests and bogs. She trained as a biomedical research technician and spent ten years working in biomedical laboratories, where she came to value careful, methodical work as a basis of impactful science. A wish to contribute to sustainable food production with more room left for nature drew her toward environmental sciences, and she completed her MSc in Environmental Soil Science at the University of Helsinki, eager to keep working on questions relating to sustainability.



Anna-Reetta began her PhD in the Soil Biology Group at Wageningen University & Research, focusing on soil organic carbon in Nordic agricultural soils and the methods used to measure it. Her thesis took a closer look at the laboratory methods and frameworks used to study soil carbon, and how methodological choices can influence the results and their interpretation. Along the way, she became increasingly interested in how soil knowledge is produced and translated into agricultural practice.

List of co-authors

The affiliations of the co-authors of the various chapters of this thesis at the time of their contribution are:

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PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 30 ECTS (= 22 weeks of activities)



Review/project proposal

- Relationship between soil organic matter fractions and soil functions in Finnish agricultural soils | 6 ECTS

In-depth / Topical / On-site Postgraduate Courses

- Soil Ecology | PE&RC | 2022 | 1.5 ECTS
- Wageningen Soil Conference | 2023 | 2.2 ECTS

Methodological / Statistical Postgraduate Courses

- Tidy data transformation and visualization with R | PE&RC | 2020 | 1.5 ECTS
- Introduction to R | PE&RC | 2020 | 0.9 ECTS
- Basic statistics | PE&RC | 2020 | 1.5 ECTS
- Generalized linear models | PE&RC | 2020 | 0.6 ECTS
- Soil Biology Lab Skills Course | WGS | 2021 | 1.5 ECTS
- Design of Experiments | WIAS & PE&RC | 2022 | 0.8 ECTS

Invited review of journal manuscripts

- Frontiers Environmental Science | Co-Designing Urban Carbon Sink Parks: Case Carbon Lane in Helsinki | 2021 | 1 ECTS
- Review of a manuscript for European Journal of Soil Science | 2022 | 1 ECTS
- Review of a manuscript for Geoderma | 2025 | 1 ECTS

Competence, skills and career-oriented activities

- Presentations for the farms involved in the Carbon Action-project | University of Helsinki | 2020, 2021 | 0.5 ECTS
- Deep Soil – seminar to farmers | Baltic Sea Action Group | 2021 | 0.5 ECTS
- Writing research proposals for Finnish Foundations (for funding) | 2020, 2025 | 4 ECTS
- Scientific Writing- course | PE&RC | 2021 | 2 ECTS
- Academic Pitching – course | University of Helsinki | 2021 | 0.5 ECTS

Scientific Integrity/Ethics in science activities

- Scientific Integrity | WGS | 2024 | 0.6 ECTS

PE&RC Retreat, PE&RC Day, and other PE&RC events

- PE&RC Day | 2021 | 0.3 ECTS
- PE&RC First Year Weekend | 2021 | 0.9 ECTS

National/local scientific meetings, seminars, and discussion groups

- Soil at risk, Helsinki | 2021 | 0.5 ECTS
- Egu Conference | 2021 | 1 ECTS

- Eurosoil | 2021 | 1 ECTS
- Talk a in webinar organised by Finnish Organic Institute | 2022 | 1 ECTS
- Discussion group for PhD's researching carbon | 2023 | 2 ECTS

International symposia, workshops and conferences

- World Congress of Soil Science | Glasgow, UK | 2022 | 2.2 ECTS
- Soil Organic Matter- 9th International Symposium | Ben Guerir, Morocco | 2024 | 2.2 ECTS

Societally relevant exposure

- Youtube video on my MSc thesis project | 2020 | 0.5 ECTS
- Webinar on deep soil SOM for farmers | 2021 | 1 ECTS
- Presentations to Finnish farmers on the carbon farming project | 2021, 2022 | 0.5 ECTS

Lecturing/Supervision of practicals/tutorials

- Soil science demos in Helsinki, Finland | 2021 | 1 day | 0.3 ECTS
- Soil structure-course in Helsinki, Finland | 2021 | 5 days | 1.5 ECTS
- Functional Agricultural Resource Management | WUR | 2023 | 4 days | 1.2 ECTS
- Plant-Soil Relations, WUR | 2025 | 3 days | 0.9 ECTS

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