



Sodium hexametaphosphate and ultrasonication – Comparing chemical and physical dispersion methods for soil organic matter fractionation

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ABSTRACT

Soil organic matter (SOM) fractionation into particulate organic matter (POM) and mineral-associated organic matter (MAOM) is widely applied to investigate soil organic carbon (SOC) stabilization and turnover, yet the choice of dispersion method strongly influences fractionation outcomes. This study tested a chemical dispersion approach using sodium hexametaphosphate (Na-HMP) and compared it with mechanical ultrasonication at 450 J mL⁻¹ (US450), previously shown to be effective. Method performance was assessed based on mass and organic carbon (OC) recovery, reproducibility, and the quality of fraction separation, the latter assessed using natural ¹³C abundance in soils transitioning from C₃ to C₄ vegetation. Both methods achieved high mass and OC recoveries (>90 %) with low variability between replicates (median coefficient of variation (CV) < 5 %), indicating that either dispersion approach can be applied reproducibly. However, significant differences in fraction allocation were observed: Na-HMP yielded higher mass and OC contributions in the POM fraction at several sites, whereas US450 consistently promoted a more MAOM-dominated distribution. The proportion of C₄-derived carbon (C) differed strongly between POM and MAOM for both methods; however, the capacity to resolve C pools based on $\delta^{13}\text{C}$ signatures was significantly higher under Na-HMP, attributed to Na-HMP weakening organo-mineral associations and mobilizing mineral-bound OC. While this reflects chemical redistribution of organic matter (OM) between fractions rather than a strictly physical size-based separation, it nonetheless sharpened the isotopic contrast between POM and MAOM. Overall, US450 is recommended for size-based separation and aggregate disruption, whereas Na-HMP is preferred for maximizing isotopic contrast and isolating pools with distinct turnover rates. The chemical mobilization of OM observed under Na-HMP suggests that mineral-associated OC may be sensitive to ionic disturbances, a pattern that warrants further investigation in the context of salt-affected or sodic soils.

1. Introduction

Soil organic matter (SOM) is fundamental for terrestrial ecosystem functioning. It continuously exchanges energy, nutrients, and carbon (C) with the biosphere, directly impacting food production, water protection, and climate resilience (Lal, 2016). Given this central role, understanding what governs SOM persistence and turnover is essential for predicting soil responses to land use and climate change. Because SOM comprises pools with widely differing persistence and turnover,

fractionation into operationally defined pools has become a key approach for disentangling the mechanisms that govern SOM stability.

Previous studies have demonstrated that the stability of SOM is influenced by a combination of biotic and abiotic factors, with mineral interactions playing a critical protective role and biochemical recalcitrance playing a secondary role in SOM persistence (Schmidt et al., 2011). Clay minerals and iron oxyhydroxides stabilize SOM through ligand exchange, electrostatic adsorption, cation bridging, hydrogen bonding, and van der Waals interactions (Carvalho et al., 2023; Lützow

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et al., 2006; Sollins et al., 2009). These minerals also contribute to soil aggregation, physically occluding organic matter (OM) and reducing microbial decomposition (Six et al., 2002). Moreover, clay activity is a key determinant of long-term organic carbon (OC) retention, as sorptive interactions with reactive clay surfaces further enhance SOM stabilization (Kleber et al., 2015).

To deepen the understanding of soil organic carbon (SOC) dynamics, research frequently focuses on OC stabilization and persistence in soils. However, the inherent heterogeneity of soils presents significant challenges for characterizing SOM stability and turnover, as well as its broader role in ecosystem functioning (Kögel-Knabner, 2002). To address this complexity, SOM fractionation is a fundamental approach for understanding and modeling SOM decomposition and stabilization processes, and for elucidating C sequestration and storage capacity in soils (Cotrufo et al., 2019; Georgiou et al., 2025). It is commonly recommended to fractionate SOM into particulate organic matter (POM) and mineral-associated organic matter (MAOM) to promote analysis of its composition and dynamics (Lavalley et al., 2020; Yu et al., 2022).

The methodology of fractionating SOM into POM and MAOM is based on the understanding that these two fractions differ fundamentally in their function, persistence, and chemical composition (Cambardella & Elliott, 1992). POM is mostly a fast-cycling, undecomposed, lightweight fraction of SOM that exists as soil particles without a permanent bond with other soil components (Lavalley et al., 2020). It is predominantly rapidly mineralized, releasing nutrients available to soil organisms and plants (Trumbore et al., 1989). In contrast, MAOM is generally characterized as slow-cycling, microscopic fraction of SOM that adheres to soil minerals, protecting the OM from microbial decomposition and enabling it to persist longer in the soil (Kleber et al., 2007; Kögel-Knabner et al., 2008; Lavalley et al., 2020; Trumbore et al., 1989). As a result, MAOM is considered stabilized OM and exhibits longer turnover times and residence durations in the soil compared to POM, which lacks persistent mineral associations and is therefore more readily decomposed (Sollins et al., 1996).

A study by Just et al. (2021) demonstrated that soil physical fractionation by sieving SOM into two particle-size fractions, $>20\ \mu\text{m}$ and $<20\ \mu\text{m}$, could effectively assess SOC fractions with different turnover rates. Although cut-offs of $30\ \mu\text{m}$ or $50\ \mu\text{m}$ are frequently used, the $20\ \mu\text{m}$ threshold is well established in soil classification systems and SOM fractionation frameworks (Hassink, 1997; Leifeld & Kögel-Knabner, 2001; Rovira et al., 2010; Six et al., 2002; Steffens et al., 2009; Tisdall & Oades, 1982). Recent evidence further indicates that most MAOM is associated with particles $<6.3\ \mu\text{m}$, suggesting that the choice between $20\ \mu\text{m}$ and larger cut-offs does not substantially affect MAOM-related conclusions (Rovira et al., 2024; Schweizer et al., 2021). The $20\ \mu\text{m}$ threshold, therefore, represents a practical and well-established compromise between conceptual relevance and analytical feasibility.

In addition to particle size, soil fractionation also enables measurement of natural ^{13}C isotope abundance to assess OC persistence in soils (Trumbore & Zheng, 1996). This approach allows researchers to differentiate between older and more recent C inputs by exploiting vegetation-driven shifts in isotopic signatures (Balesdent et al., 1987). When vegetation shifts from C_3 (Calvin cycle) to C_4 (Hatch-Slack pathway) plants, or vice versa, the resulting change in organic inputs alters the ^{13}C content of the soil, effectively labeling the OM *in situ* (Cerri et al., 1985; Farquhar et al., 1989; Lefroy et al., 1993). The stable carbon isotope ratio ($\delta^{13}\text{C}$) of plants with C_3 photosynthesis typically averages around -28‰ relative to the Pee Dee Belemnite (PDB) standard, while those from plants with C_4 photosynthesis are approximately -12‰ (PDB) (Smith & Epstein, 1971).

Both particle-size separation and isotopic tracing, however, depend critically on how soil aggregates are broken apart prior to fractionation. Several methods have been developed and widely used to isolate POM and MAOM, typically classified into fractionation schemes that employ different dispersion methods (Trumbore & Zheng, 1996). As emphasized by Just et al. (2021), the choice of dispersion method can substantially

influence soil fractionation outcomes. Kpemoua et al. (2026) further demonstrated that these differences depend on soil characteristics such as pH, clay content, and SOC, highlighting the need to harmonize data derived from different fractionation approaches. Dispersion is therefore a critical step, as soil aggregates must be disaggregated to obtain accurate particle-size fractions without altering the sample's intrinsic properties (Smith et al., 2015).

Just et al. (2021) evaluated several approaches for dispersing soil aggregates prior to physical fractionation of POM and MAOM by wet sieving. Among these, physical dispersion via ultrasonication at $450\ \text{J mL}^{-1}$ (US450) yielded the best quality indicators, producing a fractionation scheme that was fast, inexpensive, and highly reproducible (Just et al., 2021). In contrast, the fractionation approach employing sodium hexametaphosphate (Na-HMP) as a chemical dispersant was discontinued in the study by Just et al. (2021) due to their experimental design. Efforts to remove the salt with deionized water resulted in pronounced leaching of SOC, compromising the method's reliability and efficiency (Just et al., 2021). Nevertheless, the effectiveness of the widely used Na-HMP dispersion technique and its direct comparison with physical dispersion methods regarding C recovery and reproducibility remain open questions that warrant further investigation.

This study, therefore, evaluates and compares the performance of Na-HMP chemical dispersion and physical dispersion via US450. Fractionation experiments employing the chemical dispersion technique were conducted on samples from five sites representing two vegetation types (C_3/C_4), followed by wet sieving to separate the particle-size fractions. Unlike Just et al. (2021), the Na-HMP salt was not removed after chemical dispersion. Instead, it was retained within the samples throughout all subsequent analytical procedures, thereby avoiding the OC loss associated with salt removal. The outcomes were then compared with those reported by Just et al. (2021) to assess the relative effectiveness and efficiency of both dispersion approaches in soil fractionation.

The primary objective of the experiment was to determine the relative merits of Na-HMP and US450 dispersion methods, with emphasis on enhancing reproducibility while minimizing procedural complexity and labor intensity across laboratories. The dispersion methods were evaluated based on the following criteria:

- (1) Recovery - Assessment of total mass and OC losses incurred during the fractionation process.
- (2) Isotopic separation - Evaluation of $\delta^{13}\text{C}$ enrichment and the degree of separation between C_4 - and C_3 -derived C within the resulting fractions.
- (3) Reproducibility - Determination of the variability between replicates to assess the method's consistency and reliability.

2. Materials and methods

2.1. Experimental sites and soil characteristics

For a direct and accurate comparison, the soil samples used in the current experiment were identical to those employed in the previous study by Just et al. (2021), and both studies' analyses were conducted at the Soil Analysis Laboratory of the Technical University of Munich (TUM) School of Life Sciences. Two dispersion approaches were applied prior to physical fractionation: chemical dispersion with Na-HMP, performed in this study by loosening soil aggregates through 15 h of shaking on a flatbed orbital shaker, and physical dispersion via US450, applied to mechanically disrupt aggregates as described by Just et al. (2021). Both approaches were followed by wet sieving to separate the dispersed material into $>20\ \mu\text{m}$ and $<20\ \mu\text{m}$ fractions, referred to here as POM and MAOM, respectively.

The samples were from five different long-term field experiments (LTEs): Braunschweig, Hohenheim, Jyndevid, Rothalmünster, and Tännikon. The collected material included fields that had shifted from C_3

to C₄ vegetation and adjacent control plots with exclusive cultivation of C₃ vegetation (Table 1). The five sites and their two vegetation types resulted in 10 distinct sample groups for analysis. These LTEs were selected to span a range of soil types and texture gradients, providing a representative dataset of central European soils (Just et al., 2021).

As presented in Table 1, MAT had low variability with typical values for temperate regions of central Europe. In contrast, the MAP varied widely, ranging from 620 mm in Braunschweig to 1,185 mm in Tännikon. Hohenheim's SOC content was considerably higher than the other sites, at 79.9 mg g⁻¹ (C₃) and 58.0 mg g⁻¹ (C₄). This finding likely reflects the site's grassland history. Soil silt, sand, and clay proportions varied across the sites. According to the United States Department of Agriculture (USDA) soil texture classification, Jydevad was classified as Sand (S), Braunschweig as Sandy Loam (SL), Hohenheim and Rotthalmünster as Silt Loam (SiL), and Tännikon, which had the highest clay content, as Clay Loam (CL). Four out of five experimental sites were cultivated with the C₄-plant *Miscanthus x giganteus*, and, exceptionally, the Rotthalmünster site was cultivated with the C₄-plant *Zea mays*, with intermittent interruptions by millet (*Sorghum spec.*) cultivation in 2012 and 2014. At the time of sampling, sites had been under C₄ vegetation for 17–36 years following a prior period of C₃ cultivation, with adjacent C₃ plots retained as reference soils. The soil samples were collected as composite samples on ~300 m² grids at a depth of 10 cm, as described in Poeplau and Don (2013) and Just et al. (2021). Subsequently, the samples were oven-dried at 60 °C and aggregates or clods larger than 2 mm were gently broken down using a mortar and pestle before sieving to 2 mm (Just et al., 2021).

2.2. Soil fractionation and processing

To prepare the Na-HMP dispersant solution, 5 g of powder was dissolved in 1 L of distilled water, briefly mixed by hand for approximately 2 min, and then placed on a flatbed orbital shaker for 75 min at 250 rpm. The primary dataset for the Na-HMP fractionation experiment contained $n = 30$ samples [5 sites $\times$ 2 vegetation types (C₃/C₄) $\times$ 3 replications], which resulted in $n = 60$ samples after fractionation. In preparing all samples, approximately 12.5 g of dry soil was weighed into a 100 mL container before the fractionation into >20 and <20 μm , following Hassink (1997) and Six et al. (2002), as shown in the scheme in Fig. 1. The chemical dispersion was performed by adding 50 mL of 0.5% diluted Na-HMP to ~12.5 g of the soil sample and letting it shake on a flatbed orbital shaker for 15 h at 180 rpm, consistent with the Na-HMP concentration and shaking parameters reported in Cambardella and Elliott (1992), Cotrufo et al. (2019) and Sanderman et al. (2011). Unlike Just et al. (2021), the Na-HMP salt was not removed from the samples before C and $\delta^{13}\text{C}$ analyses, as its presence does not pose a risk to the analytical instruments, thereby avoiding the OC loss associated with salt-removal procedures.

The sieving protocol followed Just et al. (2021) with minor modifications: all samples were wet sieved using a 20 μm stainless steel sieve mounted on a horizontal shaking sieving machine (Analysette 03.502, Fritsch GmbH, Germany) with a frequency of ~50 Hz and an amplitude of 2–3 mm to standardize the sieving process. During fractionation,

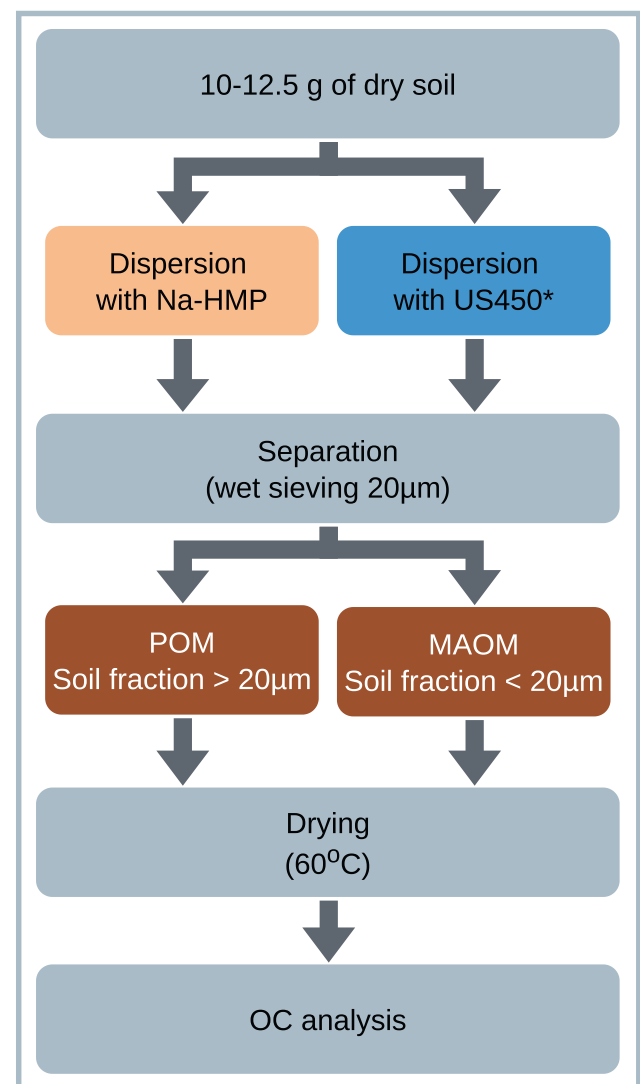


Fig. 1. Soil fractionation workflow to separate POM and MAOM for the comparison of different dispersion methods. *For the dispersion with US450, the data used were taken from the study by Just et al. (2021).

approximately 2 L of distilled water was gradually poured into the sieve until the stream exiting the sieving system was visually clear (i.e., free of suspended particles), following Poeplau et al. (2013) and Sanderman et al. (2011). Simultaneously, the sieved material was collected into pre-weighed low-density polyethylene (LDPE) containers. All material that passed through the sieve (<20 μm) was considered MAOM and collected in a 2 L container. The material remaining on the sieve (>20 μm), considered POM, was manually moved into a 250 mL container using a water spray nozzle. Subsequently, the containers with the

Table 1

Site characteristics and soil properties of the study samples. Mean annual temperature (MAT), mean annual precipitation (MAP), soil type according to the World Reference Base for Soil Resources (WRB), SOC content of C₃ reference soil (SOC_{C3}) and C₄ reference soil (SOC_{C4}), proportions of sand/silt/clay, years under C₄ vegetation, and C₄ plant species data taken from Just et al. (2021).

Experimental site	MAT [°C]	MAP [mm]	Soil Type (WRB)	SOC _{C3} [mgCg ⁻¹]	SOC _{C4} [mgCg ⁻¹]	Sand Silt Clay [%]	pH	Years with C ₄ plants	C ₄ plant
Braunschweig	8.8	620	Cambisol	18.4	17.4	67/29/4	6.2	22	Miscanthus
Hohenheim	8.7	679	Luvisol	79.9	58.0	36/54/10	7.0	21	Miscanthus
Jydevad	7.9	859	Arenosol	18.7	21.7	91/6/3	5.1	18	Miscanthus
Rotthalmünster	8.7	886	Luvisol	14.1	13.5	17/75/8	6.2	36	Maize/ Sorghum
Tännikon	8.4	1,185	Luvisol	19.5	33.2	39/32/29	7.1	17	Miscanthus

fractionated material were placed in the drying cabinet at 60 °C (Fig. S1). The dried fractions were then weighed to determine mass recoveries, removed from the LDPE containers, homogenized, and ground into a fine powder for C content analysis.

Based on the results of the C content analysis, this study investigated the dispersion method using Na-HMP as a dispersant to isolate two fractions with distinct SOM turnover times, referred to as “Na-HMP”. Then, the performance of the Na-HMP method for isolating POM and MAOM fractions was compared to the results of the physical dispersion, ultrasonication at 450 J mL⁻¹, referred to as “US450”, which had the best quality indicators among the examined methods in the study by Just et al. (2021). This comparison was possible because the soil sample material, laboratory, sieving/drying procedures, and standards were alike for both studies. A description of the US450 method follows (Just et al., 2021): dispersion was performed by ultrasonication of the soil in 150 mL deionized water at a high energy input of 450 J mL⁻¹ (Schmidt et al., 1999), using a 13-mm-diameter sonotrode at an oscillation frequency of 20 kHz and an immersion depth of 15 mm (Graf-Rosenfellner et al., 2018).

2.3. Determination of C and $\delta^{13}\text{C}$

Total C was measured in all fractions and bulk soil samples by dry combustion using a HEKATECH EuroEA 3000 elemental analyzer, with a measurement accuracy of 2 mg C g⁻¹ soil. Total inorganic carbon (IC) was determined by reaction with 4 M HCl; however, IC was not detected in any samples. Therefore, total C was assumed to represent OC concentration (Just et al., 2021).

The $\delta^{13}\text{C}$ was determined with an isotope ratio mass spectrometer (IRMS; Delta V Advantage, Thermo Fisher, Dreieich, Germany) coupled to an elemental analyzer (Euro EA, EuroVector, Milano, Italy). The standard deviation of laboratory standards was <0.1‰. Appropriate international standards were included in each analytical sequence to create a final correction of ^{13}C . The $\delta^{13}\text{C}$ values are reported relative to the Vienna Pee Dee Belemnite (V-PDB) standard, equivalent to the PDB standard referenced in earlier literature.

All measurements were performed with at least a duplicate, which was generally sufficient to achieve the required analytical precision. Additional replicates were conducted when the coefficient of variation (CV) between the first two replicates exceeded 10% for total C, or when $\delta^{13}\text{C}$ values deviated by more than 1‰ between measurements, to verify whether the variation was due to natural soil heterogeneity or analytical error.

2.4. Assessment of performance indicators

To evaluate mass recovery ($Mass_{recovery}$) of the fractionation methods, the sum of the mass contributions of the fractions ($M_{fraction}$) was divided by the sample's initial mass (M_{sample}), with the resulting values averaged across all replicates.

$$Mass_{recovery} = \left(\frac{M_{fractionPOM} + M_{fractionMAOM}}{M_{sample}} \right) \times 100 \quad (1)$$

where $M_{fractionPOM}$ and $M_{fractionMAOM}$ represent the dry mass (g) recovered in the POM and MAOM fractions, respectively, and M_{sample} represents the initial dry mass (g) of the unfractionated bulk soil sample.

To determine OC recovery ($OC_{recovery}$ (%)), the percentage was calculated as the ratio of the total OC mass recovered in the POM and MAOM fractions ($OC_{POM} + OC_{MAOM}$) to the OC mass initially present in the bulk soil sample subjected to fractionation (OC_{bulk}).

$$OC_{recovery}(\%) = \left(\frac{OC_{POM} + OC_{MAOM}}{OC_{bulk}} \right) \times 100 \quad (2)$$

where OC_{POM} and OC_{MAOM} represent the OC (g C) masses recovered in the POM and MAOM fractions, respectively, and OC_{bulk} represents the

OC mass (g C) initially present in the unfractionated bulk soil sample.

The OC mass in each fraction i was calculated as:

$$OC_i = M_{fraction_i} \times \left(\frac{C_{fraction_i}}{100} \right) \quad (3)$$

where $M_{fraction_i}$ is the recovered dry mass of fraction i (g), and $C_{fraction_i}$ is its OC concentration expressed in percent (%).

The initial OC mass in the bulk soil sample was determined as:

$$OC_{bulk} = M_{sample} \times \left(\frac{C_{bulk}}{100} \right) \quad (4)$$

where M_{sample} is the initial dry mass (g), and C_{bulk} is the OC concentration (%) of the unfractionated bulk soil.

For reporting OC contributions of individual fractions on a soil mass basis (mg C g⁻¹ soil), the contribution of fraction i was calculated as:

$$OC_{contribution_i} = \left(\frac{M_{fraction_i} \times \left(\frac{C_{fraction_i}}{100} \right)}{M_{sample}} \right) \times 1000 \quad (5)$$

The proportion of new C₄-derived C in each fraction was calculated using the following formula (Balesdent et al., 1987):

$$f_{C_4 fraction_i} = \frac{\delta^{13}\text{C}_{fraction_i}(C_4 \text{ soil}) - \delta^{13}\text{C}_{fraction_i}(C_3 \text{ soil})}{\delta^{13}\text{C}_{fraction_i}(C_4 \text{ plant}) - \delta^{13}\text{C}_{fraction_i}(C_3 \text{ soil})} \quad (6)$$

where f_{C_4} represents the proportion of C₄-derived C in the soil fraction _{i} of the C₄ vegetated soil; the terms $\delta^{13}\text{C}_{fraction_i}(C_4/C_3 \text{ soil})$ and $\delta^{13}\text{C}_{fraction_i}(C_4 \text{ plant})$ correspond to the $\delta^{13}\text{C}$ values of the SOC fraction of interest in the C₄ vegetated soil, the C₃ reference soil, and the C₄ plant grown at the site, respectively. The $\delta^{13}\text{C}$ value for the C₄ plant was fixed at -12‰ V-PDB (Menichetti et al., 2013). Thus, f_{C_4} values range from 0–1, where 1 indicates a fraction entirely composed of new C derived from C₄ plants, and 0 indicates a fraction containing no C₄-derived C.

The range of f_{C_4} between the two fractions was used to quantify the degree of isolation achieved by each dispersion treatment.

$$Range f_{C_4} = f_{C_4 max} - f_{C_4 min} \quad (7)$$

where $f_{C_4 max}$ is the maximum f_{C_4} value among the fractions of a soil sample, and $f_{C_4 min}$ is the minimum value within the same sample. The Range f_{C_4} was calculated for each replicate and then averaged across all replicates for a given soil and method. A high Range f_{C_4} therefore indicates an effective fractionation method, whereas a low Range f_{C_4} indicates a method that performs poorly at isolating the C₄- and C₃-derived C pools within a soil (Poeplau et al., 2018).

Furthermore, the reproducibility of the methods was evaluated by calculating the mean OC measurement for a given sample and then the CV of replicates for each fraction of that sample.

$$CV(\%) = \frac{SD_{replicates}}{MV_{replicates}} \times 100 \quad (8)$$

where $SD_{replicates}$ is the standard deviation (SD) of replicate measurements, and $MV_{replicates}$ is the corresponding mean OC value of the replicates. Replicate numbers differed between dispersion methods: three replicates for Na-HMP and two for US450. A smaller CV, therefore, indicates greater method reproducibility, whereas a larger CV indicates reduced reproducibility.

2.5. Data pre-processing

In this experiment, the Na-HMP salt added via the dispersant solution was estimated to contribute an additional mass of approximately 2% relative to the initial soil sample weight: assuming a homogeneous dispersant solution, 50 mL of 0.5% Na-HMP contained 0.250 g of salt.

Given its small molecular size, this dissolved salt was expected to remain associated with the MAOM (<20 μm) fraction after fractionation, rather than being retained on the sieve with the POM fraction. Previous studies have typically reported mass recoveries exceeding 98% (Sanderman et al., 2011); however, it remains unclear whether this added salt mass was accounted for in those calculations. To address this, the total mass used for recovery calculations in the present study was defined as the sum of the initial soil sample weight and 0.250 g of added Na-HMP.

Following this mass-balance approach, ten soil samples, each analyzed in triplicate (30 samples in total), were fractionated into POM and MAOM, yielding 60 fractions overall. Each fraction was then analyzed for OC content with replicate measurements, giving an initial 120 OC measurements. Where the CV within a single soil group exceeded 10%, two additional replicates were analyzed to confirm that the observed variability was not due to instrumental error, adding 12 measurements and bringing the final OC dataset to 132 values, all of which were retained for analyses.

The same 60 POM and MAOM fractions were also submitted for $\delta^{13}\text{C}$ analysis, again with replicate measurements for each fraction, giving an initial 120 results. Where replicates differed by more than 1‰, two additional replicates were analyzed for $\delta^{13}\text{C}$, this applied to 21 values. Although two soil groups - Tännikon and Jyndevad - showed higher $\delta^{13}\text{C}$ variability than the others, this variation was attributed to natural soil heterogeneity rather than analytical error, and all 141 values were retained in the final dataset.

2.6. Statistical tests

All statistical analyses were performed in R (R Core Team, Vienna, Austria). Data were visually inspected, and model residuals were evaluated for normality and homoscedasticity using diagnostic plots and formal tests. Differences in mass recovery and OC recovery between dispersion methods were assessed using Wilcoxon rank-sum tests, with site-wise comparisons adjusted for multiple testing using the Benjamini-Hochberg (BH) procedure. OC contributions between fractions were compared using unpaired and paired t-tests, with Holm correction applied for multiple testing across site and fraction comparisons. The Wilcoxon signed-rank test was applied to compare mass recovery between fractions and CV between dispersion methods.

The proportions of fC_4 and Range fC_4 were analyzed using linear mixed-effects models (LMM), with the dispersion method and fraction included as fixed effects, and site included as a random intercept. The fC_4 LMM provides information on the general isolation of fractions with different turnover rates, whereas the Range fC_4 model indicates how the choice of dispersion method affects the effectiveness of this isolation. A significance level of $p < 0.05$ was used for all tests. Residual diagnostics were performed using normal Q-Q plots and residuals vs fitted values plots, which indicated that the assumptions of normality and homoscedasticity were adequately met (Fig. S2).

3. Results

3.1. Dispersion effects on soil fractions

Mass recoveries were consistently high across all samples, ranging from 96.2 to 100.5%, with an overall mean of 97.8% (Fig. S3). Values slightly exceeding 100% are attributable to minor analytical and weighing uncertainties. An unpaired Wilcoxon rank-sum test indicated no significant overall difference in total mass recovery between dispersion methods ($W = 316$, $p = 0.46$). At the site level, however, a linear mixed-effects model with BH correction revealed significant method effects at Braunschweig ($t = -2.34$, $df = 38$, $p = 0.025$), Hohenheim ($t = 2.80$, $df = 38$, $p = 0.008$), and Jyndevad ($t = 2.28$, $df = 38$, $p = 0.029$), while Rothalmünster and Tännikon showed no significant differences ($p = 0.989$ and $p = 0.502$, respectively). At Hohenheim, Na-HMP yielded slightly higher recovery (US450: 96.5%; Na-HMP: 98.2%).

In contrast, US450 showed higher recovery at Braunschweig (US450: 98.9%; Na-HMP: 97.6%) and, numerically, at Jyndevad (US450: 99.4%; Na-HMP: 98.0%). These opposing site-specific effects, where US450 was favored at Braunschweig and Jyndevad, and Na-HMP was favored at Hohenheim, likely canceled out in the overall comparison, consistent with the non-significant overall test. Overall, absolute site-level differences were small (<2%) and did not indicate a systematic method effect (Fig. 2).

When comparing mass recovery between POM and MAOM fractions, paired Wilcoxon signed-rank tests showed no significant difference. However, the Na-HMP method yielded a higher mass in POM fractions, whereas US450 consistently produced higher mass in MAOM fractions across all sites (Fig. 2).

Regarding OC recovery, no significant overall difference between methods was detected by the Wilcoxon rank-sum test ($W = 354$, $p = 0.124$). Site-specific comparisons likewise revealed no significant method effects (Fig. S3). Although numerical differences were observed at Hohenheim ($p = 0.178$) and Rothalmünster ($p = 0.066$), these did not reach statistical significance. Effect sizes (Hedges' g) were small to moderate at most sites and larger at Hohenheim ($g = 1.61$) and Rothalmünster ($g = 1.42$), but confidence intervals were wide and largely overlapped zero, indicating substantial variability (Fig. S4). Overall, US450 exhibited slightly higher mean OC recovery than Na-HMP (Fig. 2). The lowest mean OC recovery for both methods was observed at Jyndevad (US450: 90.3%; Na-HMP: 88.4%), whereas the highest mean recovery occurred at Rothalmünster (US450: 102.0%; Na-HMP: 95.2%). The value exceeding 100% at Rothalmünster for US450 likely reflects minor weighing and analytical uncertainties, consistent with the mass recovery observations.

In contrast to overall recovery, OC distribution between fractions differed clearly by method. Na-HMP displayed a heterogeneous pattern, with a higher OC fraction contribution in the POM fraction at Hohenheim and Braunschweig, whereas US450 consistently showed higher OC concentrations in the MAOM fraction than in the POM fraction across all sites (Fig. S5). Holm-adjusted unpaired t-tests confirmed that OC contributions differed between methods depending on site and fraction (Fig. 3). For the POM fraction, contributions were significantly higher under Na-HMP at Braunschweig ($t = 5.18$, $df = 4.97$, $p = 0.021$), Hohenheim ($t = 4.30$, $df = 4.67$, $p = 0.045$), and Rothalmünster ($t = 7.22$, $df = 5.61$, $p = 0.004$), whereas for MAOM, Na-HMP contributions were significantly lower than those of US450 at Braunschweig ($t = -5.98$, $df = 5.33$, $p = 0.012$), Hohenheim ($t = -5.08$, $df = 3.30$, $p = 0.046$), Rothalmünster ($t = -11.5$, $df = 5.73$, $p < 0.001$), and Jyndevad ($t = -4.29$, $df = 7.99$, $p = 0.019$). No significant differences were observed at Jyndevad or Tännikon for POM, or at Tännikon for MAOM.

3.2. C_4 -derived OC proportion in fractions

The LMM revealed that the proportion of fC_4 differed significantly between the POM and MAOM fractions across sites ($F_{1, 42} = 121.9$, $p < 0.001$), with consistently higher fC_4 values observed in the POM fraction (Fig. 4). In contrast, neither the overall method effect ($p = 0.8236$) nor the method $\times$ fraction interaction ($p = 0.1649$) was significant, indicating that both Na-HMP and US450 preserved the isotopic signal of the C_3/C_4 vegetation shift to a comparable degree.

The analysis of Range fC_4 showed significant differences between dispersion methods. The mixed-effects model accounting for site as a random factor revealed a significant method effect ($F_{1, 19} = 21.26$, $p = 0.0002$), with Na-HMP exhibiting consistently higher Range fC_4 values than US450. This indicates a stronger differentiation between fractions under Na-HMP dispersion (Fig. 5).

3.3. Reproducibility of methods

The reproducibility of the two methods, Na-HMP and US450, was assessed by using the CV of elemental C concentrations calculated across

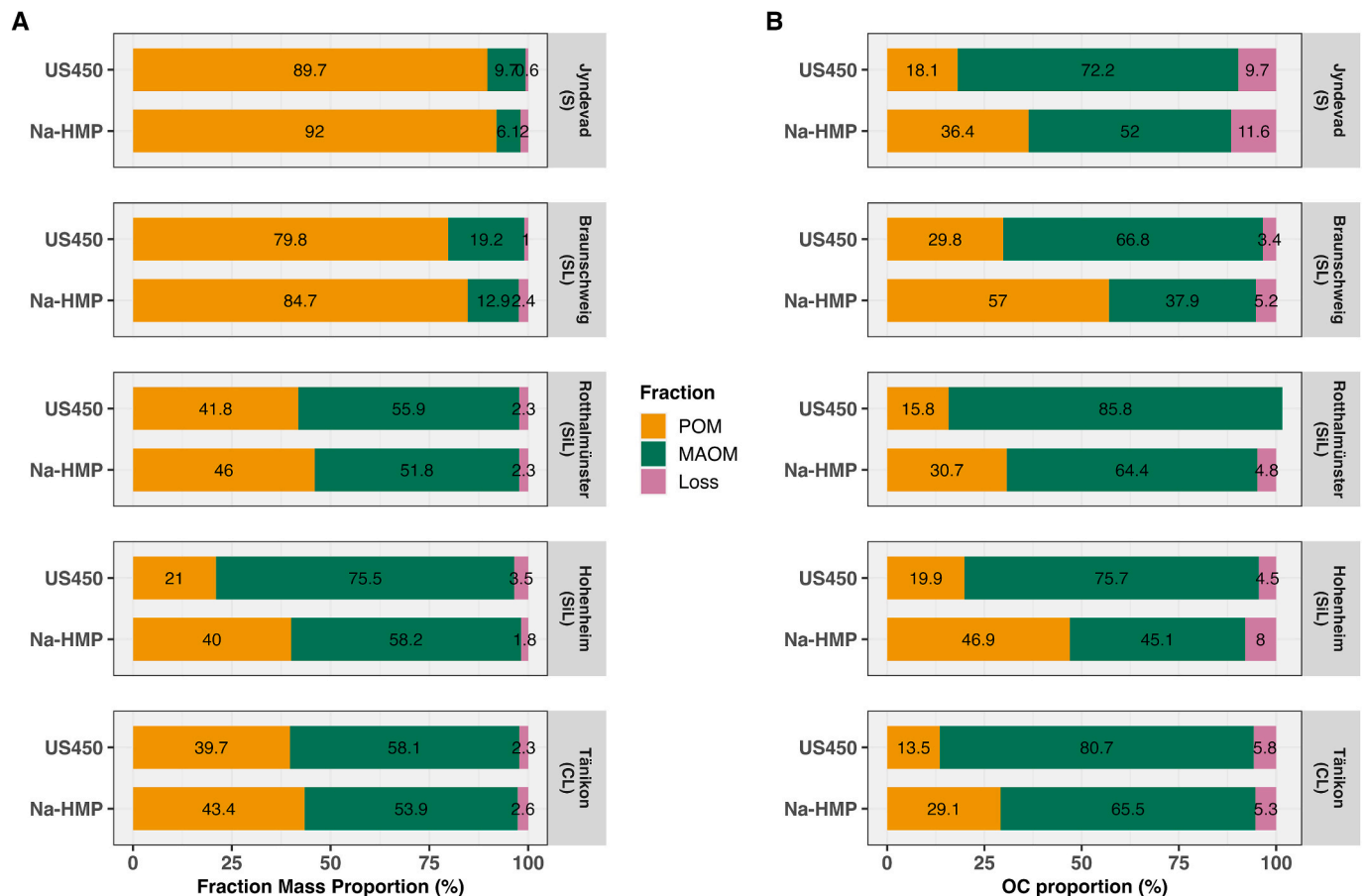


Fig. 2. Relative mass and OC distribution across sites and methods. Code in brackets refers to texture class (S = sand, SL = sandy loam, SiL = silt loam, CL = clay loam). **A:** Mean mass proportions of POM and MAOM fractions, including mass loss, by extraction method across sites. **B:** Proportional OC distribution (%) per fraction and OC loss across sites by method. Values represent the percentage of total recovered OC assigned to each fraction; absolute OC contributions in mg g^{-1} soil are shown in Fig. 3. Both panels highlight variation in fraction proportions across methods and sites.

independent fractionation replicates within each sample and fraction. Overall reproducibility was high for both dispersion methods. Across all sample groups, median CV values were 2.16% for US450 and 4.96% for Na-HMP (Fig. S6). At the fraction level, US450 exhibited lower median variability for MAOM (1.17%) than Na-HMP (2.22%), whereas POM showed substantially higher and comparable variability for both methods (US450: 9.75%; Na-HMP: 9.52%). Paired Wilcoxon signed-rank tests revealed no statistically significant differences in reproducibility between methods for MAOM or POM. When fractions were pooled, US450 showed a consistent but non-significant tendency toward lower overall CV values compared with Na-HMP ($V = 58$, $p = 0.083$).

An integrated synthesis of dispersion method effects across total mass recovery, OC recovery, OC distribution, fC_4 dynamics, and reproducibility is presented in Table 2, summarizing statistical significance, effect magnitude, site consistency, and overall robustness.

Together, these results show that total mass and OC recovery were generally high and comparable between methods overall, though mass recovery exhibited significant, opposing site-specific method effects that canceled out at the aggregate level. In contrast, the two methods differed substantially and consistently in how they distributed OC between fractions and in their capacity to isotopically resolve C pools of distinct turnover rates, with Na-HMP achieving greater isotopic separation between POM and MAOM.

4. Discussion

4.1. US450 vs. Na-HMP: mechanical and chemical dispersion methods isolate different functional SOC pools

This study revealed a clear differences between the two dispersion methods in terms of their ability to disrupt soil aggregates, mobilize OM, and isolate SOC pools with contrasting turnover rates. While US450 proved more effective at separating fractions by particle size (Fig. 2), the Na-HMP method yielded higher-quality isolation of C pools with distinct turnover rates, as reflected by consistently higher Range fC_4 values (Fig. 5). Understanding this distinction requires separating two related processes: “desorption,” referring specifically to the release of OM from mineral surfaces through competitive displacement by phosphate ions (Koorneef et al., 2023; Spohn et al., 2022), and “OM mobilization,” referring more broadly to the transfer of OM into solution and its redistribution between operationally defined fractions during fractionation (Castanha et al., 2008; Heckman et al., 2018). The mechanistic differences between methods can be understood through these two lenses.

In the US450 dispersion method, high-frequency ultrasonic waves physically break apart soil aggregates, releasing OM that was occluded within the mineral matrix while leaving organo-mineral bonds largely intact (Castanha et al., 2008; Schmidt et al., 1999). While this physical disruption is effective for size-based separation, it does not chemically alter the strong binding between OM and mineral surfaces (Kaiser & Guggenberger, 2003; Lützow et al., 2006). As a result, the isotopic composition of OM within each size fraction reflects a mixture of C pools

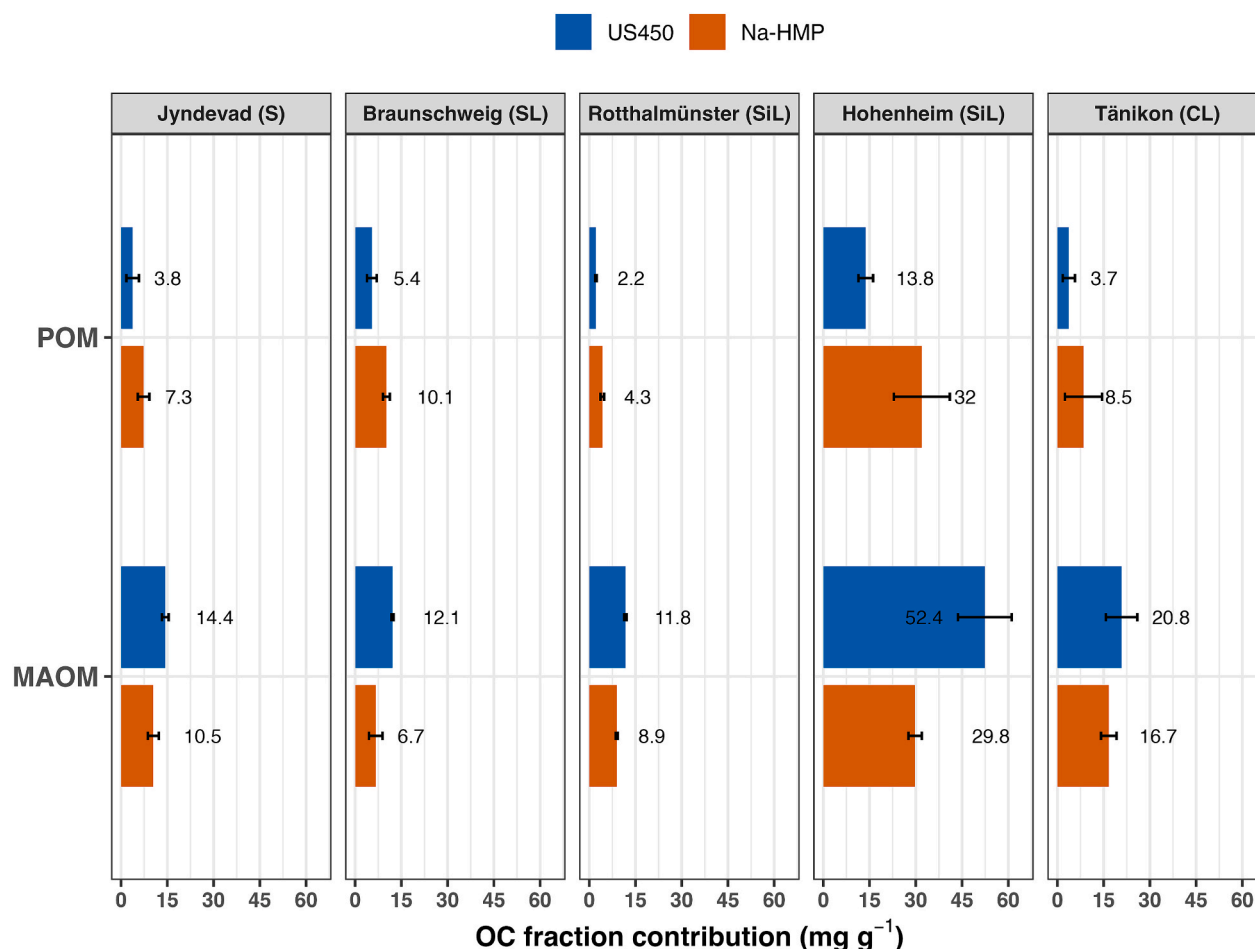


Fig. 3. Absolute OC contributions of POM and MAOM (mg g^{-1} soil) across sites and dispersion methods (Na-HMP, US450). Unlike Fig. 2, which shows proportional OC distribution (%), values here represent the actual mass of OC per gram of bulk soil recovered in each fraction. Bars represent mean $\pm$ SD. OC partitioning differed significantly between fractions and methods (method $\times$ fraction: $p < 0.001$). Code in brackets refers to texture class (S = sand, SL = sandy loam, SiL = silt loam, CL = clay loam)

with varying turnover rates, including both recently C_4 -derived inputs and older C_3 -derived material, rather than a clean separation by age or stability (Shang & Tiessen, 2000). This explains why, in the present study, US450 yielded lower Range fC_4 values than Na-HMP: physical disruption alone redistributes particles by size but does not preferentially concentrate older or newer C into distinct fractions (Shang & Tiessen, 2000; Trumbore & Zheng, 1996).

In contrast, the Na-HMP method chemically disperses soil aggregates by replacing calcium (Ca) and magnesium (Mg) cations with Na cations derived from Na-HMP (Castellini et al., 2013; Levy et al., 1991; Tyler, 1940; Wintermyer & Kinter, 1955). Unlike the mechanical action of ultrasonication, this cation exchange increases the net negative charge on clay surfaces, thereby enhancing electrostatic repulsion and promoting aggregate dispersion without the necessity of physical energy (Castellini et al., 2013; Levy et al., 1991). In addition to aggregate breakdown, the phosphate ions supplied by Na-HMP compete with negatively charged OM for mineral binding sites, facilitating partial desorption of mineral-associated OM into solution (Koorneef et al., 2023; Spohn et al., 2022). This desorption step is key to understanding the higher Range fC_4 observed under Na-HMP.

Mineral surfaces preferentially stabilize older, microbially processed OM through ligand exchange, cation bridging, and electrostatic adsorption (Kaiser & Guggenberger, 2003; Kleber et al., 2007; Lützow et al., 2006). In soils with a C_3/C_4 vegetation history, the MAOM fraction is predominantly derived from the legacy C_3 vegetation and therefore carries a more negative $\delta^{13}\text{C}$ signature (Balesdent et al., 1987;

Sanderman et al., 2013). When Na-HMP's phosphate ions competitively displace this OM from mineral surfaces, the released material, being older and C_3 -enriched by virtue of its prior mineral association, redistributes into the fine fraction during wet sieving, further enriching the MAOM fraction with C_3 -derived C (Sanderman et al., 2013; Shang & Tiessen, 2000). Simultaneously, POM retains a higher proportion of recently C_4 -derived inputs, which have not yet undergone mineral association (Lavalley et al., 2020; von Lützow et al., 2007). This selective redistribution sharpens the isotopic contrast between POM and MAOM, explaining the consistently higher Range fC_4 values observed under Na-HMP across all sites (Balesdent et al., 1987; Yu et al., 2022). However, chemical dispersants can introduce methodological artifacts by mobilizing OM between operationally defined fractions, potentially leading to re-adsorption onto finer mineral surfaces and complicating the interpretation of C origin and turnover (Castanha et al., 2008; Heckman et al., 2018; Parker et al., 2026).

The mobilization of OC by Na-HMP also has broader implications beyond fractionation methodology. Observations of increased OC leaching in Na-HMP-dispersed soils (Just et al., 2021) align with reports of enhanced OM solubilization following rewetting in sodic soils (Wong et al., 2010; Xu et al., 2025), suggesting that the ionic displacement mechanism observed here may operate under natural soil conditions as well. While drawing direct conclusions about C cycling in salt-affected soils falls outside the scope of this study, these parallels suggest that mineral-associated OC may be more sensitive to ionic disturbances than commonly assumed. Previous studies in salt-affected soils have reported

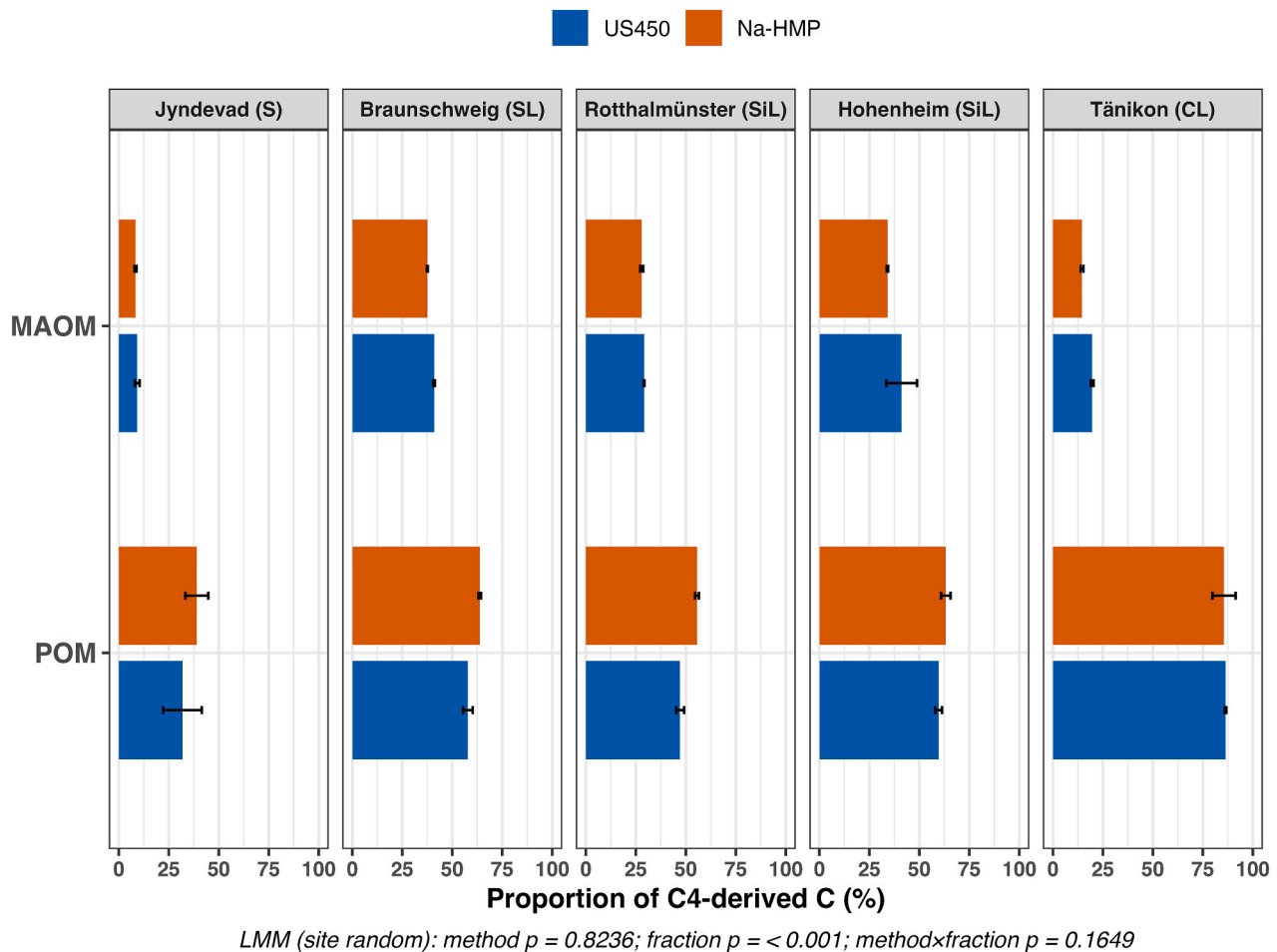


Fig. 4. Proportion of C_4 -derived carbon (fC_4 , %) in POM and MAOM fractions across sites and dispersion methods (Na-HMP, US450). Bars represent mean $\pm$ SD. fC_4 differed significantly between fractions ($p < 0.001$), but no significant method effect ($p = 0.8236$) or method $\times$ fraction interaction ($p = 0.1649$) was detected, confirming that both methods preserved isotopic signals related to C_3/C_4 vegetation shifts.

lower OC concentrations in the MAOM fraction, traditionally attributed to reduced vegetation cover and lower OM inputs (Setia et al., 2012; Wong et al., 2010; Wong et al., 2008). The present findings suggest that ionic mobilization of stabilized OC may represent an additional contributing mechanism, though this interpretation remains speculative and warrants direct experimental validation.

4.2. Na-HMP achieves greater isotopic differentiation between POM and MAOM than US450

This study aimed to evaluate the performance of two dispersion methods (Na-HMP and US450) within the context of soil physical fractionation into POM and MAOM. To assess the effectiveness of these methods, we used natural abundance $\delta^{13}C$ signatures as an independent quality indicator. By using soils with a known shift from C_3 to C_4 vegetation, we tested whether the dispersion methods could successfully separate SOM pools with distinct OC turnover rates. Shifts between C_3 and C_4 vegetation types result in characteristic changes in $\delta^{13}C$ values, enabling the estimation of C turnover and persistence in soil (Balesdent et al., 1987; Cerri et al., 1985; Lefroy et al., 1993). The proportion of C_4 -derived C (fC_4) was consistently higher in the POM fraction than in MAOM across all sites, confirming that the isotopic signals related to the C_3/C_4 vegetation shift were preserved during fractionation by both methods (Fig. 4).

Studies comparing dispersion methods have reported greater particle-size separation under Na-HMP than under ultrasonication (Dupuis et al., 2025), a pattern that contrasts with the present study,

where US450 achieved more complete size-based separation, likely reflecting differences in dispersion energy, soil type, or protocol between studies. However, particle-size separation alone does not capture the quality of isotopic differentiation between fractions. Our study extends this comparison by introducing Range fC_4 as an independent isotopic quality indicator, quantifying the difference in fC_4 between POM and MAOM within the same sample. This metric differed significantly between US450 and Na-HMP (Fig. 5), with Na-HMP consistently producing higher values. Na-HMP, therefore, more effectively separated fractions with contrasting C turnover rates: POM retained a higher proportion of recently C_4 -derived C, while MAOM retained a higher proportion of older C_3 -derived C, sharpening the resolution of age-related differences between fractions. In contrast to Just et al. (2021), who demonstrated that US450 effectively isolates SOM fractions with distinct turnover rates, the present study shows that Na-HMP, despite its lower efficiency in disaggregating soil and separating fractions by size (Just et al., 2021; Kpemoua et al., 2026), consistently achieves higher isotopic separation across all soil textures tested (Table 2, Fig. 5).

The smallest method differences were observed at Tännikon, the site with the highest clay content. This pattern is consistent with the hypothesis of Just et al. (2021) that stronger mechanical dispersion forces are more effective in clay-rich soils, where higher clay content promotes the formation of water-stable macroaggregates that are more resistant to chemical dispersion (Schweizer et al., 2019). If US450 achieves more complete aggregate disruption in clay-rich soils, its fractionation quality would be expected to converge toward that of Na-HMP. This matches the narrower Range fC_4 differences observed at Tännikon. This interpretation

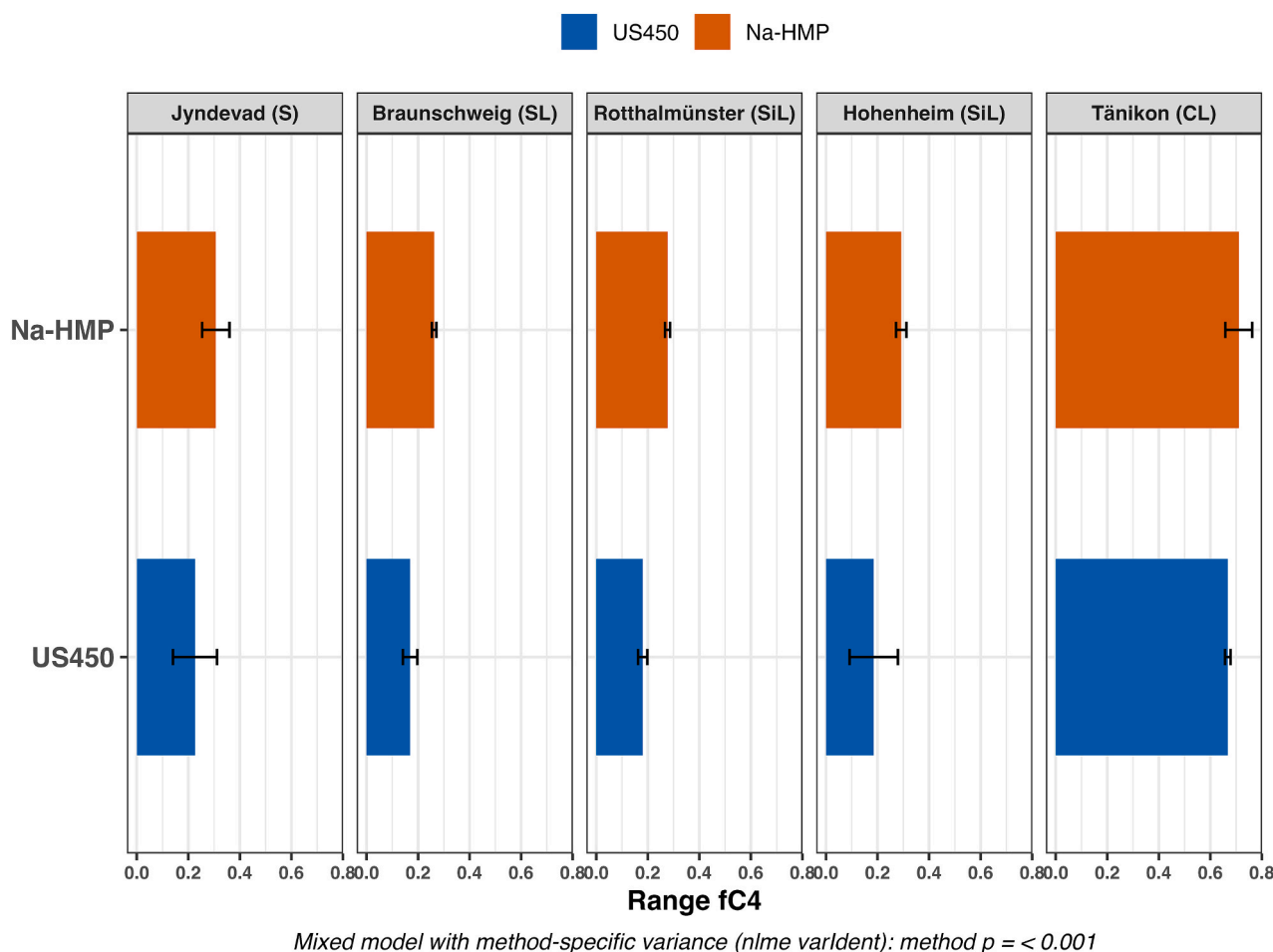


Fig. 5. Isolation quality expressed as Range fC_4 across sites and dispersion methods (Na-HMP, US450). Bars represent mean $\pm$ SD. Range fC_4 differed significantly between methods ($F_{1,19} = 21.26$, $p = 0.0002$), with higher values indicating greater separation between soil fractions with contrasting turnover rates.

is further supported by Kpemoua et al. (2026), who found that Na-HMP was more effective than low-energy ultrasonic dispersion (100 J mL^{-1}) in soils with low clay content, but that this advantage diminished with increasing clay content.

An alternative interpretation, that Na-HMP itself becomes relatively more effective in clay-rich soils, cannot be excluded. However, the convergence at Tänikon is more parsimoniously explained by improved US450 performance under higher clay content, given that Na-HMP showed broadly consistent Range fC_4 values across all sites (Fig. 5), demonstrating stable performance across clay, silty, and sandy soils. These findings suggest that method selection should be guided by the specific research objective: US450 for size-based separation and aggregate disruption, and Na-HMP, where maximizing isotopic contrast between fractions is the priority, as discussed further in the Conclusion.

4.3. Method-dependent OC redistribution: US450 shifts OC allocation towards more MAOM

Both dispersion methods achieved high mass and OC recoveries with comparable reproducibility yet produced fundamentally different outcomes in how OC was distributed between fractions, a finding that reveals more about dispersion mechanisms than about total C preservation. Mass and OC recoveries were statistically equivalent at the overall level, with no significant method effect detected (Fig. S3). However, significant site-level differences emerged at Braunschweig, Hohenheim, and Jyndevad after BH correction, with opposing directions: US450 yielded higher recovery at Braunschweig and Jyndevad, while Na-HMP yielded higher recovery at Hohenheim. These opposing

effects likely canceled out in the overall comparison, underscoring that aggregate-level statistics can mask meaningful texture-dependent behavior. Mean OC recoveries were similarly high (Na-HMP: 92.8%; US450: 95.8%), consistent with the $\sim 95\%$ benchmark reported for comparable fractionation approaches (Just et al., 2021; Poeplau et al., 2018), confirming that neither method introduced substantial C losses during processing. Reproducibility was also comparable between methods, with median CVs of 4.96% for Na-HMP and 2.16% for US450, both within established standards (Poeplau et al., 2018), and no statistically significant differences were detected for either fraction (Fig. S6).

What diverged substantially between methods was not how much C was recovered, but where it ended up. Na-HMP consistently allocated a greater proportion of mass and OC to the POM fraction, particularly in silty soils such as Hohenheim, whereas US450 produced a stronger MAOM-dominated distribution across all sites (Fig. 2). This contrast reflects fundamental differences in aggregate disruption mechanisms. Na-HMP relies on chemical dispersion via cation exchange, which may be insufficient to fully break apart tightly bound or water-stable aggregates, particularly at clay-rich and silty sites (Just et al., 2021; Kpemoua et al., 2026). US450, by contrast, applies direct ultrasonic energy that physically disrupts aggregates regardless of their chemical binding (Christensen, 1992), releasing mineral-associated material that remains occluded under chemical dispersion alone.

Two mechanisms likely drive the stronger MAOM dominance observed under US450. The first is a more complete aggregate disruption, which releases mineral-associated particles that remain physically protected under Na-HMP's milder chemical dispersion. The second is the fragmentation of coarse POM into micro-POM ($<20 \mu\text{m}$) by the kinetic

Table 2

Integrated synthesis of dispersion method effects on total mass recovery, OC recovery, OC distribution, fC_4 proportion, and reproducibility. Response variables correspond to the outcomes reported in Sections 3.1–3.3. Overall significance indicates the result of the primary overall test (Wilcoxon rank-sum or LMM); Site-level significance indicates whether method effects were detected within individual sites (Wilcoxon, BH-adjusted; LMM; or t-tests, Holm-adjusted), with significant sites named where applicable. Magnitude, effect size based on Hedges' g and observed differences: Very small/negligible ($g < 0.3$), Small (0.3–0.6), Moderate (0.6–1.0), Large/Very large ($g \geq 1.0$). Consistency reflects whether effects were detected at $\geq 4/5$ sites (Consistent), at a minority of sites (Site-specific), or at no sites (None).

Response variable	Overall Significance	Site-level significance	Magnitude	Consistency
Total mass recovery	No (Wilcoxon, $p = 0.46$)	Yes — Braunschweig, Hohenheim, Jydevad (LMM, BH-adjusted)	Very small ($< 2\%$ absolute)	Site-specific (3/5)
OC recovery	No (Wilcoxon, $p = 0.12$)	Nonsignificant	Small to large (g up to 1.61, site-dependent)	None
OC distribution between fractions	–	Yes — most sites/fractions (t-test, Holm-adjusted)	Moderate	Site-specific but repeated
fC_4 proportion (fraction effect)	Yes (LMM, $F_{1, 42} = 121.9, p < 0.001$)	–	Very large	Consistent
fC_4 proportion (method effect)	No (LMM, $p = 0.82$)	–	–	Consistent
fC_4 proportion (method $\times$ fraction interaction)	No (LMM, $p = 0.16$)	–	–	Consistent
Range fC_4	Yes (LMM, $F_{1, 19} = 21.26, p = 0.0002$)	–	Moderate-large	Consistent
Reproducibility (CV)	No (Paired Wilcoxon, $p = 0.083$)	–	Small	Consistent trend

energy of sonication, causing fine organic particles to be operationally assigned to the MAOM fraction (Just et al., 2021; Schmidt et al., 1999). Lower ultrasonic energies, such as the 100 J mL^{-1} , also tested by Just et al. (2021) as a lower-energy comparator, may offer a practical compromise, reducing POM fragmentation while still achieving adequate aggregate disruption, though at the cost of potentially incomplete dispersion in clay-rich soils. The resulting contamination of the MAOM fraction with fine POM is an inherent limitation of size-based fractionation approaches (Poeplau et al., 2025). Mass distribution data suggest that aggregate disruption is likely the dominant driver: US450 consistently produced larger absolute MAOM masses across all sites, including those with lower clay content, where POM fragmentation would be less expected (Fig. 3). POM fragmentation may represent a secondary effect, more pronounced in soils with high POM content or where coarse organic particles are more susceptible to sonication, consistent with Just et al. (2021), who showed that mass redistribution from the $>20 \mu\text{m}$ to the $<20 \mu\text{m}$ fraction with increasing dispersion intensity was most pronounced in silty and clay-rich soils, precisely the sites where the largest method-dependent differences were also observed in the present study.

A critical consequence of this redistribution is its effect on isotopic separation. Part of the OC detected as MAOM after US450 dispersion may originate from physically disrupted POM rather than from inherent

mineral-associated C, artificially increasing MAOM-OC contributions and reducing the isotopic contrast between fractions, directly reflected in the lower Range fC_4 values under US450. This mechanistic link between aggregate disruption, OC redistribution, and isotopic separation quality is a central finding of this study and highlights that fractionation outcomes are shaped as much by dispersion mechanism as by soil properties themselves.

The energy applied in this study (450 J mL^{-1}) represents a relatively high ultrasonic input. Lower energies, for example 100 J mL^{-1} , have been shown to achieve adequate aggregate disruption while reducing the risk of POM fragmentation (Just et al., 2021; Kpemoua et al., 2026). Depending on the research objective and soil type, a lower energy input may therefore represent a more appropriate compromise between dispersion efficiency and fraction integrity.

Overall, while total OC recovery was unaffected by method choice, the allocation of OC between fractions differed substantially. This shows that dispersion methods do not merely separate existing pools but can actively influence their apparent distribution. Consequently, method selection is critical and should be reported transparently, particularly in studies addressing C stabilization mechanisms or comparing datasets across sites.

5. Conclusion

This study demonstrates that sodium hexametaphosphate (Na-HMP) and ultrasonication at 450 J mL^{-1} (US450) differ fundamentally in how they disrupt soil structure, allocate OC between fractions, and isolate C pools of contrasting turnover rates. While both methods achieved high mass and OC recoveries with comparable reproducibility across soil textures, they do not produce equivalent fractionation outcomes.

US450 proved more effective at mechanical aggregate disruption and size-based separation across all sites tested, consistently promoting a stronger MAOM-dominated OC distribution, reflecting more complete release of mineral-associated material. In contrast, Na-HMP, through chemical dispersion and cation exchange, enhanced isotopic differentiation between POM and MAOM, resulting in greater separation of C_3 - and C_4 -derived C pools despite less pronounced particle-size separation. The stronger MAOM-dominated distribution observed under US450 likely stems from both more complete aggregate disruption and, secondarily, the fragmentation of POM into micro-POM by sonication energy, which causes fine organic particles to be operationally assigned to the MAOM fraction. These findings support clear methodological recommendations: US450 is recommended when size-based fractionation and structural aggregate separation are the primary objectives, particularly in clay-rich soils; Na-HMP is the more suitable choice when the goal is to maximize isotopic differentiation and isolate C pools with distinct turnover rates.

This Na-HMP-induced isotopic differentiation likely arose through partial redistribution of mineral-associated C via desorption of OM from mineral surfaces. While the isotopic evidence is consistent with this interpretation, the present results do not provide direct proof of MAOM-OC mobilization; this mechanism should therefore be regarded as a working hypothesis requiring dedicated experimental validation. The observed patterns are also aligned with implications for C dynamics in salt-affected soils, where elevated ionic concentrations may produce analogous effects on organo-mineral associations; however, this remains speculative given the scope of the present study and warrants direct investigation under field-relevant conditions.

The retention of Na-HMP salt in isolated fractions, as applied in this study to minimize OC loss during fractionation, should be considered when planning downstream analyses. Residual salt can alter pH, cation-exchange properties, microbial activity, and other analytical responses (Koorneef et al., 2023; Parker et al., 2026). Researchers should therefore evaluate whether salt removal or retention is more appropriate given their specific analytical objectives, weighing the risk of OC loss during washing against potential interference in downstream measurements.

Overall, the quality of SOM fractionation depends not only on the efficiency of aggregate disruption but also on the chemical interactions induced during dispersion. Method selection should therefore be explicitly aligned with research objectives and soil properties, and the chosen protocol should be reported transparently to ensure comparability across studies. Future work should further investigate the extent to which chemical dispersants mobilize stabilized C pools and how these processes relate to SOC dynamics under salinity and climate stress.

CRedit authorship contribution statement

Ananda Haberler: Writing – original draft. **Kaiyu Lei:** Writing – review & editing. **Franz Buegger:** Writing – review & editing. **Franziska Bucka:** Writing – review & editing. **Christopher Just:** Writing – review & editing.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) and Claude (Anthropic) to assist with refining sentence structures and manuscript preparation. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2026.118028>.

Data availability

Data will be made available on request.

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