Subsoil particulate organic matter is more responsive to ~10 years of whole--soil warming than mineral--associated organic matter in a temperate forest

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Abstract

Global average temperatures are forecast to increase by 4°C by 2100 under the Intergovernmental Panel on Climate Change SSP5--8.5 scenario. This warming could accelerate soil organic carbon (SOC) mineralization, net loss of soil carbon to the atmosphere, and consequently exacerbate global warming through a positive feedback loop. It is generally assumed that mineral---associated organic matter (MAOM) stocks are ~~is~~ less sensitive to warming compared to particulate organic matter (POM), especially in the subsoil; yet more empirical data investigating the whole--soil responses~~response~~ to warming is still required to rigorously test this assumption.

Our study was conducted in a whole--soil field warming experiment in a temperate mixed---conifer forest at Blodgett Forest Research Station, University of California, Berkeley, which had been subjected to ~~10~~ 9.5 years of warming. Soils ~~taken at~~from three depths (10–20, 40–50, and 80–90 cm) were separated into three density fractions, free POM (fPOM), occluded POM (oPOM), and MAOM. ~~and w~~We~~and we~~ then investigated the SOC concentration ~~(elemental analysis)~~ and composition of bulk soil and fractions with elemental analysis and diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. In subsoils below 50 cm, ~~We found that a~~A~~a~~a decade of experimental warming caused bulk carbon loss and shifted its~~shifted~~ ~~subsoil bulk SOC~~ composition ~~shifts~~ towards lignin and C--H aromatic bonds ~~in subsoils below 50 cm~~. W~~. w~~armed plots had significantly lower mass of the POM fractions relative to ce~~o~~ntrol plots in the ~~subsoil~~ deep soil (80–90 cm)~~.~~ with no significant difference at or ~~Conversely, ,), but there was no difference~~no quantitative POM loss occuroccurreds ~~in the topsoil~~soils above 50 cm~~, which could occur if higher decomposition losses of POM were~~

~~obscured by fresh plant inputs~~we hypothe~~i~~se ~~that this was themixed effects of the site's~~ . fPOM composition showed a significant shift towards enrichment in lignin at 40–50 cm but not in topsoils ~~fraction~~ . ~~In contrast~~Meanwhile, MAOM mass and composition shifted along the depth gradient but were not significantly ~~altered~~affected by warming~~inputs. In contrast, the mass of MAOM and its chemical composition was not different between warmed and control treatments, but did shift along the depth gradient~~. This study at Blodgett Forest ~~, particularly in the subsoil,~~ thus ~~empirically~~ supports the assumption that POM ~~is~~will be more responsive to warming in the subsoil than MAOM~~, which was particularly evident in the subsoil at Blodgett Forest.~~ .

Keywords: Soil organic carbon, density fractionation, d~~D~~iffuse r~~R~~eflectance ~~I~~infrared Fourier Transform spectroscopy, soil warming, decomposition, carbon cycling.

1. Introduction

Soil is the largest actively-cycling terrestrial carbon reservoir, storing more carbon than the atmosphere and vegetation combined (Jackson et al., 2017; Scharlemann et al., 2014; Schlesinger, 1990)~~(Jackson et al., 2017; Scharlemann et al., 2014; Schlesinger, 1990)~~. Under the SSP5-8.5 scenario, global temperatures are projected to increase by 3.3–5.7℃ by 2100 (IPCC, 2023). This warming could accelerate the decomposition of soil organic carbon (SOC) and increase soil carbon dioxide (CO_2) fluxes (Davidson and Janssens, 2006; Schlesinger and Andrews, 2000). Warming-induced soil CO_2 fluxes could further exacerbate global warming, creating a positive feedback cycle (Davidson and Janssens, 2006). Many studies have examined SOC responses to warming and reported variable outcomes, ranging from no net cumulative changes (Giardina et al., 2014; Lu et al., 2013)~~(Giardina et al., 2014; Lu et al., 2013)~~ to large SOC losses observed in more recent field warming experiments (Nottingham et al., 2020; Soong et al., 2021; Verbrigghe et al., 2022)~~(Nottingham et al., 2020; Soong et al., 2021, p.2; Verbrigghe et al., 2022)~~. The contrasting results reflect the differences in experimental approaches as well as the complexity of soil systems, where opposing mechanisms simultaneously influence SOC inputs and decomposition. As a result, bulk soil measurements alone can lack the sensitivity needed to uncover the processes driving SOC responses (Lavallee et al., 2020; Rocci et al., 2021). Instead, investigating how fractions of soil, each with distinct physical and chemical properties, respond to warming could improve our understanding of the key mechanisms that govern SOC dynamics (Georgiou et al., 2022; Lugato et al., 2021).

Soil organic carbon is typically operationally-divided by size or density fractionation into two main fractions, particulate organic matter (POM) and mineral-associated organic matter (Lavallee et al., 2020). In turn, POM can be further separated into free particulate organic matter (fPOM) and occluded particulate organic matter (oPOM);~~.~~ where, fPOM ~~is mainly~~ consists of ~~coarser,~~ partially-decomposed fragments of plant biomass stored freely within the soil, and~~, whereas~~ oPOM consists of ~~is small particles of incompletely decompose~~ plant ~~residues~~fragments that are enclosed within soil aggregates, separated after physical disturbance (Golchin et al., 1994; von Lützow et al., 2006) ~~(Poirier et al., 2005; Rocci et al., 2021; Swanston et al., 2005)~~. ~~In contrast, c~~Carbon in MAOM includes both plant-derived biomolecules ~~. POM mainly consists of coarser, partially decomposed fragments of plant biomass (Poirier et al., 2005; Rocci et al., 2021; Swanston et al., 2005). In contrast, carbon in MAOM includes both microbial derived products such as necromass and metabolites (Lehmann and Kleber, 2015; Sanderman et al., 2014; Sollins et al., 1999) and plant derived~~

biomolecules (Angst et al., 2021) and microbial-derived products such as necromass and metabolites (Lehmann and Kleber, 2015; Sanderman et al., 2014; Sollins et al., 1999) and plant derived biomolecules (Angst et al., 2021).. Due to its strong association with minerals, MAOM is considered less accessible to decomposers or their extra cellular enzymes, and consistently has longer turnover times (Heckman et al., 2022; Kaiser and Guggenberger, 2000; Lavallee et al., 2020; von Lützow et al., 2006).(Kaiser and Guggenberger, 2000; Lavallee et al., 2020; Lützow et al., 2006). Therefore, it is also often assumed to be more resistant to warming-enhanced decomposition than POM fractions (Georgiou et al., 2024; Williams et al., 2018). Nevertheless, there is limited empirical evidence to support or refute this assumption (Guan et al., 2018; Schnecker et al., 2016)(Guan et al., 2018; Schnecker et al., 2016, p.201) and few studies have investigated how the distribution of SOC amongst these fractions changes with warming (Chen et al., 2023; Schnecker et al., 2016; Soong et al., 2021).

Warming can influence not only SOC concentration and its distribution among physical fractions but also its chemical composition ((vandenEnden et al., 2021)vandenEnden et al., 2021). SOC is composed of a diverse array of biopolymers at varying stages of decay along a continuum within the soil profile, containing different organic functional groups (Kleber et al., 2021; Lehmann and Kleber, 2015) (Lehmann & Kleber, 2015; Kleber et al., 2021). SOC functional groups are specific arrangements of atoms within organic molecules that differ in their charge and reactivity, therefore influencing their potential for mineral association (Kögel-Knabner, 2002; von Lützow et al., 2006)(Kögel-Knabner, 2002; von Lützow et al., 2006). Oxygen-containing functional groups such as carboxyl functional groups can strongly bind to metal (oxyhydr)oxide surfaces through ligand exchange reactions (Kleber et al., 2021; Rowley et al., 2025; Spohn, 2024) (Kleber et al., 2021; Spohn, 2024; Rowley et al., 2025).. Warming induced shifts in the relative abundance of SOC functional groups may therefore alter the strength and mechanisms of MAOM formation, influencing SOC stabilization and persistence (Xu and Tsang, 2024).(Ofiti et al., 2021; REF). However, exactly how warming affects SOC composition throughout the entire soil profile remains poorly understood.

Warming can not only influence SOC stocks and distribution among fractions, but also carbon and microbial community composition (vandenEnden et al., 2021). Long term warming can rapidly deplete chemically less complex substrates and shift microbial communities towards decomposers of chemically complex carbon (Melillo et al., 2002; Frey et al., 2013; Stuble et al., 2019). Decomposition of these chemically complex substrates (often polymers) require multiple enzymatic steps for depolymerization (Steinweg et al., 2013)(Steinweg et al., 2013), which tend to reduce microbial carbon use efficiency (CUE;

Manzoni et al., 2012). Lower CUE means a greater fraction of carbon is respired rather than assimilated, potentially reducing microbial necromass formation and its stabilization in soil (Bradford et al., 2016; Crowther et al., 2015; Kögel-Knabner et al., 2008). Furthermore, the composition of SOC can influence its interaction with minerals, as compounds with different functional groups exhibit varying affinities for mineral surfaces (Kleber et al., 2021; Spohn, 2024). For instance, lignin monomers appeared to be selectively retained on mineral surfaces (Feng et al., 2005) and organic groups such as carboxyl group tend to adsorbed to iron (Fe) oxide surfaces via ligand exchange (Li et al., 2023) Therefore, understanding the composition of SOC, especially in different physical fractions, is essential to disentangling the nuanced shifts of SOC stabilization and destabilization processes due to warming. Therefore, understanding the composition of SOC, especially in different physical fractions, is essential to disentangling the nuanced shifts of SOC stabilization and destabilization processes due to warming.

Whole-soil warming experiments have been instrumental in advancing our understanding of the response of SOC to warming, particularly in subsoils (> 30 cm), which are estimated to contain ~50 % of SOC globally (Jobbágy and Jackson, 2000). Subsoils differ from topsoils in terms of plant inputs, microbial community, and SOC stabilization mechanisms (Fierer et al., 2003; Hicks Pries et al., 2023; Rumpel et al., 2012; Salomé et al., 2010)Although subsoil SOC is postulated to be resistant to warming.. As a large, long-term carbon reservoir(Harrison et al., 2011), its responses to warming remain largely uncertain. (Harrison et al., 2011; Rumpel and Kögel-Knabner, 2011; Sierra et al., 2024), it is imperative to understand how the whole soil profile, including the subsoil, will respond to future warming. The Blodgett Forest whole-soil warming experiment has been investigating how the top meter of soil of a temperate mixed-conifer forest responds to +4°C warming. Studies have thus far demonstrated that 4.5 years of +4°C warming at Blodgett Forest promoted decomposition and SOC loss in the subsoilssubsoil (Hicks Pries et al., 2017; Soong et al., 2021), reduced fine-root biomass, and accelerated decomposition of biochemicallychemically-complex SOC (Ofiti et al., 2021; Zosso et al., 2023). Specifically, the SOC loss in the subsoil at Blodgett Forest after xxx years ofthis short-term warming was driven by a reduction in POM, while MAOM stocks remained largely unaffected (Soong et al., 2021). Given the paucity of fresh plant inputs to deeper horizons, which limits POM inputs (Button et al., 2022; Hicks Pries et al., 2023; Jackson et al., 1996), subsoil POM losses are unlikely less likely to be promptly replenished (Button et al., 2022; Hicks Pries et al., 2023; Jackson et al., 1996).to be promptly replenished. If subsoil POM losses persist over decadal timescales, the resulting lower POM

availability could limit microbial processing and in turn, constrain further MAOM formation derived from microbial transformation of POM (Heckman et al., 2022; Witzgall et al., 2021).(Heckman et al., 2022; Witzgall et al., 2021). However, it remains unclear how prolonged warming will affect the balance between POM and MAOM and their composition at deep soil warming experiments such as Blodgett Forest and whether these SOC losses in individual fractions persist or stabilize over decadal timescales.

To address this knowledge gap, we investigated responses in the distribution of SOC and its composition across fractions and depths after 109.5 years of warming at Blodgett Forest. We fractionated soil from soil cores using density fractionation separating the free light particulate organic matter (fPOM), occluded particulate organic matter (oPOM), and MAOM fractions. We quantified bulk soil and fraction carbon contentconcentration, stable carbon isotope composition (δ^{13}C values), and quality qualifycharacterized their chemical composition using diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. We hypothesized that at 10–20 and 40–50 cm, warming would not cause substantial quantitative losses of SOC fractions because continued fresh plant inputs would offset carbon losses. Accordingly, we expected compositional changes at these depths to be limited. In contrast, in deep subsoils (80–90 cm), we expected fPOM and oPOM to decline quantitatively under warming, whereas MAOM would remain comparatively stable. We further hypothesized that warming would shift fPOM and MAOM towards relatively more aromatic signals as labile aliphatic C–H and polysaccharide components were preferentially depleted. By contrast, we expected no significant warming effect on oPOM and MAOM composition throughout the soil profile because of their inherent heterogeneity We hypothesized that under long-term warming, fPOM and MAOM would remain quantitatively stable and be characterized by aliphatic C–H due to replenishment ofthat in topsoils, fresh plant inputs which would offsets losses in topsoils. In contrast, fPOM would be depleted and MAOM would remain consistent quantitatively in subsoils under warming, and both fractions become more aromatic.(Marín-Spiotta et al., 2008; Schrumpf et al., 2013) and resistance, repectively.To address this knowledge gap, we investigated responses in the distribution of SOC and its composition across fractions and depths after 10 years of warming at Blodgett Forest. We fractionated soil from soil cores using density fractionation separating the free light particulate organic matter (fPOM), occluded particulate organic matter (oPOM), and MAOM. We quantified bulk soil and fraction carbon content, stable carbon isotope composition (δ^{13}C values), and quality using diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. We hypothesized that under long term warming, fPOM and MAOM would remain quantitatively stable and be characterized by

aliphatic C–H due to replenishment of fresh plant inputs which offsets losses in topsoils. In contrast, fPOM would be depleted and MAOM would remain consistent quantitatively in subsoils under warming, and both fractions become more aromatic.

2. Materials and methods

2.1 Study site and sampling

The whole-soil warming experiment is located inat the Blodgett Forest Research Station, run byof the University of California, Berkeley, and is located in the foothills of the Sierra Nevada near Georgetown, California (38°54'43.0"N 120°39'40.0"W; 1370 m above sea level). The climate is characterized as Mediterranean with a mean annual air temperature of 12.5°C and a mean annual precipitation of 1774 mm yr^{-1}, falling predominantly between November through April (Hicks Pries et al., 2017). The experiment is located in a mixed coniferous forest dominated by ponderosa pine (*Pinus ponderosa*), sugar pine (*Pinus lambertiana*), incense cedar (*Calocdedfrus**Calodefrus* *decurrens*), white fir (*Abies concolor*), and Ddouglas fir (*Pseudotsuga menziesii*; Rasmussen et al., 2005; Hicks Pries et al., 2017). The understory vegetation mainly consists of shrubs (*Notholithocarpus densiflorusdensiflorus)*,, grasses, and annual plants such as *Gallium triflorum*. The soil is a mesic ultic Alfisol of granitic origin, which is equivalent to a Dystric Cambisols (IUSS Working Group WRB, 2022), with fine-loamy texture.

[[To make this text accurate, I had to edit, and I think it read better to completely revise. See next paragraph]]

The warming experiment started in January 2014 and was described in detail by a previous study from this site (Hicks Pries et al., 2017) (Hicks Pries et al., 2017)(Hicks Pries et al., 2017). Briefly, it started with consists of three pairs of circular plots that are 3 m in diameter, each with surrounded by 22 vertically installed conduit pipes heating cables down to 1 2.4 m (filled with heater tape in the heated plots) and having two concentric rings of surface heating cable (warmed plots) or plain wire (control plots) installed 5 cm below the surface. In each heated/control plot pair, tEach pair contains a control plot, with the temperature in the warmed plots was elevated by +4°C compared to the control plots. The experimental setup in the between warmed and control controlplots is identical except that no heating was applied heating was not turned on for for control plots. In May 2023, two cores from each plot were collected in 10 cm depth increments down to 1 m depth. Samples were stored at -4°C until processing.

The warming experiment started in January 2014 with three pairs of control and warmed treatment plots, as described by Hicks Pries et al. (2017). The control and treatment plots have the same experimental set up. Briefly, each 3-m diameter plot is surrounded by 22 conduit pipes installed vertically down to 2.4 m. I ~~(~~in ~~heated~~warmed plots, these conduits contain heating cables~~)~~. Each warmed plot ~~and~~ has two concentric rings of surface heating cable ~~(heated plots)~~, while control plots have ~~or~~ plain wire ~~(control plots)~~ installed 5 cm below the surface. The temperature in each ~~heate~~warmed plot was elevated by +4°C compared to its paired control plot. ~~-~~In May 2023, two cores from each plot were collected in 10 cm depth increments down to 1 m depth. Samples were stored at -4°C until processing.

2.2 Soil preparation

Samples were sieved to 2 mm to separate the fine~~-~~soil fraction (< 2 mm), removing ~~)~~ ~~from~~ large rock fragments and roots. Tweezers were used to manually remove roots from the fine-soil fraction that passed the sieve. The soil samples were then freeze~~-~~dried to a constant weight. Before laboratory analysis, ~~samples~~ sub-samples from identical depth increments of the two different cores from the same plot were combined to obtain a more representative bulk sample in an effort to account for within–plot variability. A subset of these combined samples ~~was~~ was then ground with a ball mill (MM400, Retsch, Haan, Germany) for elemental analysis.

~~Unless otherwise specified, topsoil~~Topsoil and subsoil will refer to soil between 0–30 cm and 30–100 cm, respectively. Surface soil, mid-depth soil, and deep soil will respectively refer to the soil depth of 10–20, 40–50, and 80–90 cm throughout the Results and Discussion sections.

2.3 Density fractionation

Bulk soil samples from three depths (10–20, 40–50, and 80–90 cm) were fractionated by density into free POM (fPOM, large undecomposed or partially decomposed plant fragments), occluded POM (oPOM, plant fragments released by sonication), and MAOM (residual SOC bound to minerals) fractions in sodium polytungstate solution (SPT), with methods adapted from a previous study of this site ~~-~~ (Hicks Pries et al., 2018).~~Hicks Pries et al. (2018).~~ Like previous studies at this ~~study~~ site (Hicks Pries et al., 2018; Soong et al., 2021), ~~Hicks Pries et al. (2018)Hicks Pries et al. (2018) and (Soong et al., 2021)~~ we used an SPT density of 1.65 g cm^{-3}. The three depths were selected ~~in particular as~~because they

represented different soil horizons and permitted allow for comparison with previous analyses at the same experiment site, which used the same depths (Hicks Pries et al., 2017; Soong et al., 2021).

Unlike (Hicks Pries et al. (2017) and Soong et al. (2021), who used 20 g bulk soil for density fractionation, we We conducted fractionation in an 80 mL round bottom glass centrifuge tubes (Neubert-Glas, Germany) to avoid potential plasticizer contamination for the followingsubsequent lipid analysis. Briefly, we added 40 mL of 1.65 g cm^{-3} low C and N content N SPT (SPT0, TC-Tungsten Compounds Inc., Grub am Forst, Germany) were added to 8 g bulk soil in a round-bottom glass centrifuge tube with four analytical replicates per sample. The glass tube was gently shaken by hand to ensure enable full contact between the soil particles and the solution, and anyensuring that particles adhering to the tube walls were brought back into the solution. We let the samples stand for 1 h to allow for density equilibration and maximize the separation of fPOM from MAOM before centrifuging the solutions in a swinging bucket rotor for 1 h at 3130 g RCF (Megafuge 1.0, Heraeus Group, Germany). The fPOM was aspirated using a 10 mL Eppendorf pipette, filtered through a 0.8 µm polycarbonate filter (Nucleopore Track-Etch, Whatman), and rinsed with deionized water (Millipore MilliQ Advantage A10, Darmstadt, Germany, 18.2 MΩ.cm at 25 °C, 2 ppb TOC). The remaining sample was then sonicated in an ice bath at a maximum amplitude and a 50 % pulse rate for 2 mins and 26 seconds, delivering a calibrated (North, 1976) total energy input of 100 J mL^{-1} (Hicks Pries et al., 2018). The sample was then centrifuged, again at 3130 g RCF for 1 h and left to settle overnight. Subsequently, the same aspiration and rinsing procedure used for fPOM was applied to the floating material, fractionating the oPOM. To prevent the re-adsorption of oPOM onto the MAOM and to remove all the oPOMensure complete removal of oPOM, the remaining fraction mixture was centrifuged (3130 g RCF for 1 h),) aspirated, filtered, and rinsed a second time. All fractions, including the remaining MAOM, the remaining solid sample recovered in the tube, were then rinsed with deionized water until the supernatant reached the density of water. All fractions were freeze-dried at -50°C (Alpha 1–4, Martin Christ Freeze Dryers, Osterode am Harz, Germany). Then a subsample of fPOM and oPOM were ground by hand with a pestle and agate mortar and a subsample of MAOM was ground with a ball mill (MM400, Retsch, Haan, Germany) for further elemental and DRIFT analysis.

2.4 Carbon and nitrogen concentrations and carbon stable isotope compositions

Soil organic carbon concentrations (~~%~~C %) and stable carbon isotope compositions ($\delta^{13}C$ values) were analyzed in bulk soils and fractions using an elemental analyzer~~–~~-isotope ratio mass spectrometer (EA–IRMS; Flash 2000–HT Plus, linked by Conflo IV to Delta V plus isotope ratio mass spectrometer, Thermo Fisher Scientific, Bremen, Germany). Caffeine (Merck, Germany) and Chernozem standards (Harsum, Germany) were used as calibration materials. $\delta^{13}C$ values were expressed in ‰ relative to the Vienna Pee Dee Belemnite (V~~–~~PDB) standard (Coplen, 2011). At least two analytical replicates were measured for all samples. ~~Carbonates are absent at Blodgett Forest, so total carbon concentrations are assumed to represent organic carbon concentrations.~~ A p~~P~~revious study ~~has~~ measured the pH values at the same study site (Rowley et al., 2025). The soils are acidic and have a pH (H_2O) of 4.9 ± 0.1 (standard error of the mean), ranging between extremely and slightly acidic (3.7–6.2). ~~The soils are acidic and have a pH (H_2O) of 4.9±0.1 (standard error of the mean), ranging between extremely and slightly acidic (3.7-6.2; Table S14; Rowley et al., 2025).~~ Thus, carbonates are absent at Blodgett Forest, so total carbon concentrations are assumed to represent SOC concentrations.

~~The soils are acidic and have a pH ($CaCl_2$) ranging from 3.65 to 4.65 (Table. S14).Soil organic carbon concentrations (%C) and stable carbon isotope compositions ($\delta^{13}C$ values) were analyzed in bulk soils and fractions using an elemental analyzer isotope ratio mass spectrometer (EA–IRMS; Flash 2000–HT Plus, linked by Conflo IV to Delta V plus isotope ratio mass spectrometer, Thermo Fisher Scientific, Bremen, Germany). Caffeine (Merck, Germany) and Chernozem (Harsum, Germany) were used as calibration materials. $\delta^{13}C$ values were expressed in ‰ relative to the V–PDB standard (Conant et al., 2011). At least two analytical replicates were measured for all samples. Carbonates are absent at Blodgett Forest, so total carbon concentrations are assumed to represent organic carbon concentrations.~~

2.5 SOC composition analysis

To assess potential differences in SOC composition ~~caused by warming~~between treatments and across soil depths in both bulk soil and fractions, ground bulk ~~soil~~ and fraction samples were analyzed using DRIFT spectroscopy. A Bruker Invenio R spectrometer (Billerica, Massachusetts, USA) was used to record the mid~~–~~-infrared spectra with a resolution of 2 cm^{-1} between 4000 and ~~to~~ 80 cm^{-1}, collecting 64 scans for each sample. Background reflectance was determined on oven~~–~~-dried KBr (60°C) and subtracted to convert spectra to

pseudo-absorption units (log [1/R]). Background-corrected scans were checked for consistency and then averaged to produce a single spectrum.

All subsequent spectral processing was completed in R version 4.4.2 (R Core Team, 2025) using the '*prospectr*' (v0.2.8; Stevens and Ramirez-Lopez, 2025) and '*tidyverse*' (v1.3.2; Wickham et al., 2019) packages. Briefly, spectra were concatenated into a single datafile and trimmed to between 4000–400 cm^{-1} to remove spectral noise. Prior to standard normal variate normalization (SNV), the data was smoothed using a Savitzky–Golay filter to the 3rd order polynomial (Savitzky and Golay, 1964)~~(Savitzky and Golay, 1964)~~ and scaled to positive values (Fearn, 2008).~~(Fearn, 2008).~~ SNV was applied to the spectra to eliminate scattering effects and standardize spectral intensities, before applying a convex-hull rubber-band baseline correction. Area under the curve (AUC) of spectral peaks was calculated using a trapezoid area function with local baseline calculation, to ascertain the area of peaks linked to different bonding environments of SOC. The spectral band ranges assigned to different carbon functional groups were based on previous studies (Artz et al., 2008; Chatterjee et al., 2012; Ofiti et al., 2021) and are reported in Table S13. The average AUC plot for each ~~soil~~ sample type (~~bulk,~~ fPOM/~~,~~oPOM or bulk/~~,~~MAOM) was calculated and used to minimally adjust the wavenumber range of spectral bands for each peak due to slight spectral shifts caused by the mineral matrix~~, detailed in Table S13~~ (Table S13; Ellerbrock and Gerke, 2021)~~(Ellerbrock and Gerke, 2021)~~.

The ratio of peak areas between aromatic C=C/carboxylic C=O stretches (in our paper assigned as C=C aromatic2; Table S13) and aliphatic C–H stretches ~~were~~ was used to calculate the DRIFT Stability Index (Demyan et al., 2012; Haberhauer et al., 1998; Laub et al., 2020; Schiedung, 2025), ~~indicative~~ used as a proxy of the relative state of SOC decomposition. In soils devoid of carbonates such as Blodgett forest, the band range at ~3000–2800 cm^{-1} is largely unaffected by mineral-phase interference ~~in the absorbance~~ ~~The ratio of peak areas between aromatic C=C/carboxylic C=O stretches (in our paper assigned as C=C aromatic2; Table S13) and aliphatic C–H stretches were used to calculate the DRIFT Stability Index (Demyan et al., 2012; Haberhauer et al., 1998; Laub et al., 2020; Schiedung, 2025), indicative of the relative state of SOC decomposition. In soils devoid of carbonates, the band range at ~3000–2800 cm^{-1} is largely unaffected by mineral-phase interference in the absorbance~~ (Tinti et al., 2015). Other ~~disturbances~~ confounding influences on~~to~~ the spectra should be minima~~lized~~ because we always compare samples collected from close proximity with a similar parent material and texture (Reeves, 2012). We could not conduct DRIFT analysis on several POM fractions from

the deep soil due to the limited amount of material recovered, particularly in ~~warmed~~ fractions from ~~heate~~warmed plots.

2.5 Statistical analysis

All statistical analyses were performed in R version 4.4.2 (R Core Team, 2025) using ~~the~~ Rstudio (2024.12.1.563; Posit team, 2025). Plots were all created using the '*ggplot2*' ~~package~~ (v3.5.1; Wickham, 2016) and '*ggbiplot*' packages (v0.6.2; Vu et al., 2024). To test the effect of depth, treatment, and their interaction on bulk ~~soil carbon~~SOC concentration, ~~and~~ $\delta^{13}C$ values, AUC values for each bond type, and the DSI, we built linear mixed effects models ~~(LMEs)~~ in the '*nlme*' package (v3.1.166; Pinheiro et al., 2000; Pinheiro et al., 2025). We set depth, treatment, and their interaction as fixed effects, setting the ~~treatment block~~ plot pair as a random effect. Model fits were assessed using Akaike's Information Criterion (Akaike, 1998).~~(Akaike, 1998). Homoscedasticity of residuals were visually examined using conditional residual plots (Zuur et al., 2009) and normality was assessed using Q–Q plots. If model assumptions were not met, as was the case with SOC concentration, the data were log-transformed.~~ To account for autocorrelation of observations within profiles, depth class was set as a repeated measure with an autoregressive (AR1; '*nlme*') covariance structure (Grand et al., 2014). Homoscedasticity of residues were visually examined using conditional residual plots (Zuur et al., 2009) and normality was assessed using Q–Q plots. If model assumptions were not met, as was only the case with SOC concentration and AUC values, the data were log-transformed. ~~To account for autocorrelation of observations within profiles, depth class was set as a repeated measure with an autoregressive (AR1; '*nlme*') covariance structure (Grand et al., 2014).~~

For fractions, we applied linear mixed effects models~~LMEs~~ to the three fractions separately. We tested the effects of depth, treatment, and their interaction on SOC concentration in each fraction (mg ~~O~~C g^{-1} fractionated soil), distribution of ~~SOC~~ C in the density fractions (g C fraction g^{-1} bulk SOC), ~~;~~ AUC values, ~~for each bond type,;~~ and DSI, ~~and DSI~~. ~~;~~ ~~in response to treatment, depth, and their interaction as fixed effects, and block~~ Plot pairs were again ~~again~~ set as a random effect. When significant fixed-interaction effects were discovered, we ran post hoc analyses within each depth increment. The alpha level was set to $\alpha = 0.05$ in all statistical tests, where~~;~~ a $p < 0.05$ was reported as significant and a p between 0.05–0.1 as marginally significant.

To further explore trends in the bulk soil and fraction SOC composition, AUC values from the DRIFT spectra were analyzed using principal component analysis (PCA). The PCA

was performed on the correlation matrix, centered and scaled using '*prcomp*' in the R '*stats*' package (v4.4.2; R Core Team, 2025). We used 95 % confidence ellipses to visualize the approximate location of the true group centroids (mean) and the dispersion of different groups within Euclidean space (Husson et al., 2005).

3. Results

3.1 Bulk soil SOC properties

Independent of treatment, SOC concentration significantly declined with depth ($p < 0.001$; Table 1). Warming reduced SOC concentration in the soil samples from 50–100 cm, but not in the samples between 0–50 cm ($p = 0.002$; Table 1). Specifically, at 60–70 and 80–90 cm, mean SOC concentrations were on average 54 % (CI: -83, 26) and 56 % (CI: -84, 20) lower, respectively; but, the wide confidence intervals and marginally significant p-values in the post-hoc tests (0.099 and 0.086, respectively) indicate substantial variability rather than a definitive loss. There was no significant effect of warming on bulk SOC $\delta^{13}C$ values ($p > 0.1$; Table 1) but these values significantly decreased with depth ($p < 0.001$).

~~We find a statistically significant interaction between soil depth and warming on SOC concentration~~Warming reduced SOC concentration ~~was lower (with marginal significance) in the heated subsoils > 50 cm than in control plots but not in the soils above 50 cm (p ($p = 0.002$; Table 1; Table S2).), suggesting effects of warming on SOC concentration varies by depth. Warming resulted in an on average non- significant SOC concentration increase above 50 cm and decrease below 50 cm (Table S1). Specifically, at 60–70 and 80–90 cm, warming marginally decreased SOC concentrations by 54 %mean SOC concentrations were on average 54 % (CI: -83 %, 26 %; $p = 0.099$) and 56 % (CI: -84 %, 20 %; $p = 0.086$; Table S12) lower.), respectively. But the wide confidence intervals and marginally significant p-values (0.099 and 0.086, respectively) indicate substantial variability rather than a definitive loss. Independently of treatment, SOC concentration significantly declined with depth ($p < 0.001$; Table S21S2).~~

~~There was no significant interaction between soil depth and~~effect of ~~warming on bulk SOC $\delta^{13}C$ values ($p > 0.1$; Table S21) and but these values only the main effect of soil depth was significant ($p < 0.001$). In other words, depth significantly decreased $\delta^{13}C$ values across soil profile~~with depth ($p < 0.001$). ~~.~~

Table 1: Soil organic carbon (SOC) and stable carbon isotope compositions $\delta^{13}C$ values of mineral bulk soils (0–100 cm) in the control and warmed plots (mean ± SE) after 9.5 years of soil warming. $\delta^{13}C$ values are reported in ‰ relative to the Vienna Pee Dee Belemnite (VPDB) standard.

Depth	Control		Warmed	
[cm]	SOC [mg C g^{-1} soil]	$\delta^{13}C$ [‰ VPDB]	SOC [mg C g^{-1} soil]	$\delta^{13}C$ [‰ VPDB]
0–10	43.4 ± 4.7	-25.6 ± 0.2	42.9 ± 2.9	-25.6 ± 0.5

10–20	33.7 ± 6.5	-25.4 ± 0.2	46.7 ± 10.4	-25.6 ± 0.3
20–30	25.1 ± 5.8	-25.5 ± 0.1	40.6 ± 19.9	-25.5 ± 0.3
30–40	14.9 ± 3.4	-25.3 ± 0.1	21.4 ± 9.4	-25.3 ± 0.1
40–50	9.9 ± 2.6	-24.9 ± 0.1	10.8 ± 3.2	-25.1 ± 0.1
50–60	7.7 ± 1.6	-25.0 ± 0.1	5.7 ± 0.9	-25.0 ± 0.2
60–70	7.4 ± 1.1	-25.1 ± 0.3	3.5 ± 0.7	-24.6 ± 0.3
70–80	5.9 ± 1.4	-25.0 ± 0.3	2.8 ± 0.6	-24.6 ± 0.3
80–90	4.3 ± 1.4	-25.0 ± 0.3	1.8 ± 0.4	-24.9 ± 0.1
90–1~~–1~~00	3.2 ± 0.6	-25.3 ± 0.5	2.0 ± 0.6	-25.0 ± 0.3

3.2 In deep soil, fPOM concentration was ~~was~~ more responsive to warming than was ~~was~~ MAOM

fPOM concentrations significantly declined with depth ($p < 0.001$), ~~whereas warming affected these values dependent on soil depth~~while whereas and a significant interaction effect between depth and treatment was also observed ($p = 0.031$), meaning the effect of warming varied across depths (~~($p = 0.031$;~~ Fig. 1). Specifically, ~~). The interaction between soil depth and warming ($p = 0.031$; Table S3) and the main effect depth ($p < 0.001$; Table S3) had significant effects on fPOM concentrations from fractionated soil samples. Basically, fPOM concentrations significantly decreased with depth, but they were affected distinguishably by warming at different depths (Fig. 1). Specifically, AatAt 10–20 and 40–50 cm, warming on average non-significantly increased fPOM concentration by 56 % (CI: -55 %, 437 %) and 97 % (-43 %, 577 %), respectively (Table S4)~~had a non-significant positive effect on fPOM concentrations ($p > 0.1$).~~ In contrast, warming had a negative effect on ~~had adverse effects on~~reducedon fPOM concentrations~~concentration~~ in ~~subsoils~~deep soils (80–90 cm),~~,~~ marginally ~~significantly~~ reducing it by 70 % (CI: -91~~ %~~, 2.0~~ %~~; $p = 0.053$~~, Table S4~~). ~~While For~~ oPOM ~~fraction, its~~oPOM organic carbon (OC) concentrations also significantly decreased with depth ($p < 0.001$) and~~,~~ ~~the There were warming effects were marginally significant effects of the interaction betweendependent on soil depth~~the interaction effect between warming and depth was marginally significant ~~and warming~~ ($p = 0.082$~~; Table S3~~). ~~At~~At ~~) and significant effects of soil depth ($p < 0.001$; Table S3) on oPOM concentrations. oPOM fraction concentrations significantly decreased by depth and were weakly affected by warming at~~ 10–20 and 40–50 cm, there was no significant difference in oPOM concentrations ~~were identical~~ between ~~warmed and c~~control and warmed plots. ~~(Table S4). (Table S4).~~ However, warming

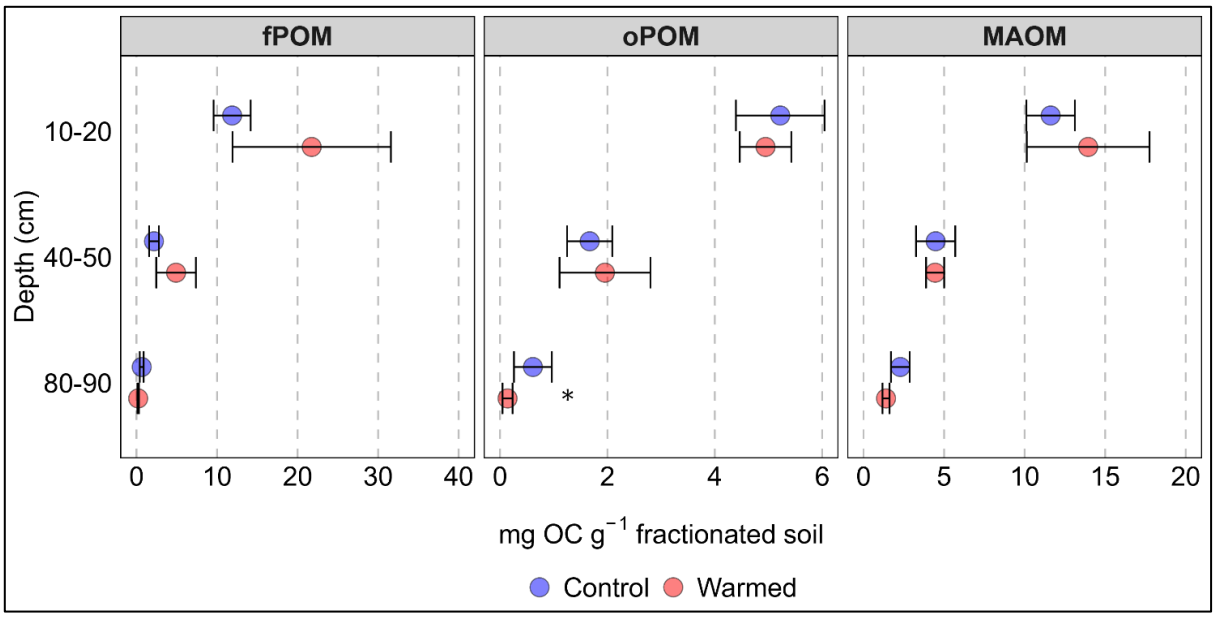


Fig. 1: Soil organic carbon (SOC) concentration in each soil fraction (mg OC g^{-1} fractionated bulk soil) at three depths (10–20, 40–50, and 80–90 cm) in control and warmed plots (n = 3), error bars represent the standard error of the mean. The asterisks indicate the significant treatment effects (p < 0.05 '*'; < 0.01 '**') on the mass of SOC in the corresponding recovered fraction.

significantly decreased oPOM concentrations at 80–90 cm, reducing its concentration by 80 % (CI: -95 %, -25 %; $p = 0.022$; Table S4). MAOM concentrations displayed a significant decline across soil depth ($p < 0.001$), but In contrast to fPOM and oPOM fractions, had no effects on MAOM concentrations ($p > 0.1$). Instead, MAOM displayed a significant decline across soil depth ($p < 0.001$; Table S3).

The proportions

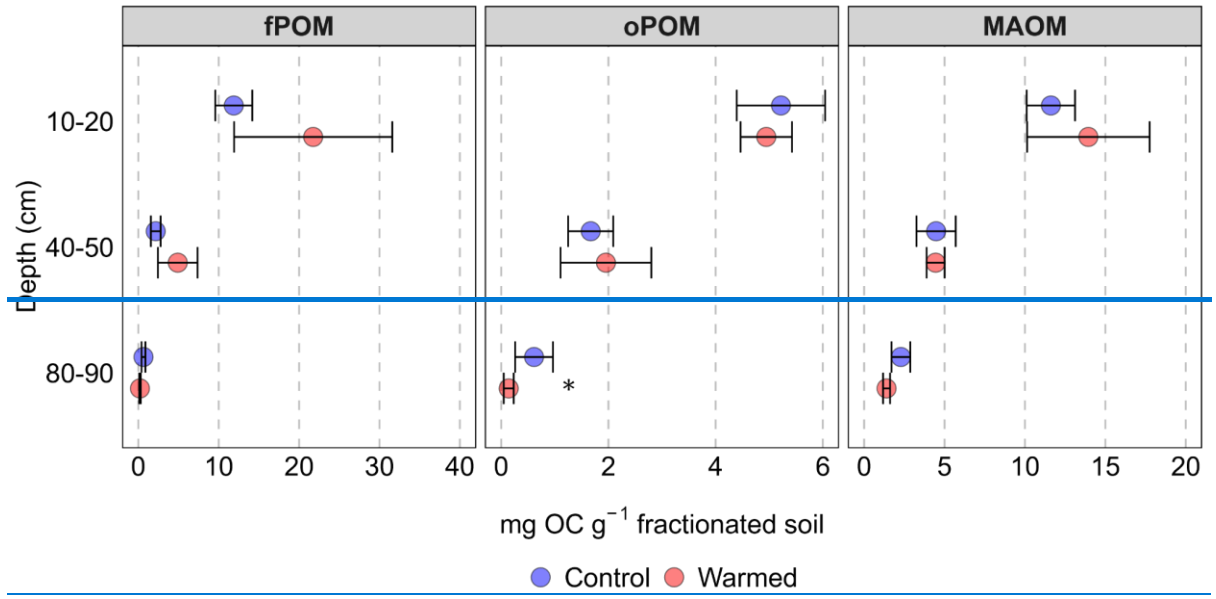
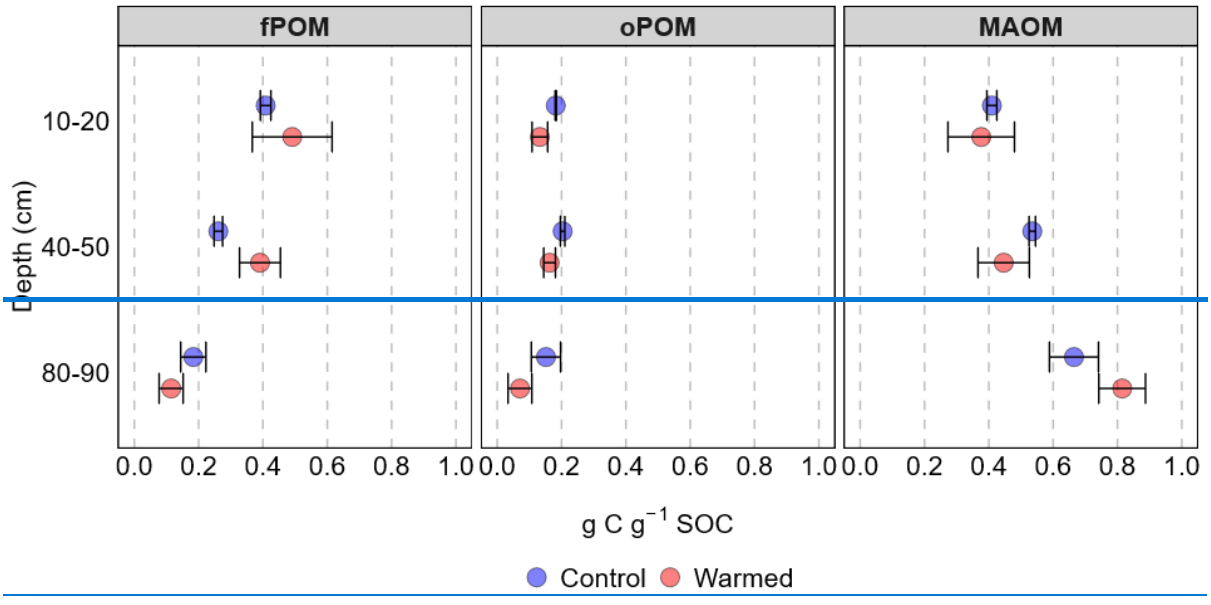


Fig. 1. Soil organic carbon (SOC) concentration in each soil fraction (mg OC g^{-1} fractionated bulk soil) at three depths (10–20, 40–50, and 80–90 cm) in control and warmed plots (n = 3), error bars represent the standard error of fPOM relative proportionsproportion to total SOC were not significantly affected by warming (p > 0.1) but(g C g^{-1} bulk SOC; Table S5). The fPOM proportion significantly decreased with depth (p = 0.002). The 032; Table S5) and on average values ranged from 0.11–0.49 g C g^{-1} bulk SOC% to 11 % in wwarmed plots and from 41 % to 180.18–0.41 g C g^{-1} bulk SOC% in ceontrol plots from 10–20 to 80–90 cm (Fig. 2). across soil depth (Fig. 2). The proportion of oPOM relative to total SOC marginally decreased by depth (p = 0.061). Particularly from, driven by a significant decrease in oPOM proportions between the 40–50 andto 80–90 cm, samples of 0.07oPOM proportions significantly decreased by 7.oPOM proportions were consistent without being significantly affected by either interaction between soil depth and warming, and main effects (Table S5). oPOM proportions remained stable across soil depth (Fig. 2 g C g^{-1} bulk SOC% (CI: -0.13.1 %, -0.01.3 %; p = 0.022; Fig. 2). The oPOM proportions relative were significantly affected by warming, independent of depth (p = 0.027; Fig. 2).). Warming caused a reduction ofin the proportion of oPOM proportion by 0.065.7 g C g^{-1} bulk SOC % (CI: -0.10.51 %, -0.01 %) across the soil profile. The consistently but non-significantly reduced oPOM proportion of MAOM at individual depths (Fig. 2), in particular at 80–90 cm, where the decline of oPOM proportion was not affected by warming (p > 0.1) (Table S5) marginally significant (p = 0.057; Table S6). There were no effects of interaction between soil depth and warming on MAOM proportion (Table S5) but its proportion significantly increased with depth (p = 0.00132032; Table S5). On average, the proportion of MAOM increased from 0.41–0.% to 66 g C g^{-1} bulk SOC% in ceontrol plots and from 0.3838–0.% to 81 g C g^{-1} bulk SOC% in warmed plots across soil profile (Fig. 2(Fig. 2).



~~Fig. 22. Distribution of total soil organic carbon (SOC) in the density fractions (g C g^{-1} total SOC) at three depths (10–20, 40–50, and 80–90 cm) in control and warmed plots (mean ± SE, n = 3~~).

~~We did not find significant effects of interaction between soil depth and warming on the fPOM proportion to total SOC (g C g^{-1} bulk SOC; Table S5). The fPOM proportion significantly decreased with depth (p = 0.032; Table S5) and on average ranged from 49 % to 11 % in warmed plots and from 41 % to 18 % in control plots across soil depth (Fig. 2). The oPOM proportions were consistent without being significantly affected by either interaction between soil depth and warming, and main effects (Table S5). oPOM proportions remained stable across soil depth (Fig. 2). Warming consistently but non-significantly reduced oPOM proportion at individual depths (Fig. 2), in particular at 80–90 cm, where the decline of oPOM proportion was marginally significant (p = 0.057; Table S6 There were no effects of interaction between soil depth and warming on MAOM proportion~~

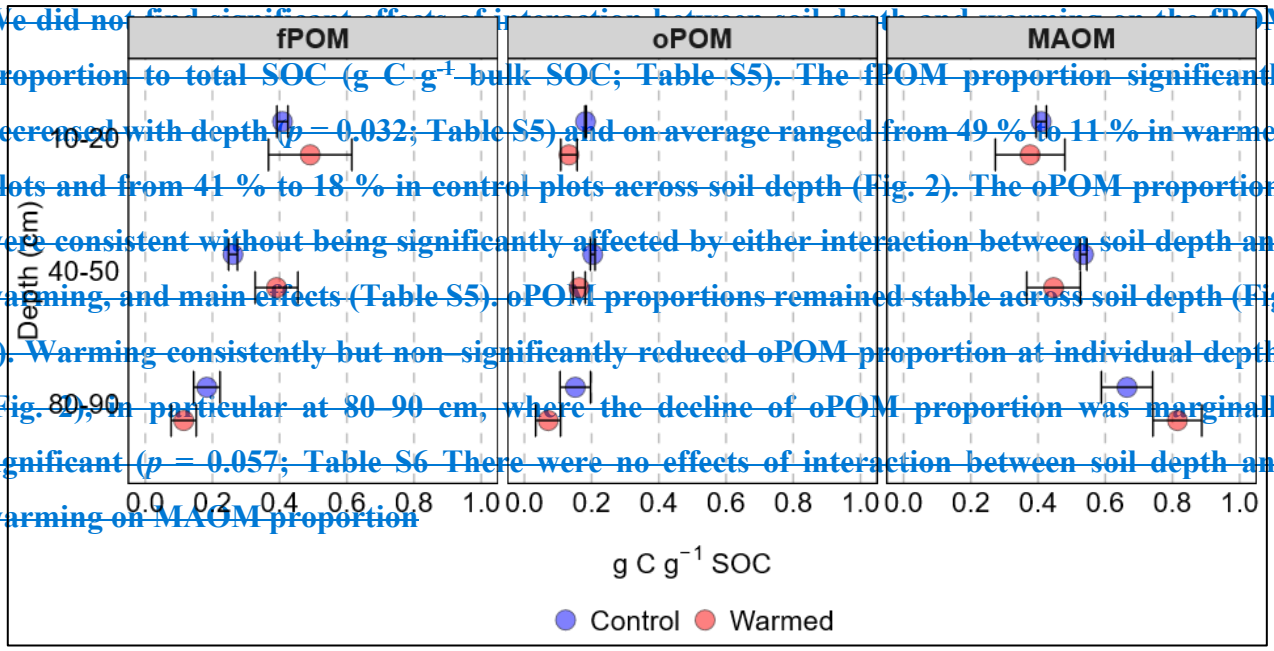


Fig. 2*:* Distribution of total soil organic carbon (SOC) in the density fractions (g C g^{-1} total SOC) at three depths (10–20, 40–50, and 80–90 cm) in control and warmed plots (mean ± SE, n = 3).

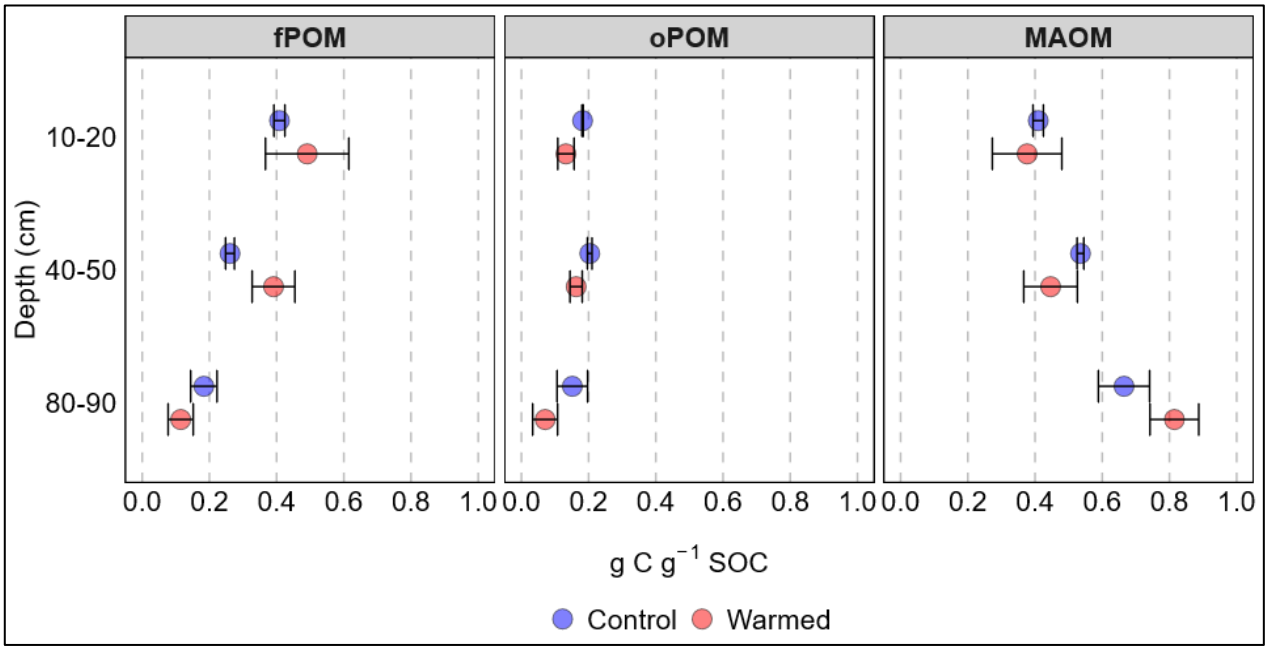
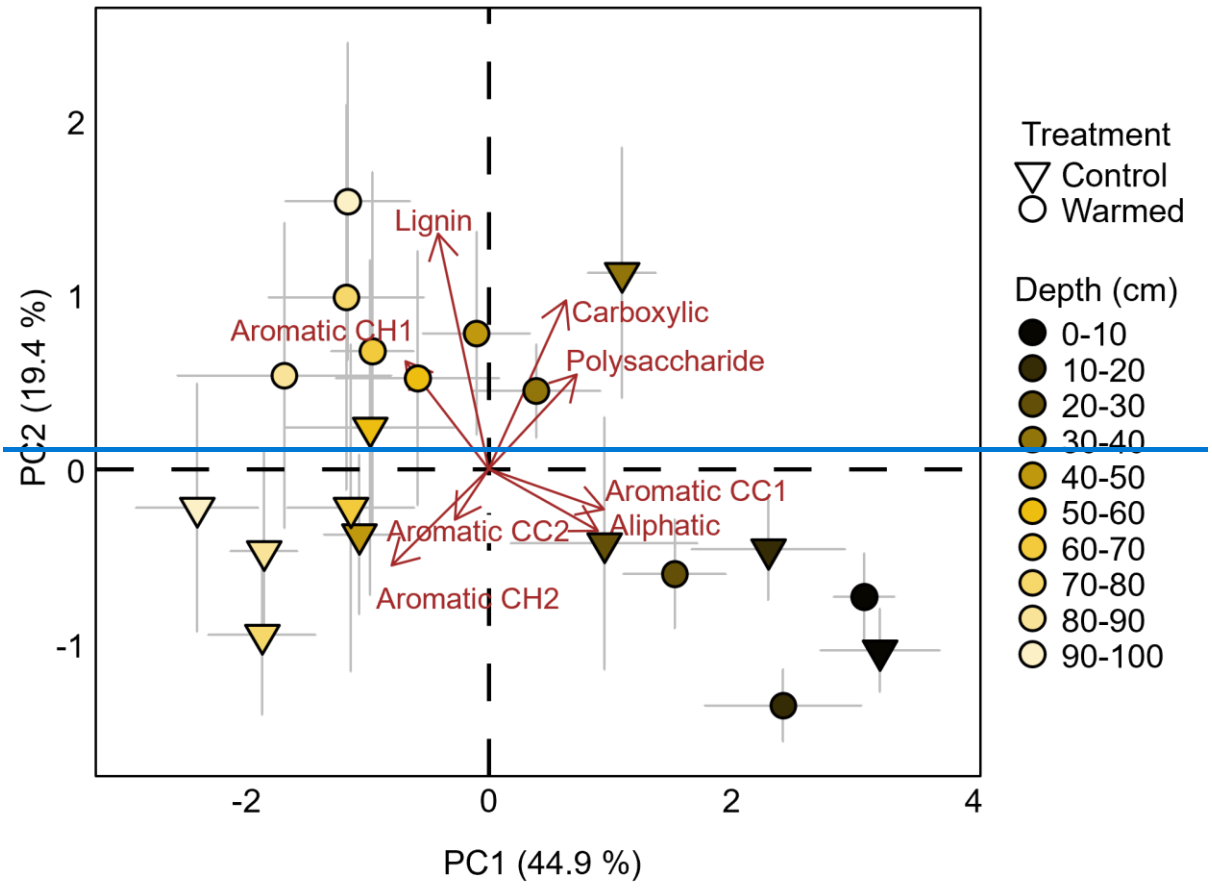


Fig. 3*:* Distribution of total soil organic carbon (SOC) in the density fractions (g C g^{-1} total SOC) at three depths (10–20, 40–50, and 80–90 cm) in ~~e~~control and ~~w~~warmed plots (mean ± SE, n = 3).

3.3 Bulk SOC composition change

Bulk SOC composition ~~changes~~ changes with depth and between warmed ~~and or~~and ~~c~~control plots are displayed in the PCA biplot (Fig. 3). The first two principal components explained 44.9 % (1st) and 19.4 % (2nd) of total variation in the bulk DRIFT AUC dataset (Fig. 3~~Fig.(Fig. 3~~). SOC composition ~~changed~~ showed a strong depth-related pattern with samples~~significantly with depth~~ moving ~~across~~ along principal component 1 and~~; the~~ topsoil samples (0–30 cm) were clearly separated from subsoil samples (> 30 cm). ~~Soil above 30~~ Topsoils~~cm cm~~ did not cluster according to treatment and was associated with relatively ~~an increased~~high AUC values for aliphatic ($p < 0.001$) and C=C aromatic (range 1; $p < 0.001$~~range1~~) regions of the DRIFT spectra (Fig. 3~~Fig. 3).(Fig. 3).~~ Samples from 30–40 cm depth contained relatively higher proportions of polysaccharide and carboxylic functional groups and showed no clear treatment-–based separation. In contrast~~t~~, at depth > 40 cm, subsoil samples tended to separate along principal component 2 according to treatment~~In contrast, spectra from subsoils > 40 cm were primarily grouped by treatment in the PCA plot and separated along principal component 2~~. Under warming, ~~Control subsoils~~subsoils below 40 cm tended to shift from ~~were characterized by an increased presence of~~ C=C aromatic (range 2) and C–H aromatic (range 2~~; Fig. 3~~; Fig. 3);~~Fig. 3~~); ~~while warmed subsoils < 40 cm displayed a trend towards~~to ~~increased AUC values for~~ C–H aromatic (range 1) and lignin-like residues (Fig. 3). However, the large standard errors of average ~~.~~



~~**Fig. 3**. Principal component analysis (PCA) of the area under the curve values from fit Diffuse Reflectance Infrared Fourier Transform spectra of bulk soil in control and warmed plots at 10 cm intervals from 0 to 100 cm. Each point represents the mean~~ PCA scores for subsoil samples indicate substantial within-group variability (~~each plot type (control and warmed, n = 3), respectively. The number after aromatic C=C/aromatic C H refers to the same C bond type assigned at different band ranges (Table~~Fig. S1), suggesting again, that these patterns should be interpreted as indicative trends with depth.~~suggesting that these patterns should be interpreted as trends rather than unambiguous compositional shifts at depth.).~~

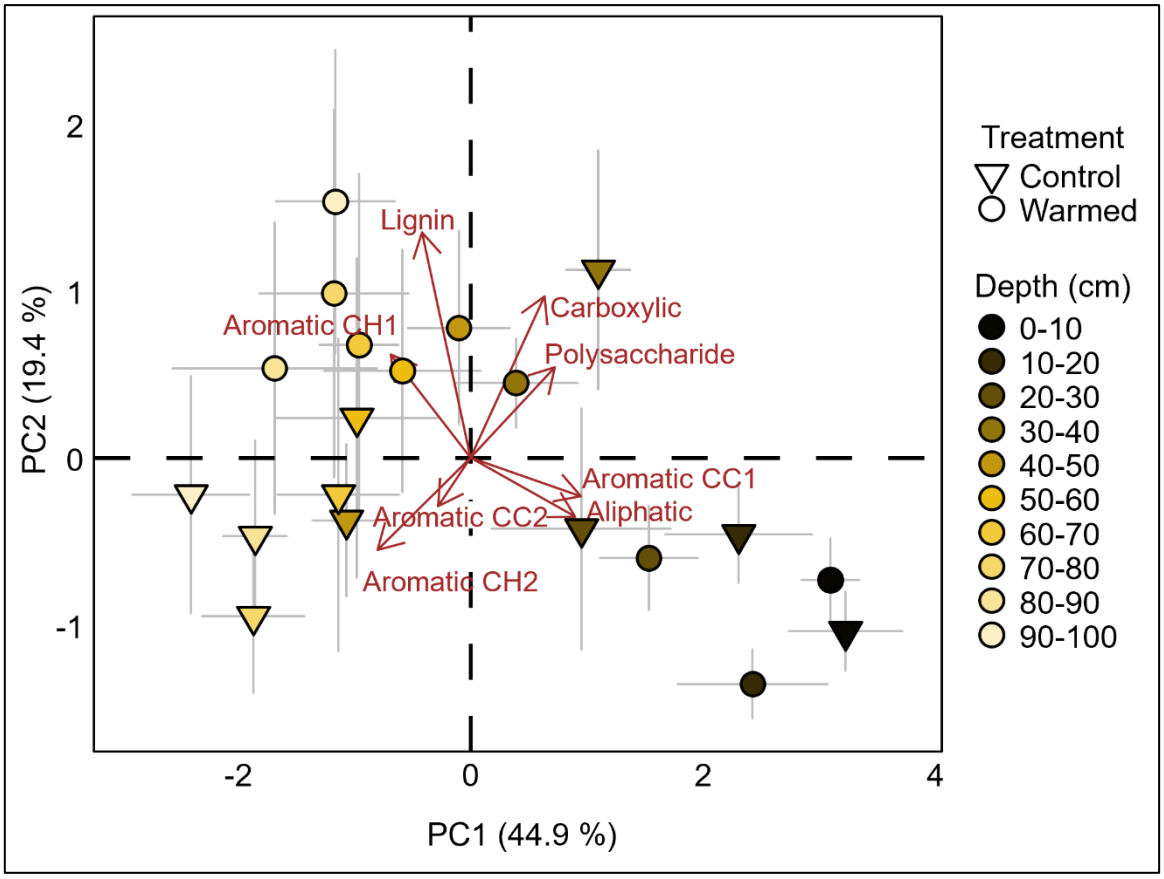


Fig. 4: Principal component analysis (PCA) of the area under the curve (AUC) values from fit ~~Diffuse~~ diffuse ~~Reflectance~~ reflectance ~~Infrared~~ infrared Fourier ~~Transform~~ transform spectra of bulk soil in control and warmed plots at 10 cm intervals from 0 to 100 cm. Each point represents the mean PCA scores of the three replicate samples per depth and treatment. The horizontal (PC1) and vertical lines (PC2) indicate the standard error of the mean of principal component scores for each plot type (control and warmed, n = 3), respectively. The number after aromatic C=C/aromatic C–H refers to the same C–bond type assigned at

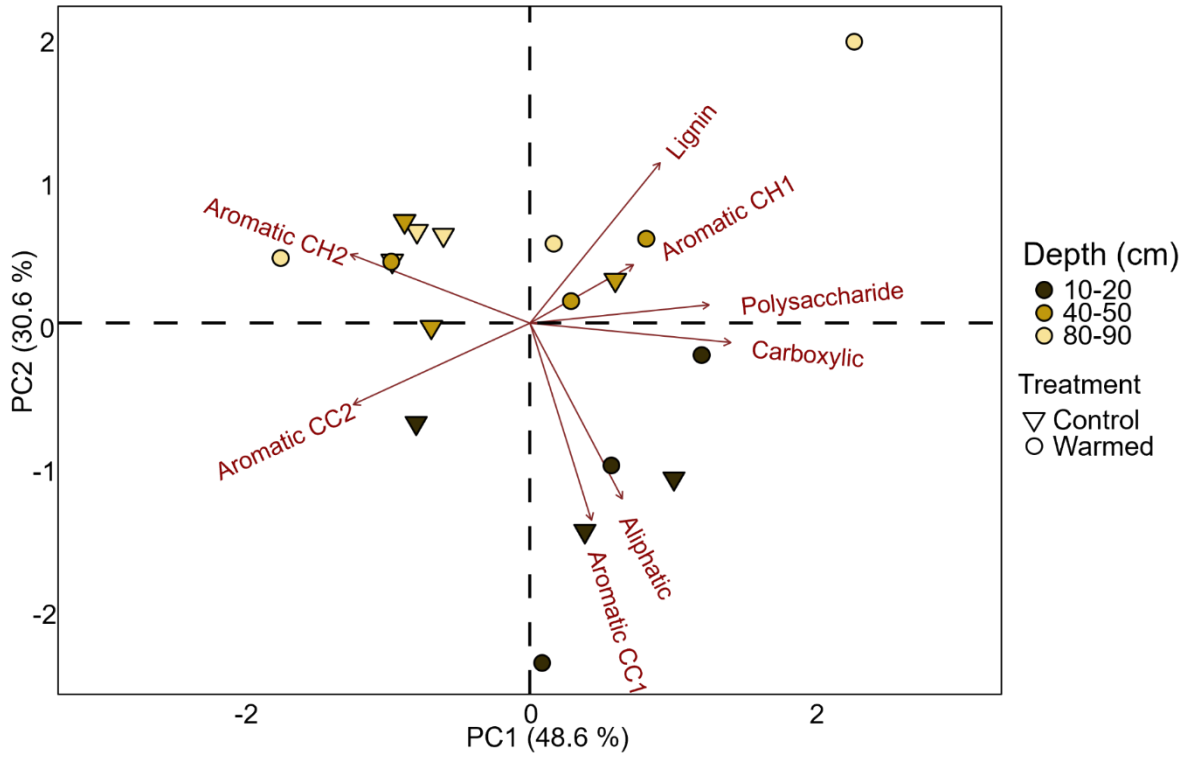
3.4 SOC compositional changes in soil density fractions

The PCA of POM fractions is displayed in the supplementary information (Fig. S2). Briefly, the main differences in POM composition were observed between the free and occluded POM fractions rather than ~~under~~ with warming or depth. Although SOC in both fractions was composed primarily of aliphatic SOC (Table S7), oPOM contained proportionally more aliphatic carbon than fPOM. Warming tended to shift fPOM composition at 40–50 cm to lignin-like residues and aromatic C–H (range 2; Fig. S2), which was confirmed by significantly increased AUC values for lignin-like residues (p = 0.002) and marginally increased aromatic C–H AUC values (range 2; p = 0.061). fPOM in topsoils and oPOM were not affected by ~~neither~~either warming ~~nor~~or depth (Fig. S2). However, warming tended to increase the variability of POM composition, as indicated by the broader dispersion of the

fPOM and oPOM samples from warmed plots along the ~~principle~~principal component 2 in the PCA plot (Fig. S2).~~s was composed primarily of aliphatic SOC (Table S7), oPOM contained relatively more aliphatic carbon than fPOM. Neither warming nor depth significantly altered the SOC composition within each fraction ((Fig. S2 marginally increased). However, warming tended to increase the variability of POM composition, as indicated by the broader dispersion of the standard errors of the mean of warmed samples in the PCA plot.~~

The first two principal components explained 48.6 % (1st) and 30.6 % (2nd) of the total variation in the fit DRIFT data of the MAOM fraction (Fig. 3~~Fig. 3).(Fig. 3).~~ SOC composition in MAOM exhibited a ~~marginal~~ depth~~-~~related trend along principal component 2, with MAOM at 10–20 cm relatively enriched in aliphatic and C=C aromatic (range 1) bonds, while those from 40–50 and 80–90 cm were more associated with lignin-like residues and C–H aromatic (range 2) bond~~ bond~~s. Warming did not alter MAOM composition at individual ~~depths, but~~depths but substantially increased the variability in ~~SOC~~ its composition at 80–90 cm and exhibited a tendency shift from C–H aromatic (range 2) to lignin-like residues and aromatic C–H (range 1; Fig. 4).~~.~~

3.5 No significant changes of aromatic/aliphatic ratio in bulk soil and fractions



We did not find significant effects of warming ($p > 0.1$), but significant effects of depth ($p < 0.001$; Fig. 5) on the DRIFT stability index (DSI) of bulk soils. Specifically, bulk soil DSI

~~Fig.~~

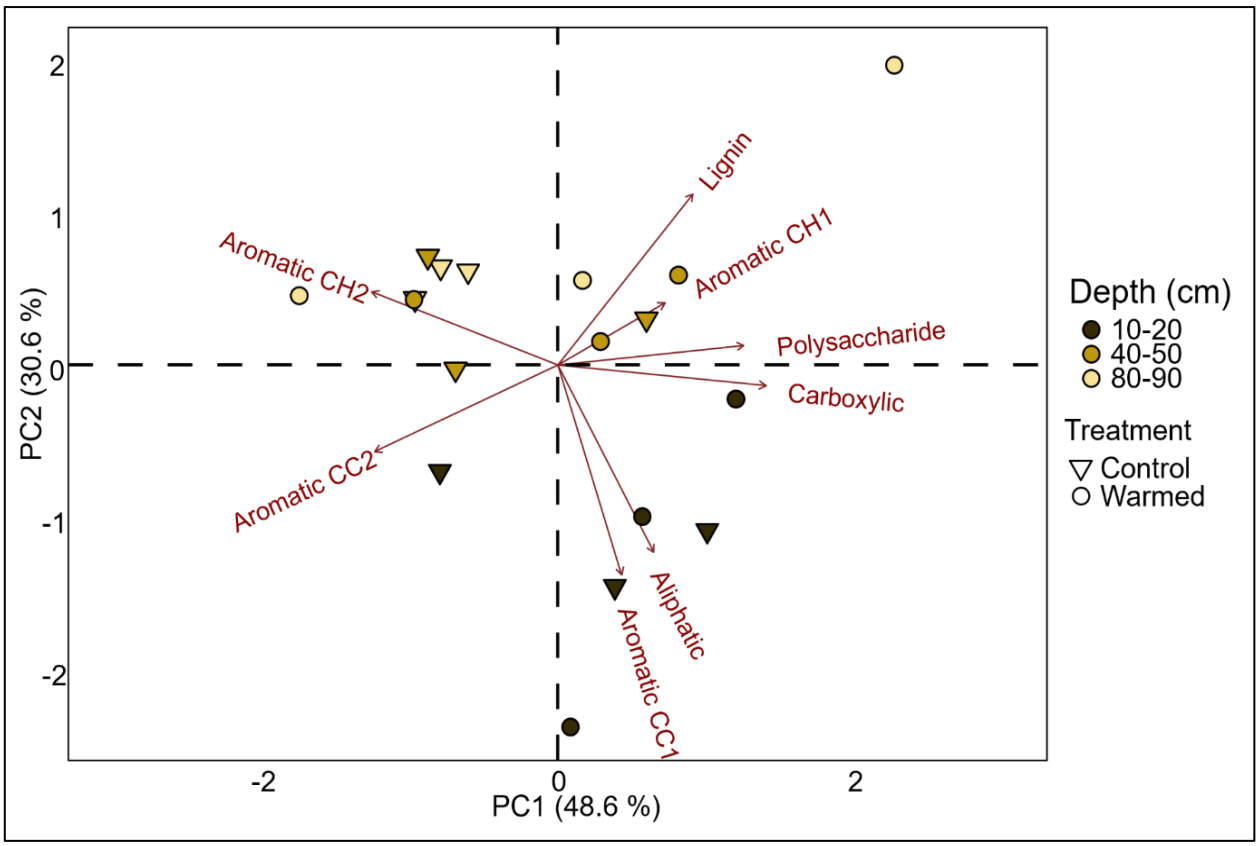


Fig. 5: Principal component analysis (PCA) of area under the curve values from fit diffuse reflectance infrared Fourier transform spectra of MAOM from control and warmed plots at three depth intervals (10–20, 40–50, 80–90 cm). Each point represents the MAOM of one sample. The number behind aromatic C=C/aromatic C–H refers to the same carbon bond type assigned at different band ranges (Table S1).

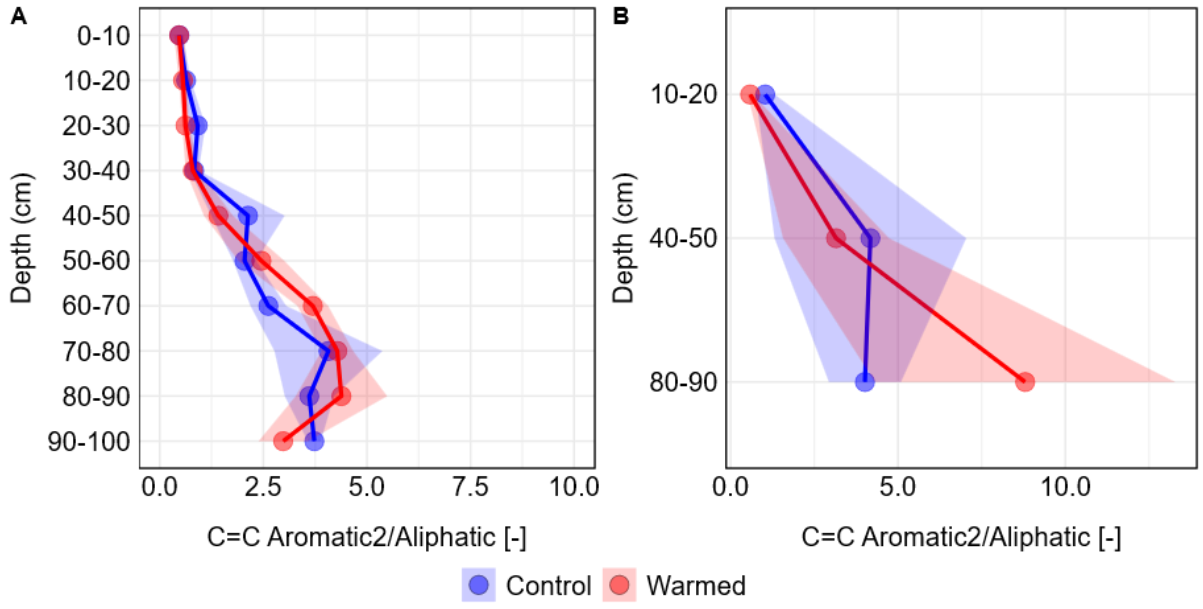
~~4. principal component analysis (PCA) of area under the curve values from fit Diffuse Reflectance Infrared Fourier Transform spectra of MAOM from control and warmed plots at three depth intervals (10–20, 40–50, 80–90 cm). Each point represents the MAOM of one sample. The number behind aromatic C=C/aromatic C–H refers to the same carbon bond type assigned at different band ranges (Table S1).~~

~~3.5 Changes of aromatic/aliphatic ratio in bulk soil and fractions~~

~~We did not find significant effects of interaction between soil depth and warming but significant effects of depth ($p < 0.001$; Table S11) on the DRIFT stability index (DSI) of bulk soils. Basically, bulk soil DSI~~ significantly increased with depth ~~from 0.5 at 0–10 cm to 3.7 at 90–100 cm in control plots~~ (Fig. 5~~Fig.~~(Fig. 5~~)~~). ~~In warmed plots, bulk soil DSI increased from 0.5 at 0–10 cm to 4.4 at 80–90 cm but slightly decreased again to 3.0 at 90–100 cm (Fig. 5). On average, warming non-significantly decreased DSI by between 5 % to 30 % in the soils above 50 cm and increased DSI by between 15 % to 43 % from 50 to 90 cm (Fig. 5).~~

~~For fPOM and oPOM, w~~There were no~~ewe did not observe either significant interaction effects between soil depth and warming, and main effects~~significant effects of warming and depth on the DSI of fPOM and oPOM ~~(~~(p > 0.1 ~~(Table 1S11~~). On average, the ~~warming non-significantly decreased~~ fPOM DSI~~ from 0.29 ± 0.07 to 0.23 ± 0.06~~ at 10–20~~ (cm and from~~ 0.27 ± 0.02) and 40–50 cm (~~ to~~ 0.18 ± 0.03~~)~~ in warmed plots were lower relative to the values at corresponding depths in ~~c~~control plots (~~cm and from~~0.29 ± 0.07 and ~~0.27 ± 0.02 to 0.18 ± 0.03 at 40–50 cm~~0.23 ± 0.06 respectively for 10–20 and 40–50 cm). ~~Simultaneously~~Similar to fPOM~~40–50 cm. Simultaneously~~, ~~warming non-significantly decreased~~ oPOM DSI exhibited lower values at 10–20 (0.18 ± 0.03) and 40–50 cm (0.14 ± 0.08) under warming compared to ambient temperature conditions (~~from 0.18 ± 0.03 to 0.14 ± 0.08 and from~~ 0.16 ± 0.01 ~~to~~ and 0.11 ± 0.02 at 10–20 and 40–50 cm, respectively~~;~~ ~~(~~Table ~~S10~~S9). However, these differences were subtle due to large standard errors.

~~). Although there were no significant effects of interaction between soil depth and warming on~~ MAOM DSI was not significantly affected by warming (p > 0.1), but~~,~~ it significantly increased with depth (p < 0.001~~; Table S11~~). Although MAOM DSI was on average larger in warmed than in ~~c~~control plots at 80–90 cm, this difference was statistically indistinguishable from zero due to high variability within the data (p > 0.1; Fig. 5).~~). On average, warming reduced MAOM DSI by 39 % (CI: -80 %, 86 %) at 10–20 cm and 5 % (CI: -69 %, 190 %) at 40–50 cm, whereas it increased MAOM DSI by 71 % (CI: -44 %, 422 %) at depth, but these effects were weak.~~



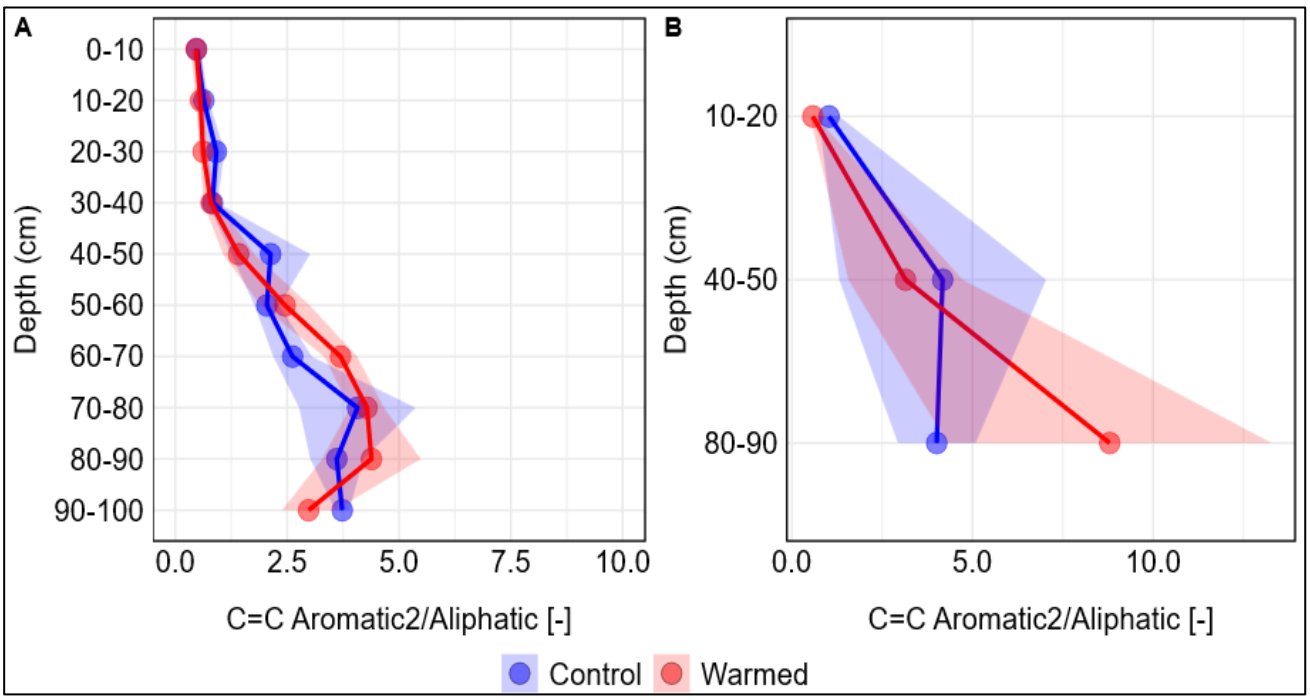


Fig. 6: The diffuse reflectance infrared Fourier transform (DRIFT) spectral stability index (DSI) or ratio between area under the curve values (AUC) of C=C aromatic (range 2) to aliphatic in bulk soil (A) at 10 depth increments from 0 to 100 cm and MAOM (B) at three soil depths (10–20, 40–50, and 80–90 cm) under control and warmed conditions (mean ± SE, n = 3). The ratio (C=C aromatic2/aliphatic) was calculated using the AUC values corresponding to the aliphatic (3020–2800 cm^{-1}) and C=C aromatic (range 2; 1670–1600 cm^{-1}) regions. The ratios of POM fractions are shown in Table S9.

~~Fi~~4. Discussion

4.1 Depth-specific shifts in SOC quantity and composition under long-term warming

4.1.1 Subsoil carbon loss to warming

There was a significant effect of depth and interaction between depth and treatment on SOC concentrations, which indicated that topsoils responded to 9.5 years of whole-soil experimental warming differently than subsoils (Table 1). This pattern of contrasting shallow versus deep responses in SOC concentration to warming is broadly consistent with earlier observations from the same site (Soong et al., 2021). However, the depth at which the warming response became evident was deeper in the more recent sampling campaign. In particular, the current study did not find a decline in SOC between 30–50 cm as was reported after 4.5 years of warming at this site (Soong et al., 2021; Zosso et al., 2021).

After nearly 10 years Ten years of whole-soil experimental warming, there were marginally caused significant, depth-dependent shiftsdifferences in SOC concentration, with indication that soils above and below 50 cm respondeding in opposite directions to warming (Table 1). This pattern of contrasting shallow versus deep responses in SOC concentration is broadly consistent with earlier observations from the same site after 2 and 4.5 years of warming (Hicks Pries et al., 2017; Soong et al., 2021). However, the depth at which the warming response reverses has shiftedwas deeper in the more recent . In particular, the current study did not find soils between 30 and 50 cm no longer exhibit the continued,a decline in significant SOC between 30-50 cm as was reported decline reported after 4.5 years of warming at this site (Soong et al., 2021); . Instead, average SOC concentrations in this interval under warming are now higher than those reported for the warmed plots in the earlier short-term studies (Zosso et al., 2021).

The shifts in depth of SOC-concentration decline between these two time points could be due to site heterogeneity or changes in rates and depths of plant inputs. Thise accumulation of SOC at 30–50 depth intervalstopsoils of warmed plots between year 4.5 and year 10 strongly points to a potentialn influx of new carbon during that intervening periodsince the last measurements. Since soils receive pPlant-derived inputs inputs exclusively from roots at to the soil temperate forest soils at these depths (insert depth) intervalare dominated by root inputs (Angst et al., 2016; Wordell-Dietrich et al., 2020). (Heckman et al., 2021; REF). we hypothesize that warming causes root growth into deeper depth after 10 years of warming, contributing to the non-significant SOC increase (Table 1). Although tThere was less a decline of root biomass in warmeheated than control plots at almost at every depth (except for 20-30 cm interval) [at all depths??] was observed by Ofiti et al. (2021) after 4.5 years (Ofiti et al. (2021). Yet,, but modeling workof the site has suggestsed that root responses may attenuate over time as plants approach a new balance between growth and maintenance costs under chroniclong-term warming (Riley et al., 2025). Recent eEmpiricalobservations from another temperate forest soil-warming experiment support a time-varying response, evidence supports this modelled observation, demonstrating postulation thatas fine root biomass in a temperate forest did not increase in the short-term (8 years), but increased significantly after with over a decade (14 years) of warming not short-term (8 years) of warming (Kwatcho Kengdo et al., 2022). We thus speculate that warming may have stimulated deeper root growth after 9.5 years of warming, heated plots did not have compensatingreduced the loss of SOC concentrations in mid-depths because stimulated deeper 9.5(**Error! Reference source not found.**). Yet, this

hypothesis requires further verification with ~~However, V verifying thisan increase in influx of deep-root carbon will require~~ additional ~~targeted,~~ depth-resolved root measurements.

~~This accumulation of SOC in the warmed plots between year 4.5 and year 10 strongly points to an influx of new carbon during that intervening period, contributing to the non-significant SOC increasebut modeling work suggest that root responses may attenuate over time as plants approach a new balance between growth and maintenance costs under chronic warming Empirical evidence supports this postulation that fine root biomass in a temperate forest increased significantly after over a decade (14 years) not short-term (8 years) of warming (Kwatcho Kengdo et al., 2022). However, verifying this influx of deep-root carbon will require targeted, depth-resolved root measurements~~

~~Ten years of whole-soil experimental warming caused significantly depth-dependent changes in SOC concentration, consistent with previous observations from the site after 2 and 4.5 years of warming (Hicks Pries et al., 2017; Soong et al., 2021). Notably, soils above and below 50 cm responded in opposite directions. 80 % of fine root biomass appeared in the top 30 cm soil at Blodgett (Hicks Pries et al., 2017), warming likely stimulated root growth into deeper soil horizons and enhanced plant inputs in the soil above 50 cm (Jackson et al., 1996; Kwatcho Kengdo et al., 2025; Leppälammi-Kujansuu et al., 2014). This new input was evidenced by on average non-significant higher SOC concentration, bulk soil DSI, and lower DSI in fPOM fraction. The enhanced inputs offset soil carbon losses due to warming-induced accelerated microbial decomposition (van Gestel et al., 2018; Wang et al., 2025).~~

~~W~~Post-hoc tests revealed that warming ~~consistently~~ marginally reduced SOC concentrations~~concentration~~ in subsoils~~subsoil~~ below 50 cm, with the strongest declines at 60–70~~,~~ and 80–90 cm (Table 1)~~, indicating subsoil carbon loss.~~ ~~This aligns with the higher average δ^{13}C values observed in warmed plots relative to controls at these depths, indicating accelerated microbial decomposition under warming (Soong et al., 2021).~~ ~~, indicating subsoil carbon loss. This agrees with previous results at this site (Hicks Pries et al., 2017; Ofiti et al., 2021; Soong et al., 2021) but contrasts findings from various field-warming studies (Jia et al., 2019; Khan et al., 2025; McGrath et al., 2022; Pegoraro et al., 2021; Wilson et al., 2016), underscoring context- and ecosystem-dependent subsoil responses (Hicks Pries et al., 2023). The observed subsoil loss, measured in the 80-90 cm depth interval, appears to be driven largely by a marginally significant declines in fPOM and a significant declines in oPOM quantity, with no detectable change in the but not by quantitatively stable MAOM fraction (Fig. 2). (Dungait et al., 2012; Fontaine et al., 2007; Salomé et al., 2010)(Henneron et al., 2022) As plant-derived biomass is the dominant, direct source of POM fractions (Lavallee et al., 2020),Given the~~

~~generally low rates of plant inputs to deep subsoils are unlikely to compensate for warming-induced carbon losses. Given limited plant inputs at depth, losses of the relatively available fPOM (Lavallee et al., 2020)are insufficiently offset by new carbon supply.~~ The ~~persistent~~ loss of subsoil carbon ~~over~~in this experiment~~ time therefore~~ highlights the vulnerability of deep SOC to long-term warming. ~~Therefore, whether this carbon loss in subsoil will persist or be further amplified over decades remains uncertain, because substrate depletion can temper warming induced increases in SOC decomposition~~ (Romero-Olivares et al., 2017)However, we hypothesize ~~since~~ that the initial stimulation of heterotrophic respiration ~~often~~may could attenuate~~s~~ over time (Riley et al., 2025), for example due to microbial acclimatization (Bradford et al., 2019)~~ and~~ or ~~as easily available~~ substrate~~s are~~ depletion~~ed~~. ~~,i~~Yet, it still~~t~~ remains uncertain whether this ~~trajectory of~~ subsoil~~-~~-carbon loss will be sustained in the longer~~er~~-term after 9.5 years of whole-soil experimental warming.~~.~~

4.1.2 Shifts in subsoil~~s~~ carbon composition

Soil carbon composition displayed marked changes with depth, with a decrease in aliphatic C–H~~ bonds~~ and an increase in aromatic bonds, which is consistent with numerous studies (Chen et al., 2018; Kögel-Knabner, 2002; Rumpel et al., 2002; Schöning and Kögel-Knabner, 2006)~~(Chen et al., 2018; Kögel-Knabner, 2002; Rumpel et al., 2002; Schöning and Kögel-Knabner, 2006)~~. ~~Yet~~~~However~~Yet~~, warming induced changes in bulk SOC composition were depth dependent. Although~~ After nearly-decadal warming, ~~we did not observe SOC composition shifts above 40 cm~~soils above 40 cm showed no clear SOC compositional shifts (Fig. 3), consistent with ~~identical~~ proportional ~~bond type~~area under the curve (AUC) contributions (Table S6) and unaltered DRIFT stability indices (DSI; Fig. 5) between treatments. ~~Collectively, warming did not change the chemical composition and stability in the top 40 cm. , other studies report contrasting results that warming enhances microbial activity, accelerating decomposition of labile, lignin , and cuticle derived carbon in a mix temperate forest (Feng et al., 2008) or hardwood forest (Pisani et al., 2015; vandenEnden et al., 2021). Similar to our study, (Schnecker et al., 2016) found that 7 years of warming did not alter soil carbon composition in topsoils, likely due to balanced effects of increased microbial degradation and root litter inputs. Although (Ofiti et al., 2021) reported decreased fine and coarse root mass primarily in subsoils > 30 under warming cm at our site, warming driven new inputs extending to 50 cm could have obscured detectable shifts in SOC composition after decadal warming.~~

Conversely, sSubsoil (below 40 cm) SOC composition tended to shift towards lignin-like residues under warming SOC compositional shifts(Fig. 3). Yet, the physical pools driving this change differed by depth.Subsoil SOC compositional shifts were consistent with prior results at this site (Ofiti et al., 2021).

At 40–50 cm, the increased bulk lignin-like signal stemmed primarily from a tendency shift in fPOM towards lignin and aromatic C–H (range 2; Fig. S2). This interpretation is further supported by respectively significant increase of lignin signal in bulk soil is primarily associated with a significant increase in lignin AUC values in fPOM (p = 0.002; Fig. S2). This relative increased signal in lignin at 1510 cm^{-1} is congruent with an marginal increase in aromatic C–H (range 2; p = 0.061) at 830 cm^{-1}, both of which are distinct peaks diagnostic for lignin spectrum (Zaccheo et al., 2002). Therefore, the chemical shifts in bulk soil at 40–50 cm towards lignin is due to a significant increase of this bond type in fPOM.increase in lignin AUC values in fPOM (p = 0.002; Fig. S2)and. This relative increased signal in lignin at 1510 cm^{-1} is congruent with an marginal marginally increased ofin lignin-like (p = 0.002) and marginal increase in in aromatic C–H AUC values (range 2; p = 0.061) AUC values,) at 830 cm^{-1}, both of which are distinct peaks diagnostic for lignin spectrumare associated with lignin spectrum (Zaccheo et al., 2002). Because fPOM comprised nearly 40 % of total SOC at this depth, (Fig. 2), this relative enrichment in lignin-like residues likely contributed substantially to the bulk soil compositional shift.Therefore, the chemical shifts in bulk soil at 40–50 cm towards lignin is due to a significant increase of this bond type in fPOM.

–Below 50 cm, bulk compositional shifts were instead associated with a general tendency towards a stronger lignin-like residues in the MAOM fraction. This trend was reflected in higher average proportion AUC contributions (Table S7), a shift of most MAOM samples towards lignin (Fig. 4), andBecauseBecause C=C aromatic (range 2) was the second most abundant bond in fPOM fraction across depths and treatments (Table S8), the depletion of fPOM fraction may have weakened its signal at bulk leve non-significantly l. higher lignin AUC values (Table S14) under warming at 80–90 cm. Given the relatively large size of the MAOM pool at depth, these average increases in lignin proportion and AUC may nevertheless have contributed to the tendency bulk compositional shifts observed in the PCA plots (Fig. 3). However, high variability among replicates at these depths, likely due to spatial heterogeneity, indicates that this trend should be interpreted with caution.level. Therefore, a marginal significant loss of fPOM fraction at depth with warming likely drove the on average non-significant decrease of DSI (Fig. 5A) and the shift from C–H/C=C aromatic (range 2) in control

~~subsoils to lignin and C–H aromatic (range 1) in warmed subsoil. With MAOM dominating the warmed subsoil (Fig. 2), bulk signals predominantly reflect mineral-associated bond signals, implying that lignin–mineral association contributes to carbon preservation at depth under warming. Collectively, warmed bulk subsoils exhibited a more advanced decomposition stage.~~

4.2 Divergent roles of different density fractions for carbon sequestration

4.2.1 fPOM as a dynamic pool of soil carbon

The fPOM is typically assumed to exhibit the strongest response to warming, due to its ~~high microbial accessibility and~~ lack of physical protection and thus high accessibility for microorganisms and the necessary components of efficient decomposition (Heckman et al., 2022; Lavallee et al., 2020; Rocci et al., 2021). However, our results demonstrated that the~~its~~ response of fPOM~~s~~ to warming vary ~~across the~~with soil depth at our site, and ~~.~~

~~T~~the ~~fPOM f~~fraction was not quantitatively altered by warming in soils above 50 cm. ~~but~~fraction ~~responded pronouncedly to warming at 80–90 cm depth but was not quantitatively altered in soils above 50 cm, , which was also observed by~~ (Soong et al., 2021)~~. It is typically assumed that fPOM should exhibit the strongest response to warming, compared to other density fractions, due to its high microbial accessibility and low degree of physical protection (Heckman et al., 2022; Lavallee et al., 2020; Rocci et al., 2021). Yet the inconsistent results from field warming studies, with no fPOM loss (Chen et al., 2023; Schnecker et al., 2016; Soong et al., 2021), or reduced fPOM (Song et al., 2012) in topsoils demonstrated that the responses of fPOM fraction to warming are not straightforward.~~

Several site-specific factors likely attenuate accelerated fPOM decomposition under warming in the top 50 cm~~, cumulatively explaining the lack of detectable changes in fPOM above 50 cm despite its generally high sensitivity to temperature~~. Firstly, the fPOM in topsoils is more easily replenished by both aboveground and belowground inputs due to its proximity to vegetation ~~(~~(Angst et al., 2016)~~(REF)~~. Second, the quality of the inputs may also contribute to this observed patterns. ~~One explanation for this discrepancy is the influence of warming on plant inputs, which are significantly correlated with fPOM (Song et al., 2012). When warming increases plant productivity (Ruehr et al., 2023), the warming-induced loss of fPOM fraction in surface soil could be offset (Liu et al., 2025). Moreover, low quality litter from m~~M~~Mixed-mixed~~ coniferous ~~forests~~litter typically has a ~~high C:N ratio~~s and lignin content~~forests~~ (Silver and Miya, 2001), which can ~~lower~~ing microbial carbon use efficiency (Manzoni et al., 2012). Consistent with this, ~~. Indeed,~~ fPOM at Blodgett Forest is N-poor with high C:N ratios (> 50

cm; Table S8; Soong et al., 2021) ~~at our site~~. ~~Furthermore, acidic soil conditions at Blodgett Forest (Rowley et al., 2025) restrict decomposition, a phenomenon widely associated with fPOM preservation (Lugato et al., 2021; Yu et al., 2022)such~~ . Finally,~~,~~ warming-induced topsoil drying (Pegoraro et al., 2026; Riley et al., 2025) likely inhibits microbial activity, particularly during summer drying season (Soong et al., 2021), thereby constraining decomposition and contributing to the unaltered fPOM quantity in the surface samples. Cumulatively, these factors suggest that continuous replenishment and environmentally constrained decomposition buffer fPOM against warming-induced losses in ~~topsoils, explaining the lack of detectable quantitative change above~~ the soils above 50 cm despite the ~~generally~~ higher temperature sensitivity of this fraction.~~, this factors likely explain the lack of detectable changes in fPOM above 50 cm in response to warming despite its generally high sensitivity to temperature Together, these factors likely buffer the topsoil fPOM pool against warming-induced losses, helping explain the lack of detectable changes in fPOM above 50 cm despite its generally high sensitivity to temperature.~~

Conversely, deep soil fPOM at 80–90 cm proved highly vulnerable. This deep fPOM quantitative loss ~~such as at Blodgett Forest could limit microbial breakdown~~ (Lavallee et al., 2020) ~~when warming augments inputs, causing fPOM fraction accrual (Crow et al., 2009). (Heckman et al., 2022) suggested that warming effects on decomposition are attenuated by depth due to substrate limitation (Ahrens et al., 2020) and thus lower microbial activity in deep soils. Yet we see loss of fPOM mass at depth at our site, which~~ was previously linked to enhanced microbial activity (Ofiti et al., 2021; Soong et al., 2021) and increased relative abundance of Actinobacteria (Zosso et al., 2021). This group of bacteria is known to utilize ~~more~~ complex carbon (Barret et al., 2011; Goodfellow and Williams, 1983). ~~Therefore, we hypothesize that the combination of increased microbial decomposition (Soong et al., 2021) and absence~~Additionally, since 80 % of root biomass resides in the top 30 cm (Hicks Pries et al., 2017), depleted substrates and a lack of fresh plant inputs could exacerbate~~s~~ this deep soil~~jointly contributed to the observed~~ fPOM decline.

The high sensitivity of this fraction~~This~~ is supported ~~as~~ in subsoils. ~~Therefore, we hypothesize that the combination of increased microbial decomposition (Soong et al., 2021) and absence of fresh plant inputs jointly contributed to the observed fPOM decline in subsoils.~~

~~a~~by its distinct depth-specific chemical composition shifts in response to warming. Specifically, fPOM composition was not affected in topsoils (Fig. S1), but ~~Consistent with changes in fPOM concentrations, warming did not significantly alter fPOM composition at 10–20 and 40–50 cm (Fig. S1). An overall lower DSI in warmed fPOM suggests a greater~~

~~contribution from fresh plant inputs compared to the control plots. These inputs likely replenished fPOM despite faster decomposition, maintaining its composition consistent even as turnover accelerated.~~ it exhibited significant relative lignin-like enrichment, a tendency of increasing C/N ratios, ~~DSI,~~ and lower DSI and $\delta^{13}C$ values in mid-depth (Fig. S8 and Fig. S9). ~~Together with trending increases in C/N ratios, DSI and aliphatic AUC, and lower $\delta^{13}C$ values, this further suggests increased root biomass at this depth.~~ At 80–90 cm, very low fPOM recovery in the warmed plots precluded compositional analysis. However, the near absence of fPOM in the warmed subsoil further supports continued and disproportionate losses of this fraction under warming (Soong et al., 2021). Overall, these findings suggest that deep-soil fPOM represents a highly temperature-sensitive carbon pool where enhanced microbial decomposition is not balanced by new plant-derived inputs. ~~At 80–90 cm, very low fPOM recovery in warmed plots precluded compositional analysis. The absence of fPOM in the warmed subsoil further supports the hypothesis that there was a disproportionate loss of this fraction under warming. Taken together~~Collectively~~together~~, ~~our results indicate that fPOM is sensitive~~is highly vulnerable ~~to warming in deep soils where enhanced decomposition occurs with depleted substrates~~sensitive ~~to warming, especially in deep soil where enhanced decomposition is not compensated by increased plant inputs.~~

4.2.2 oPOM is ~~an~~ equally dynamic, but a small pool at Blodgett

The oPOM fractions exhibited a similar depth-~~-~~specific response to warming as fPOM (Fig. 1). Soong et al. (2021) found no significant reduction in oPOM throughout the soil profile, including the 80–90 cm interval. However, our results displayed consistently proportional reduction across soil depth and quantitatively significant loss at 80–90 cm (Fig. 1 and Fig. 2). ~~These findings suggest that the prolonged~~ We speculate that decadal warming might have ~~declined increased the aggregate turnover time of~~destabilized aggregates and occluded SOC in the subsoil through time, supporting observations from ~~in several other studies~~a previous study (Poeplau et al., 2020). However, in contrast to fPOM, oPOM represented the smallest density fraction by mass and proportionally in our dataset, which was consistent with previous observations from Blodgett (Hicks Pries et al., 2017; Soong et al., 2021) and other studies (Schnecker et al., 2016; Schrumpf et al., 2013).~~,~~ ~~and~~ Thus, despite evidence that warming may destabilize oPOM, the small size of this fraction indicates that its contribution to warming-induced bulk SOC loss at Blodgett Forest is limited.~~suggested that changes to this pool are quantitatively less significant to bulk SOC.The oPOM fractions exhibited a similar depth-specific response to warming as fPOM (Fig. 1). Soong et al. (2021) found no significant~~

~~reduction in oPOM throughout the soil profile, including the 80–90 cm interval. These findings suggest that the prolonged warming might have declined the aggregate turnover time of occluded SOC in the subsoil through time, supporting observations in several other studies (Chen et al., 2023; Poeplau et al., 2020). However, in contrast to fPOM, oPOM represented the smallest density fraction by mass and proportionally in our dataset, which was consistent with previous observations from Blodgett (Hicks Pries et al., 2017, p.17; Soong et al., 2021) and other studies (Schnecker et al., 2016; Schrumpf et al., 2013), and suggested that changes to this pool are quantitatively less significant to bulk SOC.~~

The SOC composition of oPOM was highly heterogeneous and showed no clear trends with depth or warming. This is evident in the wide spread of oPOM compositions along PC1 and PC2 (Fig. S2), reflecting the heterogeneity of SOC preserved by aggregates (Wagai et al., 2009; Wiesenberg et al., 2010). This heterogeneity likely arises because oPOM comprises a mixture of ~~organic matter~~POM with different origins, decomposition states, and residence times (Dorodnikov et al., 2011; Wiesenberg et al., 2010). As particulate material becomes occluded into aggregates of varying stability, it experiences differing~~ent~~ degrees of physical protection, microbial processing, and turnover, resulting ~~is seen in other studies where oPOM is often associated with~~ a broader range in ~~$\delta^{13}C$~~ $\delta^{13}C$ and $\Delta^{14}C$ values (Marín-Spiotta et al., 2008; McFarlane et al., 2013; Rowley et al., 2021; Schrumpf et al., 2013). ~~The reason for this is the heterogenous nature of this operationally-defined fraction is likely driven by the inclusion of containing organic matter at various stages of decomposition into different aggregates, which afford varying levels of protection , which, if not further separated, can result in strongly variable chemical compositions in space and time (Dorodnikov et al., 2011; Wiesenberg et al., 2010). However, as the mass of the oPOM fraction was very limited, further separationsa quantitatively small pool of SOC at Blodgett Forest.The SOC composition of oPOM was highly heterogeneous and showed no clear trends with depth or warming. This is evident in the wide spread of oPOM compositions along PC1 and PC2 (Fig. S2), reflecting the heterogeneity of SOC preserved by aggregates (Wagai et al., 2009; Wiesenberg et al., 2010). This heterogeneity is seen in other studies where oPOM is often associated with a broader range in $\delta^{13}C$ and $\Delta^{14}C$ values (Marín-Spiotta et al., 2008; McFarlane et al., 2013; Rowley et al., 2021; Schrumpf et al., 2013). The reason for this is the heterogenous nature of this fraction containing organic matter at various stages of decomposition, which, if not further separated, can result in strongly variable chemical compositions in space and time (Dorodnikov et al., 2011; Wiesenberg et al., 2010). However, as the mass of the oPOM fraction was very limited, further~~

~~separations followed by chemical analyses were not feasible in the current study. In summary, oPOM sensitivity to warming may have increased with time to reflect that of the fPOM, but it was a quantitatively small pool of SOC at Blodgett Forest~~Yet, overall while oPOM was heterogeneous and its sensitivity to warming may have increased with time to reflect changes in fPOM, it was a quantitatively small pool of SOC at Blodgett Forest.~~.~~

4.2.3 Subsoil MAOM as a long~~-~~-term carbon preservation pool

The mineral~~-~~associated organic matter was consistently less responsive to warming than fPOM and oPOM across the soil profile (Fig. 1 and Fig. 2~~Figs. 1-2~~). ~~This~~ Our results provide~~s~~ among the first fraction~~-~~-resolved evidence that subsoil MAOM remained ~~consistent~~ stable in both mass and chemical composition under nearly-decadal whole-soil warming. This pattern aligns with other warming studies (Schindlbacher et al., 2009; Schnecker et al., 2016) but contrasts with ~~(~~Chen et al.~~,~~ (2023), who documented warming~~-~~induced ~~decreased inputs and suppressed decomposition leading to a~~ significant MAOM loss. In our warmed topsoils, we hypothesize that greater plant inputs (Castellano et al., 2015) combined with warming-elevated microbial activity could have offset MAOM destabilization and loss (Cotrufo et al., 2013; Islam et al., 2022), resulting in constant MAOM.~~The mineral-associated organic matter was consistently less responsive to warming than fPOM and oPOM across the soil profile (Figs. 1-2). This provides among the first fraction-resolved evidence that subsoil MAOM remained consistent in both mass and chemical composition under decadal warming.~~

In ~~Direct sorption and microbial assimilation are the major drivers of MAOM formation in topsoils (Mikutta et al., 2019)~~(Mikutta et al., 2019). ~~Sufficient fPOM supplies sufficient substrates that can be sorbed to minerals via *in vivo* pathway (Cotrufo et al., 2013; Liang et al., 2017; Witzgall et al., 2021). In our warmed topsoils, greater plant inputs (Castellano et al., 2015) with elevated microbial activity likely offset MAOM loss (Cotrufo et al., 2013; Islam et al., 2022), resulting in constant MAOM. This pattern aligns with other warming studies (Schindlbacher et al., 2009; Schnecker et al., 2016) but contrasts with (Chen et al., 2023), who documented warming-induced decreased inputs and suppressed decomposition leading to a significant MAOM loss. Given the continuous exchange of MAOM and bulk SOC in topsoil, reflected by similar DSI of both, we infer that warming accelerated turnover and shortened MAOM residence time (Mikutta et al., 2019)~~(Mikutta et al., 2019), ~~especially relative to subsoil MAOM. In~~ our deep subsoils (> 50 cm), we hypothesized~~sise~~ that MAOM remains stable likely because the accelerated decomposition of fPOM and oPOM ~~decomposition~~ provided substrates that ~~can~~ were subsequently~~be~~ adsorbed to mineral

surfaces~~.minerals.~~ Another possible reason would be that warming can also enhance vertical transport of ~~DOC~~ dissolved organic matter (Soong et al., 2021), which can be efficiently associated with minerals (Kramer et al., 2012; Mikutta et al., 2019; Villarino et al., 2021; Yu et al., 2022). This apparent stability of MAOM stocks under warming is further supported by its largely unchanged chemical composition and on average increased DSI in subsoils.~~(Kramer et al., 2012; Mikutta et al., 2019; Villarino et al., 2021; Yu et al., 2022).~~

MAOM composition remained consistent in subsoils under warming, characterized mainly by both C=C aromatic (range 2) and ~~lignin polyphenolic~~ lignin-like peaks~~signals~~ (Table S8), ~~which did not hold at bulk level~~a pattern not observed in the bulk soil (Fig. 3). This ~~affirmed~~ supports the substantial loss of C=C aromatic compounds (range 2) from fPOM under warming and~~, which~~ aligns with significant loss of aromatic~~-binding containing~~ polymers documented at this site ~~by~~ (Zosso et al., 2023)~~ at this site,~~. ~~a~~Although ~~although~~ ~~these bonding types~~such compounds are typically considered ~~represent~~ chemically complex ~~that typically~~ and were previously thought to accumulate during microbial decomposition (Calderón et al., 2013; Demyan et al., 2012; Haberhauer et al., 1998), their decline~~. Its decline points to~~ suggests that mineral protection rather than intrinsic recalcitrance ~~as the key process for~~governs SOC ~~preservation~~persistence (Marschner et al., 2008). ~~Such o~~Organo-~~organo~~ mineral associations may account for the pronounced lignin-like signal ~~signal~~ and higher average DSI in warmed deep soil, ~~because~~ as phenolic-rich lignin derivatives are readily stabilized by ~~Fe/Al oxides and to some extent Ca~~different geochemical actors (Kögel-Knabner et al., 2008; Rowley et al., 2025) ~~(Kögel-Knabner et al., 2008; Rowley et al., 2024)~~. ~~Concurrently, a consistent decrease in aliphatic signals in MAOM in deep soil under warming was also observed, indicating that the strength of organo–mineral associations may shape its warming responses (Conant et al., 2011; Kaiser et al., 1996; Lützow et al., 2006). However, resolving the underlying mechanisms was beyond the scope of the current study and warrants targeted future work.~~ Collectively, the small changes in its concentration and composition support the notion that MAOM is a slow-cycling carbon pool at Blodgett, which buffers soil carbon loss under nearly-decadal whole-soil warming ~~and can be regarded as a carbon reservoir to help mitigate climate change~~.

5. Conclusion

Our study is the first to investigate the SOC composition across different soil fractions within a whole-soil field warming experiment after ~~nearly 10~~9.5 years of warming. Our findings highlight that bulk soil and SOC fractions responded differently to long-term warming, with fPOM and oPOM being more responsive than MAOM over a nearly~~a~~-decadal

timescale. The loss of dynamic fPOM fraction in part drives the consistent ~~and marginally significant~~ decrease ~~of~~ in SOC concentrations at certain depths in subsoil. This has direct implications for carbon–climate feedback modeling, as initial warming–enhanced CO_2 fluxes (Hicks Pries et al., 2017; Soong et al., 2021) may be attenuated over decadal timescales by the depletion of this labile POM ~~,~~ in ~~particular in~~the subsoil. MAOM showed much lower responses to warming than the POM fraction, ~~and aromatic compounds in association with minerals is a key mechanism for SOC preservation at Blodgett~~and was enriched in aromatic and lignin-like compounds.~~.~~ We demonstrate that combining DRIFT spectroscopy with density fractionation provides a more powerful approach for better understanding ~~of~~ SOC dynamics under warming than analyzing bulk soil alone. ~~Therefore, f~~ Future whole-soil warming experiments should prioritize studying the responses of subsoils and distinct SOC fractions to better resolve depth-specific carbon dynamics and improve predictions of soil carbon–climate feedbacks.~~uture long -term whole- soil warming experiments should place particular emphasis on subsoils and individual SOC fractions to better understand depth -specific carbon dynamics.~~

Competing interests

The authors declare that they have no conflict of interest.

Code/Data availability

All data from this study is available on ESS-Dive (doi:10.15485/3024414).

Author contributions

BS conducted all the lab work, contributed to field work, wrote the original draft, conducted statistical analysis, and created the figures. GLBW supervised BS, contributed to methods, conceptualization, data interpretation and validation, edited, and reviewed the manuscript. MWIS obtained funding for Zurich costs, and contributed to conceptualization, data interpretation, writing reviews, and editing. EP led the field work, and contributed to statistical analysis, writing review. MST designed and is PI of the Blodgett whole soil warming experiment, contributed to field work, and edited the manuscript. MCR developed the DRIFT spectra analysis pipeline for spectral analysis and calculation, contributed to statistical analysis, conceptualization, data interpretation and validation, edited and reviewed the manuscript.

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