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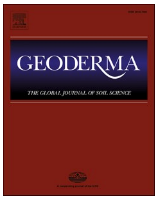

Glued forever: incomplete dispersion hampers POM-MAOM fractionation in soils with high SOC and metal (hydr)oxide content

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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 μm) from MAOC (<50 μm). 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 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 μm) present in the coarse fraction (>50 μm) 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 g MAOC kg^{-1} soil (range: 0–42 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 °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.

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

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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 μm) that theoretically primarily contains POC and a fine fraction (often < 50 μm) 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 (Lavallee 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 Ca^{2+}, 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 h, 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 μm) 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.

2. Materials and methods

2.1. Definitions

In this manuscript, the terms ‘fine fraction’ and ‘MAOC’ (mineral associated organic carbon) are used to describe the <50 μm 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 μm 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 Lavallee et al. (2020).

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 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.

2.3. Soil analyses

All soil analyses were performed on homogenised soils, sieved over 2 mm and dried at 40 °C for 48 h. Soil pH was measured in 0.01 M $CaCl_2$ after shaking for 2 h 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 h. The presence of inorganic carbon (IC) was assessed by visual inspection of CO_2 development after adding droplets of 12 M 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 h 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 h 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 $BaCl_2$ 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 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.

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 h. 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 h. 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:

$$\text{SOC recovery (\%)} = \frac{\text{OCfine*DWfine} + \text{OCcoarse*DWcoarse}}{(\text{DWfine} + \text{DWcoarse})\text{*SOC}}\text{*100} \tag{1}$$

in which SOC, OCfine and OCcoarse are the organic carbon contents (%) in the bulk soil, the fine fraction and the coarse fraction, respectively, and DWfine and DWcoarse the dry weights (g) of the fine and the coarse fraction, respectively. DWfine and OCfine 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.

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 12 M 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) H_2O_2 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 H_2O_2 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 μS m^{-1}. Finally, the solutions were decanted and samples were stored in demi water until further analysis (Supplementary Information 1).

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, Eqs. (2)–(4); Fig. 1). The first measure of underdispersion (Underdispersion 1; Und1) represents the percentage of fine particles in the coarse fraction:

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

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:

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

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:

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

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.

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

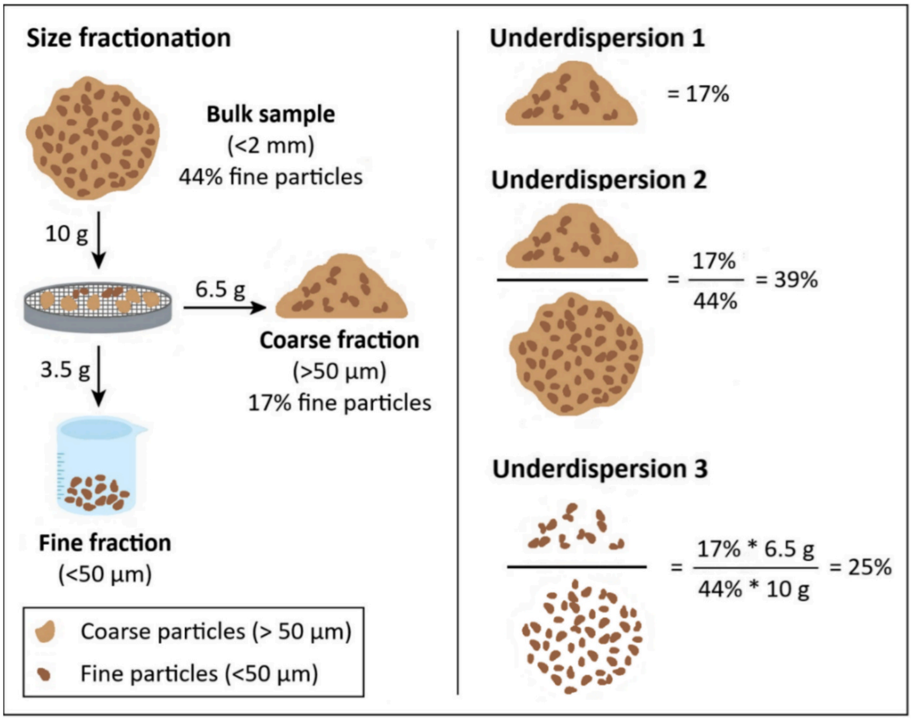

Fig. 1. Schematic overview of the size fractionation procedure (left) and the quantification of underdispersion (right). In this example, the coarse fraction constitutes 65% of the bulk soil and contains 17% fine particles, compared to 44% of fine particles in the bulk soil.

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).

3. Results

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 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 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 (ρ = 0.50–0.73), CEC (ρ = 0.36–0.67), silt (ρ = 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 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 (Fig. 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 (Fig. 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 $soil^{-1}$). 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 ($\rho = 0.85$; Fig. 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 (Fig. 2, middle row). The MAOC in the coarse fraction correlated most strongly with Und3 ($\rho = 0.95$; Fig. 2, bottom row).

Table 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
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
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
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

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 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; Fig. 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 3) and residuals were still normally distributed.

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 1). The RMC was positively correlated with all measures of underdispersion (Table 2; Fig. 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 (ρ

Table 2
Spearman correlation coefficients (ρ) among underdispersion and soil parameters. RMC = residual moisture content. Asterisks indicate values are significant ($p <$ 0.05).

	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*

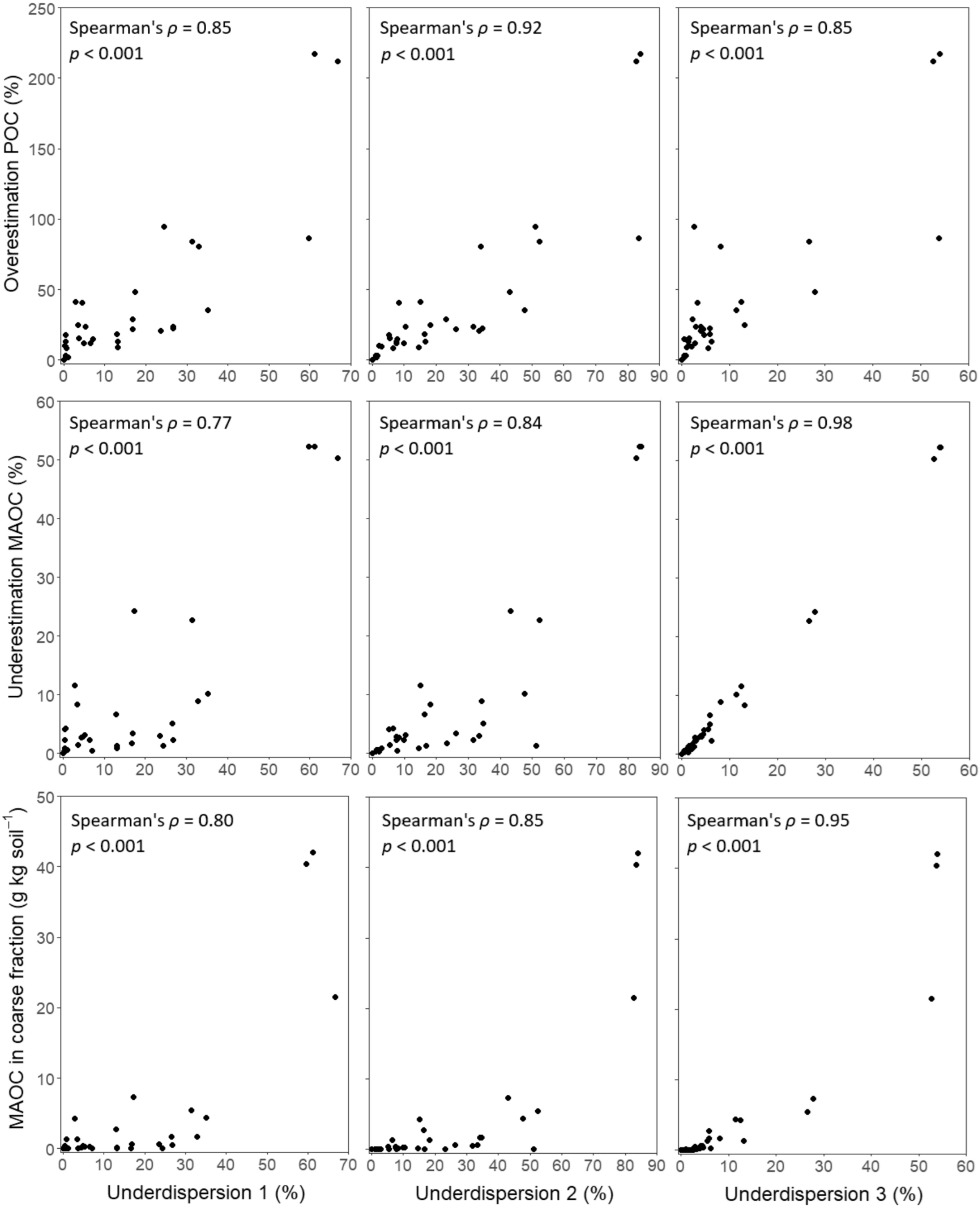

Fig. 2. Relative overestimation of POC (top), relative underestimation of MAOC (middle) and absolute amounts of MAOC found in the coarse fraction (bottom) as a function of Underdispersion 1 (left), 2 (middle) and 3 (right).

Table 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

= 0.48) and the relation seemed curvilinear.

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 μm) 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 g MAOC kg^{-1} soil and 2.9% of SOC). This furthermore will partially be compensated by POC ending up in the fine

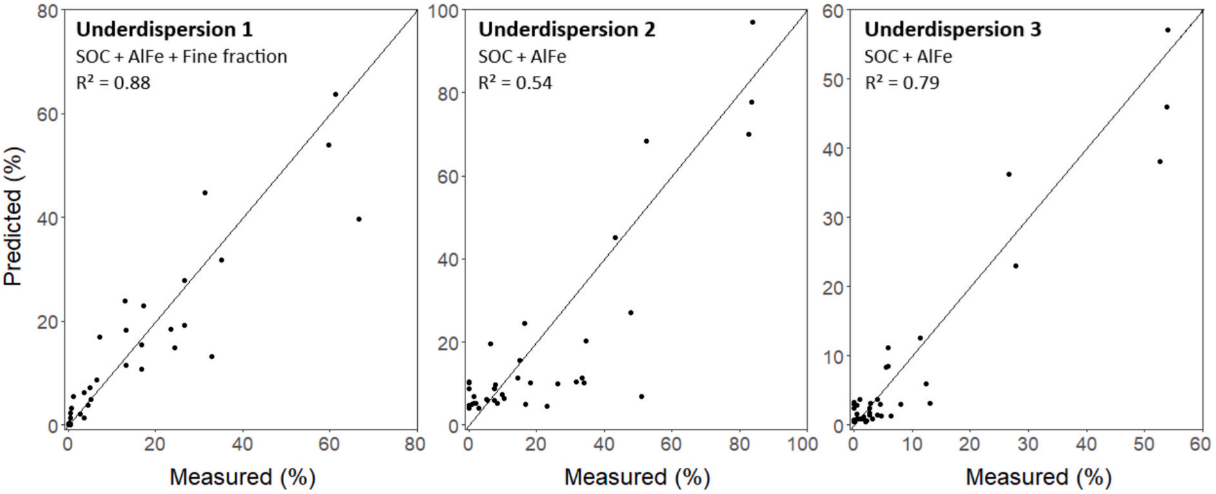

Fig. 3. Predicted versus measured Und1 (left), Und2 (middle) and Und3 (right) and the 1:1 lines. Predicted values are based on the reduced models (Table 3) and were squared for back-transformation to the original scale.

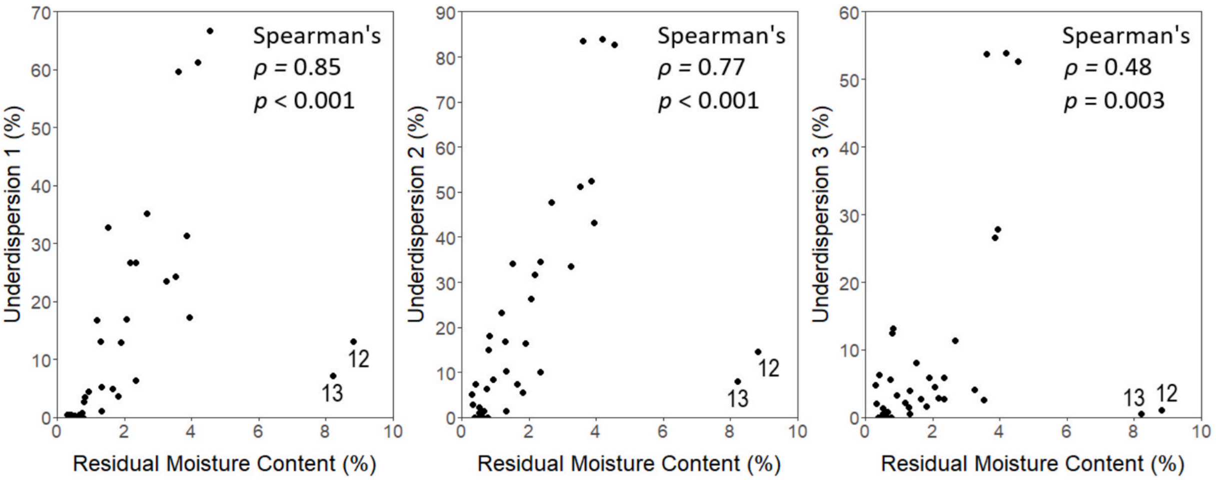

Fig. 4. Underdispersion 1, 2 and 3 plotted against the residual moisture content. Numbers refer to the soils in Table 1 and are included for discussion purposes.

fraction (Poeplau et al., 2025).

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.

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.

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).

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.

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 1; Fig. 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 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 cmol $+$ kg^{-1} versus $<$ 0.41 cmol $+$ kg^{-1} for all other selected soils; Table 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 cmol $+$ 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 (Fig. 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 1), we recommend future studies to include soils covering a larger variety in carbonate contents to assess its potential effect on underdispersion.

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 2; Fig. 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 4.5). The high RMC of soils 12 and 13 could be related to a combination of high clay (Fig. S2) and high exchangeable Mg and Ca contents (Table 1; Fig. 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.

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. 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 μm). 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 °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 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.

Credit authorship contribution statement

M.S. Breure: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **A.J. van der Sluijs:** Conceptualization, Writing – original draft, Methodology, Investigation. **G.J. Koorneef:** Writing – review & editing, Supervision, Methodology, Conceptualization. **A-R. Salonen:** Writing – review & editing. **M.M. Pulleman:** Writing – review & editing. **M. Hagens:** Writing – review & editing,

Conceptualization. **W.D.C. Schenkeveld:** Writing – review & editing, Supervision, Methodology, Conceptualization. **D.P. Di Lonardo:** Writing – review & editing, Supervision, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2026.117704.

Data availability

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

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