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Mineral associated organic carbon correlates with soil health in agricultural fields

Erin D Lane · Kathryn White · Alissa White · Julia Siegel · Heather Darby · Caitlin Hicks Pries

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Abstract Increasing terrestrial carbon storage can reduce atmospheric carbon dioxide levels as a climate mitigation strategy. Agricultural soil offers potential for persistent soil carbon sequestration. Mineral-associated organic carbon (MAOC) is generally more persistent than particulate organic carbon (POC), but it is unclear how much influence soil health and management can have on MAOC relative to climate and edaphic conditions. Soil samples from 196 agricultural fields on 77 farms throughout Vermont were fractionated by size to understand how these factors affect the amount and proportions of POC and MAOC. Our results reveal a significant effect of crop type, climate, soil texture, and aggregate stability on these carbon fractions. Hay and pasture fields had on average 69% more POC and 30% more MAOC than corn and vegetable fields. Both POC and MAOC increased with improved soil health scores suggesting that healthier soils store more carbon in both fractions. MAOC dynamics also varied with soil texture. In clay-rich soils, MAOC increases with mean annual temperature (MAT), particularly in regions with higher mean annual precipitation (MAP). However, this effect is the opposite in soils with lower clay content in which MAOC decreases with higher MAT and MAP. Overall, our findings demonstrate that MAOC can be influenced by management, highlighting how effective soil management strategies aimed at increasing soil health like planting perennials can also enhance long-term carbon sequestration in agricultural soils.

Keywords Particulate organic carbon · Mineral-associated organic carbon · Agricultural soil · Soil health · Aggregate stability · Size fractionation · Climate mitigation strategy

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E. D. Lane (✉)
USDA Forest Service Northern Research Station, 271 Mast Rd, Durham, NH 03824, USA
e-mail: erin.d.lane@usda.gov

E. D. Lane · J. Siegel · C. Hicks Pries
Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA

E. D. Lane
Ecology, Evolution, Environment, and Society Program, Dartmouth College, Hanover, NH 03755, USA

K. White
Department of Crop and Soil Sciences, Agroecology Lab, North Carolina State University, Raleigh, NC 27695, USA

A. White · H. Darby
Department of Agriculture, Landscape, and Environment, University of Vermont, Burlington, VT 05405, USA

A. White
American Farmland Trust, 2000 Pennsylvania Avenue NW, Washington, D.C. 20006, USA

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Introduction

The more carbon that is stored on land, the less carbon dioxide there is in the atmosphere acting as a greenhouse gas (GHG). Increasing the terrestrial carbon sink complements efforts to reduce fossil fuel combustion, which is important given that achieving immediate reduction in anthropogenic GHG emissions is not entirely feasible (IPCC 2022; Kalghatgi 2020). Soil carbon accounts for ~ 70–80% of the terrestrial carbon sink (Friedlingstein et al. 2022; Scharlemann et al. 2014). Land management decisions can affect terrestrial carbon causing either the release of carbon dioxide (CO_2), contributing to climate change, or an increase in carbon sequestration (Lal 2004; Paustian et al. 2016). Managing farms to increase soil carbon sequestration could play a substantial role in reducing the impacts of climate change (Finzi et al. 2020; Griscom et al. 2017; Minasny et al. 2017; Poeplau & Don 2015).

The factors affecting the capacity of agricultural soil to serve as a carbon sink are complex and uncertain, in part due to the heterogeneous nature of soil (Lehmann & Kleber 2015). To comprehensively assess soil carbon on farms, scientists need to account for this heterogeneity through sampling and statistical methodologies (Basile-Doelsch et al. 2020). Soil carbon can be conceptually split into pools and physically separated into fractions, which can help explain its formation and persistence amidst complexity (Heckman et al. 2022; Stanley et al. 2023). Two common empirical fractions—particulate organic carbon (POC) and mineral-associated organic carbon (MAOC)—form differently, react independently, and vary in persistence (Lavallee et al. 2020). Because it is more available to microbes, POC is more responsive to management and more vulnerable to loss (Rocci et al. 2021; Wooliver & Jagadamma 2023; Zhang et al. 2024). Through interactions with soil minerals, MAOC is protected from microbes and consequently can be more resistant to change (Canisares et al. 2023; Cotrufo et al. 2019). Generally microbially processed, MAOC is less sensitive to temperature-induced losses than POC though MAOC has been shown to increase with increasing soil temperature because of increased decomposition of POC. (Benbi et al. 2014; Dignac et al. 2017; Prairie et al. 2023; Rocci et al. 2023; van Wesemael et al. 2019).

Generally, the more persistent soil carbon is MAOC. The potential of soil carbon for GHG mitigation depends on its persistence. Because MAOC is more durable than POC, it remains in the soil longer, delaying release to the atmosphere (Heckman et al. 2022). Therefore, the amount of MAOC is a key attribute for determining the contribution of soil to climate mitigation. Identifying what drives the amount of carbon in each fraction on farms can help us understand the capacity of agricultural soil to sequester carbon long-term.

The formation and persistence of POC and MAOC are controlled by soil biology, edaphic factors, and climate. Carbon inputs from plant residues and root exudates contribute to POC and MAOC, respectively (Heckman et al. 2023; Villarino et al. 2021). Microbial activity can increase POC decomposition and MAOC formation (Canisares et al. 2023; Córdova et al. 2018; Sokol et al. 2019) via stabilization of microbial necromass (Creamer et al. 2019). Soil texture, like clay, facilitates the formation of stable aggregates, which can stabilize both POC and MAOC (Song et al. 2022). Sensitivity to changes in pH affects mineral solubility and thus the capacity of MAOC to sorb carbon (Bailey et al. 2019; Song et al. 2022; Ye et al. 2018). The capacity to sorb carbon is also affected by the type, crystallinity, and weathering status of soil minerals (Mikutta et al. 2006; Torn et al. 1997; Xu et al. 2022). Since MAOC formation can be limited by the availability and suitability of minerals, soils can become saturated with MAOC (Georgiou et al. 2022). In terms of climate, POC is less protected from soil microbes and is more sensitive to warming induced losses from increased microbial decomposition than MAOC (Benbi et al. 2014; Rocci et al. 2023; Soong et al. 2021; van Wesemael et al. 2019). In contrast, MAOC may be more sensitive to soil moisture conditions (Heckman et al. 2022) and organo-mineral associations can be destabilized in wet soils (Huang & Hall 2017). Some, but not all, of these controls can be affected by management.

Multiple studies have identified the relationship between land management and soil carbon (Minasny et al. 2017; Paustian et al. 2016), but there is less information on how land management practices affect the partitioning of carbon among soil fractions. Use of nitrogen fertilizers generally increases POC and MAOC through increased plant productivity (Ramesh et al. 2019; Rocci et al. 2021).

Disturbed cropland soils can have reduced MAOC (Panchal et al. 2022; Sokol & Bradford 2019). No-till and cover crops have long been assumed to build soil carbon, but the observed effects on soil organic carbon (SOC) stocks and mineral associations are variable (Jilling et al. 2020; Poeplau & Don 2015; Powlson et al. 2011; Wooliver & Jagadamma 2023). The slow rates of change in MAOC can make it difficult to observe direct management effects (Plaza-Bonilla et al. 2014). However, the available evidence suggests that the same management practices that increase total soil carbon can increase MAOC. In pastures, MAOC is more abundant with intensive rotational grazing compared to continuous grazing (Bai et al. 2019; Grandy et al. 2009; Mosier et al. 2022). Prairie et al. (2023) found cropping intensification, no-till adoption, and crop livestock integration increased MAOC by 20%.

Soil health indicators, which are commonly measured to evaluate soil management practices, may help us better link soil carbon fractions with soil management practices (Abbas et al. 2020; Dignac et al. 2017). Soil health is theoretically defined as the capacity of the soil to function and sustain life (Lal 2016), but in practice it is an aggregated numerical score of several empirically measured soil physical, chemical and biological attributes such as aggregate stability, nutrient content, active carbon, and soil respiration (e.g., Cornell's Comprehensive Assessment of Soil Health; (Moebius-Clune et al. 2016)). Given that soil health assessments are a more commonly performed and accessible measurement than soil fractionation, it is important to know whether POC and MAOC fractions can be inferred from individual soil health indicators and aggregated soil health scores (Christy et al. 2023; Culman et al. 2012). However, how POC and MAOC relate to soil health indicators or overall soil health scores is largely unknown (Cotrufo et al. 2019; Lavallee et al. 2020). Since the amount of soil organic matter is a key soil health metric, it is likely that POC will be directly related to overall soil health scores (Guo 2021; Moebius-Clune et al. 2016). The extent to which MAOC relates to soil health scores is less clear, but MAOC is likely positively associated with physical indicators like soil texture and aggregate stability that affect the capacity of minerals to sorb carbon (C) and the ability of microbes to access C, respectively (Basile-Doelsch et al. 2020; Cotrufo et al. 2013).

We quantified POC and MAOC in 196 agricultural fields across Vermont, USA to explore their relationships with climate, edaphic conditions, management, and soil health indicators. This information will help us understand the capacity of regional agricultural soil to store MAOC (Georgiou et al. 2022) and farmers' ability to influence MAOC. We expect pastures to have more MAOC than other crop types because they are often less disturbed by tillage, contain perennial crops, and receive manure inputs, allowing for more microbial activity that transfers carbon into the MAOC pool. We expect soils with higher clay content to have more MAOC because the increased proportion of clay-sized particles provides more mineral surface area for organic matter sorption. We predict that POC concentrations will decrease with increased mean annual temperature, but MAOC will not be affected. Lastly, we anticipate a positive correlation between POC and soil health scores due to the relationship between total soil carbon and soil health (Lal 2016), but relationship between soil health scores and MAOC will be difficult to discern, since MAOC responds slowly to soil health practices.

Methods

We separated soil organic matter samples by size into particulate (> 53 μm) and mineral-associated (< 53 μm) fractions as part of the State of the Soil Health project (White et al. 2022). We assessed soil characteristics, climate, soil health indicators, and soil carbon concentrations across agricultural systems. In addition to soil samples, we collected information about the farms including location, crop type, and field management history (available for 27% of the fields). Our specific focus within this broader project was to investigate the effects of management, soil texture, and climate on the fractions of soil carbon, as an indicator of soil carbon persistence.

Study sites

We sampled soil from 196 agricultural fields on 77 farms throughout Vermont. Sites were chosen based on participation in existing research projects, and several farms were added to extend the geographic extent (Fig. 1). Each field was categorized by crop type, defined as: corn (silage), hay, pasture, wheat,

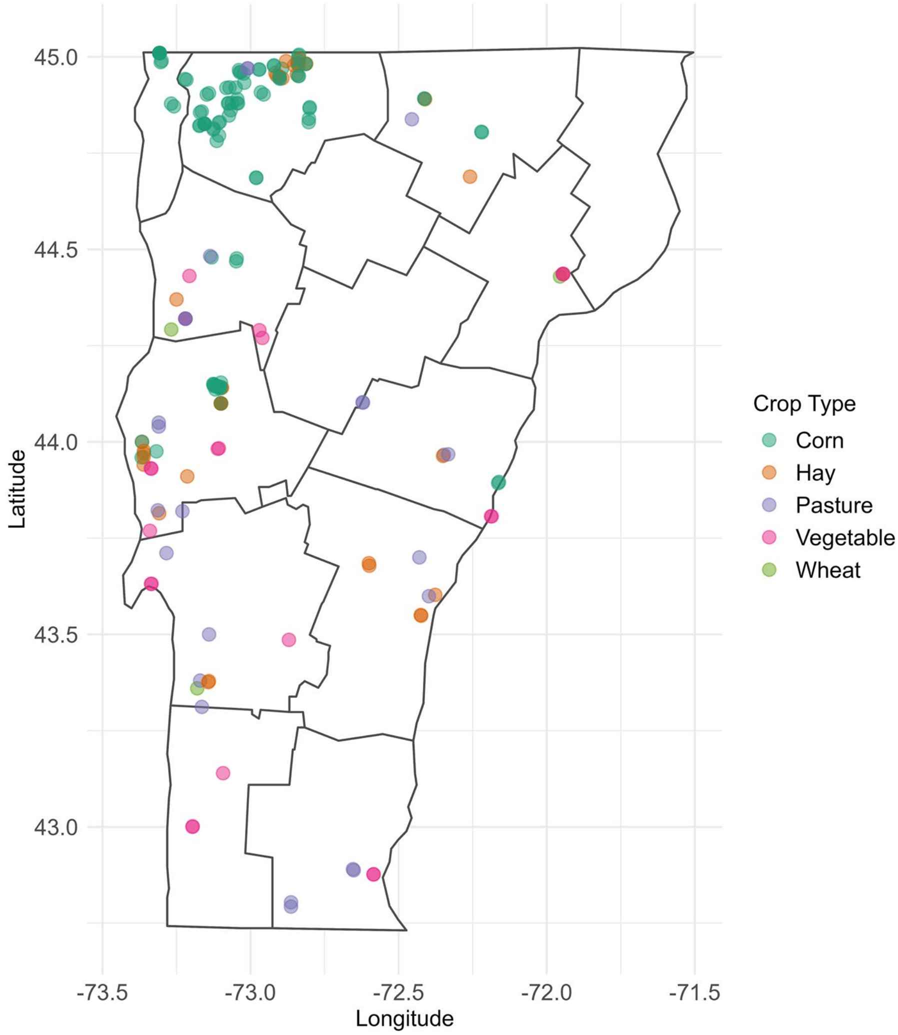

Fig. 1 Locations of sample points labeled by crop type

or vegetable (examples: strawberries, kale, brassicas, garlic, and mixed vegetables). Only two samples came from wheat fields, so wheat fields were excluded from crop type analyses due to low sample size. Fields were classified as perennial vegetation if they were in hay or pasture at the time of sampling or classified as annual vegetation if corn, wheat or vegetables were planted. Farmers reported their tillage practices for the previous year, indicating either no-till or the depth of tillage. We received reports on tillage for 52% of the fields we sampled.

Climate data for each field was derived from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) using geographic coordinates and the data explorer. Data for mean annual temperature and precipitation are interpolated averages of monthly normals for that location between 1991 and 2020 at 800 m resolution (PRISM Climate Group 2024). Overall, the farm fields span a range of soil characteristics, crop types, and climatic conditions found in Vermont. Soil carbon concentrations (to 30 cm) ranged from 1.45 to 8.68% (mean 4.28%). Soil clay content ranged from 6.20 to 53.7% (mean 21.31%). Mean annual temperature (MAT) over the past 30 years ranged from 4.56 to 8.56 °C (mean 7.18 °C) and mean annual precipitation (MAP) ranged from 93 to 141 cm (mean 106 cm).

Soil sampling and CASH

During the spring, summer, and fall of 2021, we collected soil using the Comprehensive Assessments of

Soil Health (CASH) protocol for sample collection (Moebius-Clune et al. 2016). This protocol consists of collecting ten subsamples of the top 15 cm of soil (organic litter removed from the surface) in a zig-zag pattern across each field using a sharp trowel. The subsamples were composited and homogenized, then portioned for the standard CASH analysis from Cornell University Soil Health Lab (Ithaca, NY) and soil fractionation at Dartmouth College's Hicks Pries Lab (Hanover, NH). All soil samples for fractionation were kept frozen at −20 °C until processing. During sampling of each field, researchers used a 2-inch diameter slide hammer probe to collect three 30 cm deep cores along a transect. These samples were processed by the University of Vermont Agricultural and Environmental Testing Laboratory to determine dry mass and percentage carbon (White et al. 2022). Following standard laboratory procedures, soil samples were homogenized and sieved to 2 mm to obtain the fine earth component and coarse fragments (> 2 mm) were separated and accounted for volumetrically. Bulk density was calculated using the oven-dry mass (< 2 mm) and the measured core volume, with correction for coarse fragments (Soil Survey Staff 2014). For the subset of samples used in the fractionation analysis for which both bulk density and percent carbon data were available (n = 165), soil carbon stocks (*SOC*, kg C m^{-2}) to 30 cm depth were calculated by multiplying carbon concentration (*C*, %) by bulk density (*BD*, g cm^{-3}) and depth (30 cm) with a factor of 10 for unit conversions.

$$SOC = \frac{C}{100} x BD x 30 x 10$$

The overall soil health score is a unitless index from the CASH framework (Fine et al. 2017) that integrates ratings from biological, chemical, and physical metrics (SI, Table S1). The scores are derived from comparisons of these metrics to previously analyzed samples with similar edaphic and regional attributes in the CASH database. The CASH training manual (Moebius-Clune et al. 2016) describes each part of the CASH process including rationale for including each metric with links to the Standard Operating Procedures for processing and analyzing the samples. The metrics used in our statistical models were active carbon, aggregate stability, pH, soil texture, and the overall soil health score. Soil texture was determined by particle size analysis and clay defined as particles < 2 microns. We did not determine clay mineralogy in our analysis. The active carbon procedure is especially relevant to our study, so we describe it here. Permanganate-oxidizable carbon (PoxC) is included in the soil health protocol as a measure of active carbon. The soil was treated with a potassium permanganate ($KMnO_4$) solution (0.02 M), which oxidizes and loses color proportionally as it reduces. The change in color was measured in comparison to $KMnO_4$ standards with a spectrophotometer. The proportion of C oxidized was determined based on the stoichiometric oxidation of 0.75 mol C per mol $KMnO_4$.

Fractionation

Soil was physically fractionated based on size: POC is larger (> 53 µm) and MAOC smaller (< 53 µm). While either size or density can be used to fractionate soil carbon (Cotrufo et al. 2019; Six et al. 2002), we chose size fractionation because it is quicker and less labor-intensive given our 196 samples (Cambardella & Elliott 1993). First, each sample was sieved to 2 mm to remove rocks and roots and break up clods. Then the samples were dried at 90 °C for at least 48 h or until the sample yielded a consistent mass. We then placed approximately 20 g (± 8 g) of the prepared sample into a bottle in which we added a 0.5% sodium hexametaphosphate (SHMP) solution. The bottle was then placed on a shaking table for 24 h to disperse and disrupt aggregates (Keskinen et al. 2024). Samples were then wet-sieved using MilliQ water (Merck Millipore) and a 53-micron sieve to separate the larger particulate fraction (> 53 µm) remaining on the top of the sieve from the smaller mineral-associated fraction (< 53 µm) falling through the sieve. We acknowledged that, as is typical for this fractionation method, the mass of SHMP added is included in the MAOC fraction. We determined the SHMP contributed approximately 2.5% of the total sample mass and that this mass increase was only marginally significantly different from zero as determined by a one-sided *t*-test ($p = 0.052$). The methodology we used for size fractionation disrupts macroaggregates prior to the finer sieving, so fine, mineral-associated carbon from within aggregates was included in our MAOC fraction and any larger SOC from within aggregates was included in our POC fraction. Each fraction was dried at 90 °C for 48 h before being finely ground and weighed (20 to 40 mg) into tin capsules for carbon and nitrogen analysis using an ECS4010 elemental analyzer (Costech Analytical Technologies, Valencia, CA). Inorganic carbon, in addition to the organic carbon we are trying to measure, could be included with this type of combustion measurement. High pH is an indicator that inorganic carbon may be a substantial soil component and only 19% of our samples had pH greater than 7. To assess the potential for impact of inorganic carbon on our results, the presence of inorganic carbon was qualitatively tested in these samples. Hydrochloric acid (HCl, 1 mol/L) was added to 1–2 g of ground sample. The observation of bubbles within 5 min was noted as soils containing carbonate (Nelson 1996). For those samples, we removed the inorganic carbon using HCl (~10 ml HCl added to ~10 g sample) and then re-analyzed the sample on the elemental analyzer for carbon and found the change in carbon detected to be negligible.

Data analysis

Statistical analyses and plotting were conducted using R software version 2024.04.2 (R Core Team 2024) and results were considered statistically significant at an alpha level of 0.05. Response variables of interest were the concentrations of POC and MAOC (mg g^{-1} dry soil) and the proportion of carbon as MAOC as calculated by the amount of MAOC divided by the sum of POC and MAOC. The proportion of carbon as MAOC was transformed using the logit function to meet statistical assumptions.

We first compared the effects of crop type on soil carbon fractions and soil stocks to 30 cm with an Analysis of Variance (ANOVA). The ANOVA was performed using the generalized least squares (gls) function [nlme package (Pinheiro 2021)] in R so that we could include a variance structure by crop type because the model assumption of homoscedasticity was not met. Post-hoc pairwise comparisons using Tukey's Honestly Significant Difference (HSD) test were used to assess differences among crop types. Next, we assessed how potential predictor variables varied among crop types using the same ANOVAs and post hoc tests. The predictor variables included MAT, MAP, pH, clay content and the soil health indicators, aggregate stability and active carbon (PoxC), as well as the overall soil health score. These predictors were chosen because they are related to mechanisms of soil carbon formation and persistence.

Next, we performed multiple linear regressions using the gls function (Pinheiro 2021) in R to analyze the influence of the aforementioned predictors on concentrations of soil carbon fractions and the proportion of carbon as MAOC. A Pearson correlation matrix was constructed to examine the potential for multicollinearity between these predictors using the corrplot function in R (Wei & Simko 2024). All predictors exhibited correlation coefficients less than 0.49, so we included them all in the models. Expert knowledge and visualizations of the relationships between sets of predictor variables were used to identify potential interactions. Interactions that could be visually detected informed the model structure. The models included an interaction between clay, MAT, and MAP for MAOC and for the proportion of carbon as MAOC, and between MAT and MAP for POC. We tested for potential spatial autocorrelation (SI, Figure S1) by comparing models with and without an exponential spatial autocorrelation structure using semivariograms. The model with spatial autocorrelation did not improve the semivariogram, which showed limited evidence of autocorrelation to begin with (SI, Figure S2). For POC only, the model with spatial autocorrelation improved the Akaike Information Criterion (AIC) by 9 units, but its results were practically the same as the model without it (SI, Table S3). Thus, we chose to use the simpler model for all response variables. Lastly, we used stepwise backward selection by dropping predictors that did not improve the AIC values to select the most parsimonious model for each fraction. Estimates of the regression coefficients, their standard errors, t-values, and associated p-values were used to determine if the effects of the remaining predictors on the response variable were significantly different from zero. Response variables were plotted against the statistically significant drivers using ggpredict from the package ggeffects (Lüdecke 2020). Model assumptions were checked by examining residuals for violations of normality and homoscedasticity and by creating partial residual plots to confirm linearity. Model performance was evaluated by calculating a pseudo coefficient of determination (r^2) of the relationship between the data and the model predictions. Finally, linear regressions were used to assess the relationship between soil health metrics and POC, MAOC, and the proportion of SOC as MAOC using the lm function in base R. Significant relationships based on p-values ($\alpha < 0.05$) of regression coefficients were visualized with scatterplots.

Results

Crop type had a significant effect on the concentration of POC ($F_{3,189} = 17.28$, $p < 0.0001$), the concentration of MAOC ($F_{3,189} = 5.53$, $p = 0.001$), and the proportion of carbon as MAOC ($F_{3,189} = 3.98$, $p = 0.008$; Table 1) in the top 15 cm of soil. Post-hoc comparisons indicated perennial hay and pasture fields had higher carbon concentrations in both fractions than annual corn and vegetable crops (Table 1). Hay and pasture fields had 69% greater concentration of POC than corn silage and vegetable fields ($p < 0.001$). Similarly, there were significantly higher MAOC concentrations in hay fields compared to corn fields ($p = 0.0048$). Pastures had a significantly lower proportion of carbon as MAOC compared to corn fields ($p = 0.0062$). Carbon stocks to 30 cm also differed with hay fields having 17% more carbon than corn silage fields ($p = 0.038$), 32% more carbon than vegetable fields ($p = 0.011$). Soil carbon stocks did not significantly differ between hay fields and pastures.

Differences among field types may be due to differences in management. Among the 52% of farms that reported, 95% of pastures and 59% of hay fields were in no-till management consistent with typical regional practices for established perennial systems; reported tillage may reflect establishment phase. In contrast, no-till management was far less common in annual cropping systems, occurring in only 7.5% of corn silage fields and 4.5% of vegetable fields (SI, Figure S3). Carbon stocks to 30 cm showed increased stocks in fields under perennial crops (SI, Figure S4). Aggregate stability was 83% greater in fields under perennial crops than annual crops ($F_{1,189} = 65.41$, $p < 0.0001$) (SI, Figure S5). Mineral-associated organic carbon concentration was less affected by crop type (Table 1) though MAOC in hay fields was 33% greater than in soils under silage corn (SI, Table S3). Likewise, crop type had little effect on the proportion of carbon as MAOC. However, because of the significant increase in pasture POC and the relatively smaller effect on pasture MAOC compared to corn silage fields, the proportion of carbon as MAOC in pastures was lower (Table 1).

On the other hand, differences among crop types may also be driven by differences in predictors related to mechanisms of soil carbon formation and persistence. We found no significant difference in MAT among crop types ($F_{3,187} = 2.45$, $p = 0.652$), but MAP did differ significantly ($F_{3,187} = 4.92$, $p = 0.0026$; SI, Table S4). Pastures had higher MAP than corn silage fields ($p = 0.018$). Clay also differed significantly ($F_{3,187} = 2.78$, $p = 0.042$; SI, Table S4) with pastures having greater clay content than vegetable fields ($p = 0.043$). Among soil health metrics, active carbon did not differ among fields ($F_{3,187} = 0.86$, $p = 0.463$; SI, Table S4), but aggregate stability ($F_{3,187} = 17.63$, $p < 0.0001$) and pH did ($F_{3,187} = 6.04$, $p < 0.0001$). Aggregate stability was significantly greater in perennial field types (hay and pasture) than annual (corn

Table 1 Mean ± standard error of POC, MAOC and proportion of carbon as MAOC in the top 15 cm and carbon stocks to 30 cm

Crop type	n Fractions *stocks*	POC (mg C g^{-1} soil)	MAOC (mg C g^{-1} soil)	Proportion of carbon as MAOC	Carbon stocks to 30 cm (kg C m^{-2})
Hay	33 *29*	10.32 ± 1.10^{bc}	20.12 ± 1.37^{b}	0.66 ± 0.024^{ab}	12.9 ± 0.673^{b}
Pasture	24 *15*	12.91 ± 1.15^{c}	19.49 ± 1.67^{ab}	0.60 ± 0.029^{b}	12.2 ± 0.670^{ab}
Corn silage	114 *102*	6.45 ± 0.273^{a}	15.11 ± 0.628^{a}	0.69 ± 0.011^{a}	11.0 ± 0.293^{a}
Vegetable	22 *17*	8.38 ± 1.31^{ab}	16.54 ± 1.63^{ab}	0.67 ± 0.022^{ab}	9.77 ± 0.863^{a}

Within a column, letters not shared are significantly different ($\alpha < 0.05$). The top value for n is the sample size for soil fractionations and bottom n in italics is the sample size assessed for total carbon stocks to 30 cm silage and vegetable) ($p < 0.001$). Vegetable fields had a higher pH than corn silage fields ($p = 0.001$) and pastures ($p = 0.003$). Soil health scores differed significantly ($F_{3,187} = 5.92$, $p < 0.0001$), but only in that hay fields scored more highly than corn silage fields ($p = 0.001$; SI, Table S4).

Overall, our multiple regression model (SI, Table S3) explained more of the variability in the concentration of POC ($n = 194$, $r^2 = 0.48$) and the concentration of MAOC ($n = 194$, $r^2 = 0.36$), than the variability in the proportion of carbon as MAOC ($n = 194$, $r^2 = 0.11$). Concentrations of POC and MAOC significantly varied with management (crop type), edaphic, and climatic variables, as well as soil health scores (Table 2). Aggregate stability ($t_{194} = 4.89$, $p < 0.0001$), active carbon ($t_{194} = 7.46$, $p < 0.0001$), and the interaction between temperature and precipitation ($t_{194} = -3.36$, $p = 0.0007$) were significant predictors for the concentration of POC. Increased active carbon and aggregate stability were predictors of higher concentrations of POC (Fig. 2a, b), with a wide range of POC concentrations observed when aggregate stability was high. At lower MAT, an increase in precipitation increased POC concentrations, while at higher MAT, precipitation had little effect on POC (Fig. 3a). Clay content, pH, and location were not significant predictors of POC in the model, and they were dropped.

As with POC, aggregate stability ($t_{194} = 4.29$, $p < 0.0001$) and active carbon ($t_{194} = 6.77$, $p < 0.0001$) were significant predictors of MAOC. More stable aggregates and higher active carbon concentrations resulted in increased MAOC concentrations (Fig. 2c, d). The interaction between MAP, soil texture (clay content), and MAT was also a significant predictor of MAOC ($t_{194} = 1.99$, $p = 0.048$; Fig. 3b). In clay-rich soils, MAOC increased with increasing temperature, especially for farms that received higher precipitation. However, the effect diminished in soils with lower clay content. In soils with less clay and higher precipitation, there was no relationship between MAOC and temperature (Fig. 3b). As with POC, pH was not a significant predictor of MAOC concentrations in the model and was dropped.

The proportion of carbon as MAOC was not significantly affected by aggregate stability ($t_{194} = 0.67$, $p = 0.506$) or predicted by active carbon ($t_{194} = -1.17$, $p = 0.242$). Instead, clay content ($t_{194} = 2.25$, $p = 0.025$) and interactions of MAT and MAP with clay ($t_{194} = 2.47$, $p = 0.015$) were significant predictors. Higher percentages of clay were associated with greater proportions of carbon as MAOC. The effect of precipitation on MAOC proportion varied with clay content and temperature. In clay-rich soils, the proportion of carbon as MAOC increased steeply with an increase in temperature, but this was only true at high MAP (Fig. 3c). When MAP was lower, the proportion of carbon as MAOC increased slightly with increased temperature, regardless of clay content. Again, pH was not a significant predictor of proportion of carbon as MAOC and was removed from the model.

Overall, there was a positive relationship between soil health scores and POC concentrations ($r^2 = 0.28$, $F_{192} = 74.64$, $p < 0.0001$, slope = 0.26 mg POC soil health score unit^{-1}). Similarly, the positive relationship between overall soil health score and MAOC concentrations was significant ($r^2 = 0.26$, $F_{192} = 68.13$, $p < 0.0001$, slope = 0.38). However, the proportion of carbon as MAOC exhibited a weaker yet significant negative relationship to soil health score ($r^2 = 0.021$, $F = 4.173$, $p = 0.042$, slope = −0.008), indicating reduced MAOC as a proportion of total C with increasing soil health scores (Fig. 4). The relationship between POC and MAOC concentrations is also positive ($r^2 = 0.09$, $F_{194} = 21.29$, $p < 0.0001$; Fig. 5). Concentration of MAOC as a function of clay and silt content (slope = 0.054, $p = 0.064$) is less than global capacity (slope = $0.86^{\pm}9$) (Georgiou et al. 2022) (Fig. 6). Lastly, carbon stocks (to

Table 2 Summary of results from statistical analyses including multiple regression and comparisons among crop types

Factor	POC	MAOC
Active carbon	↑	↑
Aggregate stability	↑	↑
pH	—	—
Hay compared to corn crop type	↑	↑
Pasture compared to corn and vegetable crop types	↑	—
Temperature in high MAP	↓	↑ (in high clay)
Temperature in low MAP	↑	↑
Soil health score	↑	↑

Arrows pointing up represent positive relationships and arrows pointing downward, negative, while a long dash is no relationship

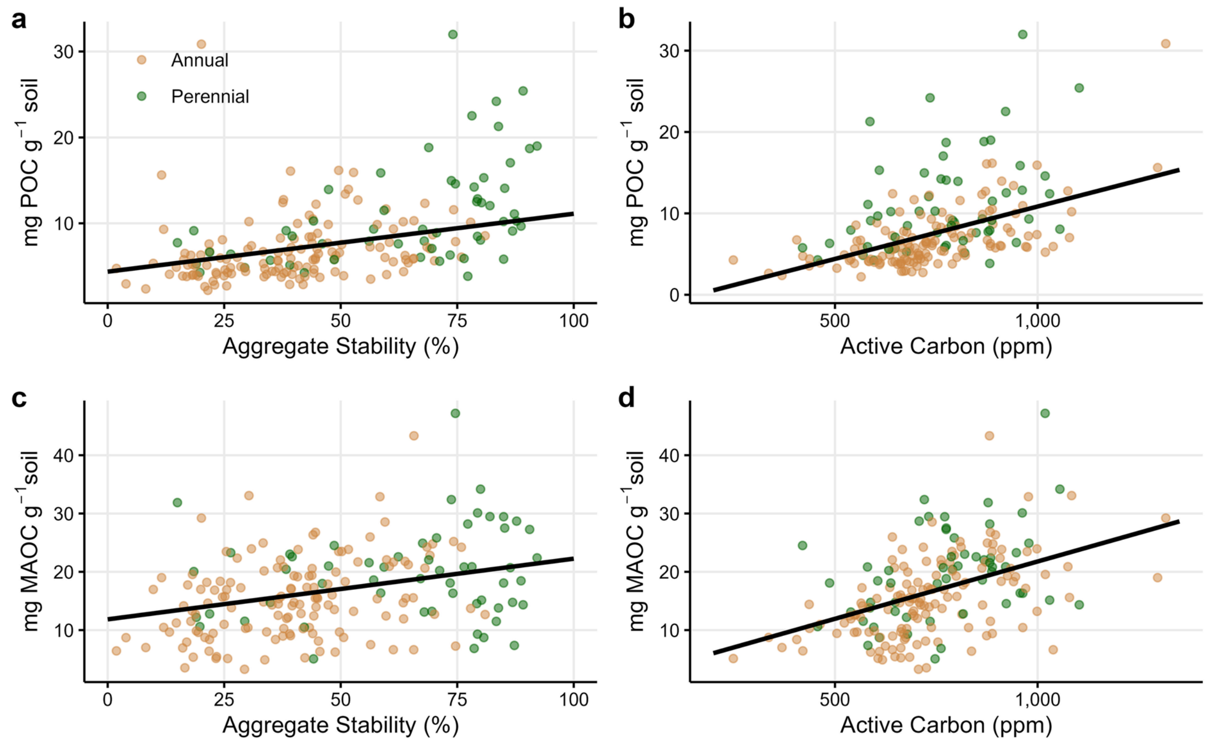

Fig. 2 Relationship between predicted changes in particulate organic carbon (POC) and: **a** aggregate stability (slope = 0.0679 mg g^{-1} soil %$^{-1}$; POC increases by 8.32% per 10% increase) **b** active carbon (slope = 0.0132 mg g^{-1} soil ppm^{-1}; POC increases by 16.12% per 100 ppm increase).The relationship for mineral-associated organic carbon (MAOC) shows: **c** aggregate stability (slope = 0.1078 mg g^{-1} soil %$^{-1}$; MAOC increases by 4.05% per 10% increase) **d** active carbon (slope = 0.0195 mg g^{-1} soil ppm^{-1}; MAOC increases by 6.43% per 100 ppm increase). Points represent individual soil samples (n = 196), and slopes were calculated with other predictors held constant in the models

30 cm) also increased significantly with soil health score ($F_{1,163}$ = 249.1, r^2 = 0.60, $p < 0.001$). For each unit increase in the soil health score, carbon stock increased by 0.25 kg C m^{-2} (estimate = 0.247 ± 0.016 SE; Fig. 7).

Discussion

Our findings demonstrate a relationship between crop type, soil health metrics, environmental conditions, and soil fractions. We considered how the significant drivers of carbon fractions indicate the degree to which farmers have control over the amount of carbon protected by mineral-associations and the practical implications for management of similar agricultural soils in mesic temperate climates. Overall, we found that both POC and MAOC concentrations correlated positively to increased soil health metrics and overall soil health scores. In particular, POC and MAOC concentrations were greater in hay and pasture fields as opposed to row crops like corn and vegetables, potentially due to greater aggregate stability under perennial management, which often involved no till practices. This understanding suggests that soil management strategies aimed at enhancing carbon sequestration can increase the persistent MAOC fraction.

Management

The first evidence that management affects the concentrations of POC and MAOC was the significant differences among crop types in our study. We found greater concentrations of POC and MAOC in perennial hay and pasture systems relative to annual silage corn and vegetable crops. Similarly, carbon stocks were higher in hay fields than corn and vegetable fields and stocks in pastures were intermediate

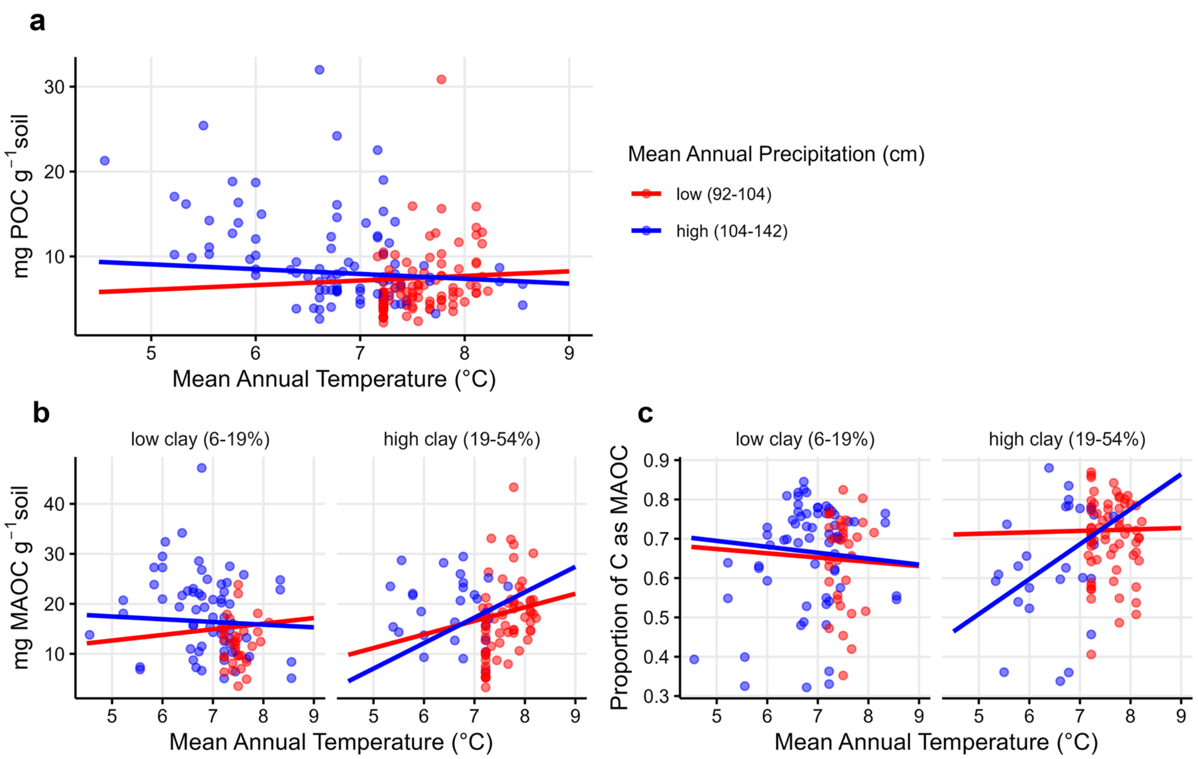

Fig. 3 The relationship between the concentration of POC (**a**) and the interaction of mean annual precipitation and mean annual temperature. Interactions between precipitation, temperature, and clay content related to MAOC concentration (**b**) and the proportion of carbon as MAOC (**c**). Points are the data and lines show the model predictions which are extrapolated beyond the range of sampled conditions

between hay and the annual crops (White et al. 2022). In agricultural fields, carbon inputs to soil organic matter from roots outnumber inputs from aboveground plant tissues by a ratio of 8:1 (Jackson et al. 2017). The perennial plants in pastures and hay fields produce more root biomass compared to annual cropping systems (Follett & Reed 2010) contributing to greater amounts of SOC, POC, and MAOC (Jackson et al. 2017; Puget & Drinkwater 2001; Rasse et al. 2005; Rossi et al. 2020). In addition, corn and vegetable fields are more likely to receive annual tillage disrupting macroaggregates and increasing SOC respiration losses (Six et al. 2000; Smith et al. 2015). Because most perennial fields in our study were also under no till management, our data does not allow us to definitively attribute the greater SOC to one practice or the other. Our findings are consistent with previous results in agricultural studies (Cates et al. 2022; Ferreira et al. 2020; Niu et al. 2024) including one study showing pastures have higher POC concentrations relative to tilled annual crop fields due to perennial vegetation, deeper roots, greater plant diversity, and manure inputs (Franzluebbers & Stuedemann 2002).

Generally, crop types had similar proportions of carbon as MAOC (60–69%). When a crop type had a greater proportion of carbon as MAOC, this was a consequence of relatively less POC rather than more MAOC. For example, corn fields had a higher proportion of MAOC than pastures, even though pastures contained higher concentrations of both POC and MAOC. The higher MAOC proportion in corn silage was potentially caused by tillage disturbance and lower residue inputs decreasing POC and leaving the remaining soil carbon relatively enriched in MAOC. A similar result was found in croplands globally where almost 80% of soil carbon was in mineral-associations, a greater proportion than other land cover types (Heckman et al. 2022). These results reveal that the dynamic portion of SOC, POC, is more negatively

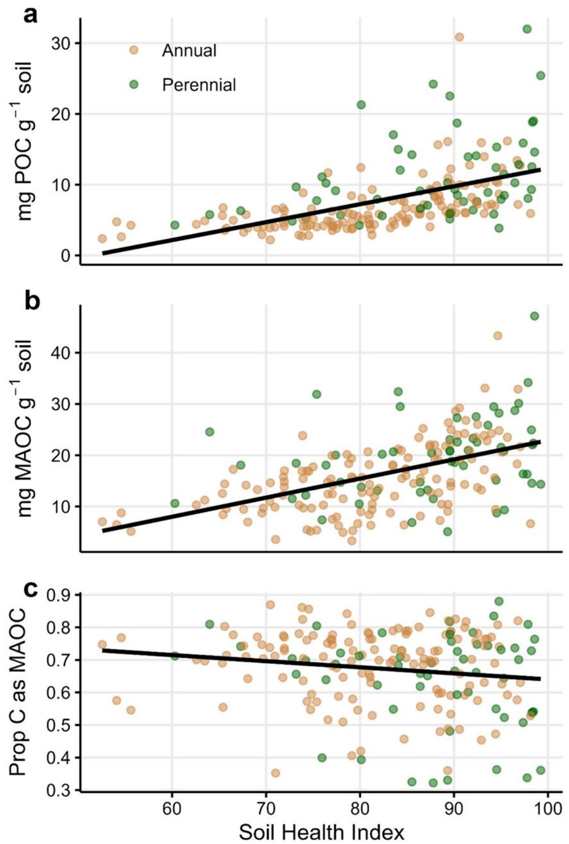

Fig. 4 The relationship between soil health score (index) and a) POC concentration (slope = 0.25, $p < 0.0001$), b) MAOC concentration (slope = 0.37, $p < 0.0001$), and c) proportion of carbon as MAOC (slope = −0.008, $p = 0.03$) Points indicate individual soil samples (one per field, n = 196) and lines represent best fit in a linear regression. For every 1 unit increase in soil health score, concentrations of POC increased by 3.15% or 2.57 mg g^{-1} soil. For every 1 unit increase in soil health score, concentrations of MAOC increased by 2.25% or 3.77 mg g^{-1} soil. For every 1 unit increase in soil health score proportions of carbon as MAOC decreased by 1.05%

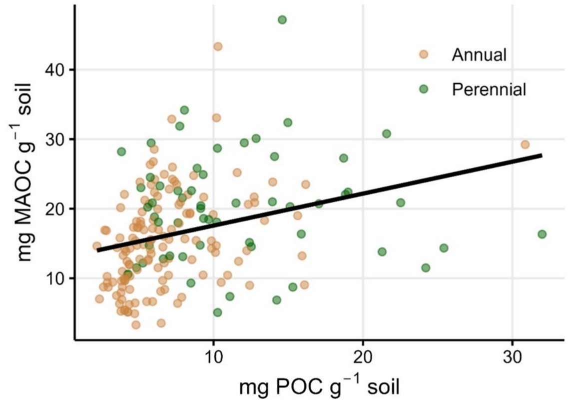

Fig. 5 Relationship between POC and MAOC (slope = 0.45, $p < 0.0001$). Points indicate individual soil samples (one per field, n = 196) and the line represents the best fit in a linear regression. For every 1 mg increase in POC, concentrations of MAOC are 0.47 mg greater

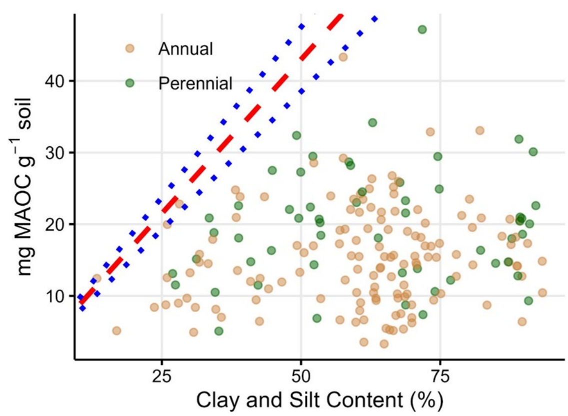

Fig. 6 Relationship between clay and silt content (%) and the concentration of MAOC. The dashed red line is the maximum slope (95th quartile) representing the threshold at which minerals become saturated with carbon according to a recent meta-analysis (from Georgiou et al 2022)

influenced annual cropping systems in this mesic, temperate region than MAOC.

The more persistent MAOC fraction was also responsive to perennial crop management as it was greatest in hay and pasture fields, compared to corn fields (Table 1). Rhizosphere interactions increase MAOC formation (Sokol & Bradford 2019) and perennial systems may have greater root inputs and residue incorporation (Lal 2004; Martens 2000). Manure inputs may increase microbial decomposition of POC and provide substrate to favor conversion to MAOC (Guo 2021). While we may assume that pastures receive manure inputs, we were not able to evaluate the effects of organic amendments on POC and MAOC in this study. As with POC, perennial crops, lack of tillage, and greater root inputs all contribute to the greater MAOC concentrations in the hay and pasture fields.

In any observational study of agricultural soil, it can be difficult to disentangle whether differences are due to management practices or inherent environmental properties that led the farmers to manage the soils differently in the first place. While field types

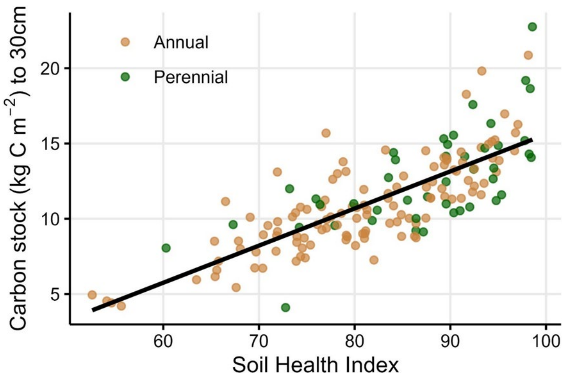

Fig. 7 Relationship between soil health score (index) and soil carbon stock to 30 cm depth across annual and perennial fields. Each point represents a field, colored by crop type (Annual or Perennial). The black line shows the linear regression across all fields

significantly differed from one another in key properties like soil texture and MAP, these cannot explain our results. For example, pastures had the highest MAP, but the effects of MAP on soil carbon varied by MAT, which did not differ among sites. Vegetable fields had the lowest clay content but did not have the lowest MAOC concentrations as might be expected. Hay and corn had significantly different MAOC concentrations but similar clay content. The only consistently significant difference among the crop types that explain the greater MAOC and POC concentrations in the perennial hay fields and pastures is aggregate stability, which is a soil property that is highly responsive to management (Mikha et al 2021, Sithole et al 2019, Bronick and Lal 2005, Six et al 2000).

Environmental factors

Environmental drivers of POC and MAOC (climate, soil texture, and their interactions) had significant effects on POC and MAOC, despite the relatively small variations in climate observed throughout the state of Vermont. The amount of carbon in each fraction is affected by input quantity and quality and decomposition rates, both of which are influenced by climate effects on plant productivity and microbial activity (Lehmann & Kleber 2015). Concentrations of POC increased with MAT at lower MAP and decreased with increased MAT at higher MAP (Fig. 3a, b). Higher microbial activity at warmer temperatures increases decomposition, especially of POC (Benbi et al. 2014; Davidson & Janssens 2006). Precipitation plays a key role as a limiting factor for plant growth and for POC decomposition. With greater MAP, increased decomposition rates may override the effect of increased inputs on POC. With less moisture, decomposition is reduced so POC increases with temperature, perhaps reflecting the effect of MAT on plant inputs. This is best observed in environments where MAP is sufficient for plant growth as in this temperate mesic region.

The interaction between MAT and MAP also emerged as a significant predictor of MAOC, in a three-way interaction with clay content (Fig. 3b, c). In clay-rich soils, MAOC increased with MAT, particularly in regions with higher precipitation. We found similar results predicting the proportion of carbon as MAOC, except for a lack of temperature effect in higher clay soils with lower precipitation. Under these conditions, lower precipitation limits POC decomposition and a higher ratio of POC: MAOC results. The greater surface area of clay particles allows sorption of dissolved organic carbon compounds released by POC decomposition (Stewart et al. 2007) likely driving these relationships. When enough clay is present, the interactive effects of MAT and MAP increase plant inputs and support high microbial activity leading to the stabilization of dissolved organic carbon and microbial byproducts on clay-sized surfaces (Kalbitz et al. 2005). In contrast, the interaction between MAT and MAP has the opposite effect in lower clay soils. With less sorptive surface area stabilizing dissolved organic carbon, high microbial activity and POC decomposition leads to increased carbon loss via leaching or mineralization. These results indicate clay content may be the limiting factor in MAOC formation rather than climate conditions in mesic, temperate soils. Our results agree with many studies that show clay-rich soils may have a greater capacity to stabilize organic matter (Haddix et al. 2020; Luo et al. 2010; Rabot et al. 2024). Research suggests that POC can accumulate indefinitely (Castellano et al. 2015), while MAOC stabilization on mineral surfaces may reach a saturation point (Georgiou et al. 2022; Stewart et al. 2007). We found the agricultural soil in our study to be below saturation, which represents an opportunity to build more MAOC through management. Our results suggest that perennial crops maximize both POC and MAOC and are an effective strategy to increase and stabilize soil carbon until the mineral surfaces are saturated. Though subsequent additions to POC would be less persistent, continued management (e.g., perennial crops, no-tillage) could maintain or build overall soil carbon stocks.

We expected that more acidic soils would have lower MAOC proportions due to reduced microbial activity and increased mineral solubility limiting the opportunity for forming mineral associations, and that acidity might promote POC accumulation due to slower decomposition (Kemmitt et al. 2006). In previous research over a broad geographic range, increasing pH was negatively correlated with overall soil carbon (Zhou et al. 2017). However, our study, with a much narrower pH range (5.4–7.6, mean: 6.5), found no significant relationships between pH and either POC or MAOC. While vegetable fields had a significantly higher pH than corn fields and pastures, perhaps due to amendments, this did not translate to differences in carbon fractions. Lime applications that maintain soil pH within crop production guidelines likely prevent soils from crossing thresholds where pH affects carbon dynamics (Rasmussen et al. 2018). Therefore, on the farms included in this study, climate and texture have more control over POC and MAOC concentrations than pH.

Individual soil health indicators

In our study, aggregate stability emerged as a key factor positively influencing both POC and MAOC storage, highlighting its role in protecting carbon from decomposition. The methodology used for measuring aggregate stability in the CASH analysis (the capacity of soil to resist degradation under force) primarily assesses the stability of macroaggregates ($>250\ \mu$m). The macroaggregates physically protect carbon compounds, sheltering them from microbes (Balesdent et al. 2018), leading to decreased POC losses (Chaplot & Cooper 2015).

Aggregate stability is tightly connected to management (Boniface et al. 2025), and our results provide insight into management effects based on crop type. Aggregate stability was greater in perennial crop types than annual likely due to less tillage in those crop types. The soil stability resulting from lack of tillage and the extensive root system of perennial vegetation in hay fields and pastures has been shown to increase aggregate stability (Milne and Haynes 2004). Particulate organic carbon also accumulates compared to annual production systems where plant residue removal and tillage usually occur. Organic inputs such as plant residues, animal manures, compost, and root exudates-common in pastures and hay fields- encourage aggregate formation by facilitating bonds between particles through increased microbial activity (Mizuta et al. 2015)increasing aggregate stability (Dignac et al. 2017). These same practices are aligned with increasing POC as demonstrated in the results of our research (Grandy & Robertson 2007; Mirsky et al. 2008; Six et al. 2002). Additionally, stable aggregates also protect MAOC from decomposition. Microbial activity that promotes aggregation also results in greater microbial necromass for MAOC formation (Heckman et al. 2023; Sokol et al. 2019). Our results indicate that management practices that increase aggregate stability will in turn increase MAOC and potential long-term soil carbon storage.

Active carbon, measured as PoxC, (Weil et al. 2003) is used for early tracking of improved soil health because it is sensitive to changes in management (Hurisso et al. 2016)(Mirsky et al. 2008). Often examined as a proxy measurement for POC (Mirsky et al. 2008), it is unclear precisely what PoxC measures. Some claim PoxC represents a labile, microbially-available food source (Magdoff et al. 2021). Others suggest that the PoxC method is reactive to complex biomolecules including lignin (Christy et al. 2023; Woodings & Margenot 2023) or that PoxC can represent both processed (Culman et al. 2012) and plant-derived (Christy et al. 2023) organic matter. Given this range, it is not surprising that PoxC was positively related to both POC and MAOC concentrations in our study. These results corroborate the findings of a previous study in a nearby pasture in New Hampshire, USA (Teixeira et al. 2023). Rather than a proxy for just POC, it seems PoxC measures a subset of active carbon that is not wholly POC nor MAOC. It is worth noting that in our study, we found larger differences in POC and MAOC among crop types than in PoxC, making PoxC the least responsive carbon metric to management.

Overall soil health

Understanding the relationship between the CASH soil health score and soil carbon fractions can provide insights into how these scores translate to climate mitigation benefits. The CASH scoring system favors soil in optimal conditions for agricultural production using scoring curves established from a database of samples. In our study, soil health scores had a positive linear relationship with both POC and MAOC, but a weak negative relationship with the proportion of carbon as MAOC (Fig. 4). This indicates that while higher absolute amounts of MAOC and POC are related to greater soil health scores, the relative proportion of carbon as MAOC does not necessarily correlate with overall soil health scores. It should be noted that total soil organic matter content and active carbon were included in the scoring for the CASH soil health overall score, which lends a circular nature to the linear relationship since POC and MAOC are soil organic matter fractions and are correlated with active carbon. Consistent with previous research, our results support the strong link between POC and soil health (Cambardella & Elliott 1993; Lal 2016). In addition, our results demonstrate that higher levels of active carbon and more stable soil aggregates are associated with greater MAOC storage, emphasizing the role of these soil health indicators as predictors of MAOC. These findings align with the broader understanding that healthy soils with stable aggregates are better able to retain organic matter (Cotrufo et al. 2019). Our results demonstrate that management practices promoting soil health (e.g. perennial cropping systems) lead to greater POC formation and should increase MAOC stabilization under the framework that POC is a precursor to MAOC (Cotrufo et al. 2015; Lavallee et al. 2020). Our results further demonstrate that climate and soil factors that increase POC also increase MAOC (Fig. 5). In contrast, despite the positive relationship between soil health score and MAOC concentration, soil health scores had a weak negative relationship with the proportion of carbon as MAOC. We expected that due to its more complex formation process (Jilling et al. 2021), MAOC may be a lagging indicator of management compared to POC. However, our data show MAOC increasing with soil health slightly faster than POC (steeper slope shown in the slope coefficients and Fig. 4a, b). This agrees with other recent research showing MAOC as a strong predictor of soil health (Beckstrom et al 2025). However, given their high r^2 value (0.60), carbon stocks are still a superior predictor of soil health scores than either fraction ($r^2 = 0.26–0.28$). Another expectation was that with high soil health scores, MAOC could become saturated while POC continues to accumulate. However, our analysis shows that fields in both annual and perennial production appear to be largely unsaturated representing opportunities to increase MAOC across crop types (Fig. 6; SI, Figure S6).

Greater soil health can enhance nutrient cycling and availability. This leads to increased plant growth resulting in greater crop residue and root inputs particularly in perennial forage and pasture systems. The positive relationship between soil health scores and total soil carbon stocks is expected, as soil carbon both contributes to and is included in soil health assessments. Our study confirmed this association, showing that higher soil health scores correspond to greater carbon stocks. Importantly, we extend this understanding by demonstrating that soil health is also positively associated with concentrations of both POC and MAOC. Roots and root exudates are particularly important inputs to microbial activity and increased biomass, increasing both active pools like POC and, because such inputs are efficiently processed, more stabilized pools like MAOC (Sokol et al. 2019). This follows the Microbial Efficiency-Matrix Stabilization framework that efficiency leads to MAOC formation (Cotrufo et al. 2013).

Implications

Our results show that management practices that increase soil health, such as enhancing aggregate stability, are associated with increased POC and MAOC, underscoring the mechanistic link between soil carbon storage and persistence. Management was important even though POC and MAOC concentrations were also dependent on management-independent factors such as climate and clay content. Site context influences potential fraction concentrations, but management decisions, like crop type, whether to plant perennials or to till, affect the realized concentrations. While our scope of inference is limited to mesic, cool temperate climates, our results align with studies investigating the effect of management on fractions from vastly different regions. A study in a semi-arid agricultural system similarly found POC and MAOC both responded to management, in this case prolonged perennial vegetation (Arije et al. 2024). A meta-analysis of studies throughout Europe and the Americas found cover cropping increased POC by 15% and MAOC by

5.6% on average (Wooliver and Jagadamma 2023). While a greater responsiveness of POC than MAOC to management is a common theme among studies worldwide, it is important to note that MAOC often responds significantly to management (Liu et al. 2024; Prairie et al. 2023). Furthermore, our study and others (e.g., Arije et al. 2024) demonstrate that MAOC remains well below saturation, suggesting continued potential to increase persistent soil carbon through management. Our data indicate that hay fields (SI, Figure S6) with cooler MAT and higher MAP may have the most potential for building MAOC (Fig. 3).

Our study found a clear relationship between soil health scores and the concentrations of both POC and MAOC. To our knowledge, this is the second study specifically investigating the relationship between soil health scores and MAOC; the first study, in Hawaiian Andisols, also found a strong relationship between the two (Beckstrom et al. 2025). Beyond the comprehensive soil health score, POC and MAOC were positively associated with widely used individual soil health indicators like POX-C and aggregate stability as seen in previous studies (e.g., Blanco-Moure et al. 2013; Chaplot & Cooper 2015; Teixeira et al. 2023). Collectively, these results suggest that building and maintaining both soil health and long-term soil carbon are likely more aligned than they are in conflict. Managing to maintain and increase soil health contributes to more soil carbon in both labile and persistent fractions.

Building MAOC is an effective approach to increase long-term carbon storage in soils as a strategy for climate mitigation. Several plans for climate mitigation identify increasing agricultural soil carbon as an important strategy to mitigate climate change. The POC and MAOC measurements on farms in this study serve as an important foundation for providing farmers with contextualized information about how soil health goals align with carbon sequestration goals and can contribute to long-term monitoring or future experimental designs as an established baseline from which to grow knowledge (Franzluebbers et al. 2020; Fujisaki et al. 2018; Luo et al. 2020). Further research can explore how additional factors, such as soil amendments, cultivar selection, crop rotations, and irrigation, influence soil carbon fractions and deepen our understanding of soil carbon persistence (Lugato et al. 2021; Mackey et al. 2013; Zhang et al. 2024).

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Author contributions Erin Lane: conceptualization, data curation, methodology, investigation, formal analysis, visualization, writing-original draft, writing- review and editing; Kathryn White: visualization, writing- review and editing; Alissa White: project administration, writing- review and editing; Julia Siegel: investigation, writing-review and editing; Heather Darby: project administration, funding acquisition, writing-review and editing; Caitlin Hicks Pries: conceptualization, project administration, funding acquisition, investigation, formal analysis, methodology, supervision, resources, validation, visualization, writing-review and editing.

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Data availability Hicks Pries, Caitlin; Lane, Erin (2026). Size Fractions and Soil Health in Agricultural Soils Across Vermont. figshare. Dataset. https://doi.org/10.6084/m9.figshare.31329652.

Declarations

Conflict of interest We have no conflicts of interest to disclose. Caitlin Hicks Pries reports financial support was provided by Natural Resources Conservation Service. Heather Darby and

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