

Soluble and insoluble fractions of mineral-associated organic carbon differ in composition, accumulation patterns, and environmental controls

Hongfei Liu^a, Carson Thompson^b, Hai-Ruo Mao^b, Chao Liang^{c,d}, Amy M. McKenna^e, Fu Chen^f, Edward A.G. Schuur^{g,h}, Andrea Jillingⁱ, Mengqiang Zhu^{a,b,*}

^a Department of Geological, Environmental, and Planetary Sciences, University of Maryland, College Park, MD, 20742, USA

^b Department of Ecosystem Science and Management, University of Wyoming, Laramie, WY, 82071, USA

^c State Key Laboratory for Development and Utilization of Forest Food Resource, College of Environmental and Resource Sciences, Zhejiang A&F University, Hangzhou, 311300, China

^d Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, 110016, China

^e National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL, 32310, USA

^f Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, 20742, USA

^g Center for Ecosystem Science and Society, Northern Arizona University, Flagstaff, AZ, USA

^h Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, USA

ⁱ Department of Environmental Health Sciences, University of South Carolina, Columbia, SC, USA

ARTICLE INFO

Keywords:

Mineral-associated organic matter
Soluble fraction
Insoluble fraction
Chemical composition
Carbon sources
Radiocarbon age

ABSTRACT

Mineral-associated organic carbon (MAOC) is one of the largest and most persistent soil C reservoirs, with major implications for soil functioning and climate mitigation. However, bulk MAOC is chemically heterogeneous and may contain organic matter with contrasting solubility, chemical composition, source-related signatures, and environmental controls, which remain poorly understood. Here, we used archived surface and subsurface soils from 43 National Ecological Observatory Network sites across North America to isolate MAOC and partition it into soluble fractions, which were released by repeated hydrofluoric acid (HF) extraction followed by water rinsing, and insoluble fractions which comprised the extraction residue. Across sites, the soluble fraction accounted for 11–96% of total MAOC, whereas the insoluble fraction accounted for 5–89%. The soluble fraction exhibited a Langmuir-type accumulation pattern and was enriched in phenolic and carboxyl functional groups, as well as lignin, tannin, and condensed aromatic-like molecules. In contrast, the insoluble fraction increased nonlinearly without an apparent asymptote across the observed MAOC range and was enriched in alkyl C and non-aromatic molecular classes, including lipids, carbohydrates, and proteins. Composition-based estimates indicated stronger plant-associated contributions in soluble MAOC and stronger microbial-associated contributions in insoluble MAOC. Despite these chemical contrasts, radiocarbon values did not differ detectably between the soluble and insoluble MAOC fractions; instead, $\Delta^{14}\text{C}$ declined with soil depth and increased with mean annual precipitation. Environmental analyses showed that a higher contribution of soluble MAOC to bulk MAOC was associated with deeper soils and wetter, more acidic, Fe/Al-rich conditions. These findings suggest that bulk MAOC contains operationally separable fractions with distinct solubility, accumulation behavior, chemical composition, source-related signatures, and environmental associations. Accounting for this heterogeneity may improve conceptual and process-based models of MAOC dynamics.

1. Introduction

Soil organic matter (SOM) is a major global carbon stock that supports soil fertility and regulates climate, yet it is vulnerable to climate and land-use change (Davidson and Janssens, 2006; Lehmann and

Kleber, 2015). A large share of SOM is stabilized through association with minerals as mineral-associated organic matter (MAOM), which stores about 65% of global soil organic carbon (SOC) and turns over on decadal-to-millennial timescales (Torn et al., 1997; Kleber et al., 2015; Sokol et al., 2022). Although minerals can also catalyze abiotic

* Corresponding author. Department of Geological, Environmental, and Planetary Sciences, University of Maryland, College Park, MD, 20742, USA.

E-mail address: mqzhu@umd.edu (M. Zhu).

<https://doi.org/10.1016/j.soilbio.2026.110287>

Received 8 June 2026; Received in revised form 11 August 2026; Accepted 12 August 2026

Available online 13 August 2026

0038-0717/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

degradation, mineral protection generally increases activation-energy barriers and constrains microbial access, yielding a net stabilizing effect in upland soils (Kleber et al., 2015, 2021; Hemingway et al., 2019; Zhou et al., 2024). Understanding how organic carbon becomes associated with minerals, and how different forms of mineral-associated organic carbon (MAOC) vary in composition and persistence, is therefore central to predicting SOC dynamics and improving soil-carbon management.

Organic matter associated with minerals is chemically and physically heterogeneous. Organic inputs entering MAOM range from soluble molecules, such as plant leachates, root exudates, microbial metabolites, soluble products of litter depolymerization, cell-lysis products, and extracellular polymeric substances, to less soluble or particulate materials, such as fine plant residues, microbial necromass fragments, and hydrophobic compounds including lipids (K. Kalbitz et al., 2000; Kleber et al., 2015; Cotrufo and Lavalée, 2022; Whalen et al., 2022). These materials differ in polarity, molecular size, functional-group chemistry, and physical form, all of which can influence how they interact with minerals. Soluble organic compounds sorb to mineral surfaces at the molecular scale, either through direct attachment to reactive mineral surfaces or through co-precipitation with metal cations (Kaiser and Guggenberger, 2000; Kleber et al., 2015, 2021). These interactions can produce surface-conditioning layers through organo-mineral bonding and multilayered or patchy coatings through subsequent organo-organic associations (Kleber et al., 2007; Underwood et al., 2024). Less soluble, hydrophobic, or particulate organic materials may instead be retained in mineral-associated fractions through organo-organic contacts, physical proximity to mineral particles, occlusion within fine mineral-organic structures, or other solid-solid associations (Totsche et al., 2018; Possinger et al., 2020; Witzgall et al., 2021; Kang et al., 2024). These association modes are not mutually exclusive, and no routine fractionation method can isolate them as pure mechanistic endmembers. Nevertheless, separating MAOC into operationally soluble and insoluble fractions provides a tractable way to test whether these contrasting forms of MAOC differ in abundance, chemical composition, source-related signatures, radiocarbon characteristics, and environmental controls.

Most empirical studies and process-based SOC models have emphasized MAOM formation through the sorption of dissolved organic matter to mineral surfaces. In many models, the process is represented using Langmuir-type functions, in which dissolved organic matter accumulates until available sorption sites approach saturation (Woolf and Lehmann, 2019; Ahrens et al., 2020; Yu et al., 2020). This framework is powerful for describing sorptive stabilization of soluble compounds, but it may underrepresent mineral-associated organic matter that is stabilized primarily as hydrophobic or particulate organic materials through solid-solid associations. Some models have therefore included additional routes for the formation of insoluble organic materials in MAOC (Ahrens et al., 2020; Zhang et al., 2021). However, empirical datasets that quantify both soluble and insoluble MAOC fractions across broad environmental gradients remain limited. This gap restricts our ability to evaluate whether MAOC fractions with contrasting solubility follow different accumulation patterns, respond to different environmental controls, or require separate representation in SOC models.

Distinguishing soluble and insoluble MAOC fractions may also help clarify the debated origins of MAOC. Current SOC theory holds that MAOC derives from both plant and microbial sources, with little contribution from secondary synthesis (Schmidt et al., 2011; Lehmann and Kleber, 2015; Sokol et al., 2019; Lavalée et al., 2020), yet the relative contributions of these sources remain debated and the pathways by which they enter MAOC are poorly resolved. Amino-sugar biomarkers and chemical assays suggest that microbial-derived C contributes 12–87% of MAOC (mean: 39%), with the remainder attributed to plant-derived C, varying across climates, soil depths, and land uses (Angst et al., 2021). This contrasts with microbially centric models that predict microbial dominance in MAOC formation (Cotrufo et al., 2013;

Kallenbach et al., 2016), a view supported in part by solid-state ^{13}C NMR studies reporting low aromatic C in MAOC (Kögel-Knabner et al., 2008). However, the low aromatic signal may partly result from hydrogen fluoride (HF) pretreatment, which is commonly used to remove paramagnetic Fe before nuclear magnetic resonance (NMR) analysis but also dissolves oxides and phyllosilicates that sorb dissolved organic matter. Because HF can preferentially remove aromatic, likely plant-derived compounds from soils, it may bias inferred MAOC composition toward an apparently greater microbial contribution (Wen et al., 2021). In some acidic forest soils, HF removed up to 76% of clay-associated C, much of it aromatic (Eusterhues et al., 2007; Spielvogel et al., 2008).

Here, we used archived soils from National Ecological Observatory Network (NEON) sites across North America to separate MAOC into two operational fractions with contrasting solubility in aqueous solution and to evaluate how these fractions differ in relative abundance, chemical composition, source-related signatures, radiocarbon characteristics, and environmental controls. We define the soluble MAOC fraction as the total organic C released into the aqueous phase during both HF demineralization and subsequent water rinsing. The insoluble MAOC fraction is defined as the organic C remaining in the extraction residue, representing organic C that remains insoluble under the same circum-neutral conditions after mineral associations are disrupted. These fractions are interpreted as operational pools with contrasting solubility, rather than as pure mechanistic endmembers of MAOC formation. Accordingly, these terms describe the aqueous behavior of the organic matter after mineral dissolution and do not imply that soluble MAOC existed in dissolved form in the intact soil solution. We hypothesized that the soluble fraction would be enriched in reactive, aromatic compounds with stronger plant-associated signatures, whereas the insoluble fraction would be enriched in less aromatic compounds with stronger microbial-associated signatures, and that the relative importance of these two pools would vary systematically across environmental gradients. To test these hypotheses, we isolated MAOM by size-density fractionation, partitioned MAOC into soluble and insoluble fractions using repeated HF extraction and water rinsing, and characterized bulk MAOC and its fractions using C K-edge X-ray absorption near-edge structural (XANES), solid-state ^{13}C NMR spectroscopy, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), and radiocarbon analysis. This approach provides a cross-ecosystem assessment of chemical and source-related heterogeneity within MAOC.

2. Materials and methods

2.1. Study sites and sampling

Soil samples were obtained from NEON soil archive, encompassing 43 terrestrial sites across the United States that span broad climatic, edaphic, and ecological gradients (Fig. S1). At each site, soils were collected from a single NEON “Megapit” following standardized NEON protocols (Thorpe et al., 2016). This study focused on mineral soils from A and B horizons, excluding C horizons due to low organic carbon contents. For each site, chemical extractions were conducted on all available A- and B-horizon samples from all sites ($n = 180$), while detailed chemical characterization was performed on the uppermost A or B horizon only ($n = 74$).

Soil physical and chemical properties, litter biomass, and root biomass were obtained from the NEON data portal (Text S1). Soil properties included texture, pH, elemental composition, cation-exchange properties, and other standard NEON soil variables. Concentrations of Fe and Al in short-range-ordered (SRO) mineral phases and organo-metal complexes (termed Fe_{ox} and Al_{ox}) were quantified in our laboratory using ammonium oxalate extraction (Schwertmann and Jackson, 1964). Mean annual temperature (MAT) data were obtained from the NEON data portal, and mean annual precipitation (MAP) and potential evapotranspiration (PET) data were derived using ClimateNA v5.10 following established procedures (Wang et al., 2016). Effective

moisture was calculated as MAP – PET.

2.2. Isolation of MAOC and separation of soluble and insoluble MAOC fractions

Mineral-associated organic matter (MAOM) was first isolated from bulk soil by density and size fractionation (Fig. 1). The density/size fractionation protocol with sodium polytungstate (SPT) was used to isolate MAOM (Leuthold et al., 2022). Air-dried soil (<2 mm; 5 g) was

first shaken with deionized water for 15 min to remove water-extractable organic matter. The suspension was centrifuged at 5000 rpm for 30 min, and the supernatant was filtered through a 0.45- μm polytetrafluoroethylene membrane. Particles retained on the filter were returned to the residual soil. The residual soil was then mixed with 25 mL sodium polytungstate (SPT; density = 1.85 g cm^{-3}) and 12 glass beads, shaken for 18 h to disperse aggregates, and centrifuged at 5000 rpm for 30 min. The floating material was collected as the light particulate organic matter fraction (light POM; density $<1.85\text{ g cm}^{-3}$).

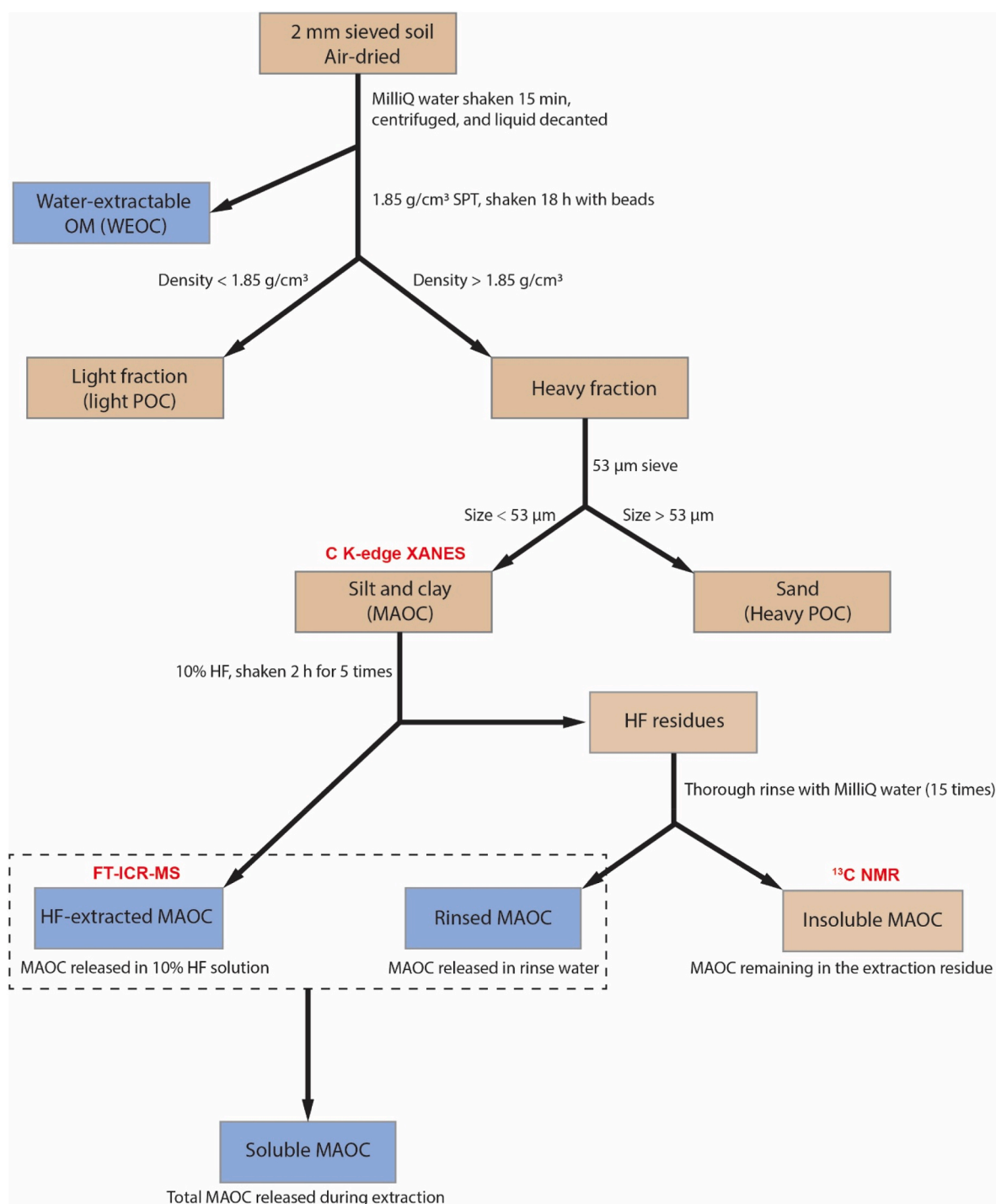


Fig. 1. Workflow for the SOM fractionation into bulk MAOC, HF-extracted MAOC, soluble MAOC, and insoluble MAOC. WEOC, water-extractable organic carbon; POC, particulate organic carbon; MAOC, mineral-associated organic carbon; HF-extracted MAOC, the OC released into 10% hydrofluoric acid (HF) solution; soluble MAOC: a combination of HF-extracted OC and OC released during subsequent rinsing; insoluble MAOC: residual C remaining after water rinsing. The chemical composition of soluble MAOC was derived by mass balance from the C K-edge XNAES and the ^{13}C NMR of insoluble MAOC.

The heavy fraction (density $>1.85 \text{ g cm}^{-3}$) was wet-sieved at $53 \mu\text{m}$ to separate heavy POM (density $>1.85 \text{ g cm}^{-3}$, size $>53 \mu\text{m}$) from MAOM, which was operationally defined as the silt + clay-sized heavy fraction (density $>1.85 \text{ g cm}^{-3}$, size $<53 \mu\text{m}$). All fractions were dried at 60°C and ground before further analysis. Total mass recovery was monitored for each sample. Samples were retained only when total mass recovery was 75–120% of the initial dry soil mass; observed recoveries ranged from ~80% to 110%.

MAOM was subsequently separated into soluble and insoluble fractions using repeated HF extraction followed by extensive Milli-Q water rinsing (Eusterhues et al., 2003) (Fig. 1). HF extraction effectively dissolves metal oxides and phyllosilicates and releases mineral-associated organic carbon into solution, allowing MAOM to be separated operationally into soluble and insoluble fractions under the extraction conditions used here (Roberson and Hem, 1969; Hossner, 1996; Eusterhues et al., 2007; Spielvogel et al., 2008). Briefly, 1 g of dried MAOM was treated with 10 mL of 10% HF and shaken for 2 h at room temperature. The suspension was centrifuged at $5000 \times g$ for 2 h, and the supernatant was collected. This HF extraction was repeated five times. The combined HF supernatants were stored at -20°C and are referred to as HF-extracted MAOC, representing the fraction of MAOC only released into the 10% HF solution. The remaining residue was then rinsed 15 times with 10 mL Milli-Q water (pH 7.5) per rinse. The rinsing step removed residual HF and recovered an additional fraction (~7%) of MAOM (Fig. S2) that was not soluble under acidic HF conditions (10% HF, pH ≈ 3) but was released at circumneutral pH (Milli-Q water, pH ≈ 7.5). The organic C released during the HF extraction (i.e., HF-extracted MAOC) and subsequent rinsing was combined to quantify the soluble MAOC fraction. The extraction residue remaining after HF extraction and water rinsing was defined as the insoluble MAOC fraction.

Carbon contents of bulk MAOC and insoluble MAOC were measured by elemental analysis. Inorganic C was removed from calcareous samples by HCl fumigation before elemental analysis (Harris et al., 2001). Total soluble MAOC concentration (g/Kg silt + clay) was calculated by mass balance as the difference between bulk MAOC and insoluble MAOC:

$$[\text{Soluble MAOC}] = \frac{[\text{bulk MAOC}] - [\text{insoluble MAOC}] \times f_{\text{insoluble}}}{(1 - f_{\text{insoluble}})}$$

Where $f_{\text{insoluble}}$ is the mass ratio of insoluble MAOC to bulk MAOC. In addition, carbon concentrations in HF-extracted MAOC were measured using a total organic carbon analyzer.

2.3. Characterization of MAOC chemical composition

Carbon K-edge X-ray absorption near-edge structural (XANES) and solid-state ^{13}C cross-polarization magic-angle spinning (CPMAS) NMR spectroscopy were used to characterize the C functional group composition of bulk MAOC and insoluble MAOC, respectively. Because XANES and CPMAS NMR differ in detection principles, sample preparation, and analytical sensitivity, cross-method mass-balance reconstruction was restricted to broad functional groups detectable by both techniques and was interpreted cautiously.

The XANES data were collected at the SGM beamline at the Canadian Light Source, following established protocols (Mao et al., 2025) (Text S2). Finely ground samples were pressed into pellets, and partial fluorescence yield spectra were collected, normalized, and energy-calibrated using standard procedures. Functional groups were quantified by spectral deconvolution into quinonic, aromatic, phenolic, aliphatic, carboxyl/amide, O-alkyl, and carbonyl components using Gaussian peak fitting.

NMR data were collected from 36 uppermost A-horizon and 15 uppermost B-horizon insoluble MAOC samples (Text S3). Spectra were processed and integrated into seven chemical shift regions

corresponding to major carbon functional groups. A molecular mixing model was applied to estimate the relative contributions of six biochemical classes: carbohydrates, proteins, lignin, lipids, carbonyls, and condensed aromatics (Baldock et al., 2004; Nelson and Baldock, 2005). Because condensed aromatics are underrepresented in CPMAS NMR due to rapid relaxation effects (Smernik et al., 2002), a $2\times$ correction factor was applied to the condensed aromatic fraction prior to calculation of relative biochemical abundances (Hockaday et al., 2009).

The functional-group composition of soluble MAOC was reconstructed by mass balance from the measured composition of bulk MAOC and insoluble MAOC:

$$P_{\text{soluble}} = \frac{(P_{\text{bulk}} \times \text{bulk MAOC} - P_{\text{insoluble}} \times \text{MAOC})}{\text{soluble MAOC}}$$

Where P_{soluble} , P_{bulk} , and $P_{\text{insoluble}}$ represent the relative abundance of a given functional group in the organic material of soluble MAOC, bulk MAOC, and insoluble MAOC, respectively. Bulk MAOC, insoluble MAOC, and soluble MAOC represent OC concentrations (g/kg soil) in the corresponding MAOC fractions.

To estimate the combined proportion of lignin and condensed aromatics in soluble MAOC and bulk MAOC, we first used the NMR dataset to establish an empirical relationship between the molecular mixing model estimate of lignin + condensed aromatics and the corresponding aromatic + phenolic C functional-group abundance (Fig. S3). This relationship was then applied to bulk MAOC and soluble MAOC, for which functional-group composition was available. This approach may underestimate aromatic compounds in soluble MAOC because it excludes non-aromatic functional groups associated with aromatic structures, particularly in soluble MAOC where carboxyl C is abundant (mean 50.0%, range 41.8–61.1%).

The molecular composition of HF-extracted MAOC was characterized by FT-ICR-MS. HF-extracted MAOC samples from uppermost A and B horizons were purified using solid-phase extraction with PPL cartridges to remove salts and metals before mass spectrometric analysis (Dittmar et al., 2008) (Text S4). Samples were analyzed using a 21 T electrospray FT-ICR-MS at the National High Magnetic Field Laboratory (Hendrickson et al., 2015; Smith et al., 2018). Molecular formulas were assigned using PetroOrg software based on exact mass, elemental constraints, and mass-error thresholds. Peaks with signal-to-noise ratios ≥ 6 were retained. Molecular intensities were normalized to total signal intensity (Sleighter et al., 2010), and compound classes were assigned in van Krevelen space based on H/C and O/C ratios (Boye et al., 2017). Because electrospray ionization and solid-phase extraction can introduce selectivity, FT-ICR-MS data were interpreted as relative molecular fingerprints rather than absolute compound concentrations. Potential HF-induced changes in the detected molecular fingerprint were evaluated separately by comparing Suwannee River fulvic acid before and after exposure to 10% HF using FT-ICR-MS; experimental details and results are provided in Text S7 and Table S4.

2.4. Estimation of plant and microbial contributions

Microbial- and plant-associated contributions in bulk MAOC and its subfractions were assessed using chemical-composition-based approaches (Text S5). These estimates should not be interpreted as definitive source partitioning, because many compound classes can originate from both plant and microbial sources (Whalen et al., 2022).

For XANES-based estimates, lignin and condensed-aromatic-like components were treated as indicators of predominantly plant-associated or pyrogenic contributions, whereas non-aromatic components were treated as primarily microbial-derived. This approach reflects a limitation common to compound class-based source estimates, because aromatic compounds can also be produced by microbes, including melanin and phenolic metabolites, and non-aromatic compounds such as carbohydrates, proteins, and lipids can derive from both

plant and microbial sources (Fernandez et al., 2019; Whalen et al., 2022). For FT-ICR-MS- and NMR-based estimates, compounds were assigned to plant or microbial-associated classes according to biochemical class following the criteria described in Text S5. Contributions to bulk MAOC were estimated using either XANES alone or a combined FT-ICR-MS and ^{13}C NMR data (Table S1). Contributions to the insoluble MAOC fraction were estimated from ^{13}C NMR data, whereas those in the soluble MAOC fraction and HF-extracted MAOC were estimated from XANES/NMR mass-balance reconstruction and FT-ICR-MS data, respectively (Table S1).

2.5. Radiocarbon analysis

Radiocarbon contents of bulk MAOC and insoluble MAOC were measured to evaluate whether the soluble and insoluble MAOC fractions differed in radiocarbon signatures. Samples were selected from 19 NEON sites spanning broad gradients in MAP and MAT. The dataset included uppermost A-horizon samples from 14 sites and paired uppermost A- and B-horizon samples from five additional sites. For these samples, soluble MAOC accounted for 27.3–61.5% of bulk MAOC. This range was selected to avoid samples in which either soluble MAOC or insoluble MAOC overwhelmingly dominated total MAOC, because such samples would make mass-balance estimation of the soluble fraction radiocarbon signature highly uncertain. Bulk MAOC and insoluble MAOC were analyzed directly using a Mini Carbon Dating System at Northern Arizona University. Results were reported as $\delta^{14}\text{C}$ (‰). The radiocarbon signature of soluble MAOC was estimated by C-weighted mass balance using fraction modern values:

$$F_{\text{soluble}} = \frac{(F_{\text{bulk}} \times \text{bulk MAOC} - F_{\text{insoluble}} \times \text{insoluble MAOC})}{\text{soluble MAOC}}$$

where F_{soluble} , F_{bulk} , and $F_{\text{insoluble}}$ are the fraction modern values of soluble MAOC, bulk MAOC, and insoluble MAOC, respectively. Bulk MAOC, insoluble MAOC, and soluble MAOC represent OC concentrations (g/kg soil) in the corresponding MAOC fractions. The calculated F_{soluble} values were then converted to $\delta^{14}\text{C}$. Additional details of radiocarbon analysis are provided in Text S6.

2.6. Statistical analyses

All statistical analyses were performed in R, with statistical significance evaluated at $\alpha = 0.05$. Data normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene's tests, respectively. When necessary, data were transformed to meet parametric assumptions. Differences between A and B horizons in the soluble MAOC proportion and selected chemical properties were evaluated using one-way ANOVA followed by Tukey's honestly significant difference test. Differences among MAOC fractions in functional-group composition, biochemical-class abundance, and source-related signatures were evaluated using ANOVA, with Tukey's test for post hoc comparisons where appropriate. Uncertainty associated with the mass-balance reconstruction of soluble MAOC composition was evaluated using Monte Carlo uncertainty propagation. Measured C concentrations and compound-class relative abundances were randomly sampled from normal distributions based on their observed means and standard deviations, and soluble MAOC compound-class abundances were recalculated over 10,000 iterations.

Relationships between bulk MAOC concentration and soluble MAOC, insoluble MAOC, or the soluble MAOC proportion were evaluated using linear and nonlinear models. Candidate models included linear, Langmuir-type, quadratic, exponential, power-law, and logarithmic functions. Models were compared using Akaike information criterion (AIC), and the model with the lowest AIC was selected unless competing models differed by less than two AIC units, in which case the simpler model was retained. Environmental controls on the soluble MAOC

proportion and MAOC chemical composition were evaluated using regression-based approaches. Multicollinearity among candidate predictors, including soil physicochemical properties, climate variables, and vegetation variables, was first assessed using variance inflation factors (VIFs) with the R package *car*. Variables with $\text{VIF} > 5$ were removed before model selection. Backward stepwise regression using the R package *MASS* was then used to identify predictors of the soluble MAOC proportion, aromatic compound abundance in HF-extracted MAOC, and aromatic compound abundance in insoluble MAOC. Partial correlation analyses were used to visualize relationships between selected environmental predictors and aromatic compound abundance after accounting for covariation among predictors (Grace et al., 2016).

Structural equation modeling was conducted using the R package *piecewiseSEM* to evaluate direct and indirect relationships among effective moisture, soil pH, Fe_{ox} and Al_{ox} , soil depth, and the soluble MAOC proportion. Candidate paths were specified based on prior knowledge of relationships among climate, soil geochemistry, mineral-associated Fe and Al, and MAOC solubility. Non-significant paths were sequentially removed when doing so improved model parsimony without reducing overall model fit. Final model fit was evaluated using Fisher's C statistic, and standardized path coefficients were reported. Pairwise Levene's tests were used to compare the variability of the soluble MAOC proportion with that of MAOC chemical-composition variables across environmental gradients. P-values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure.

Within each horizon, differences in $\delta^{14}\text{C}$ among soluble MAOC, insoluble MAOC, and bulk MAOC were evaluated using one-way ANOVA followed by Tukey's honestly significant difference test. Relationships between $\delta^{14}\text{C}$ and environmental variables were evaluated using Spearman rank correlations.

3. Results

3.1. Relative abundance and accumulation patterns of soluble and insoluble MAOC fractions

The soluble and insoluble MAOC fractions varied substantially across ecosystems and soil horizons (Fig. 2A). Across all A- and B-horizon samples ($n = 180$), soluble MAOC accounted for 10.8–95.5% of bulk MAOC, with a mean contribution of 52.5%. Insoluble MAOC accounted for 4.5–89.2% of bulk MAOC, with a mean contribution of 47.5%. The relative contribution of soluble MAOC was significantly greater in B horizons than in A horizons, whereas insoluble MAOC showed the opposite pattern (Fig. 2A).

Both soluble and insoluble MAOC concentrations increased with increasing bulk MAOC concentration, expressed as g C kg^{-1} silt + clay (Fig. 2B). However, the two fractions showed different accumulation patterns. Among the candidate models tested, a Langmuir-type model best described the relationship between soluble MAOC and bulk MAOC concentration, whereas insoluble MAOC was best described by an accelerating nonlinear regression and did not show an apparent asymptote within the observed MAOC range. Consequently, the relative contribution of soluble MAOC to bulk MAOC declined with increasing bulk MAOC concentration, while the relative contribution of insoluble MAOC increased (Fig. 2C). This shift was more pronounced in wetter ecosystems, defined here as sites with $\text{MAP/PET} > 0.65$.

3.2. Environmental associations with the soluble MAOC contribution

Structural equation modeling identified soil depth and effective moisture ($\text{MAP} - \text{PET}$) as key predictors of the relative contribution of soluble MAOC to bulk MAOC (Fig. 2D). The soluble MAOC/MAOC ratio increased with soil depth. Effective moisture was indirectly associated with the soluble MAOC/MAOC ratio through its relationships with soil pH and oxalate-extractable Fe and Al. Specifically, effective moisture was positively associated with $\text{Fe}_{\text{ox}} + 1/2\text{Al}_{\text{ox}}$ and negatively associated

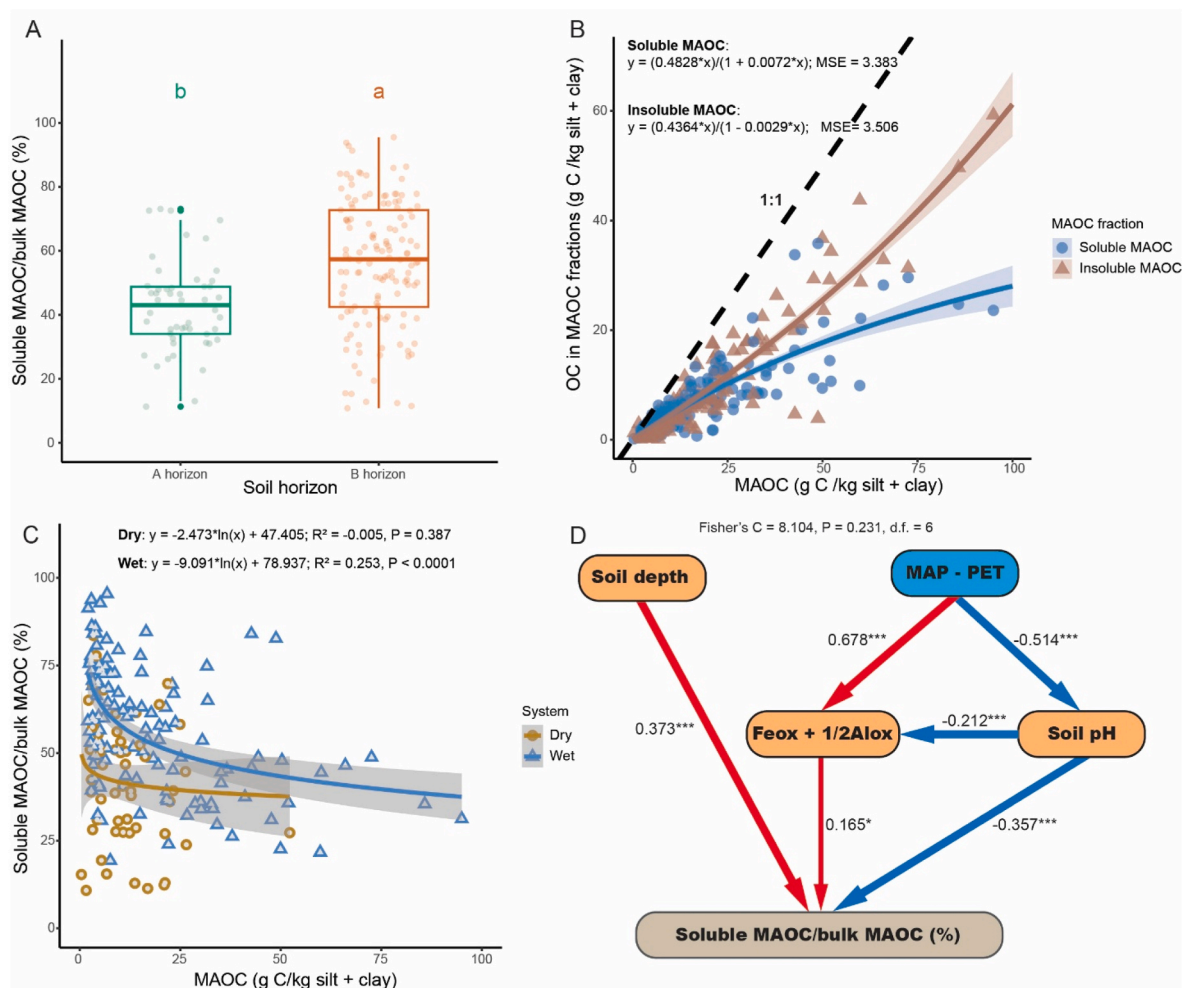


Fig. 2. The relative contributions of soluble and insoluble MAOC fraction to bulk MAOC, and their key controlling environmental drivers. (A) The relative contribution of soluble MAOC (soluble MAOC/bulk MAOC, %) in the A and B horizon soils ($n = 180$); The correlations between soluble or insoluble MAOC concentrations (B) or soluble MAOC/bulk MAOC ($n = 180$) (C) and MAOC concentrations (g C/kg silt + clay) ($n = 180$); (D) A structural equation model illustrating the environmental factors influencing soluble MAOC/bulk MAOC ($n = 180$). In the boxplot, the box represents the first and third quartiles, the whiskers indicate the minimum and maximum values, the horizontal line represents the median, and different lowercase letters denote statistically significant differences. In panel B, the blue line represents a Langmuir adsorption model fit, and brown a fit with a modified model based on Langmuir. In panel C, the blue and brown lines show logarithmic fits, while the gray band represents the 95% confidence intervals. In panel D, solid arrows represent significant pathways, with red and blue arrows indicating positive and negative piecewise relationships, respectively. The width of the arrows corresponds to the strength of the causal relationships. Numbers on the arrows denote standardized path coefficients, with significance levels represented as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. MSE: mean square error; MAP: mean annual precipitation; PET: potential evapotranspiration; Fe_{ox} + 1/2Al_{ox}: a sum of oxalate-extractable Fe and Al, and only half of the extracted Al is considered. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

with soil pH. In turn, the soluble MAOC/MAOC ratio was positively associated with Fe_{ox} + 1/2Al_{ox} and negatively associated with soil pH. Soil pH was also negatively associated with Fe_{ox} + 1/2Al_{ox}. The final SEM showed acceptable model fit based on Fisher's C statistic (Fisher's C = 8.104, $P = 0.231$, d. f. = 6).

3.3. Chemical composition of bulk MAOC, soluble MAOC, HF-extracted MAOC, and insoluble MAOC

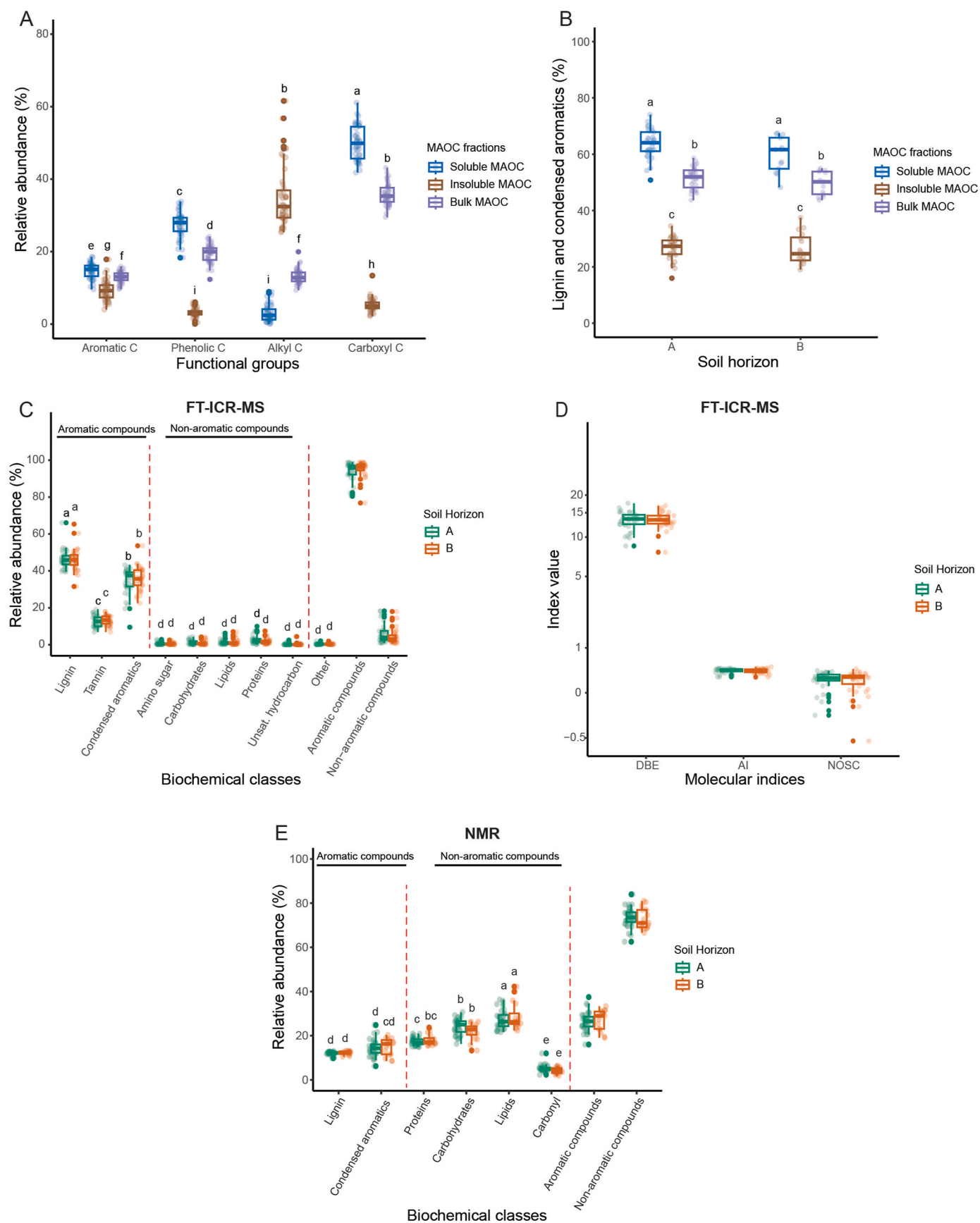
The chemical composition of bulk MAOC and its soluble and insoluble fractions differed markedly (Fig. 3). Based on C K-edge XANES analysis, bulk MAOC was dominated by carboxyl C and phenolic C. Carboxyl C accounted for 29.4–43.2% of bulk MAOC, with a mean of 35.7%, and phenolic C accounted for 12.3–24.2%, with a mean of 19.6% (Fig. 3A). Aromatic C and alkyl C were less abundant, accounting for 9.6–16.0% and 9.3–20.0% of bulk MAOC, respectively. Molecular mixing model estimates based on functional-group composition indicated that lignin and condensed aromatics together accounted for

43.7–58.7% of the organic material in bulk MAOC, with a mean of 51.0% (Fig. 3B).

Monte Carlo uncertainty propagation indicated that the mass-balance reconstruction of soluble MAOC functional-group composition was robust to propagated uncertainty (Table S1). Across 10,000 simulations, all reconstructed compound classes had positive 95% confidence intervals, and no simulations produced negative values.

Soluble MAOC was enriched in phenolic and carboxyl C relative to insoluble MAOC (Fig. 3A). Phenolic C accounted for 18.3–33.8% of soluble MAOC, with a mean of 27.5%, while carboxyl C accounted for 41.8–61.1%, with a mean of 50.0%. Molecular mixing model estimates indicated that lignin and condensed aromatics together accounted for 48.2–74.0% of the organic molecules in soluble MAOC, with a mean of 62.5% (Fig. 3B).

Insoluble MAOC showed a contrasting composition. It was depleted in phenolic C, carboxyl C, and aromatic C relative to soluble MAOC, while being enriched in alkyl C (Fig. 3A). Phenolic C accounted for 0.04–6.0% of insoluble MAOC, with a mean of 3.1%, and carboxyl C



(caption on next page)

Fig. 3. Chemical composition and properties of soluble MAOC, HF-extracted MAOC, insoluble MAOC, and bulk MAOC. (A) Relative abundance of functional groups in soluble MAOC, insoluble MAOC, and bulk MAOC ($n = 51$). (B) Relative abundance of lignin and char in soluble MAOC, insoluble MAOC, and bulk MAOC in A- and B-horizon soils ($n = 51$). (C) Relative abundance of biochemical classes in HF-extracted MAOC, identified by FT-ICR-MS in A- and B-horizon soils ($n = 74$). (D) Chemical indices of HF-extracted MAOC calculated from FT-ICR-MS data ($n = 74$), including double bond equivalents (DBE), aromaticity index (AI), and nominal oxidation state of carbon (NOSC). (E) Relative abundance of biochemical classes in insoluble MAOC ($n = 51$), derived from a mixing model. Boxes represent the first and third quartiles; whiskers indicate minimum and maximum values; and solid dots mark outliers. Different letters indicate statistically significant differences among treatments ($P < 0.05$).

accounted for 2.2–13.4%, with a mean of 5.2%. Aromatic C accounted for 0.04–17.9%, with a mean of 9.3%, whereas alkyl C accounted for 25.3–61.6%, with a mean of 32.9%. At the biochemical-class level, insoluble MAOC was dominated by lipids, carbohydrates, and proteins, which together accounted for 56.7–79.1% of the organic material, with a mean of 68.6% (Fig. 3E). Lignin and condensed aromatics were less abundant in insoluble MAOC, accounting for 16.4–37.5% of the organic material, with a mean of 26.5%.

The HF-extracted MAOC fraction, which represented the major component of total soluble MAOC (87%), was further characterized using FT-ICR-MS (Fig. 3C). Aromatic compounds, defined as lignin, tannin, and condensed aromatics, dominated the HF-extracted MAOC fraction and together accounted for 76.8–99.0% of the assigned molecular formulas, with a mean of 94.2%. Lignin was the most abundant class, accounting for 31.6–66.1%, with a mean of 46.1% of the assigned molecular formulas, followed by condensed aromatics, which accounted for 9.5–53.6%, with a mean of 35.1%, and tannin, which accounted for 6.8–19.1%, with a mean of 13.0%.

3.4. Environmental associations with the chemical composition of MAOC fractions

Because the chemical composition of soluble MAOC was reconstructed by mass balance from XANES and NMR data, analyses of environmental controls focused on directly measured HF-extracted MAOC and insoluble MAOC composition. Backward stepwise regression identified soil pH and MAP as retained predictors of the relative abundance of aromatic compounds in HF-extracted MAOC. The relative abundance of aromatic compounds in HF-extracted MAOC increased with MAP and decreased with soil pH (Fig. 4A–D). For insoluble MAOC, backward stepwise regression retained soil pH, clay content, and MAT as predictors of aromatic compound abundance. The relative abundance of aromatic compounds in insoluble MAOC increased with soil pH, clay content, and MAT (Fig. 4E–H). These relationships were based on partial regression analyses that accounted for covariation among retained predictors.

3.5. Plant and microbial contributions to MAOC

Chemical composition-based estimates indicated that bulk MAOC contained both plant- and microbial-associated organic matter (Fig. S4;

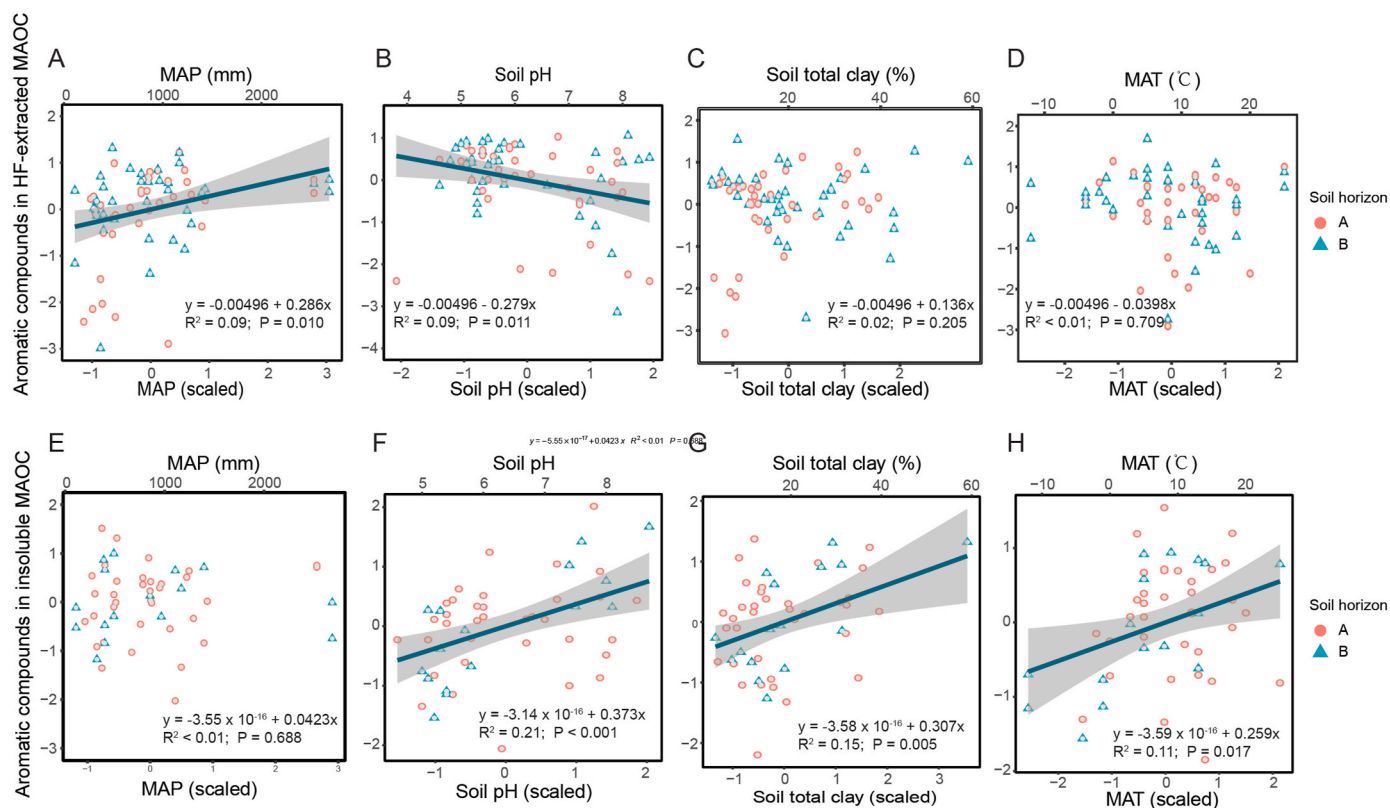


Fig. 4. Key environmental factors showing the relative abundance of aromatic compounds in HF-extracted MAOC fraction ($n = 74$) and insoluble MAOC fraction ($n = 51$). Multivariate partial plots showing correlations between the relative abundance of aromatic compounds in HF-extracted MAOC or insoluble MAOC and environmental factors, including soil pH, clay, mean annual precipitation (MAP) and temperature (MAT). Aromatic compounds are defined as the sum of lignin, tannin and condensed aromatics. Green lines indicate linear fits; gray bands represent 95% confidence intervals. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table S2). Based on XANES-derived estimates, plant-associated or pyrogenic components accounted for about 44–59% of the organic material in bulk MAOC, with a mean of about 51%, while microbial-associated or non-aromatic signatures accounted for about 41–56%, with a mean of about 49%. Estimates based on combined FT-ICR-MS and NMR data yielded broader and slightly higher plant-associated contributions in bulk MAOC, ranging from about 40% to 81% of the organic material, with a mean of about 59% (Fig. S5; Table S2).

The soluble and insoluble MAOC fractions showed contrasting source-related signatures. Soluble MAOC had a stronger plant-associated signature, with XANES/NMR estimates indicating that plant-associated contributions accounted for about 48–74% of organic molecules, with a mean of about 62% (Fig. S4; Table S2). FT-ICR-MS analysis of HF-extracted MAOC showed an even stronger dominance of lignin-, tannin-, and condensed-aromatic-like molecular classes, which accounted for about 77–99% of the organic molecules, with a mean of 94% (Fig. 3C; Fig. S6; Table S2). In contrast, insoluble MAOC had a stronger microbial-associated contribution. Based on NMR-derived biochemical-class estimates, plant-associated contributions accounted for about 27–46% of the organic material in insoluble MAOC, with a mean of about 36%, while microbial-associated contributions accounted for about 54–73%, with a mean of about 64% (Fig. S4; Fig. S7; Table S2).

3.6. Radiocarbon signatures of MAOC fractions

Across the radiocarbon dataset, $\Delta^{14}\text{C}$ values of soluble MAOC, insoluble MAOC, and bulk MAOC ranged from -384‰ to 146‰ (Fig. 5A). Within each horizon, soluble MAOC, insoluble MAOC, and bulk MAOC did not differ significantly in $\Delta^{14}\text{C}$. Radiocarbon values differed more clearly by soil horizon than by MAOC fraction. For soluble MAOC, insoluble MAOC, and bulk MAOC, $\Delta^{14}\text{C}$ values were significantly higher in A horizons than in B horizons (Fig. 5A). Spearman correlation analyses showed that $\Delta^{14}\text{C}$ values were positively associated with MAP and negatively associated with soil depth (Fig. 5B). These relationships were observed for soluble MAOC, insoluble MAOC, and bulk MAOC. The positive correlations with MAP were significant at $P < 0.05$ for all three pools. The negative correlations with soil depth were significant at $P < 0.05$ for soluble MAOC and at $P < 0.01$ for insoluble MAOC and bulk MAOC. Thus, variation in radiocarbon

signatures was more strongly aligned with horizon/depth and climate than with the operational soluble versus insoluble fraction distinction.

4. Discussion

4.1. Environmental controls on the relative abundance of soluble MAOC

The large variation in soluble and insoluble MAOC fractions across NEON soils indicates that the recoverable MAOC solubility is strongly shaped by environmental context. Effective soil moisture, a climate factor, and soil depth were strongly associated with the soluble MAOC proportion, likely through their relationships with soil pH and Fe/Al phase abundance. The higher soluble MAOC contribution in acidic, Fe/Al-rich soils is consistent with stronger retention of soluble organic compounds through sorption and metal complexation. Such acidic mineral soils were also low in the total organic C concentration (Fig. S8). These conditions further suppress microbial activity (Fierer and Jackson, 2006), hence the production of microbial necromass for insoluble MAOC formation (Totsche et al., 2018). As a result, DOC, once entering the acidic mineral soils, is quickly adsorbed onto mineral surfaces rather than microbially assimilated to produce microbial necromass (Sokol et al., 2019). Moreover, DOC readily percolates into subsoil layers in wet environments (Karsten Kalbitz et al., 2000; Kaiser and Kalbitz, 2012), potentially increasing the contribution of soluble organic matter retained in deeper mineral horizons. In contrast, dry ecosystems typically have a low net primary productivity, limited moisture, and low abundance of Fe and Al oxides in soils, restricting DOC production, vertical transport, and sorption (Schimel, 2010; Kaiser and Kalbitz, 2012). In summary, soluble MAOC contributed more strongly in deeper, lower-SOC soils and wetter climates, whereas insoluble MAOC contributed more strongly in surface, higher-SOC soils and drier regions. These patterns are consistent with, but do not directly prove, differences in dominant retention processes.

4.2. Distinct accumulation pattern of soluble and insoluble MAOC

Both soluble MAOC and insoluble MAOC increased with total MAOC concentration, but their accumulation patterns differed markedly (Fig. 2B), suggesting distinct accumulation behavior. Soluble MAOC followed a Langmuir-type relationship with total MAOC, approaching

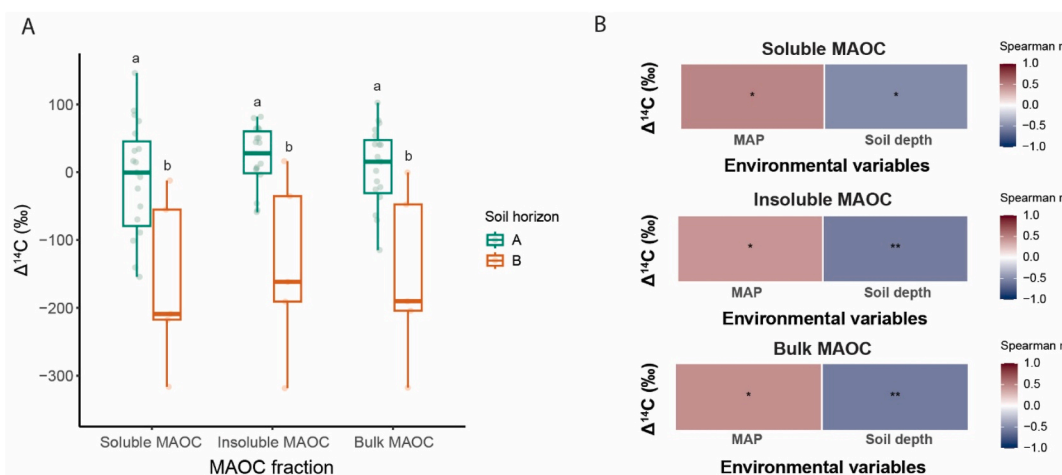


Fig. 5. $\Delta^{14}\text{C}$ (‰) of soluble MAOC, insoluble MAOC, and bulk MAOC. (A) $\Delta^{14}\text{C}$ (‰) of soluble MAOC, insoluble MAOC, and bulk MAOC in the uppermost A soil horizons from 19 NEON sites ($n = 19$) and B soil horizons from five NEON sites ($n = 5$). (B) Heatmap of Spearman correlation coefficients (r) between $\Delta^{14}\text{C}$ (‰) of soluble MAOC, insoluble MAOC, and bulk MAOC and environmental variables across 19 NEON sites ($n = 24$). Box plots display the interquartile range (25th to 75th percentile), with whiskers indicating the minimum and maximum values, the horizontal line denoting the median, and different lowercase letters indicating statistically significant differences ($P < 0.05$). In the heatmap, red and blue colors indicate positive and negative correlations, respectively; color intensity reflects correlation strength. Numerical values within cells represent correlation coefficients, with significance indicated by asterisks (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

an apparent upper limit at high MAOC concentrations. This saturation-like behavior is consistent with the idea that retention of soluble organic compounds is constrained by finite reactive mineral surfaces, metal-binding capacity, or available organo-mineral interaction sites (Gu et al., 1994; Hassink, 1997; Kaiser and Guggenberger, 2000). Such behavior aligns with adsorption-based representations of MAOM formation in some SOC models, where dissolved organic matter accumulates on mineral surfaces until sorption capacity becomes limiting (Woolf and Lehmann, 2019; Ahrens et al., 2020; Yu et al., 2020).

Insoluble MAOC showed a different pattern, increasing nonlinearly without an apparent asymptote across the observed MAOC range. This pattern suggests that the accumulation of less soluble or particulate organic matter is not described well by a simple Langmuir-type sorption model over the range of soils examined here. Instead, insoluble MAOC may accumulate through processes involving physical proximity to minerals, incorporation into fine mineral-organic structures, and organo-organic interactions with pre-existing organic matter (Totsche et al., 2018; Possinger et al., 2020; Witzgall et al., 2021; Kang et al., 2024). Recent nanoscale and experimental studies show that organo-organic interfaces and existing mineral-associated organic matter can promote additional C accumulation beyond direct sorption to previously unoccupied mineral surfaces (Possinger et al., 2020; Kang et al., 2024; King and Sokol, 2025). Although insoluble MAOC did not show an apparent asymptote across the observed range, its accumulation is ultimately finite. As insoluble organic matter continues to accumulate in MAOM, increasing organic loading is expected to reach a threshold concentration above which the organic-rich microaggregates destabilize, particularly during MAOM isolation, due to the weak organo-organic/particle-particle interactions (Kögel-Knabner et al., 2008; Georgiou et al., 2025). Thus, both fractions are expected to have upper limits of accumulation, but only soluble MAOC showed saturation-like behavior within the observed MAOC range.

These contrasting accumulation dynamics imply a shift in the dominant MAOC fraction as MAOC increases. Soluble MAOC appears to contribute more strongly during the initial phase of MAOC accumulation, whereas insoluble MAOC becomes progressively more important as MAOC builds up, particularly in wetter ecosystems (MAP/PET > 0.65). This transition may reflect declining availability of reactive mineral or metal-associated sites for additional soluble organic matter retention, coupled with enhanced litter inputs and microbial processing, which promote the accumulation of less soluble or particulate organic matter under conditions of greater organic matter supply. Consistently, a recent study shows that increasing plant-derived DOC inputs promotes transformation of DOC into microbial necromass, thereby shifting MAOC accumulation from DOC sorption onto mineral surfaces to the accrual of microbial residues onto pre-existing MAOC via organo-organic associations (King and Sokol, 2025).

4.3. Chemical composition of soluble and insoluble MAOC

The soluble and insoluble MAOC differed markedly in chemical composition (Fig. 3). The high phenolic to aromatic C and carboxyl to alkyl C ratios of soluble MAOC based on the XANES and NMR analyses (Fig. 3A) and the dominance of aromatic compound classes in HF-extracted MAOC based on the FT-ICR-MS analysis (Fig. 3C) together suggest extensive oxidation of aromatic rings (phenolic), and side chains (carboxyl), of dissolved aromatic compounds. These findings are consistent with previous observations that organic compounds rich in phenolic and carboxyl groups are generally polar and can interact strongly with mineral surfaces or metal cations (Scheel et al., 2008; Kleber et al., 2015).

The strong enrichment of aromatic C in soluble MAOC observed in the present study was also found in previous studies. A recent FT-ICR-MS study reported that aromatic compounds accounted for more than 75% of the organic molecules in soluble MAOC recovered via sequential extractions using sodium pyrophosphate, hydroxylamine-HCl, and

dithionite-HCl (Yang et al., 2024). Moreover, another study applying HF extraction to MAOC and characterizing soluble MAOC using Fourier transform infrared spectroscopy (FTIR) similarly found that soluble MAOC was dominated by aromatic C (Wen et al., 2021). Furthermore, previous work showed that HF-extraction residues were poor in aromatic and carboxylic carbon relative to soluble fraction from boreal forest mineral soils based on bulk soils and a ^{13}C NMR analysis (Warren and Ziegler, 2017). The convergence of these results across fundamentally different analytical approaches — including FT-ICR-MS, FTIR, and ^{13}C NMR — supports the interpretation that the soluble MAOC recovered here is enriched in aromatic compounds, in spite of potential methodological biases associated with FT-ICR-MS regarding sample preparation, and data acquisition and interpretation (Dittmar et al., 2008; Ohno et al., 2016; Raeke et al., 2016).

Insoluble MAOC had contrasting chemical characteristics with lower phenolic, carboxyl, and aromatic C but higher alkyl C at the functional group level, and by depletion in lignin and condensed aromatics but enrichment in lipids, carbohydrates, and proteins at the molecular level. These chemical features indicate that insoluble MAOC is relatively depleted in oxidized and polar functional groups and relatively enriched in hydrophobic molecular features. The chemical contrast between soluble and insoluble MAOC therefore supports the broader conclusion that bulk MAOC contains chemically distinct subfractions that would be obscured if MAOC were treated as a single homogeneous pool.

Despite the fact that the relative contributions of soluble or insoluble fractions to total MAOC are ecosystem specific, the chemical composition of each fraction surprisingly remained relatively conserved across ecosystems (Table S3). The relatively conservative nature of their chemistry reflects joint controls of microbial processing, mineral sorption, and intrinsic chemical properties of organic compounds. Microbes may preferentially decompose non-aromatic substrates (e.g., polysaccharides and amino acids) compared to lignin and tannins (Berg and McClaugherty, 2008; Wilkinson et al., 2014), enhancing aromatic contributions to SOM (Mao et al., 2025). Depolymerization and oxidation of lignin and tannin during further decomposition produce soluble hydroxyl- and carboxyl-rich aromatic compounds that are preferentially sorbed to mineral surfaces compared to non-aromatic compounds (Kleber et al., 2015; Lehmann and Kleber, 2015; Lv et al., 2016; Hu et al., 2024). Together, microbial processing and mineral interactions may preferentially retain soluble, oxidized aromatic compounds, whereas insoluble MAOC tends to consist of larger, hydrophobic, and weakly oxidized organic materials.

Environmental controls on chemical composition were still detectable but modest. The relative abundance of aromatic compounds in HF-extracted MAOC increased with MAP and decreased with soil pH, consistent with stronger sorption of aromatic compounds under wetter, more acidic, Fe/Al-rich conditions (Eusterhues et al., 2011; Coward et al., 2018; Hu et al., 2024). In contrast, the share of aromatic compounds in insoluble MAOC increased with soil pH, clay content, and MAT. The mechanisms behind these relationships are less direct and may involve differences in litter input (Fig. S9), microbial processing, clay-associated physical protection, and pyrogenic C abundance (Kögel-Knabner et al., 2008; Islam et al., 2022; Leuthold et al., 2025). Because the statistical relationships were modest, these environmental correlations should not be overinterpreted as strong causal controls. Additionally, soil mineralogy can be a key factor influencing the chemical composition of both soluble and insoluble MAOC (Kögel-Knabner et al., 2008). In future work, we will characterize soil mineralogy using X-ray diffraction (XRD) to better elucidate its role in shaping the chemical composition of these MAOC fractions.

4.4. Source-related signatures of soluble and insoluble MAOC

It is challenging to quantify C sources in soils (Whalen et al., 2022), and various approaches have been employed to estimate the quantities of plant and microbial C, including chemical composition-based

methods. Source assignments based on chemical composition must be interpreted cautiously. Aromatic compounds can originate from plant tissues, pyrogenic inputs, or microbial products such as melanin and phenolic metabolites, while non-aromatic compounds such as carbohydrates, lipids, and proteins can have both plant and microbial origins (Fernandez et al., 2019; Whalen et al., 2022). Within this limitation, our estimates of microbial-associated contributions in bulk MAOC based on chemical composition are largely consistent with previous reports based on amino sugar biomarkers, the most widely used methods for quantifying microbial C (Angst et al., 2021). The combined FT-ICR-MS and NMR analyses yielded plant-associated compositional estimates of about 40–80% (mean: about 59%) for the organic material in bulk MAOC, similar to previous reports (13–88%, mean: 61%) based on biomarker analyses (Angst et al., 2021). The XANES-based approach yielded a slightly lower plant-associated estimate than FT-ICR MS/NMR estimate, likely because only aromatic C is considered as plant-derived C. However, our results do not align with prior studies using ^{13}C NMR spectroscopy, which reported that bulk MAOM primarily consisted of polysaccharides and aliphatic compounds (Guggenberger et al., 1994; Kögel-Knabner et al., 2008), potentially because a large portion of aromatics had already been removed by HF treatment prior to NMR measurement.

Potential sources for the soluble MAOC include DOC derived from highly decomposed aboveground litter, sloughed root tissues, and root exudates (K. Kalbitz et al., 2000; Sokol and Bradford, 2019) and soluble microbial metabolites, such as phosphate-containing compounds that sorb strongly to mineral phases (Swenson et al., 2015; Spohn, 2024). The insoluble MAOC is consistent with contributions from microbial necromass and extracellular polymeric substances (EPS) (Totsche et al., 2018). Nitrogen-rich microbial necromass can form stable associations with minerals, especially poorly crystalline iron phases, whereas EPS facilitate aggregation by binding mineral particles and organic matter (Totsche et al., 2018; Zhao et al., 2025). The non-microbial C in insoluble MAOC likely includes fine-sized decaying plant litter and root debris, and poorly oxidized pyrogenic C (Hardy et al., 2017; Witzgall et al., 2021). However, direct identifications of these source materials are warranted in future studies.

4.5. Radiocarbon signatures of soluble and insoluble MAOC

Our radiocarbon results showed no detectable difference in $\Delta^{14}\text{C}$ between soluble and insoluble MAOC in a given soil, suggesting that these two fractions did not differ clearly in mean radiocarbon signature, despite their distinct chemistry and source-related signatures. Because $\Delta^{14}\text{C}$ reflects the mean age and mixing history of carbon in a pool rather than intrinsic stability, these results should not be interpreted as direct evidence for equal persistence or vulnerability. One might expect the higher abundance of reactive functional groups in the soluble fraction to favor stronger mineral interactions; however, the measured $\Delta^{14}\text{C}$ values did not provide evidence for older mean carbon ages in this fraction (Bölscher et al., 2025). The absence of detectable $\Delta^{14}\text{C}$ differences between the two fractions may reflect several non-exclusive processes. On one hand, the soluble fraction receives fresh DOC leached from overlying litter layers, which can decrease measured ^{14}C age of the soluble fraction (Kaiser and Kalbitz, 2012; Ahrens et al., 2020). On the other hand, the insoluble fraction may retain old carbon through repeated microbial recycling without mineralization. High microbial carbon use efficiency can promote successive assimilation and resynthesis of organic substrates, resulting in the incorporation of older carbon into newly formed microbial biomass and necromass (Rethemeyer et al., 2005; Rumpel and Kögel-Knabner, 2011; Kästner et al., 2021). If microbial residues contribute substantially to insoluble MAOC, this recycling can maintain the older carbon even if insoluble MAOC is more accessible to microbial decomposition than soluble MAOC. Together, these findings are consistent with previous work demonstrating that radiocarbon measurements constrain the mean age of carbon within a

pool but do not directly quantify its stability or vulnerability, i.e., decoupling ^{14}C age from intrinsic stability (Rethemeyer et al., 2005; Rumpel and Kögel-Knabner, 2011).

Our results indicate that MAOC $\Delta^{14}\text{C}$ values decreased with increasing soil depth and increased with increasing MAP across ecosystems. These findings were also observed in previous studies. Heckman et al. (2023) reported similar depth-related patterns across NEON sites. Subsoils receive less fresh organic matter and support lower microbial biomass and activity than surface horizons (Fontaine et al., 2007; Rumpel and Kögel-Knabner, 2011), limiting both the replacement of older MAOC and the formation of new MAOC, thereby increasing MAOC persistence (Ahrens et al., 2020; Heckman et al., 2023). Consistent with our findings, faster MAOC turnover was found under warmer and wetter climatic conditions (Zhou et al., 2024). Higher moisture enhances plant productivity, fresh carbon inputs and DOC transport, while stimulating microbial activity (Kalbitz et al., 2000; Rustad et al., 2001; Heckman et al., 2023). These processes accelerate new MAOC formation and the replacement of older MAOC, and may increase priming-driven destabilization of MAOC (Jilling et al., 2021; Heckman et al., 2023).

4.6. Utility and limitations of HF extraction for separating soluble and insoluble MAOC

Our results suggest that the 10% HF extraction followed by water rinsing can recover a substantial soluble fraction of MAOM while causing limited compositional alteration under the conditions tested. Previous studies used oxalate, pyrophosphate, and dithionite to extract mineral-associated organic matter associated with specific mineral phases (Heckman et al., 2018; Yang et al., 2024). However, these extractants only dissolve certain types of metal oxides and phyllosilicates, unlike HF that dissolves all phyllosilicates and metal oxides regardless of their crystallinity and chemical composition (Roberson and Hem, 1969; Hossner, 1996). The minimal alteration of SOC by 10% HF, as demonstrated with fulvic acid and FT-ICR-MS analysis in the present study (Text S7; Table S4), is expected. Fluoride is not generally expected to be the primary driver of organic-matter alteration under these conditions. It is the acid of the solution that alters the organics through acidic hydrolysis of some labile proteins and polysaccharides (Skjemstad et al., 1994; Craig and Collins, 2000; Zegouagh et al., 2004). However, previous studies indicated that such alterations are minor and HF pretreatment largely preserves bulk SOC molecular composition (Schmidt et al., 1997; Rumpel et al., 2006; Fang et al., 2010; Hall et al., 2020; Zhao and Pan, 2023). Thus, HF extraction followed by water rinsing provides a useful operational approach for separating the soluble and insoluble MAOC fractions for further characterization, provided that potential extraction-induced alteration and method-specific selectivity are explicitly considered.

5. Conclusion

Our study provides a cross-ecosystem assessment of soluble and insoluble MAOC fractions across diverse North American soils. These fractions varied widely in their relative contributions to bulk MAOC and differed strongly in chemical composition. Soluble MAOC showed saturation-like accumulation with increasing total MAOC and was enriched in oxidized aromatic compounds, consistent with stronger plant-associated or pyrogenic compositional signatures. In contrast, insoluble MAOC showed no apparent saturation over the observed MAOC range and was enriched in less oxidized, non-aromatic molecular classes, consistent with stronger microbial-associated compositional signatures. These contrasting patterns indicate that bulk MAOC is not a chemically uniform pool, but instead contains fractions with distinct solubility, accumulation behavior, chemical composition, and source-related signatures.

The unique but relatively conservative C chemistry associated with

each fraction reflects chemical convergence, likely resulting from joint controls of microbial processing, mineral interaction, and intrinsic chemical properties of organic compounds. Our study further suggests that inferred plant-versus microbial-associated contributions vary with the relative contribution of soluble and insoluble MAOC, which in turn is shaped by environmental context. Despite marked differences in C chemistry and inferred source-related signatures, soluble and insoluble MAOC did not differ detectably in $\Delta^{14}\text{C}$ within horizons. Together, these findings integrate MAOC chemistry, source-related signatures, persistence of MAOC, and environmental controls, providing a foundation for understanding MAOC heterogeneity, stability, and accrual across ecosystems.

Our findings provide empirical evidence useful for refining process-based SOC models focused on MAOM dynamics. Models, such as COMMISSION 2.0 and JSM, treat MAOM formation as a single unified sorption process (Woolf and Lehmann, 2019; Ahrens et al., 2020; Yu et al., 2020). Our results suggest that MAOC dynamics may be better represented by accounting for soluble organic matter retention and less soluble or particulate organic matter (fine-sized, < 53 μm) accumulation as distinct but interacting processes, rather than treating MAOC as a single homogeneous sorption pool. These fractions may require different parameterization of accumulation kinetics, chemical composition, source-related inputs, and environmental sensitivities, as partly reflected in models such as Millennial model V2 and MEMS 2.0 model (Zhang et al., 2021; Abramoff et al., 2022). Soluble MAOC may be represented using Langmuir-type functions linked to oxidized aromatic compounds, whereas insoluble MAOC may require alternative accumulation functions linked to microbial turnover, particulate inputs, and organo-organic or particle-particle interactions. Implementing this separation in SOC models may allow for better understanding of MAOC formation, destruction, and saturation (Georgiou et al., 2025), improving projections of SOC responses to environmental change and management.

CRedit authorship contribution statement

Hongfei Liu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Carson Thompson:** Data curation, Methodology. **Hai-Ruo Mao:** Data curation. **Chao Liang:** Conceptualization, Writing – review & editing. **Amy M. McKenna:** Data curation, Methodology. **Fu Chen:** Data curation, Methodology. **Edward A.G. Schuur:** Data curation, Methodology. **Andrea Jilling:** Conceptualization, Writing – review & editing. **Mengqiang Zhu:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interests

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.

Acknowledgements

This work was partially supported by National Science Foundation under DEB 2027284. We thank Professor M. Francesca Cotrufo (Colorado State University) for her insightful comments and stimulating discussion that improved the manuscript. We also thank Prof. Jingdong Mao (Old Dominion University) for the discussion on the potential alteration of organic matter by HF and interpretation of the NMR data. The National Ecological Observatory Network (NEON) is a program sponsored by the U.S. National Science Foundation and operated under cooperative agreement by Battelle. This material uses specimens and/or samples collected as part of the NEON Program. The 21 T FT-ICR mass spectrometer at the National High Magnetic Field Laboratory is located in the Ion Cyclotron Resonance user facility, which is supported by the

United States of America National Science Foundation Division of Materials Research and Division of Chemistry through DMR-2128556 and the State of Florida. The carbon K-edge XANES data were collected at the Canadian Light Source, supported by the Natural Sciences and Engineering Research Council of Canada, the National Research Council Canada, the Canadian Institutes of Health Research, the Province of Saskatchewan, Western Economic Diversification Canada, and operated by the University of Saskatchewan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2026.110287>.

References

- Abramoff, R.Z., Guenet, B., Zhang, H., Georgiou, K., Xu, X., Viscarra Rossel, R.A., Yuan, W., Clais, P., 2022. Improved global-scale predictions of soil carbon stocks with millennial version 2. *Soil Biology and Biochemistry* 164, 108466. <https://doi.org/10.1016/j.soilbio.2021.108466>.
- Ahrens, B., Guggenberger, G., Rethemeyer, J., John, S., Marschner, B., Heinze, S., Angst, G., Mueller, C.W., Kögel-Knabner, I., Leuschner, C., Hertel, D., Bachmann, J., Reichstein, M., Schrumpp, M., 2020. Combination of energy limitation and sorption capacity explains 14C depth gradients. *Soil Biology and Biochemistry* 148, 107912. <https://doi.org/10.1016/j.soilbio.2020.107912>.
- Angst, G., Mueller, K.E., Nierop, K.G.J., Simpson, M.J., 2021. Plant- or microbial-derived? A review on the molecular composition of stabilized soil organic matter. *Soil Biology and Biochemistry* 156, 108189. <https://doi.org/10.1016/j.soilbio.2021.108189>.
- Baldock, J.A., Masiello, C.A., Gélina, Y., Hedges, J.I., 2004. Cycling and composition of organic matter in terrestrial and marine ecosystems. New approaches in marine organic biogeochemistry: a tribute to the life and science of John I. Hedges 92, 39–64. <https://doi.org/10.1016/j.marchem.2004.06.016>.
- Berg, B., McLaugherty, C., 2008. *Plant Litter: Decomposition, Humus Formation, Carbon Sequestration*. Springer.
- Bölscher, T., Cardon, Z.G., Garcia Arredondo, M., Grand, S., Griffen, G., Hestrin, R., Imboden, J., Jamoteau, F., Lacroix, E.M., Pérez Castro, S., Persson, P., Riley, W.J., Keiluweit, M., 2025. Vulnerability of mineral-organic associations in the rhizosphere. *Nature Communications* 16, 5527. <https://doi.org/10.1038/s41467-025-61273-4>.
- Boye, K., Noël, V., Tfaily, M.M., Bone, S.E., Williams, K.H., Bargar, J.R., Fendorf, S., 2017. Thermodynamically controlled preservation of organic carbon in floodplains. *Nature Geoscience* 10, 415–419. <https://doi.org/10.1038/ngeo2940>.
- Cotrufo, M.F., Lavalée, J.M., 2022. Chapter one - soil organic matter formation, persistence, and functioning: a synthesis of current understanding to inform its conservation and regeneration. In: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 1–66. <https://doi.org/10.1016/bs.agron.2021.11.002>.
- Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology* 19, 988–995. <https://doi.org/10.1111/gcb.12113>.
- Coward, E.K., Ohno, T., Plante, A.F., 2018. Adsorption and molecular fractionation of dissolved organic matter on iron-bearing mineral matrices of varying crystallinity. *Environmental Science and Technology* 52, 1036–1044. <https://doi.org/10.1021/acs.est.7b04953>.
- Craig, O.E., Collins, M.J., 2000. An improved method for the immunological detection of mineral bound protein using hydrofluoric acid and direct capture. *Journal of Immunological Methods* 236, 89–97. [https://doi.org/10.1016/S0022-1759\(99\)00242-2](https://doi.org/10.1016/S0022-1759(99)00242-2).
- Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440, 165–173. <https://doi.org/10.1038/nature04514>.
- Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnology and Oceanography: Methods* 6, 230–235. <https://doi.org/10.4319/lom.2008.6.230>.
- Eusterhues, K., Rennert, T., Knicker, H., Kögel-Knabner, I., Totsche, K.U., Schwertmann, U., 2011. Fractionation of organic matter due to reaction with ferrihydrite: coprecipitation versus adsorption. *Environmental Science and Technology* 45, 527–533. <https://doi.org/10.1021/es1023898>.
- Eusterhues, K., Rumpel, C., Kleber, M., Kögel-Knabner, I., 2003. Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. *Organic Geochemistry* 34, 1591–1600. <https://doi.org/10.1016/j.orggeochem.2003.08.007>.
- Eusterhues, K., Rumpel, C., Kögel-Knabner, I., 2007. Composition and radiocarbon age of HF-resistant soil organic matter in a podzol and a cambisol. *Organic Geochemistry* 38, 1356–1372. <https://doi.org/10.1016/j.orggeochem.2007.04.001>.
- Fang, X., Chua, T., Schmidt-Rohr, K., Thompson, M.L., 2010. Quantitative ^{13}C NMR of whole and fractionated Iowa mollisols for assessment of organic matter composition.

- Geochimica et Cosmochimica Acta 74, 584–598. <https://doi.org/10.1016/j.gca.2009.10.008>.
- Fernandez, C.W., Heckman, K., Kolka, R., Kennedy, P.G., 2019. Melanin mitigates the accelerated decay of mycorrhizal necromass with peatland warming. *Ecology Letters* 22, 498–505. <https://doi.org/10.1111/ele.13209>.
- Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences* 103, 626–631. <https://doi.org/10.1073/pnas.0507535103>.
- Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450, 277–280. <https://doi.org/10.1038/nature06275>.
- Georgiou, K., Angers, D., Champigny, R.E., Cotrufo, M.F., Craig, M.E., Doetterl, S., Grandy, A.S., Lavalley, J.M., Lin, Y., Lugato, E., Poeplau, C., Rocci, K.S., Schweizer, S.A., Six, J., Wieder, W.R., 2025. Soil carbon saturation: what do we really know? *Global Change Biology* 31, e70197. <https://doi.org/10.1111/gcb.70197>.
- Grace, J.B., Anderson, T.M., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., Hautier, Y., Hillebrand, H., Lind, E.M., Pärtel, M., Bakker, J.D., Buckley, Y.M., Crawley, M.J., Damschen, E.I., Davies, K.F., Fay, P.A., Firm, J., Gruner, D.S., Hector, A., Knops, J.M.H., MacDougall, A.S., Melbourne, B.A., Morgan, J.W., Orrock, J.L., Prober, S.M., Smith, M.D., 2016. Integrative modelling reveals mechanisms linking productivity and plant species richness. *Nature* 529, 390–393. <https://doi.org/10.1038/nature16524>.
- Gu, B., Schmitt, J., Chen, Z., Liang, L., McCarthy, J.F., 1994. Adsorption and desorption of natural organic matter on iron oxide: mechanisms and models. *Environmental Science and Technology* 28, 38–46. <https://doi.org/10.1021/es00050a007>.
- Guggenberger, G., Christensen, B.T., Zech, W., 1994. Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. *European Journal of Soil Science* 45, 449–458. <https://doi.org/10.1111/j.1365-2389.1994.tb00530.x>.
- Hall, S.J., Ye, C., Weintraub, S.R., Hockaday, W.C., 2020. Molecular trade-offs in soil organic carbon composition at Continental scale. *Nature Geoscience* 13, 687–692. <https://doi.org/10.1038/s41561-020-0634-x>.
- Hardy, B., Leifeld, J., Knicker, H., Dufey, J.E., Deforce, K., Cornélis, J.-T., 2017. Long term change in chemical properties of preindustrial charcoal particles aged in forest and agricultural temperate soil. *Organic Geochemistry* 107, 33–45. <https://doi.org/10.1016/j.orggeochem.2017.02.008>.
- Harris, D., Horváth, W.R., Van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. *Soil Science Society of America Journal* 65, 1853–1856.
- Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant and Soil* 191, 77–87. <https://doi.org/10.1023/A:1004213929699>.
- Heckman, K., Lawrence, C.R., Harden, J.W., 2018. A sequential selective dissolution method to quantify storage and stability of organic carbon associated with Al and Fe hydroxide phases. *Geoderma* 312, 24–35. <https://doi.org/10.1016/j.geoderma.2017.09.043>.
- Heckman, K.A., Possinger, A.R., Badgley, B.D., Bowman, M.M., Gallo, A.C., Hatten, J.A., Nave, L.E., SanClements, M.D., Swanson, C.W., Weiglein, T.L., Wieder, W.R., Strahm, B.D., 2023. Moisture-driven divergence in mineral-associated soil carbon persistence. *Proceedings of the National Academy of Sciences* 120, e2210044120. <https://doi.org/10.1073/pnas.2210044120>.
- Hemingway, J.D., Rothman, D.H., Grant, K.E., Rosengard, S.Z., Eglinton, T.I., Derry, L.A., Galy, V.V., 2019. Mineral protection regulates long-term global preservation of natural organic carbon. *Nature* 570, 228–231. <https://doi.org/10.1038/s41586-019-1280-6>.
- Hendrickson, C.L., Quinn, J.P., Kaiser, N.K., Smith, D.F., Blakney, G.T., Chen, T., Marshall, A.G., Weisbrod, C.R., Beu, S.C., 2015. 21 tesla fourier transform ion cyclotron resonance mass spectrometer: a national resource for ultrahigh resolution mass analysis. *Journal of the American Society for Mass Spectrometry* 26, 1626–1632. <https://doi.org/10.1007/s13361-015-1182-2>.
- Hockaday, W.C., Masiello, C.A., Randerson, J.T., Smernik, R.J., Baldock, J.A., Chadwick, O.A., Harden, J.W., 2009. Measurement of soil carbon oxidation state and oxidative ratio by ¹³C nuclear magnetic resonance. *Journal of Geophysical Research, Biogeosciences* 114. <https://doi.org/10.1029/2008JG000803>.
- Hossner, L.R., 1996. Dissolution for total elemental analysis. In: *Methods of Soil Analysis, SSSA Book Series*, pp. 49–64. <https://doi.org/10.2136/sssabookser5.3.c3>.
- Hu, Z., McKenna, A.M., Wen, K., Zhang, B., Mao, H., Goual, L., Feng, X., Zhu, M., 2024. Controls of mineral solubility on adsorption-induced molecular fractionation of dissolved organic matter revealed by 21 T FT-ICR MS. *Environmental Science and Technology* 58, 2313–2322. <https://doi.org/10.1021/acs.est.3c08123>.
- Islam, M.R., Singh, B., Dijkstra, F.A., 2022. Stabilisation of soil organic matter: interactions between clay and microbes. *Biogeochemistry* 160, 145–158.
- Jilling, A., Keiluweit, M., Gutknecht, J.L.M., Grandy, A.S., 2021. Priming mechanisms providing plants and microbes access to mineral-associated organic matter. *Soil Biology and Biochemistry* 158, 108265. <https://doi.org/10.1016/j.soilbio.2021.108265>.
- Kaiser, K., Guggenberger, G., 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. *Organic Geochemistry* 31, 711–725. [https://doi.org/10.1016/S0146-6380\(00\)00046-2](https://doi.org/10.1016/S0146-6380(00)00046-2).
- Kaiser, K., Kalbitz, K., 2012. Cycling downwards – dissolved organic matter in soils. *Soil Biology and Biochemistry* 52, 29–32. <https://doi.org/10.1016/j.soilbio.2012.04.002>.
- Kalbitz, K., Solinger, S., Park, J.-H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: a review. *Soil Science* 165, 277–304. <https://doi.org/10.1097/00010694-200004000-00001>.
- Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications* 7, 13630. <https://doi.org/10.1038/ncomms13630>.
- Kang, J., Qu, C., Chen, W., Cai, P., Chen, C., Huang, Q., 2024. Organo-organic interactions dominantly drive soil organic carbon accrual. *Global Change Biology* 30, e17147. <https://doi.org/10.1111/gcb.17147>.
- Kästner, M., Miltner, A., Thiele-Bruhn, S., Liang, C., 2021. Microbial necromass in soils—linking microbes to soil processes and carbon turnover. *Frontiers in Environmental Science* 9, 756378. <https://doi.org/10.3389/fenvs.2021.756378>.
- King, A.E., Sokol, N.W., 2025. Soil carbon formation is promoted by saturation deficit and existing mineral-associated carbon, not by microbial carbon-use efficiency. *Science Advances* 11, eadv9482. <https://doi.org/10.1126/sciadv.adv9482>.
- Kleber, M., Bourg, I.C., Coward, E.K., Hansel, C.M., Myneni, S.C.B., Nunan, N., 2021. Dynamic interactions at the mineral-organic matter interface. *Nature Reviews Earth & Environment* 2, 402–421. <https://doi.org/10.1038/s43017-021-00162-y>.
- Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P.S., 2015. Chapter one - mineral-organic associations: formation, properties, and relevance in soil environments. In: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 1–140. <https://doi.org/10.1016/b.s.agron.2014.10.005>.
- Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry* 85, 9–24. <https://doi.org/10.1007/s10533-007-9103-5>.
- Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K., Leinweber, P., 2008. Organo-mineral associations in temperate soils: integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science* 171, 61–82. <https://doi.org/10.1002/jpln.200700048>.
- Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K., Leinweber, P., 2008. Organo-mineral associations in temperate soils: integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science* 171, 61–82. <https://doi.org/10.1002/jpln.200700048>.
- Lavalley, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global Change Biology* 26, 261–273. <https://doi.org/10.1111/gcb.14859>.
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature* 528, 60–68. <https://doi.org/10.1038/nature16069>.
- Leuthold, S., Haddix, M., Lavalley, J., Cotrufo, M., 2022. Physical fractionation techniques. Reference Module in Earth Systems and Environmental Sciences. Elsevier, pp. 1–13.
- Leuthold, S.J., Soong, J.L., Even, R.J., Cotrufo, M.F., 2025. Decadal persistence of grassland soil organic matter derived from litter and pyrogenic inputs. *Nature Geoscience*. <https://doi.org/10.1038/s41561-025-01638-y>.
- Lv, J., Zhang, S., Wang, S., Luo, L., Cao, D., Christie, P., 2016. Molecular-scale investigation with ESI-FT-ICR-MS on fractionation of dissolved organic matter induced by adsorption on iron oxyhydroxides. *Environmental Science and Technology* 50, 2328–2336. <https://doi.org/10.1021/acs.est.5b04996>.
- Mao, H.-R., Hart, S.C., Peak, D., McKenna, A.M., Zhu, M., 2025. Microbial transformation and mineral adsorption control chemical evolution of soil organic matter during semi-arid ecosystem development. *Geochimica et Cosmochimica Acta*. <https://doi.org/10.1016/j.gca.2025.08.041>.
- Nelson, P.N., Baldock, J.A., 2005. Estimating the molecular composition of a diverse range of natural organic materials from solid-state ¹³C NMR and elemental analyses. *Biogeochemistry* 72, 1–34. <https://doi.org/10.1007/s10533-004-0076-3>.
- Ohno, T., Sleighter, R.L., Hatcher, P.G., 2016. Comparative study of organic matter chemical characterization using negative and positive mode electrospray ionization ultrahigh-resolution mass spectrometry. *Analytical and Bioanalytical Chemistry* 408, 2497–2504. <https://doi.org/10.1007/s00216-016-9346-x>.
- Possinger, A.R., Zachman, M.J., Enders, A., Levin, B.D.A., Muller, D.A., Kourkoutis, L.F., Lehmann, J., 2020. Organo-organic and organo-mineral interfaces in soil at the nanometer scale. *Nature Communications* 11, 6103. <https://doi.org/10.1038/s41467-020-19792-9>.
- Raeke, J., Lechtenfeld, O.J., Wagner, M., Herzsprung, P., Reemtsma, T., 2016. Selectivity of solid phase extraction of freshwater dissolved organic matter and its effect on ultrahigh resolution mass spectra. *Environmental Science: Processes & Impacts* 18, 918–927. <https://doi.org/10.1039/C6EM00200E>.
- Rethemeyer, J., Kramer, C., Gleixner, G., John, B., Yamashita, T., Flessa, H., Andersen, N., Nadeau, M.-J., Grootes, P.M., 2005. Transformation of organic matter in agricultural soils: radiocarbon concentration versus soil depth. *Mechanisms and Regulation of Organic Matter Stabilisation in Soils* 128, 94–105. <https://doi.org/10.1016/j.geoderma.2004.12.017>.
- Roberson, C.E., Hem, J.D., 1969. *Solubility of Aluminum in the Presence of Hydroxide, Fluoride, and Sulfate*. US Government Printing Office.
- Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant and Soil* 338, 143–158. <https://doi.org/10.1007/s11104-010-0391-5>.
- Rumpel, C., Rabia, N., Derenne, S., Quenea, K., Eusterhues, K., Kögel-Knabner, I., Mariotti, A., 2006. Alteration of soil organic matter following treatment with hydrofluoric acid (HF). *Organic Geochemistry* 37, 1437–1451. <https://doi.org/10.1016/j.orggeochem.2006.07.001>.
- GCTE-NEWS, Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* 126, 543–562. <https://doi.org/10.1007/s004420000544>.

- Scheel, T., Haumaier, L., Ellerbrock, R.H., Rühlmann, J., Kalbitz, K., 2008. Properties of organic matter precipitated from acidic forest soil solutions. *Organic Geochemistry* 39, 1439–1453.
- Schimel, D.S., 2010. Drylands in the Earth System. *Science* 327, 418–419. <https://doi.org/10.1126/science.1184946>.
- Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kögel-Knabner, I., 1997. Improvement of ¹³C and ¹⁵N CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. *European Journal of Soil Science* 48, 319–328. <https://doi.org/10.1111/j.1365-2389.1997.tb00552.x>.
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56. <https://doi.org/10.1038/nature10386>.
- Schwertmann, U., Jackson, M.L., 1964. Influence of hydroxy aluminum ions on pH titration curves of hydronium-aluminum clays. *Soil Science Society of America Journal* 28, 179–183. <https://doi.org/10.2136/sssaj1964.03615995002800020016x>.
- Skjemstad, J., Clarke, P., Taylor, J., Oades, J., Newman, R., 1994. The removal of magnetic materials from surface soils—a solid state ¹³C CP/MAS NMR study. *Soil Research* 32, 1215–1229. <https://doi.org/10.1071/SR9941215>.
- Sleighter, R.L., Liu, Z., Xue, J., Hatcher, P.G., 2010. Multivariate statistical approaches for the characterization of dissolved organic matter analyzed by ultrahigh resolution mass spectrometry. *Environmental Science and Technology* 44, 7576–7582. <https://doi.org/10.1021/es1002204>.
- Smernik, R.J., Baldock, J.A., Oades, J.M., Whittaker, A.K., 2002. Determination of T1ρH relaxation rates in charred and uncharred wood and consequences for NMR quantitation. *Solid State Nuclear Magnetic Resonance* 22, 50–70. <https://doi.org/10.1006/snmr.2002.0064>.
- Smith, D.F., Podgorski, D.C., Rodgers, R.P., Blakney, G.T., Hendrickson, C.L., 2018. 21 tesla FT-ICR mass spectrometer for ultrahigh-resolution analysis of complex organic mixtures. *Analytical Chemistry* 90, 2041–2047. <https://doi.org/10.1021/acs.analchem.7b04159>.
- Sokol, N.W., Bradford, M.A., 2019. Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. *Nature Geoscience* 12, 46–53. <https://doi.org/10.1038/s41561-018-0258-6>.
- Sokol, N.W., Sanderman, J., Bradford, M.A., 2019. Pathways of mineral-associated soil organic matter formation: integrating the role of plant carbon source, chemistry, and point of entry. *Global Change Biology* 25, 12–24. <https://doi.org/10.1111/gcb.14482>.
- Sokol, N.W., Whalen, E.D., Jilling, A., Kallenbach, C., Pett-Ridge, J., Georgiou, K., 2022. Global distribution, formation and fate of mineral-associated soil organic matter under a changing climate: a trait-based perspective. *Functional Ecology* 36, 1411–1429. <https://doi.org/10.1111/1365-2435.14040>.
- Spielvogel, S., Priezel, J., Kögel-Knabner, I., 2008. Soil organic matter stabilization in acidic forest soils is preferential and soil type-specific. *European Journal of Soil Science* 59, 674–692. <https://doi.org/10.1111/j.1365-2389.2008.01030.x>.
- Spohn, M., 2024. Preferential adsorption of nitrogen- and phosphorus-containing organic compounds to minerals in soils: a review. *Soil Biology and Biochemistry* 194, 109428. <https://doi.org/10.1016/j.soilbio.2024.109428>.
- Swenson, T.L., Bowen, B.P., Nico, P.S., Northen, T.R., 2015. Competitive sorption of microbial metabolites on an iron oxide mineral. *Soil Biology and Biochemistry* 90, 34–41. <https://doi.org/10.1016/j.soilbio.2015.07.022>.
- Thorpe, A.S., Barnett, D.T., Elmendorf, S.C., Hinkley, E.-L.S., Hoekman, D., Jones, K.D., LeVan, K.E., Meier, C.L., Stanish, L.F., Thibault, K.M., 2016. Introduction to the sampling designs of the national ecological observatory network terrestrial observation system. *Ecosphere* 7, e01627. <https://doi.org/10.1002/ecs2.1627>.
- Torn, M.S., Trumbore, S.E., Chadwick, O.A., Vitousek, P.M., Hendricks, D.M., 1997. Mineral control of soil organic carbon storage and turnover. *Nature* 389, 170–173. <https://doi.org/10.1038/38260>.
- Totsche, K.U., Amelung, W., Gerzabek, M.H., Guggenberger, G., Klumpp, E., Knief, C., Lehtondorf, E., Mikutta, R., Peth, S., Prechtel, A., Ray, N., Kögel-Knabner, I., 2018. Microaggregates in soils. *Journal of Plant Nutrition and Soil Science* 181, 104–136. <https://doi.org/10.1002/jpln.201600451>.
- Underwood, T.R., Bourg, I.C., Rosso, K.M., 2024. Mineral-associated organic matter is heterogeneous and structured by hydrophobic, charged, and polar interactions. *Proceedings of the National Academy of Sciences* 121, e2413216121. <https://doi.org/10.1073/pnas.2413216121>.
- Wang, T., Hamann, A., Spittlehouse, D., Carroll, C., 2016. Locally downscaled and spatially customizable climate data for historical and future periods for North America. *PLoS One* 11, e0156720.
- Warren, J., Ziegler, S.E., 2017. A solid phase extraction method for isolating HF-extractable soil organic matter for NMR analysis. *Organic Geochemistry* 111, 1–8. <https://doi.org/10.1016/j.orggeochem.2017.04.012>.
- Wen, Y., Wen, J., Wang, Q., Bai, L., Wang, Y., Su, S., Wu, C., Zeng, X., 2021. Organic carbon preservation promoted by aromatic compound-iron complexes through manure fertilization in red soil. *Journal of Soils and Sediments* 21, 295–306. <https://doi.org/10.1007/s11368-020-02769-y>.
- Whalen, E.D., Grandy, A.S., Sokol, N.W., Keiluweit, M., Ernakovich, J., Smith, R.G., Frey, S.D., 2022. Clarifying the evidence for microbial- and plant-derived soil organic matter, and the path toward a more quantitative understanding. *Global Change Biology* 28, 7167–7185. <https://doi.org/10.1111/gcb.16413>.
- Wilkinson, A., Hill, P.W., Farrar, J.F., Jones, D.L., Bardgett, R.D., 2014. Rapid microbial uptake and mineralization of amino acids and peptides along a grassland productivity gradient. *Soil Biology and Biochemistry* 72, 75–83. <https://doi.org/10.1016/j.soilbio.2014.01.026>.
- Witzgall, K., Vidal, A., Schubert, D.I., Höschen, C., Schweizer, S.A., Buegger, F., Pouteau, V., Chenu, C., Mueller, C.W., 2021. Particulate organic matter as a functional soil component for persistent soil organic carbon. *Nature Communications* 12, 4115. <https://doi.org/10.1038/s41467-021-24192-8>.
- Woolf, D., Lehmann, J., 2019. Microbial models with minimal mineral protection can explain long-term soil organic carbon persistence. *Scientific Reports* 9, 6522. <https://doi.org/10.1038/s41598-019-43026-8>.
- Yang, Z., Ohno, T., Singh, B., 2024. Effect of land use change on molecular composition and concentration of organic matter in an oxisol. *Environmental Science and Technology* 58, 10095–10107. <https://doi.org/10.1021/acs.est.4c00740>.
- Yu, L., Ahrens, B., Wutzler, T., Schrumpp, M., Zaehle, S., 2020. Jena soil model (JSM v1.0; revision 1934): a microbial soil organic carbon model integrated with nitrogen and phosphorus processes. *Geoscientific Model Development* 13, 783–803. <https://doi.org/10.5194/gmd-13-783-2020>.
- Zegouagh, Y., Derenne, S., Dignac, M., Baruiso, E., Mariotti, A., Largeau, C., 2004. Demineralisation of a crop soil by mild hydrofluoric acid treatment: influence on organic matter composition and pyrolysis. *Journal of Analytical and Applied Pyrolysis* 71, 119–135. [https://doi.org/10.1016/S0165-2370\(03\)00059-7](https://doi.org/10.1016/S0165-2370(03)00059-7).
- Zhang, Y., Lavalley, J.M., Robertson, A.D., Even, R., Ogle, S.M., Paustian, K., Cotrufo, M. F., 2021. Simulating measurable ecosystem carbon and nitrogen dynamics with the mechanistically defined MEMS 2.0 model. *Biogeosciences* 18, 3147–3171. <https://doi.org/10.5194/bg-18-3147-2021>.
- Zhao, Q., Bell, S., Kukkadapu, R., Richardson, J., Cliff, J., Bowden, M., Leitchy, S., Hofmockel, K.S., 2025. Accumulation of soil microbial necromass controlled by microbe-mineral interactions. *Environmental Science and Technology* 59, 17558–17570. <https://doi.org/10.1021/acs.est.5c01482>.
- Zhao, T., Pan, Y., 2023. An evaluation of the effect of hydrofluoric acid (HF) treatment on keratins. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 340, 377–384. <https://doi.org/10.1002/jez.b.23173>.
- Zhou, Z., Ren, C., Wang, C., Delgado-Baquerizo, M., Luo, Y., Luo, Z., Du, Z., Zhu, B., Yang, Y., Jiao, S., Zhao, F., Cai, A., Yang, G., Wei, G., 2024. Global turnover of soil mineral-associated and particulate organic carbon. *Nature Communications* 15, 5329. <https://doi.org/10.1038/s41467-024-49743-7>.