



Lignin and fungal abundance modify manganese effects on soil organic carbon persistence at the continental scale

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ABSTRACT

Manganese (Mn) may play an outsized role in soil biogeochemical cycles relative to its abundance. The role of Mn-facilitated oxidation of biomacromolecules during litter decomposition is well-established, but the balance between Mn-promoted soil organic carbon (SOC) oxidation and long-term SOC protection in mineral soils is unknown, especially in subsoils. In this study, we used soils collected across the US National Ecological Observatory Network (NEON) to assess the distribution of Mn and relationships between Mn abundance and SOC concentration, potential mineralization, and persistence at a continental spatial scale. Total reducible Mn was not spatially correlated to site moisture (Spearman's $\rho = 0.24$), highlighting that Mn abundance may influence SOC cycling independently from other moisture-driven soil chemical properties (e.g., reactive iron and aluminum). However, Mn effects on SOC cycling depended on depth, soil, or site-level properties. In particular, fungal:bacterial biomass ratio, proportion of SOC in the free light fraction, lignin abundance, and/or proportion of undegraded organic matter mediated the effect of Mn on SOC cycling metrics. For example, the effect of Mn on SOC concentration in subsoils shifted from positive (approximately +270 % relative to mean subsoil SOC) to negative (−125 %) with increasing fungal:bacterial ratio. We propose that convergence of high Mn, lignin-rich substrates, and fungal:bacterial ratio amplifies lignin mineralization in surface soils, but does not result in higher net SOC turnover due to fungal biomass stabilization. In contrast, we suggest that Mn abundance promotes smaller, but more persistent SOC stocks in subsoils by accelerating SOC transformation from particulate to microbial biomass pools.

1. Introduction

Manganese (Mn) plays a key role in soil biogeochemical cycles by driving transformations of oxide minerals and organic litter inputs (Guha et al., 2001; Negra et al., 2005; Berg et al., 2015; Li et al., 2021). While Mn abundance in soil is low relative to other reactive metals, Mn may have a disproportionate effect on soil organic carbon (SOC) cycling

processes due to rapid and environmentally-relevant Mn redox transformations (Negra et al., 2005). However, in mineral soils, counter-acting Mn-mediated SOC stabilizing and de-stabilizing processes complicate the prediction of net Mn effects on SOC turnover (Li et al., 2021). The role of Mn abundance in SOC turnover in deeper (>20–30 cm) mineral subsoils is especially uncertain, but may constitute a novel soil chemical control on the persistence of the subsoil terrestrial SOC

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reservoir.

Manganese-mediated oxidative depolymerization of biopolymers, especially lignin, is a known control on litter decomposition at an ecosystem scale (Berg, 2000; Berg et al., 2015; Keiluweit et al., 2015; Stendahl et al., 2017). Faster organic matter (OM) decomposition with increasing Mn content has been identified in litter and organic soil horizon incubation and litter bag experiments (Berg et al., 2015; Sun et al., 2019). A number of abiotic and biotic mechanisms have been linked to oxidation and often oxidative depolymerization of OM, including Mn(II) oxidation, formation of Mn(III)-organic oxidants, and direct oxidation at Mn(III/IV) oxide surfaces (Sunda and Kieber, 1994; Chorover and Amistadi, 2001; Allard et al., 2017; Jones et al., 2018; Li et al., 2021). In addition to direct oxidation, birnessite (nominally Mn(III/IV) $O_2 \cdot nH_2O$) has also been shown to catalyze abiotic protein fragmentation, resulting in depolymerization of proteins to soluble peptides (Reardon et al., 2016). In the forest floor and organic soils, fungal Mn-peroxidase (MnP) enzymes are important for biotic lignin mineralization, influencing both litter decomposition rates and forest floor OM loss as a function of Mn availability (Keiluweit et al., 2015; Kranabetter, 2019). While lignolytic enzymes are also produced by soil bacteria (Singh et al., 2013), the ubiquity of MnP and other peroxidase-promoted oxidation pathways in fungi (e.g., basidiomycetes, including white rot fungi) suggest fungal processes may disproportionately contribute to Mn-mediated OM oxidation.

Despite the known importance of available Mn as a control on surface litter decomposition, the impact of Mn availability on SOC mineralization in mineral soils remains a gap in our understanding of SOC turnover. In mineral subsoils (e.g., deeper than 20–30 cm), the effects of Mn availability on SOC mineralization are largely unknown, especially at large (e.g., continental) spatial scales. Using a depth gradient from surface leaf litter to A-horizon mineral soils, Jones et al. (2020) suggest that shifts in the relative abundance of Mn and reactive iron (Fe) species may decrease the impact of Mn-driven oxidation with depth. In addition, the role of Mn in depolymerization of relatively high molecular-weight, unaltered plant residues (e.g., in the forest floor) suggests that Mn oxidation may not contribute appreciably to bulk SOC mineralization in subsoils, which are often depleted in particulate SOC (i.e., SOC in the free light fraction) relative to surface soils (Schrumpf et al., 2013; Liebmann et al., 2020).

While biotic or abiotic Mn-mediated SOC oxidation could increase SOC decomposition rates in mineral soils, these effects may be counteracted by SOC stabilization processes directly or indirectly linked to reactive Mn (Li et al., 2021). After depolymerization, smaller organic substrates can be directly mineralized to CO_2 , but dissolved organic carbon (DOC) or organic compounds assimilated into microbial biomass can also be protected from mineralization by partitioning to persistent SOC pools (Cotrufo et al., 2013; Kleber et al., 2015). Manganese oxide minerals may directly contribute to SOC-mineral protection via a range of potential surface interaction mechanisms (e.g., electrostatic interactions, ligand exchange), or through physical occlusion within Mn-oxide coatings, concretions, and precipitates (Stuckey et al., 2018; Li et al., 2021).

In contrast to gradients in reactive Fe and aluminum (Al) and base cation-facilitated SOC stabilization (Rasmussen et al., 2018; Possinger et al., 2021), the spatial variation in reactive Mn effects on SOC cycling across soil types are relatively unknown. While gradients in reactive Fe and Al are generally closely tied to soil pH (Rasmussen et al., 2018) and moisture availability (Kramer and Chadwick, 2018), spatial variation in Mn abundance is driven in part by spatially-isolated Mn hotspots (e.g., due to rare manganiferous parent materials, Mn-hyperaccumulating vegetation, or anthropogenic sources) (Bourgault and Rabenhorst, 2011; Richardson, 2017). In addition, soil properties impacting Mn abundance do not clearly align across spatial or soil chemical gradients. For example, while many Fe-bearing primary minerals that are more abundant in low-pH systems are also enriched in Mn (Gilkes and McKenzie, 1988), less-persistent, soluble Mn(II) forms are also favored

at lower pH (Negra et al., 2005). While total Mn (by acid digestion) is modeled and mapped across the conterminous US (Smith et al., 2019), the spatial variation of more operationally “reactive” forms of Mn is less well known, including reducible (citrate-dithionite-extractable) and organically-complexed (pyrophosphate-extractable) Mn forms. In particular, the faster cycling and higher reactivity of organic Mn (including Mn(II) in plant residue-derived SOC and putative Mn(III)-organic complexes) suggests that this form of Mn may have a disproportionate role in Mn-mediated SOC oxidation (Keiluweit et al., 2015; Jones et al., 2018; Li et al., 2021), but the spatial distribution and relative impact of organic Mn abundance on SOC turnover are unknown at large scales, particularly in subsoils.

In this work, we assessed impacts of reactive Mn abundance on depth-resolved SOC cycling across a continental-scale network, using A-horizon (~0–15 cm-depth), upper B-horizon (~25–50 cm-depth), and lower B-horizon (~70–100 cm-depth) soils collected from 35 terrestrial US National Ecological Observatory Network (NEON) sites. Leveraging the unique combination of soil and SOC composition metrics available for subsoils up to ~100 cm in depth, we tested the predictive power of Mn abundance on SOC concentrations and long-term persistence of bulk, mineral-associated, and particulate SOC pools (via density separation and radiocarbon ($\Delta^{14}C$) abundance). For a subset of sites ($n = 26$), we assessed Mn effects on potential mineralization (via incubation experiments) in A- and upper B-horizons. Due to the role of Mn in the oxidation of plant biomacromolecules (especially by fungi), we hypothesized that Mn effects on SOC mineralization would be amplified by lignin-rich SOC composition and higher fungal:bacterial ratio. In addition, due to the likelihood of lower particulate SOC and Mn at depth relative to surface soils, we hypothesized that SOC oxidation effects would result in net faster SOC turnover in surface horizons but contribute to SOC persistence in subsoils.

2. Materials and methods

2.1. Overview of data sources

Soil OC concentration, bulk radiocarbon, site-level climate, and basic soil characterization data for this study were derived from a published database of soil physicochemical, SOC, and site-level data for terrestrial, non-permafrost US NEON sites ($n = 35$ sites; Nave et al., 2021a) (Table 1, Table S1) (2.2). Detailed information regarding soil classification and land-use are included in Nave et al. (2021b), Heckman et al. (2021), and Possinger et al. (2021). Dense fraction (DF) and free light fraction (FLF) SOC radiocarbon measurements were derived from Heckman et al. (submitted) (2.2.2.1, 2.2.2.2). In addition, abundance of lignin and relative SOM degradation (via amino acid:lignin (AA:LIG) ratio) were derived from alkaline cupric oxidation data (Weiglein et al., 2022) (2.2.2.3). We modified published soil incubation data available for a subset of sites ($n = 26$) (Possinger et al., 2021; Weiglein et al., 2022) to assess potential mineralization of SOC during rapid, intermediate, and slower incubation phases (0–3, 3–20, and 20–90 d, respectively) (2.2.2.4) (Fig. S1, Fig. S2). We also generated site-level average fungal:bacterial biomass data (new to this study) derived from the NEON phospholipid fatty acid (PLFA) data product, when available ($n = 24$ out of 35 sites; NEON, 2021a) (2.3) (Fig. S2).

2.2. NEON soil characterization database

Soil core collection, processing, and soil sample preparation are described in detail in Nave et al. (2021b). Briefly, 5 soil cores (to 2-m, bedrock, or refusal) were collected within the NEON site eddy covariance flux tower footprint. Cores were stored under refrigeration (3 °C) until description by genetic horizon. Horizons were pooled by pedogenic features to generate one composite A-horizon (~0–15 cm), upper B-horizon (~25–50 cm), and lower B-horizon (~70–100 cm) bulk sample per site. Composite samples were air-dried, root-picked (3 min), and

Table 1

National Ecological Observatory Network (NEON) study sites. UNDERC = University of Notre Dame Environmental Research Center; LBJ = Lyndon B. Johnson National Grassland.

Site ID	Site name	Soil order	Site ID	Site name	Soil order
ABBY	Abby Road	Inceptisol	OAES	Klemme	Inceptisol
BART	Bartlett	Spodosol	ONAQ	Onaqui-Ault	Aridisol
	Experimental Forest			Steppe	
BLAN	Blandy	Alfisol	ORNL	Oak Ridge	Ultisol
	Experimental Farm				
CLBJ	LBJ/Caddo	Alfisol	OSBS	Ordway-Swisher	Entisol
CPER	Central Plains	Mollisol	RMNP	Rocky Mtn.	Mollisol
	Exp. Range			National Park	
DCFS	Dakota-Coteau	Mollisol	SCBI	Smithsonian	Alfisol
				Cons. Biol. Institute	
DELA	Dead Lake	Ultisol	SERC	Smithsonian	Ultisol
				Environ. Res. Center	
DSNY	Disney	Spodosol	SJER	San Joaquin	Alfisol
GRSM	Great Smoky Mtn. Natl. Park	Inceptisol	SOAP	Soaproot	Alfisol
				Saddle	
HARV	Harvard Forest	Inceptisol	SRER	Santa Rita	Entisol
JORN	Jornada	Aridisol	STEI	Steigerwalt	Spodosol
KONA	Konza	Mollisol	STER	Sterling	Mollisol
	Agriculture				
KONZ	Konza Core	Mollisol	TALL	Talladega	Ultisol
LENO	Lenoir Landing	Inceptisol	TREE	Treehaven	Spodosol
MLBS	Mountain Lake	Entisol	UNDE	UNDERC	Spodosol
MOAB	Moab	Aridisol	WOOD	Woodworth	Mollisol
NOGP	Northern Great Plains Res. Lab.	Mollisol	WREF	Wind River	Andisol
NIWO	Niwot Ridge	Mollisol			

sieved (2-mm mesh).

2.2.1. Soil physicochemical properties

For the soil database, soil physicochemical properties (extractable cations, selective dissolutions, soil pH, and particle size distribution) were determined as described in Heckman et al. (2021). Briefly, extractable base cations (Ca^{2+} and Mg^{2+}) were determined by ammonium (NH_4^+) acetate extraction following standard protocols (Soil Survey Staff, 2014). Parallel selective dissolution using citrate-dithionite (CD), acid NH_4^+ oxalate (AO), and sodium pyrophosphate (SP) was conducted for Fe, Al, and Mn following USDA NRCS protocols (Soil Survey Staff, 2014). For selective dissolution data, upper and lower bound values derived from Nave et al. (2021a) were averaged as appropriate for the element detection limit and instrument accuracy. Soil pH was determined in water (1:2 soil:water ratio). Soil texture was determined by laser diffraction (adjusted $< 0.6 \mu\text{m}$ clay particle diameter cutoff) (Fisher et al., 2017; Yang et al., 2019).

In this study, parallel selective dissolution extraction data were used to represent operationally defined pools of Mn. Here, we defined SP-extractable Mn to be “organically-complexed Mn” (Mn_{ORG}), with high affinity for Mn(III)-organic complexes (Nico and Zasoski, 2001; Jones et al., 2020), CD-extractable Mn to be “reducible Mn” (Mn_{RED}), and AO-extractable Mn to be “Mn oxides” (Mn_{OX}), inclusive of citrate-dithionite reducible and minor contributions of non-reducible Mn (Jarvis, 1984; Gilkes and McKenzie, 1988; Negra et al., 2005; Jones et al., 2020) (Table 2).

2.2.2. Soil organic carbon characterization

2.2.2.1. Density fractionation. We used SOC radiocarbon abundance data for the DF (i.e., mineral-associated SOM) and FLF (i.e., particulate SOM) (Heckman et al., submitted). Density fractionation methods are described in Weiglein et al. (2022) and Possinger et al. (2021) and

Table 2

Summary of operationally defined reactive manganese (Mn) pools targeted by citrate-dithionite, sodium (Na) or potassium (K) pyrophosphate, and acid ammonium oxalate dissolution.

Reference	Extractant	Operational Definition
<i>Citrate-dithionite</i>		
This study	0.4 g Na-dithionite in 25 mL 0.57 M Na-citrate	Reducible Mn
Jarvis (1984)	0.4 % Na-dithionite in 1.0 M NH_4 acetate, pH 7	Reducible Mn
Jones et al. (2020)	Citrate-dithionite + HCl	Total reducible Mn
<i>Sodium pyrophosphate</i>		
This study	0.1 M Na-pyrophosphate, pH 10	Organically-complexed
Jarvis (1984)	0.1 M K-pyrophosphate	Organically-complexed
Jones et al. (2020)	0.1 M Na-pyrophosphate, pH 10	Organically complexed
Keiluweit et al. (2015)	0.1 M Na-pyrophosphate, pH 10	Soluble and organically-complexed
<i>Ammonium oxalate</i>		
This study	0.2 M ammonium oxalate, pH 3	Mn oxides
Jarvis (1984)	Acid ammonium oxalate	Mn associated with reactive oxides

followed standard protocols published in Golchin et al. (1994a, 1994b), Strickland and Sollins (1987), and Swanston et al. (2005). Sodium polytungstate solution was adjusted to a density of 1.65 g cm^{-3} and sonication applied at $750 \text{ J g soil}^{-1}$.

2.2.2.2. Total elemental and bulk radiocarbon analysis. For the bulk soil and density fractions, total organic C (TOC) and N concentrations (Nave et al., 2021a) were determined by combustion using an IsoPrime 100 elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) (Iso-prime ltd, Cheadle, UK). When IRMS measurements were not available, EA data only were used to calculate TOC and molar C:N ratio in this study. Bulk radiocarbon depletion measurements were obtained from Nave et al. (2021a). All radiocarbon abundance ($\Delta^{14}\text{C}$, per mil) measurements (bulk soil, FLF, and DF) were determined by conversion to graphite followed by measurement by accelerator mass spectrometry (AMS) according to standard protocols (Vogel et al., 1987; Davis et al., 1990; Beverly et al., 2010), following methods described in detail in Heckman et al. (2021). Briefly, measurements were conducted at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory (LLNL) and the WM Keck Carbon Cycle Accelerator Mass Spectrometry facility at the University of California Irvine (UCI). Samples were graphitized prior to radiocarbon measurement through combustion and reduction to graphite in the presence of hydrogen gas and a Fe catalyst. Samples containing carbonates were acid fumigated prior to graphitization (Harris et al., 2001). Corrections for mass-dependent fractionation were conducted using independently measured $\delta^{13}\text{C}$ values at LLNL (Stuiver and Polach, 1977) and inline AMS measurements at UCI.

2.2.2.3. Alkaline cupric oxide oxidation. Estimates of bulk soil lignin content were determined using alkaline cupric oxide (CuO) oxidation products (published in Weiglein et al., 2022), following Goñi and Montgomery (2000) and Hatten et al. (2012). The CuO oxidation approach provides yields of hydrolyzable vanillyl, syringyl, and cinnamyl phenols, para-hydroxy benzenes, diacids, fatty acids, amino acids, and benzoic acids. In this study, we used the sum of lignin phenols (i.e., vanillyl, syringyl, and cinnamyl) normalized to total SOC content ($\mu\text{g lignin g OC}^{-1}$) as a metric of total lignin abundance. Because of the expectation that lignin phenols are limited to plant-derived sources (Hatten et al., 2012), the ratio of amino acid-to-lignin phenol oxidation products was used as a proxy for the degree of bulk SOM degradation.

2.2.2.4. Potential SOC mineralization (incubation experiments). The

potential mineralization of bulk SOC was determined using aerobic incubation experiments, described in Weiglein et al. (2022) and Possinger et al. (2021). Briefly, incubation experiments were conducted using A and upper-B horizons from a subset of sites ($n = 26$) selected to represent the breadth of soil physicochemical properties across the 35-site archive (Fig. S1, Fig. S2). For each site-by-horizon combination, 10–20 g soil samples (air-dry, <2-mm) were incubated in polypropylene specimen cups within ~1 or 0.5-L glass Mason jars fitted with air-tight butyl rubber septa ($n = 1$ jar per incubation experimental unit). For incubation experiments, soil texture was estimated using USDA NRCS Web Soil Survey or NEON Megapit soil texture measurements (Soil Survey Staff, 2018; NEON, 2018). The bulk density of A horizon samples was adjusted to approximately 1.0 g cm^{-3} , and the bulk density of upper-B horizon samples was adjusted to approximately 1.2 g cm^{-3} .

In this study, we used incubation data from incubation units maintained at site mean summer temperature (MST) and field capacity moisture ($\sim 33 \text{ kPa}$) for up to 90 days. Incubation temperature was consistently maintained ($\pm 0.5^\circ \text{C}$) in 566-L incubator units (VWR Model 10753–894; VWR International, LLC, Radnor, PA, USA), set within an average 0.4°C ($\pm 1.3^\circ \text{C}$) of site MST for each temperature bin. Moisture content was maintained at $\sim 33 \text{ kPa}$ throughout the incubation using soil texture-specific pedotransfer functions (Saxton et al., 1986). To prevent drying, <10 mL DI water was maintained in the base of each jar.

Incubation headspace CO_2 measurements are described in Weiglein et al. (2022). Briefly, evolved CO_2 during incubation was determined by headspace gas sampling at variable time intervals optimized to keep headspace CO_2 concentration within the instrumental analytical range (~ 370 – $10,000 \text{ ppm CO}_2$). Headspace samples were collected using glass, gas-tight gas chromatography (GC) vials, and CO_2 concentration was measured using a GC-2010 instrument fitted with a methanizer and flame ionization detector (Shimadzu Scientific Instruments, Columbia, MD, USA). After each incubation interval, jars were vented to ambient air, and initial ambient CO_2 (~ 370 – 400 ppm CO_2) was subtracted from final headspace CO_2 to determine evolved CO_2 . To correct for abiotic CO_2 evolution, solid-phase and periodic headspace gas $\delta^{13}\text{C}$ measurements were made using an IsoPrime 100 EA-IRMS (IsoPrime Ltd, Cheadle, UK) and Thermo Scientific Delta V IRMS (Thermo Fisher Scientific, Waltham, MA), respectively. A three end-member $\delta^{13}\text{C}$ mixing model was used to correct for abiotic CO_2 evolution (Tamir et al., 2011) when carbonates were present, as indicated by hydrochloric acid effervescence (“k” horizon designation).

In this study, we used CO_2 mineralization curves to determine cumulative specific respiration (CSR) for 0–3, 3–20, and 20–90 d incubation phases. Here, we consider these incubation time steps to qualitatively represent rapidly oxidized SOC, intermediate SOC, and more slowly decomposed SOC, respectively (Schädel et al., 2020). At each measurement timepoint, CSR was determined by normalizing CO_2 -C mineralized by initial SOC ($\text{mg CO}_2\text{-C g initial SOC}^{-1}$). Due to variable measurement intervals, we linearly interpolated CSR between each timepoint (time resolution = 0.05 d) using the “approx” function in the base R stats package (R Core Team, 2020) in RStudio (RStudio Team, 2018) to determine CSR at exactly 3.0, 20.0, and 90.0 d timepoints.

2.2.3. Climate data

Net primary production (NPP) ($\text{kg C m}^{-2} \text{ yr}^{-1}$) was determined using MODIS data (ORNL DAAC, 2018; Running et al., 2018). Average (over 1961–1990) site-level mean annual temperature (MAT) and aridity index (i.e., mean annual precipitation – Hargreaves reference evaporation, $\text{MAP} - \text{E}_{\text{ref}}$) were obtained from the ClimateNA_MAP database (Wang et al., 2012). PRISM data were used to determine site mean summer temperatures (MST) used for incubation temperature bins (PRISM Climate Group, 2016).

2.3. Site average fungal:bacterial biomass

In addition to published data and modifications of published data

described in Section 2.2, the NEON microbial biomass data product (DP1.10104.001) (NEON, 2021a) was used to estimate relative differences in the site-level average ratio of fungal:bacterial phospholipid fatty acid (PLFA) biomarkers in mineral soils (up to approximately 30 cm-depth). Soil PLFA extraction and analysis was conducted following internal NEON protocols (NEON, 2021a). We used extracted mass (ng g soil^{-1}) of 18:2 ω 6 and cis18:1 ω 9 PLFAs to represent fungal biomarkers, and the sum of iso15:0, anteiso15:0, iso16:0, iso17:0, anteiso17:0, 10Me16:0, cyclo17:0, cis18:1 ω 7, cyclo19:0, cis15:0, and cis16:1 ω 7 to indicate bacterial biomass, following Contosta et al. (2015) (note that trans16:1 ω 7 is not reported by NEON). To verify that this approach was able to resolve fungal:bacterial biomass ratio trends across sites, we used the NEON soil physical and chemical property (periodic) data product (DP1.10086.001) (NEON, 2021b) to correlate site-level fungal:bacterial ratio to paired soil pH and C:N ratio values (Fig. S3). The C:N ratio and pH relationships were broadly consistent with expectation from the literature: e.g., increasing fungal:bacterial ratio with increasing C:N (Waring et al., 2013) and increasing fungal:bacterial ratio in generally more acidic soils (e.g., in forests) (Joergensen and Wichern, 2008). As a result, we limited usable PLFA data to mineral soils with concomitant pH measurements ($n = 24$ sites). The distribution of extractable Mn and TOC for these sites was comparable to the full sample archive (Fig. S2). All PLFA ratios were averaged across all sampling periods (2017–2019) and periodic sampling plot locations (1–3 measurements site $^{-1}$ year $^{-1}$) to obtain a site-level average fungal:bacterial ratio.

2.4. Statistical analyses

All statistical analysis was conducted in R (v.4.0.2) in RStudio (v1.1.423) using the base R stats package unless otherwise noted (RStudio Team, 2018; R Core Team, 2019).

2.4.1. Spatial and depth correlations

Relationships among extractable Mn abundance, climate variables, and soil properties were assessed by Spearman Rank correlation. Correlations were performed separately for A, upper-B, and lower-B horizons ($n = 35$ observations for each horizon type). In addition, Mn:Fe ratio and key SOC composition (C in the FLF and alkaline cupric oxidation products) depth trends were assessed by Spearman Rank correlation.

2.4.2. Multiple linear regression

To test Mn effects on SOC cycling parameters as a function of SOC composition and biotic variables, we used linear regression models with CSR (0–3, 3–20, and 20–90 d), bulk soil, DF, and FLF fraction $\Delta^{14}\text{C}$ abundance, or total OC concentration as response variables. Due to the strong dependence of SOC concentration and $\Delta^{14}\text{C}$ abundance on depth alone (Heckman et al., 2021) and within-site nesting of horizon type, we tested Mn effects on SOC cycling parameters within horizons separately, using ln-transformed Mn (DC or SP-extractable) and horizon midpoint depth as fixed effects. We also tested for interaction effects between Mn abundance and C:N ratio, proportion of C in the FLF, site-level fungal:bacterial ratio, lignin content (normalized to SOC), and amino acid: lignin (AA:LIG) ratio. For SOC content and $\Delta^{14}\text{C}$ abundance, we used the full sample set ($n = 35$ sites), consisting of A-, upper-B, and lower-B horizons. For CSR (0–3, 3–20, and 20–90 d), incubated sites and horizons ($n = 26$ sites; only A- and upper-B horizons) were used. In some cases, missing predictor or response variable data points (more common in deeper horizons) resulted in variable full combinations of observations; exact degrees of freedom are reported in Supplementary Data S1 and S2. Response variables were ln-transformed when transformation increased the normality of model residuals. All interaction terms were ln-transformed. Model analysis of variance (ANOVA) F-tests (“anova” in base R) was used to determine the main effects of Mn content, horizon midpoint depth, and the interaction between Mn content and SOC composition metrics.

When interaction effects were detected (F-test interaction $p < 0.1$) (Table S2), we visualized conditional effects of Mn on CSR, $\Delta^{14}\text{C}$ abundance, or total OC using the *interplot* package (Solt and Hu, 2021) for multiple linear regression models. If Mn_{ORG} but not Mn_{RED} interactions were significant, or vice versa, we plotted both to contrast the direction and magnitude of SOC composition interactions as a function of extractant. The “interplot” function provides information about the direction and magnitude of Mn effects and the strength of their dependence on conditioning variables (i.e., the model interaction term) by simulating marginal effects and 95 % confidence intervals over the full range of each conditioning variable (Solt and Hu, 2021).

3. Results

3.1. Reactive Mn forms

Median total reducible Mn in the mineral soil was low relative to other extractable metals (e.g., <1 % of CD-extractable Fe), and ranged from close to zero up to 0.25 % Mn (by weight) in outlying high-Mn sites (Fig. 1). Across sites and horizons, acid NH_4 oxalate (AO) and citrate dithionite (CD)-extractable Mn were nearly equivalent (Fig. S4). Organically-complexed Mn (by proxy of sodium pyrophosphate-extractable Mn) accounted for, on average, approximately 50 % of

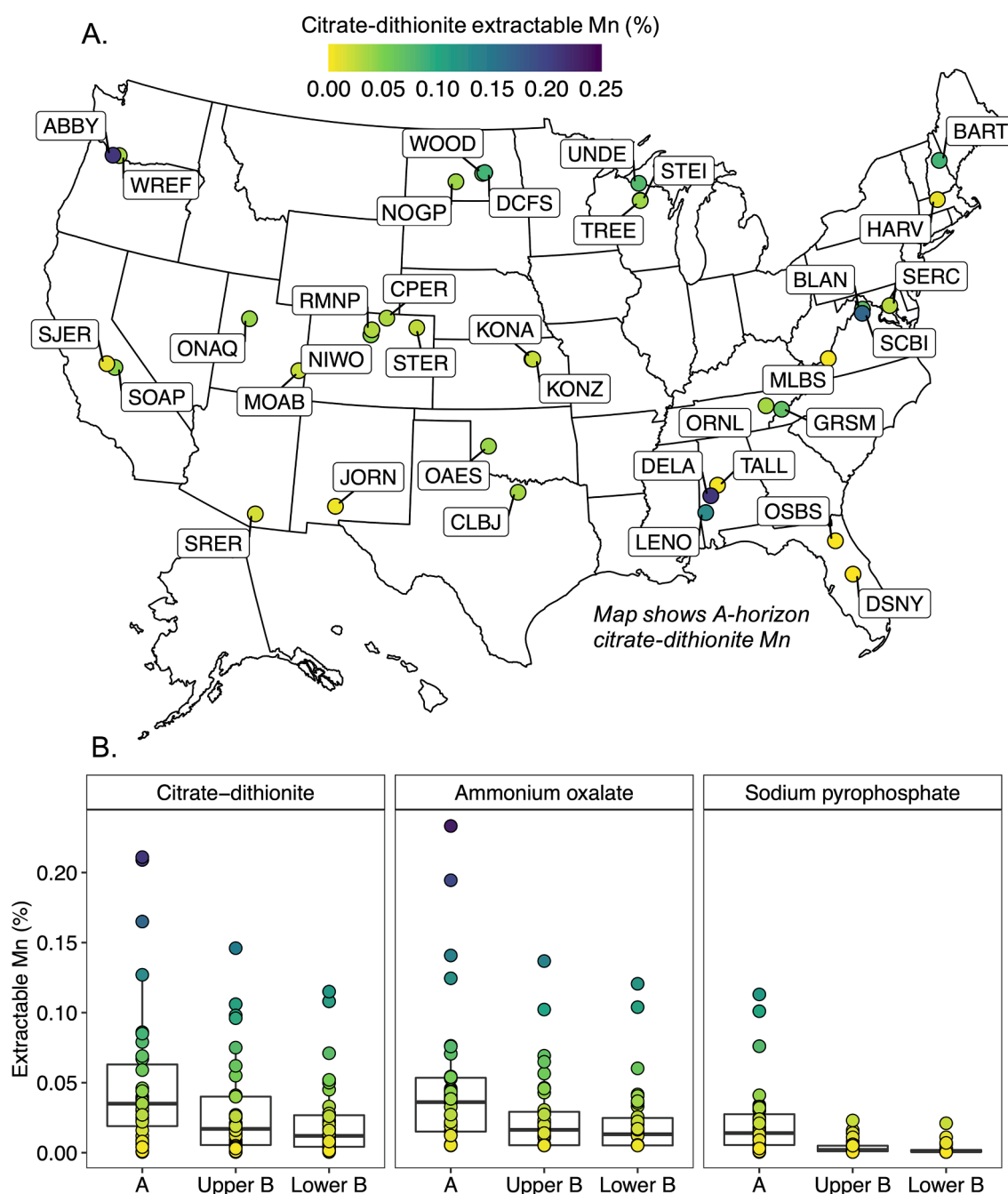


Fig. 1. Distribution of extractable manganese (Mn) across terrestrial National Ecological Observatory Network (NEON) sites. (A) Spatial distribution of A-horizon total reducible Mn (by proxy of citrate-dithionite extraction). (B) Distribution of extractable Mn across horizon type and by selective dissolutions targeting reducible Mn (citrate-dithionite), Mn oxides (ammonium oxalate), and organically-complexed Mn (sodium pyrophosphate). Points show individual observations. Lower and upper edges of boxes show first and third quartiles (25th and 75th percentiles) and lower and upper whiskers show the smallest and largest value no further than $1.5 \times$ interquartile range (IQR) of the box edges, while points outside whiskers indicate outliers beyond $1.5 \times$ IQR.

total reducible Mn in A horizons, and 15 % in B horizons (Fig. 1, Fig. S4). Due to the 1:1 relationship between AO and CD-extractable Mn, all further analyses are presented for CD-extractable Mn (“reducible Mn”, Mn_{RED}) and sodium pyrophosphate (SP)-extractable Mn (“organically-complexed Mn”, Mn_{ORG}) only.

3.2. Spatial and depth distribution of Mn and soil properties

Continental-scale distribution of Mn_{RED} in A and upper-B horizons was not significantly correlated (Spearman $p > 0.1$) to site moisture availability (MAP, $MAP-E_{ref}$) or metrics of site-level organic inputs (e.g., NPP) at any depth (Fig. 2, Fig. S5). However, spatial patterns of Mn_{RED} were weakly correlated to latitude and MAT (Spearman $Rho \sim 0.2$ – 0.4) (Fig. S5). In addition to latitudinal patterns similar to Mn_{RED} , Mn_{ORG} in A-horizons was positively correlated to variables associated with gradients in moisture driving above-ground productivity and organic inputs, including MAP, $MAP-E_{ref}$, NPP, and longitude (Fig. S5). In deep subsoils (lower B-horizons), no correlations between climate variables and either Mn_{RED} or Mn_{ORG} were detected.

Clay content was positively correlated to Mn_{RED} and Mn_{ORG} abundance (Fig. S5) in surface and upper B-horizons. While Mn_{ORG} was not correlated to pH, higher Mn_{RED} was weakly correlated (Spearman $Rho < 0.3$) with higher pH in subsoils (Fig. S5). Both Mn_{RED} and Mn_{ORG} abundance were generally positively correlated to exchangeable base cations and reactive metals in surface soils (Spearman $Rho > 0.3$) (Fig. 2, Fig. S5).

The concentration of Mn_{RED} relative to CD-extractable Fe significantly decreased with depth (Spearman $Rho = -0.35$, $p < 0.001$) (Fig. 3). The proportion of SOC in the FLF significantly decreased with depth (Spearman $Rho = -0.49$, $p < 0.001$), but in some cases contributed up to approximately 30 % of total SOC in deep subsoils (~80–100 cm-depth) (Fig. 3). The abundance of lignin (via the sum of lignin phenols) relative to total SOC did not change significantly with depth (Spearman $Rho = 0.10$, $p = 0.39$). However, AA:LIG ratio (a proxy indicating higher degree of total SOM degradation and greater contribution of microbially-processed SOM) increased significantly with depth (Spearman $Rho = 0.63$, $p < 0.001$) (Fig. 3).

3.3. Effects of Mn abundance on SOC cycling metrics

3.3.1. SOC concentration

Overall, total Mn_{RED} was not a significant predictor of SOC concentration (Fig. 4A, Supplementary Data S1). In contrast, Mn_{ORG} and SOC were positively related, consistent with targeted extraction of organic

Mn pools (Fig. 4C, Supplementary Data S1) (Jarvis, 1984). The relationship between Mn and SOC concentration did not depend on SOC composition in A-horizons. However, in subsoils, the direction and magnitude of Mn effects on SOC concentration depended on SOC composition and soil biotic properties, including fungal:bacterial ratio, C:N ratio, and AA:LIG ratio (i.e., degree of OM degradation) (Table S2). We detected significant (ANOVA F-test $p < 0.10$) interactions between Mn effects on SOC and SOC composition in subsoils, particularly upper B-horizons (Table S2, Fig. 4B, D, Supplementary Data S2). When fungal:bacterial PLFA ratio, C:N ratio, and abundance of undegraded SOM (i.e., low AA:LIG ratio) were high, increasing Mn_{RED} was correlated to lower SOC concentrations (Fig. 4B). The abundance of undegraded SOM also reversed the relationship between Mn_{ORG} and SOC concentration (i.e., when AA:LIG ratio was low, higher Mn_{ORG} was related to SOC loss) (Fig. 4D).

3.3.2. SOC potential mineralization

No overall relationship between either Mn_{RED} or Mn_{ORG} and CSR was detected at any incubation phase (0–3, 3–20, or 20–90 d) (Fig. 5A, Fig. S6, Supplementary Data S1). Soil OC composition-mediated effects of Mn on CSR were distinct between the rapid (0–3 d) and later (3–20 and 20–90 d) incubation phases (Table S2, Fig. 5B, D, Fig. S7, Supplementary Data S2). In upper B horizons, higher C:N ratio and fungal:bacterial ratio depressed Mn effects on SOC potential mineralization during the rapid incubation phase (Table S2, Fig. S7). In contrast, in A horizons, increasing proportion of particulate SOC, higher fungal:bacterial ratio, and abundance of undegraded SOM amplified Mn effects on SOC potential mineralization during later incubation phases (Table S2, Fig. 5B, D).

3.3.3. SOC persistence (radiocarbon abundance)

Higher Mn_{RED} was associated with either higher SOC persistence (i.e., lower $\Delta^{14}C$) (in A- and upper B-horizons), or had a net neutral relationship with SOC persistence (in lower-B horizons) (Fig. 6A, Fig. S8, Supplementary Data S1). In contrast, Mn_{ORG} effects on SOC persistence varied across SOC pools and depths (Fig. S8, Supplementary Data S1). While Mn_{ORG} was correlated to higher persistence of particulate SOC in A and upper B-horizons, this relationship was reversed in lower B-horizons, particularly in the mineral-associated (i.e., dense) fraction (Fig. S8, Supplementary Data S1). In surface horizons, Mn_{RED} and Mn_{ORG} enhancement of SOC persistence was amplified by high lignin content and higher AA:LIG ratio (Table S2, Fig. 6B, D, Supplementary Data S2). However, in subsoils, higher Mn_{RED} and Mn_{ORG} decreased the persistence of particulate SOC when soil lignin abundance was high

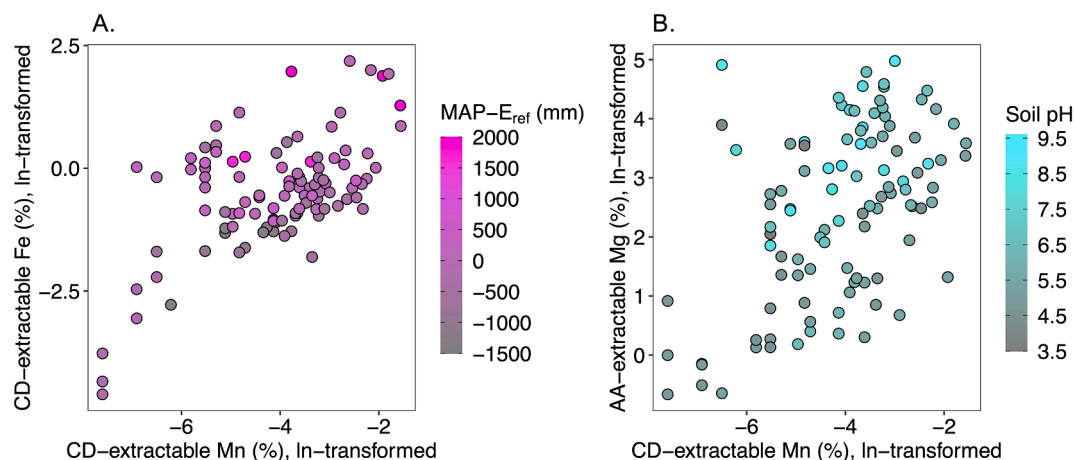


Fig. 2. Correlation between total reducible manganese (via citrate-dithionite (CD) extraction) and CD-extractable iron (Fe) (A) or ammonium acetate (AA)-extractable magnesium (B). $MAP-E_{ref}$ = mean annual precipitation – Hargreaves reference evaporation. Correlation coefficients for Mn and Fe, Mg, $MAP-E_{ref}$, and pH are shown in Fig. S5.

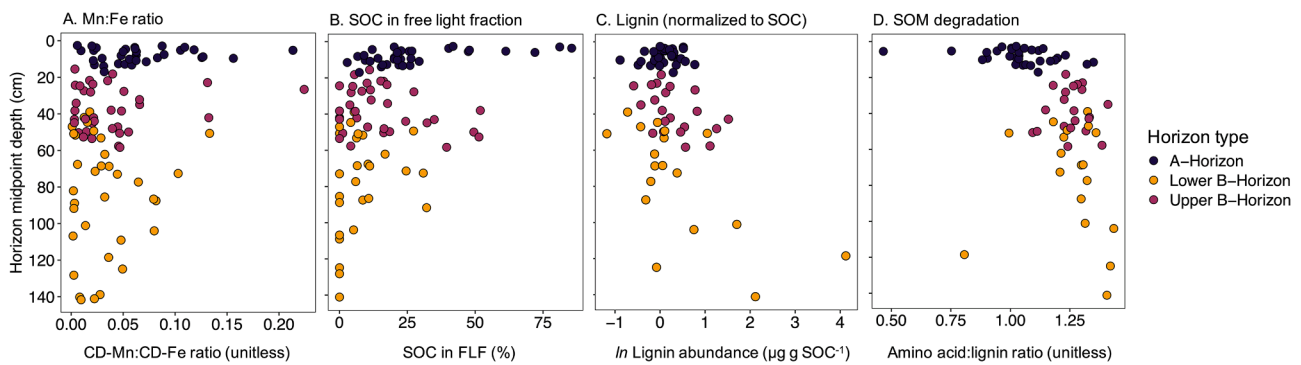


Fig. 3. Depth distribution of manganese (Mn) and key soil properties. (A) Citrate-dithionite (CD)-extractable Mn-to-CD-extractable iron (Fe) ratio. (B) Proportion soil organic carbon (SOC) in the free-light fraction (FLF) (i.e., particulate SOC). (C) Lignin abundance (sum of lignin phenols) relative to total SOC. (D) Relative degradation of bulk soil organic matter (SOM) via the metric of amino acid-to-lignin ratio.

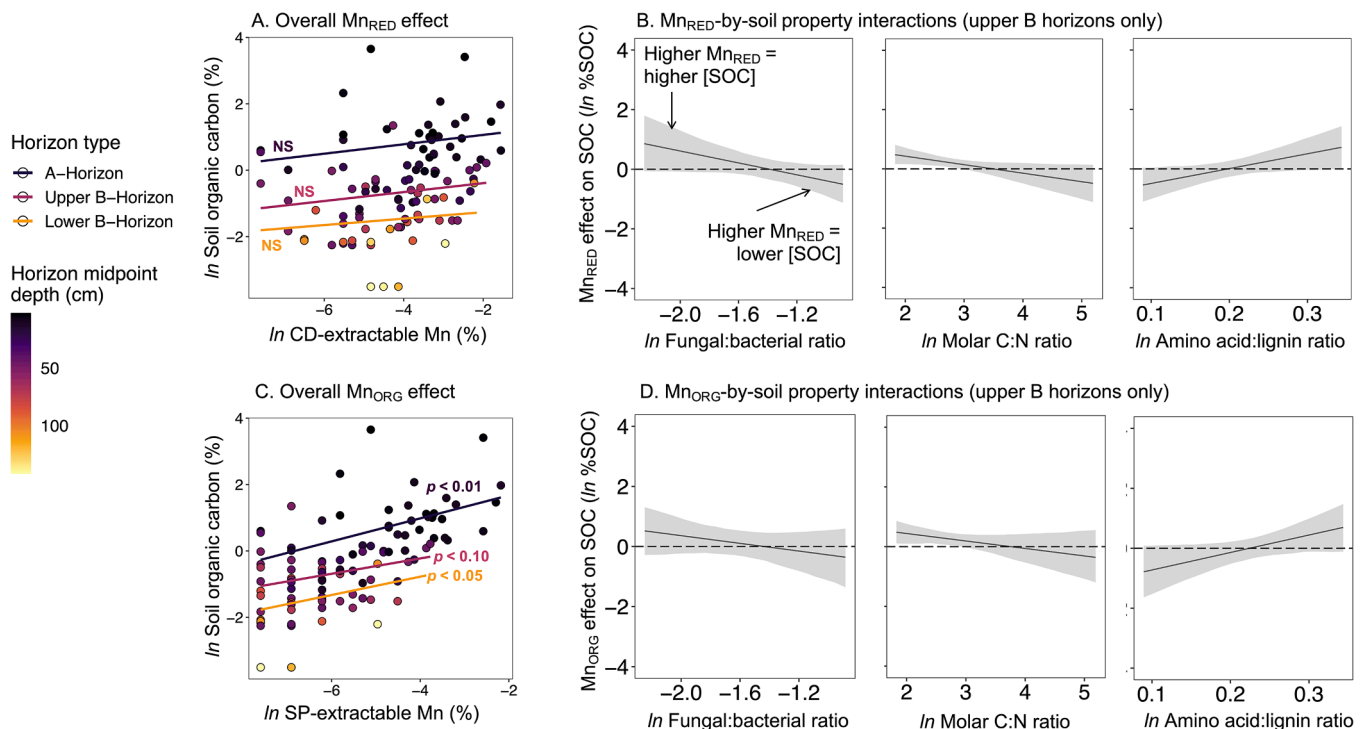


Fig. 4. Direction and magnitude of Mn effects on soil organic carbon (SOC) concentration depend on soil fungal:bacterial ratio, C:N ratio, and degree of organic matter degradation (amino acid:lignin ratio). (A, C) SOC concentration (%) as a function of reducible (Mn_{RED} , citrate-dithionite extractable) or organically-complexed (Mn_{ORG} , sodium pyrophosphate-extractable) manganese (Mn) concentration (%). Best-fit lines are for visualization only and are derived from linear multiple regression using horizon type as a fixed effect. P-values are derived from a linear model using Mn concentration and horizon midpoint depth as fixed effects (NS = not significant at $\alpha = 0.10$) (Supplementary Data S1). (B, D) Visualization of how soil SOC composition and biotic properties modify the relationship between Mn and SOC concentration in upper B horizons. Plots show simulated marginal effects and 95 % confidence intervals (shaded area) of Mn on SOC concentration as a function of fungal:bacterial ratio, C:N ratio, and amino acid:lignin ratio.

(Table S2, Fig. 6B, D, Supplementary Data S2).

4. Discussion

4.1. Total reducible Mn distribution is independent of moisture-driven soil chemistry gradients

The shift from reactive metal to exchangeable base cation-mediated SOC stabilization that co-occurs with a shift from high to low moisture availability has been proposed as a key framework for understanding controls on SOC amount (Rasmussen et al., 2018), turnover (Heckman et al., 2021), and sensitivity to climate drivers (Possinger et al., 2021). While Mn_{ORG} was spatially linked to moisture availability, this

relationship did not extend to the total reducible Mn pool, which was not spatially correlated to MAP, MAP- E_{ref} , or NPP, and only weakly correlated to pH (Fig. 2). Moreover, reducible Mn abundance was positively correlated to both reactive metals and exchangeable divalent cations, which are themselves generally anti-correlated (Rasmussen et al., 2018). As such, the identified spatial trends in total reactive Mn abundance appear to be independent of other moisture-driven gradients in soil chemistry, especially deeper in the soil profile.

In lieu of climate-driven distribution, the spatial patterns of reducible Mn may be linked to co-occurrence of Mn- and Fe-bearing parent materials (evidenced by Mn-to-Fe and/or Mn-to-Al correlations), as well as clay-mineral cation exchange (e.g., retention of Mn^{2+}) (evidenced by Mn-clay and/or base cation correlations) (Fig. 2). While a weak

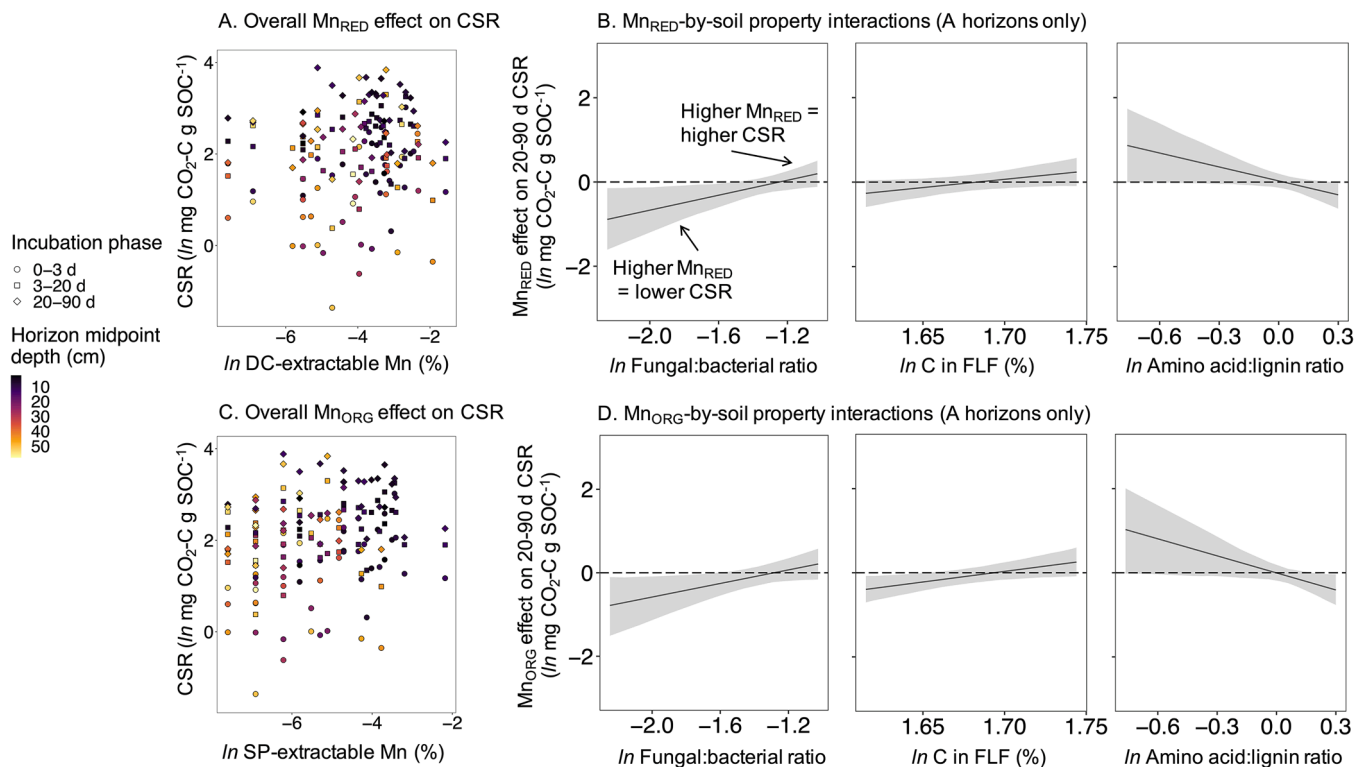


Fig. 5. Direction and magnitude of Mn effects on soil organic carbon (SOC) potential mineralization depend on soil fungal:bacterial ratio, percent C in the free light fraction (FLF), and degree of organic matter degradation (amino acid:lignin ratio). (A, C) Potential mineralization of SOC as cumulative specific respiration (CSR) in 0–3, 3–20, and 20–90 d incubation phases as a function of reducible (Mn_{RED} , citrate-dithionite extractable) or organically-complexed (Mn_{ORG} , sodium pyrophosphate-extractable) manganese (Mn) concentration (%). No individual Mn-by-CSR relationship was significant (i.e., for each combination of incubation phase and horizon) (Fig. S6; Supplementary Data S1); consequently, no best-fit lines are shown in A and C. (B, D) Visualization of how soil SOC composition and biotic properties modify the relationship between Mn and CSR in A horizons. Plots show simulated marginal effects and 95% confidence intervals (shaded area) of Mn on SOC concentration as a function of fungal:bacterial ratio, proportion C in the FLF, and amino acid:lignin ratio.

latitudinal gradient was detected, we also detected high spatial heterogeneity in Mn enrichment (Richardson, 2017), supported by the order of magnitude or greater difference in total reducible Mn between relatively co-located sites (e.g., ABBY vs WREF, and DELA vs TALL) (Fig. 1). Consequently, as a spatially-independent and heterogeneous soil property, accounting for Mn abundance across wide variation in soil types may not be redundant to other reactive metals and soil properties, and therefore contribute to improved representation of mineralogy in modeling and mapping of SOC.

Accounting for up to 50 % of Mn_{RED} in A-horizons in this study, Mn_{ORG} (putatively including redox-active Mn(III)-organic complexes, see Jones et al., 2020), may constitute a meaningful Mn pool for Mn-driven SOC processes, especially in surface soils. Spatial patterns of Mn_{ORG} were distinct from reducible Mn only in their correlation to climate variables associated with sufficient moisture for high NPP and organic C input (e.g., higher precipitation), consistent with an overall positive relationship between Mn_{ORG} and total SOC (Jarvis, 1984). In most cases, we detected similar SOC composition interaction effects on Mn_{RED} and Mn_{ORG} -SOC relationships. However, Mn_{ORG} was related to lower SOC persistence in deep subsoils (Fig. S8) (an effect opposite to Mn_{RED}), potentially linked to more recent SOC and associated organic Mn derived from root inputs at depth. This effect supports further evaluation of Mn_{ORG} sources, oxidation states, and bonding environments within the mineral soil Mn_{ORG} pool (Li et al., 2021), especially in subsoils.

4.2. SOM composition and biota mediate Mn effects on SOC cycling

Our work suggests that, at the continental scale, reactive Mn concentration on its own has a net neutral relationship with SOC cycling: i.

e., Mn oxidizing and stabilizing processes are balanced, preventing identification of consistent directional trends at macroscales. However, the lack of continental-scale Mn-SOC relationships underscores the emerging view that the primary control on SOC turnover for a given location is dependent on ecosystem context, precluding the existence of universally applicable trends between soil properties and SOC metrics. For example, recent work has pointed to divergence in the primary controls on SOC cycling metrics across soil orders (Kögel-Knabner and Amelung, 2021; Nave et al., 2021b) and bioclimatic subsystems (Heckman et al., 2021; Possinger et al., 2021). We suggest that the ecosystem context in which Mn-promoted SOC turnover may be the most relevant is where fungal decomposition of relatively undegraded, lignin-rich SOC predominates. Conversely, Mn-mediated SOC stabilization may be the dominant process when SOC is composed of more microbially-processed, soluble materials low in lignin. For example, the effect of Mn on SOC concentration switched from positive ($\sim +270$ % relative to mean subsoil SOC) to negative (-124 %) as site-average fungal:bacterial ratio increased (Fig. 4). This shift in direction and magnitude of effects underscores the need to account for subsystem-specific Mn effects. Moreover, increasing abundance of reactive Fe and Al or extractable base cations (both positively correlated with Mn content) generally decreases potential SOC mineralization, an opposite effect to Mn-promoted oxidation (Possinger et al., 2021; Weiglein et al., 2022). This reversal of effect direction highlights that representation of Mn may target unique SOC cycling processes that are underrepresented in current SOC conceptual or biogeochemical models primarily based on reactive Fe and Al or base cation content (e.g., Rasmussen et al., 2018; Kramer and Chadwick, 2018; Hall et al., 2020; Heckman et al., 2021; Yu et al., 2021).

The conditional Mn effects on mineral soil SOC cycling identified

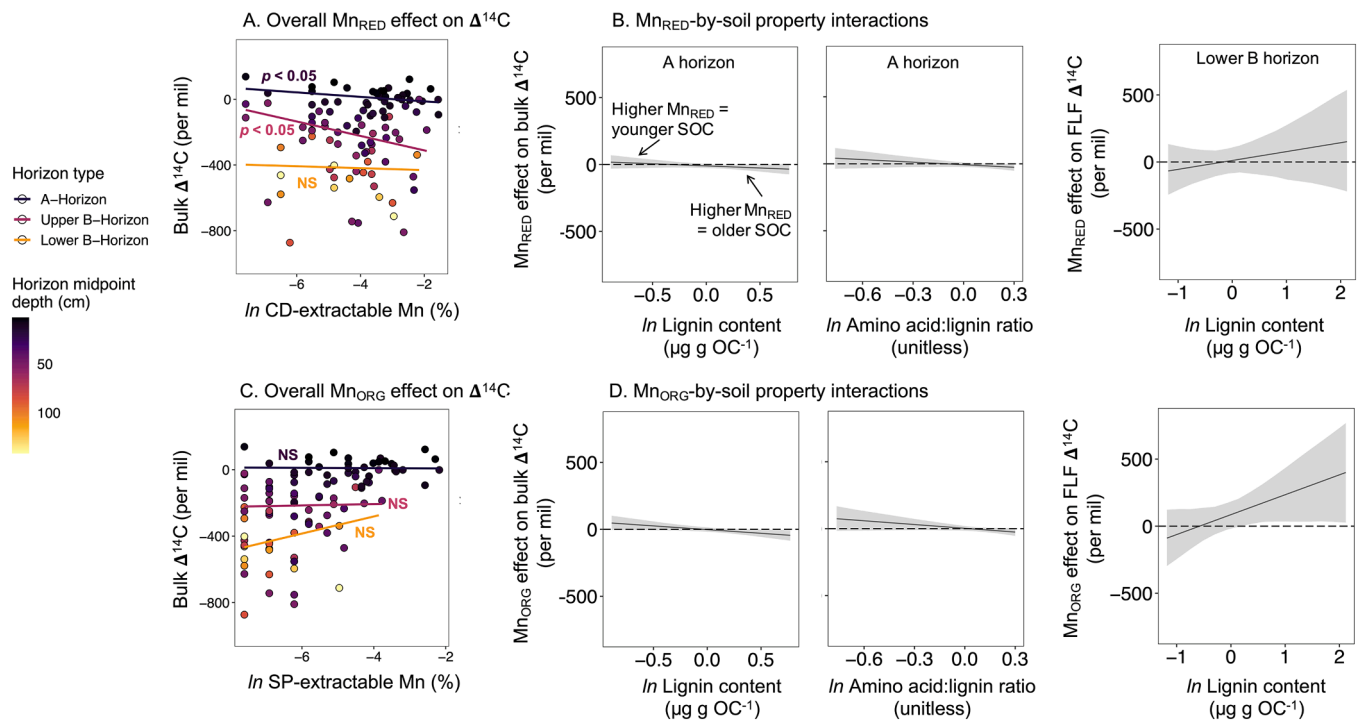


Fig. 6. Direction and magnitude of Mn effects on soil organic carbon (SOC) persistence (via radiocarbon abundance, $\Delta^{14}\text{C}$) depend on lignin abundance. (A, C) Bulk soil $\Delta^{14}\text{C}$ as a function of reducible (Mn_{RED} , citrate-dithionite extractable) or organically-complexed (Mn_{ORG} , sodium pyrophosphate-extractable) manganese (Mn) concentration (%). Best-fit lines are for visualization only and are derived from linear multiple regression using horizon type as a fixed effect. P-values are derived from a linear model using Mn concentration and horizon midpoint depth as fixed effects (NS = not significant at $\alpha = 0.10$). (B, D) Visualization of how lignin abundance modifies the relationship between Mn and bulk $\Delta^{14}\text{C}$ (A horizons) and free light fraction (FLF) $\Delta^{14}\text{C}$ (lower B horizons). Plots show simulated marginal effects and 95 % confidence intervals (shaded area) of Mn on $\Delta^{14}\text{C}$ as a function of lignin abundance (relative to total SOC).

here provide an opportunity to conceptually incorporate Mn into our understanding of mineral soil SOC cycling, accounting for both the SOC composition- and depth-specific nature of potential Mn effects. However, inclusion of Mn as a predictor in large-scale conceptual and quantitative modeling of SOC is complicated by divergence in both the magnitude and direction of Mn effects on different SOC metrics (e.g., SOC concentration vs persistence) and depth. Here, we propose a concept of both depth- and SOC composition-specific Mn effects to provide a starting point for further work to incorporate Mn into large-

scale assessment of SOC cycling (Fig. 7).

In surface soils, we detected lignin and fungal biomass-dependent links between Mn abundance and higher SOC potential mineralization in later stages of incubation (Fig. 5B), consistent with the known importance of Mn availability in fungal-mediated lignin oxidation (Berg et al., 2007; Whalen et al., 2018). The role of lignin abundance in Mn effects on decomposition is consistent with incubation experiments by Sun et al. (2019), who identified Mn-promoted decomposition in high-lignin litter during late phases of incubation. However, in contrast to

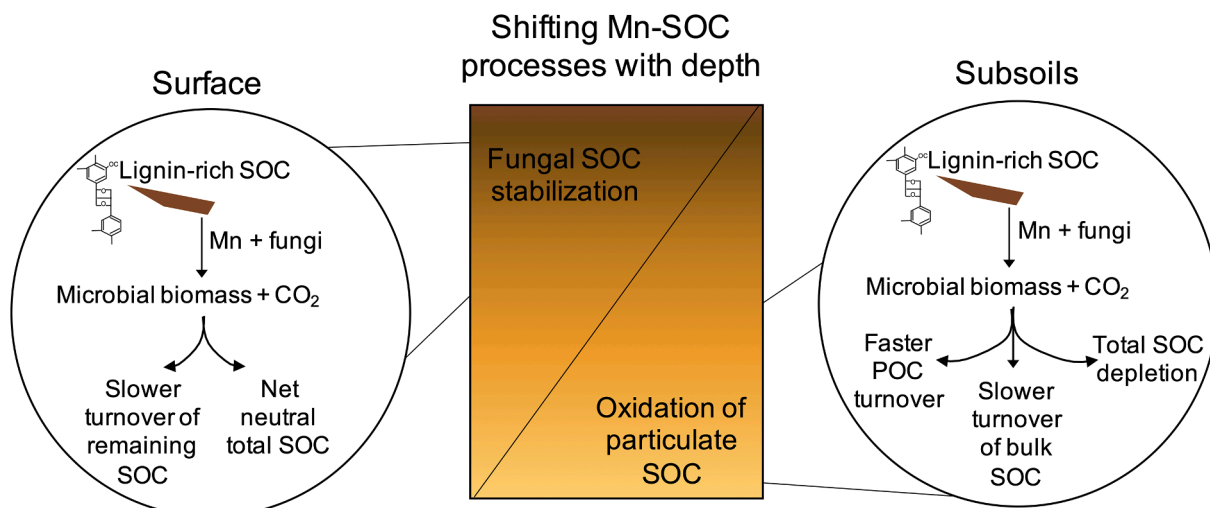


Fig. 7. Conceptual summary of proposed divergence in manganese (Mn) effects on soil organic carbon (SOC) between surface and subsoils. In surface soils, we suggest that while in later stages of decomposition Mn may accelerate mineralization of lignin-rich SOC, this results in net neutral SOC turnover and neutral-to-slower turnover of the bulk SOC pool (possibly via fungal biomass SOC-stabilizing pathways). In subsoils, we suggest that Mn may stimulate faster turnover of lignin-rich particulate SOC (POC) and formation of smaller, but more persistent, bulk SOC pools.

our hypothesis that SOC oxidation effects would result in faster *in-situ* SOC turnover in surface soils, these effects did not extend to bulk SOC persistence. Higher SOC persistence was correlated to higher Mn concentrations in surface soils, and amplified by both higher lignin abundance and higher AA:LIG ratio (Fig. 6B), consistent with a shift towards microbial communities more readily able to degrade lignin. Mn_{RED} had no relationship to SOC concentrations regardless of SOC composition in A-horizons. As such, the limited range of conditions in which Mn oxidation effects were present suggests that the accumulation of lignin decomposition products in microbially-processed persistent SOC (e.g., in fungal necromass) (Liang et al., 2019) counteracts Mn-driven SOC oxidation in this scenario (Fig. 7). The decoupling between Mn effects on late-phase decomposition and bulk soil persistence are consequently in line with the emerging view that stabilization of microbial residues is a key pathway for formation of persistent SOC (Cotrufo et al., 2013; Kallenbach et al., 2016). In future studies, changes to microbial biomass pools and allocation to stabilized (e.g., mineral-associated) SOC pools as a function of Mn and lignin abundance would provide further mechanistic support for this proposed pathway. In addition, microbial enzyme assays targeted to lignin degradation would inform mechanistic links among microbial communities, SOC composition, Mn abundance, and net SOC persistence.

In subsoils, increasing fungal:bacterial biomass ratio at the site level amplified Mn-promoted decreases in SOC concentration (Fig. 4), but did not modify Mn effects on SOC potential mineralization or persistence. Further, while generally related to higher SOC persistence in subsoils, Mn abundance was related to lower persistence of particulate (i.e., FLF) SOC when soil lignin content was high (Fig. 6B, D). Together, these findings suggest that increasing Mn abundance accelerates partitioning from particulate SOC to smaller, but more persistent microbial biomass (particularly fungal) pools in subsoils (Fig. 7).

Our work suggests that the well-established role of Mn in facilitating litter decomposition (Berg et al., 2015) may also extend to lignin-rich particulate SOC regardless of its location in the soil profile. While the proportion of SOC in particulate pools generally decreases with depth, in this study, up to 30 % of total SOC was in particulate form in subsoils (Fig. 3), consistent with Liebmann et al. (2020). Moreover, lignin (normalized to total SOC) was high in subsoils relative to surface soils (Fig. 3). Though particulate SOC may also be subject to biophysical protection within the mineral soil matrix (e.g., by aggregate occlusion) (Schrumpf et al., 2013), we suggest the conversion of particulate SOC to microbial (particularly fungal) biomass pools – possibly mediated by Mn-facilitated lignin oxidation – may result in higher overall persistence of SOC in mineral soils. As such, lignin-dependent Mn effects on the turnover of the particulate SOC pool in subsoils may be a significant process impacting subsoil SOC persistence, particularly when subsoils receive high inputs of lignin-rich particulate SOC.

5. Conclusions

In this work, we show that soil Mn abundance may influence SOC cycling independently from other key soil chemical properties (e.g., soil pH), both with respect to spatial distribution and effect direction and magnitude. However, Mn effects were dependent on ecosystem context and depth, particularly in subsoils. The divergence in effect direction and magnitude across variation in SOC composition metrics highlights the need for system-specific representation of potential Mn effects at larger scales. The analogous role of Mn in SOC oxidation between surface litter and particulate SOC at depth also motivates further consideration of how known environmental controls on surface litter decomposition propagate to subsoils as a function of particulate SOC abundance. While this work does not probe physicochemical or biological mechanisms of Mn-SOC dynamics, we provide a starting point to justify further exploration of large-scale Mn trends within the context of interacting SOC composition-mineralogy-ecosystem controls on SOC cycling. Future work should therefore emphasize gradients in Mn

composition (e.g., redox speciation and ligand-stabilized Mn forms) both across depth and across gradients in SOC composition, both at large scales (e.g., across subsystems) and microscales (e.g., across particulate SOC-mineral soil interfaces) to test the proposed particulate SOC-fungal stabilization pathway proposed here.

Declaration of Competing Interest

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

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Competing interests

The authors do not declare competing interests.

Data availability

The bulk soil and site characterization data used for assessment of Mn distribution and effects on SOC cycling in the study are available through the Environmental Data Initiative (Nave et al., 2021a). Additional data are available on request to the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2022.116070>.

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