



Conversion of native grassland to coniferous forests decreased stocks of soil organic carbon and microbial biomass

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Abstract

Aims Encroachment of woody species into grasslands is a global phenomenon that affects ecosystem services, including soil organic carbon (SOC) storage and microbial community structure. We determined stocks of SOC and soil microbial biomass as affected by conversion of grasslands to coniferous forests.

Methods We examined SOC and soil $\delta^{13}\text{C}$ signatures under three vegetation covers: native grasslands, eastern redcedar (*Juniperus virginiana*), and ponderosa pine (*Pinus ponderosa*), at six soil depths (0–10, 10–30, 30–100, 100–170, 170–240, 240–300 cm). Fatty acid methyl esters (FAMES)

were used to quantify soil microbial biomass (MB) and as biomarkers for bacteria, arbuscular mycorrhizal fungi (AMF), and saprophytic fungi (SF) which includes ectomycorrhizal fungi (ECM).

Results Native grasslands had higher overall SOC and MB stocks in the top 300 cm soil depth (7.39 kg SOC m^{-2} and 65.6 mmol MB m^{-2}) than converted cedar (6.80 kg SOC m^{-2} and 38.4 mmol MB m^{-2}) or pine (5.87 kg SOC m^{-2} and 39.9 mmol MB m^{-2}) forest. Losses of AMF biomass occurred on conversion of native grasslands to pine forest. Stocks of SF biomass only differed with vegetation type in topsoil.

Conclusions Conversion of native grasslands to cedar or pine forest increased topsoil (0–10 cm) SOC storage due to needle litter accumulation. Conversion of native grasslands to forests decreased subsoil (10–300 cm) SOC stocks and had replaced, by increasing depth, 30 to 19% and 45 to 26% of grassland C with cedar and pine C, respectively. Changes in soil pH, nutrients, and soil microbial communities accelerated SOC turnover.

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Keywords C_4 – C_3 vegetation change · $\delta^{13}\text{C}$ signature · Mean residence time · Arbuscular mycorrhizal fungi · Saprophytic fungi · Ectomycorrhizal fungi

Abbreviations

SOC	Soil organic carbon
MRT	Mean residence time
MB	Microbial biomass

FAME	Fatty acid methyl ester
AMF	Arbuscular mycorrhizal fungi
SF	Saprophytic fungi
ECM	Ectomycorrhizal fungi

Introduction

Invasive species threaten biodiversity and alter ecosystem functions worldwide (Gurevitch and Padilla 2004). Globally, substantial proportions of grasslands were lost to the encroachment of woody species in the past decades (Arasumani et al. 2021; Bora et al. 2021; Hynson et al. 2013; van Mantgem et al. 2021). These alterations can reduce grasslands ecosystem resilience and resistance and increase susceptibility to climate change, fire, and further encroachment (Arasumani et al. 2021; Bora et al. 2021; Lubetkin et al. 2017). Conversion of native grasslands to wooded areas alters microclimate and reduces light intensity and temperature for understory communities, and thus impacts grass cover and productivity (Bora et al. 2021; Chikowore et al. 2021; Mazis et al. 2021). Encroachment also affects soil hydrological regime, due to increased evapotranspiration and decreased soil water availability relative to grasslands (Awada et al. 2013; Curtis et al. 2019; Kormos et al. 2017). Also, coniferous species can decrease soil pH and bulk density and alter nutrient availability and soil organic carbon (SOC) storage (Hou et al. 2019; Podwika et al. 2020).

Encroachment of coniferous species into native grasslands increases aboveground plant biomass (Hoeksema et al. 2020) and has been proposed as a potential strategy to increase litter input to soil (Lugato et al. 2021), thereby directly increasing SOC storage in topsoil (i.e., A horizon). However, much less is known about the effects of conifer encroachment on subsoil (i.e., below A horizon) SOC storage and turnover (Nuñez et al. 2021). Although subsoils are usually considered as the limited access/low activity region for microbes (Kleber and Lindsley 2022), they are highly relevant for SOC storage, considering that 77% of SOC is estimated to be stored in the depth of 30–300 cm globally (Lal 2018). Encroachment of coniferous species and associated changes in the quantity, quality, and distribution of C input, rooting depth, and soil microbial community, can either decrease or increase SOC storage and turnover

(Hoeksema et al. 2020; Nuñez et al. 2021). Increased input of new C can stimulate losses of old SOC stored in deep soil (Fontaine et al. 2007) or lead to net increases in SOC due to compensation (Jilkova et al. 2019; Liang et al. 2018). Additionally, conversion of native grasslands to wooded areas can alter soil microbial communities and therefore SOC turnover. Encroachment of eastern redcedar into grasslands is facilitated by forming mutualisms with native grassland arbuscular mycorrhizal fungi (AMF) that shifts over time to AMF species that are better adapted to changes in soil edaphic properties as tree density increases (Liang et al. 2017). Pine species encroachment frequently results in co-invasion of ectomycorrhizal fungi (ECM), of which pines are obligate mutualists (Hynson et al. 2013; Moyano et al. 2019). Conversion of grasslands to pine plantations could shift the dominance from AMF to ECM (Hoeksema et al. 2020; Liang et al. 2017), thereby accelerating SOC decomposition since ECM are generally more capable of decomposing SOC compared to AMF (Hoeksema et al. 2020; Zak et al. 2019).

To study SOC turnover and its mean residence time (MRT), $\delta^{13}\text{C}$ signatures can be used as a tool to examine the contribution of different sources to SOC. In the scenario of C_4 – C_3 vegetation change, the SOC derived from the original C_4 plants and new C_3 plants can be differentiated based on plant $\delta^{13}\text{C}$ signature. The uptake of ^{13}C by C_4 and C_3 plants via photosynthesis are 4‰ and 19‰ less than the ^{13}C in the atmosphere, respectively (Zacháry 2019). Consequently, C_4 plant litter is more enriched in ^{13}C compared to C_3 plant litter. Isotope signatures also stratify by soil depth. Surface soil $\delta^{13}\text{C}$ signatures are generally consistent with current vegetation cover, whereas deep soil $\delta^{13}\text{C}$ signatures usually reflect previous vegetation cover (Gautam et al. 2017).

Grasslands are one of the most threatened and least protected ecosystems globally, with few intact grassland ecosystems remaining (Scholtz and Twidwell 2022). The semi-arid, C_4 dominated grasslands of the Nebraska Sandhills remain one of the relatively intact temperate grasslands in the world (Jacobson et al. 2019; Scholtz and Twidwell 2022). Invasion or spread of coniferous species into this grassland ecosystem may strongly affect SOC storage and turnover, but studies evaluating the long-term consequences of this on subsoil SOC below 30 cm are lacking. To address this knowledge gap, we measured SOC, soil

^{13}C natural abundance, soil physical and chemical properties, and soil microbial biomass using fatty acid methyl esters (FAMES) to a soil depth of 300 cm under a native grassland dominated by C_4 grasses as the benchmark, and under two C_3 forests planted on native grassland. Our goal was to assess whether conversion of native grasslands to coniferous forests decreased SOC stocks and the changes in SOC storage that could, in part, be explained by changes in soil microbial biomass and microbial community structure.

Materials and methods

Site selection, experiment design, and soil sampling

Study site was located at the Nebraska National Forest (NNF) at Halsey (865 m elevation; 41°51'45" N, 100°22'06" W), a 25,000-ha experimental forest established in the Sandhills grasslands in 1902. It is currently the largest man-made forest in the United States (Adane and Gates 2015). The forest was hand-planted with various coniferous species, including eastern redcedar (*Juniperus virginiana*) and ponderosa pine (*Pinus ponderosa*), in the 1930s. Hereafter and throughout the manuscript, eastern redcedar and ponderosa pine are respectively referred as cedar and pine for simplicity. The climate is semiarid continental (Singh et al. 2021), with a mean annual temperature of 8.5 °C and mean annual precipitation of 570 mm. The soils are loose, well-drained Valentine (Mixed, Mesic Typic Ustipsamments), containing greater than 90% of sand. See soil texture in Table S1. The texturally uniform soils, similar plantation ages, well-recorded site history, and the presence of native grasslands in the area provide an ideal proxy for studying the effects of conifer encroachment into native grassland on SOC.

Two forested (pine and cedar) and one native grassland site were selected at NNF for the study. The grasslands are dominated by perennial C_4 grass communities, including sand bluestem (*Andropogon hallii*), prairie sandreed (*Calamovilfa longifolia*), sand dropseed (*Sporobolus cryptandrus*). Photosynthesis of the C_4 C fixation pathway discriminates less against the ^{13}C isotope in CO_2 , compared to the C_3 C fixation pathway of cedar and pine, so grassland litter is less depleted in ^{13}C than cedar or pine litter. The

grassland SOC therefore contains a $\delta^{13}\text{C}$ signature, from which cedar- or pine-derived C can be differentiated. The stand age, average tree density, and height for cedar and pine were 65 and 82 years, 356 and 375 tree ha^{-1} , and 9 and 17 m, respectively (Mazis et al. 2021; Mellor et al. 2013). Pine and cedar stands contain understory vegetation of grasses, forbs, shrubs, and cactus (Mazis et al. 2021). Soil samples were collected in June 2021. Three transects were established within each vegetation type. The transects were 40 m long and ~40 m apart from each other to comply with permitting restrictions. Four soil cores were sampled along each transect and the sampling locations were ~13 m apart from each other. Soil cores were taken using a hydraulic auger (Conservation and Survey Division, University of Nebraska-Lincoln) to the soil depth of 300 cm. Soil cores were shipped in coolers with ice and then stored in freezer before processing. Each soil core was processed and analyzed individually. The intact soil cores were divided into three genetic horizons: A, AC, and C, approximately corresponding to the depth increments of 0–10 cm, 10–30 cm, and 30–300 cm, respectively. The C horizon was further divided into four increments: 30–100, 100–170, 170–240, and 240–300 cm, resulting in six depth increments in total. See specific sampling depth for each sample in the archived data (<https://github.com/lilidong1111/SOC>). Overall, our experiment had two factors: vegetation type and soil depth. Each combination of factors had $n=12$: three replicates (i.e., transects), and each replicate had four subsamples (i.e., cores per transect) for a total of 216 soil samples. Due to small amount of soil for some surface horizons, $n \leq 12$ for some analyses. The litter layer consisted of decomposed and partially decomposed plant materials, so the litter layer was sampled separately from the soils. We collected the plant materials that did not pass through a 2 mm sieve in the litter layer.

Laboratory analyses

The C concentrations and $\delta^{13}\text{C}$ values of litter and soil were analyzed at the Water Sciences Laboratory in the Nebraska Water Center according to Mellor et al. (2013). Litter was washed with deionized water. Litter and soil were air-dried and ground to pass a 200 μm sieve for C determination by dry combustion using an Elemental Analyzer (Carlo Erba NA 1500; Milan,

Italy). When soil pH > 7.2, soil samples were acid-washed to remove the inorganic carbon before SOC measurement. Litter and soil $^{13}\text{C}/^{12}\text{C}$ values were determined using an Elemental Analyzer coupled with an Isotope Ratio Mass Spectrometer (GV Isochrom; Manchester, UK). The percentage of the original grassland SOC replaced by tree litter C under the cedar and pine stands (C_{replaced}) was calculated using the $\delta^{13}\text{C}$ value of the grasslands soil ($\delta^{13}\text{C}_{\text{grassland}}$), the cedar and pine soils ($\delta^{13}\text{C}_{\text{forest}}$), and the cedar and pine litter ($\delta^{13}\text{C}_{\text{litter}} = -27.8\text{‰}$ and -27.2‰ , respectively): $C_{\text{replaced}} = (\delta^{13}\text{C}_{\text{forest}} - \delta^{13}\text{C}_{\text{grassland}}) / (\delta^{13}\text{C}_{\text{litter}} - \delta^{13}\text{C}_{\text{grassland}}) \times 100\%$ (Zacháry 2019). The MRT of grasslands SOC in the cedar and pine soils was calculated by the decay rate constant (k) and the time since vegetation change (t): $\text{MRT} = 1/k = -t / \ln(1 - C_{\text{replaced}})$ (Zacháry 2019). The calculation of MRT was based on several assumptions (Mellor et al. 2013), so we consider these MRT values as a rough estimation rather than a precise dating approach. The assumptions were that (1) SOC decomposition followed first-order kinetics, (2) C_3 and C_4 derived SOC decomposed at the same rate, and (3) ^{13}C concentrations in different plant components were the same. Although these assumptions may be not met, our study was based on two tree species with similar $\delta^{13}\text{C}$ signatures planted at approximately the same time, which allowed us to conduct a comparative evaluation between the contributions of the two tree species to SOC turnover.

Microbial fatty acid methyl esters (FAMES) were extracted from 10 g air-dried soil using 0.2 M KOH in methanol (Grigera et al. 2007). Details are described in supplementary material. Thirty of the most dominant FAMES found in microorganisms were used as a proxy for soil microbial biomass (nmol FAMES g^{-1} soil), sixteen FAMES were used to represent bacterial biomass and community structure (Bååth and Anderson 2003), C18:2cis 9,12 was used to represent SF biomass, including ECM biomass (Bååth and Anderson 2003), and C16:1cis11 was used to represent AMF biomass (Olsson 1999).

Bulk density was calculated by dividing dry soil mass by soil core volume. Soil texture was determined by the hydrometer method (Bouyoucos 1951) and the ultrasonication method (Greenberg et al. 2019; Remus et al. 2018). Soil pH was measured using the 1:1 soil to water slurry method. Nitrate-N was measured by the cadmium reduction method

using potassium chloride as extraction agent (Gelderman and Beegle 1998). Soil P was measured by the Olsen method (Frank et al. 1998). Soil K, Ca, and Mg were measured using ammonium acetate (Warncke and Brown 1998). Soil S was measured using mono-calcium phosphate by the turbidimetric procedure (Combs et al. 1998). Soil Zn, Fe, and Mn were measured using diethylenetriaminepentaacetic acid (Whitney 1998).

The equivalent soil mass method

The equivalent soil mass (ESM) method was used to account for significant differences in soil masses due to impacts of land use change on bulk density (Poelplau and Don 2013; von Haden et al. 2020; Wendt and Hauser 2013). Fixed depth concentrations of SOC and plant-available nutrients were transformed to fixed mass stocks using a cubic spline interpolation method developed by von Haden et al. (2020) (RStudio-2022.12.0-353.dmg, PBC, Boston, MA, USA). Grassland soil masses were used as the references for pine and cedar soils in our calculations. The reported stocks in our study are based on ESM. Even though the stocks are presented by sampled depth, they are actually based on the ESM depth rather than the sampled depth; Therefore, the ESM stocks do not necessarily pertain to the sampled depth, but instead they are the estimated values that would have been obtained if the soils were sampled according to the reference soil mass rather than the sampled depth (von Haden et al. 2020).

Statistical analyses

A two-way mixed model ANOVA was used to determine the main and interaction effects of vegetation type and soil depth on SOC and the other soil properties (Glimmix procedure; SAS 9.4, SAS Institute Inc., Cary, NC, USA). Vegetation type and soil depth were the fixed factors, with soil depths as repeated measures, and transect and soil core were the random factors. Least Squares Means were compared by Fisher's Least Significant Difference. Data were log-transformed to achieve normal distribution when necessary. The univariate procedure was used for checking normality of residuals. Normality was determined by the Shapiro-Wilk's test. Equal variance was determined by the Levene's test. Significance was set at

a 0.05 level. Results were reported as untransformed mean $\pm$ arithmetic standard error. Treatment effects on 14 bacterial FAMES (nmol%) by depth interval to 100 cm were explored using canonical discriminant analysis (CDA) to identify changes in bacterial community structure (CANDISC procedure; SAS 9.4, SAS Institute Inc., Cary, NC, USA). Two bacterial FAMES, a10MeC18:0 and cyC19:11,12, were removed for CDA due to detection of loss at depth.

Results

Stocks of SOC

Vegetation type and soil depth had an interaction effect on SOC stock ($p < 0.01$, Fig. 1). In the 0–10 cm depth, grasslands had lower SOC stock than cedar and pine forests (0.91 ± 0.09 kg m⁻² vs. 1.53 ± 0.16 and 1.50 ± 0.15 kg m⁻², $p = 0.047$). In the 10–240 cm depths, grasslands had higher SOC stock than cedar and pine forests. In the 240–300 cm depth, SOC stocks were not significantly different under the three vegetation types ($p = 0.20$). Throughout the 0–300 cm depth, grassland had the highest overall SOC stock, $88 \pm 1\%$ of which was in the 10–300 cm depth (Tables 1 and 2).

Soil $\delta^{13}\text{C}$ values and mean residence time (MRT)

Vegetation type and soil depth had an interaction effect on soil $\delta^{13}\text{C}$ values ($p < 0.01$, Fig. 2). In the 0–10 cm depths, grassland soil had significantly less depleted $\delta^{13}\text{C}$ values than cedar and pine forest soils. This trend was consistent with that in the litter layer (Table S2). In the 10–100 cm depths, grassland soil still had less depleted $\delta^{13}\text{C}$ values than cedar and pine forest soils. In the 100–170 cm depth, grassland soil had less depleted $\delta^{13}\text{C}$ values than pine forest soil but more depleted than cedar forest soil. In the 170–240 cm depth, soil $\delta^{13}\text{C}$ values were not different under these three vegetation types. In the 240–300 cm depth, grassland soil had more depleted $\delta^{13}\text{C}$ values than cedar and pine forest soils. Soil $\delta^{13}\text{C}$ values significantly increased in the 10–300 cm depth compared to the 0–10 cm depth regardless of vegetation type. The MRT of the original grassland SOC under cedar and pine stands was lower in the 0–10 cm depth (73–149 years)

than that in the 10–300 cm depths (158–352 years, Table 3). The MRT was lower under pine (73–306 years) than that under cedar (149–352 years).

Stocks of soil microbial biomass

Grassland had the highest overall microbial biomass stock to 300 cm (65.7 ± 11 mmol FAMES m⁻²) followed by similar stocks in pine (39.9 ± 10 mmol FAMES m⁻²) and cedar (38.9 ± 4 mmol FAMES m⁻²) (Table 4). The percent distribution of microbial biomass by depth interval was similar among vegetation types in surface soil but declined under cedar at depths below 30 cm (Table 4). In contrast, the combined biomass of SF and ECM varied by vegetation type across depth intervals except in surface soil where grassland $\geq$ cedar $\geq$ pine (Fig. 3D). The CDA analysis of bacterial FAMES to 100 cm resulted in six significant canonical functions with the first two accounting for 62% of the variation in soil bacterial communities. All nine treatment combinations were different from each other ($p < 0.0001$).

Discussion

Topsoil SOC storage increases on conversion of native grassland to cedar or pine forest

In topsoil (0–10 cm), the conversion of native grassland to cedar and pine forest increased SOC storage by $69 \pm 7\%$ and $65 \pm 6\%$, respectively (Fig. 1). Cedar and pine produce larger amounts of aboveground biomass and therefore input more litter to the topsoil than grass. Indeed, the topsoil $\delta^{13}\text{C}$ values under cedar, pine and grass reflected the $\delta^{13}\text{C}$ signatures in the plant litter (Fig. 2 and Table S2), indicating significant input of C from plant litter to topsoil. Cedar- and pine-derived C contributed to $40 \pm 4\%$ and $70 \pm 4\%$ of the SOC storage in the topsoil (Table 3). The greater accumulation of pine litter in the topsoil may result from higher tree density and height compared to cedar, as well as higher C concentration of pine litter (Table S2). The higher C concentration of pine litter can decelerate decomposition (Craig et al. 2018; Fernandez et al. 2020; Lin et al. 2017).

Table 1 Stocks of soil plant-available nutrients and soil pH

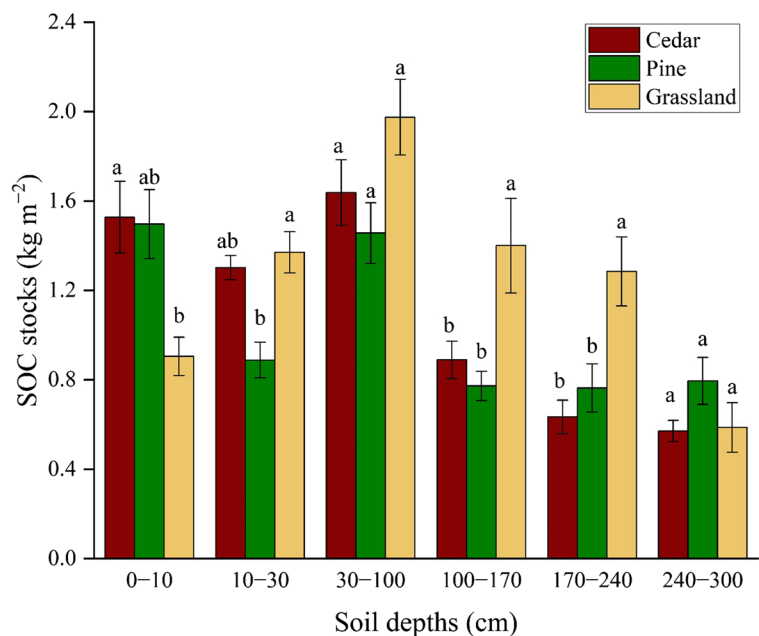
Depth (cm)	Vegetation	Nitrate-N (g m ⁻²)	P (g m ⁻²)	K (g m ⁻²)	Ca (g m ⁻²)	Mg (g m ⁻²)	S (g m ⁻²)	Zn (g m ⁻²)	Fe (g m ⁻²)	Mn (g m ⁻²)	pH
0–10	Cedar	0.06±0.02 a	2.03±0.37 a	14.0±1.2 ab	145.5±15.2 a	11.1±0.4 a	0.83±0.09 a	0.10±0.01 a	2.23±0.20 b	1.05±0.17 b	6.63±0.16 a
	Pine	0.08±0.03 a	1.69±0.58 a	9.2±2.2 b	57.7±1.8 b	8.9±0.6 a	0.61±0.07 a	0.22±0.08 a	9.67±1.79 a	2.49±0.45 a	5.36±0.14 c
	Grassland	0.09±0.02 a	1.69±0.10 a	15.1±1.0 a	64.8±2.2 b	9.8±0.4 a	0.62±0.02 a	0.18±0.03 a	2.89±0.22 b	0.89±0.08 b	6.06±0.07 b
10–30	Cedar	0.04±0.02 b	3.80±0.27 a	25.6±1.5 b	137.4±10.3 a	29.7±1.3 a	1.98±0.11 a	0.09±0.01 ab	5.90±0.28 a	0.75±0.06 b	5.67±0.17 b
	Pine	0.08±0.03 b	2.07±0.25 b	18.9±1.1 c	167.9±42.2 a	23.9±0.7 ab	0.99±0.06 b	0.06±0.01 b	5.82±0.16 a	1.86±0.15 a	5.66±0.09 b
	Grassland	0.15±0.03 a	3.63±0.32 a	31.8±1.5 a	148.6±8.1 a	22.0±1.3 b	1.66±0.21 a	0.15±0.03 a	6.09±0.30 a	1.35±0.22 b	6.17±0.04 a
30–100	Cedar	0.03±0.01 b	8.91±0.88 a	99.2±5.7 b	531.1±37.4 b	126.4±6.6 a	3.90±0.36 a	0.29±0.02 b	17.89±1.29 a	2.68±0.33 b	5.57±0.11 b
	Pine	0.03±0.08 b	3.26±0.35 c	89.3±5.4 b	639.2±31.0 ab	100.1±4.0 b	2.50±0.15 a	0.21±0.01 b	11.75±0.35 b	3.15±0.31 ab	6.71±0.07 a
	Grassland	0.62±0.13 a	4.90±0.56 b	132.2±15.1 a	775.7±79.9 a	119.0±9.8 a	2.93±0.30 a	0.38±0.06 a	15.68±1.08 a	4.01±0.58 a	6.66±0.06 a
100–170	Cedar	0.30±0.11 b	11.54±5.26 a	103.9±7.2 b	650.7±30.6 b	113.7±5.0 b	2.42±0.76 a	0.34±0.04 b	11.80±0.51 b	3.08±0.26 b	6.18±0.10 b
	Pine	0.38±0.12 b	3.03±0.14 c	74.7±3.5 c	579.7±15.8 b	90.7±3.1 c	1.85±0.21 a	0.18±0.02 c	10.23±0.94 b	2.69±0.14 b	6.87±0.05 a
	Grassland	1.07±0.28 a	7.03±0.85 b	141.1±9.7 a	1056±69.4 a	147.6±8.2 a	1.58±0.32 a	0.45±0.07 a	16.32±1.71 a	4.69±0.63 a	6.91±0.09 a
170–240	Cedar	0.07±0.02 b	5.57±0.48 ab	93.2±5.9 b	629.3±41.4 b	98.1±5.9 b	0.89±0.22 b	0.29±0.05 ab	9.66±0.31 b	2.51±0.16 b	6.61±0.05 b
	Pine	0.20±0.06 b	4.18±0.19 b	70.3±2.5 c	536.0±18.3 b	84.4±2.6 b	1.79±0.26 a	0.24±0.03 b	9.55±0.70 b	2.64±0.27 b	6.87±0.07 a
	Grassland	0.72±0.19 a	8.61±1.42 a	169.2±16.0 a	1152±117.3 a	159.9±16.1 a	0.92±0.28 b	0.34±0.04 a	16.21±2.04 a	5.09±0.70 a	7.06±0.08 a
240–300	Cedar	0.05±0.02 b	5.45±0.31 b	86.0±4.2 b	553.3±23.4 b	82.7±3.5 b	0.67±0.19 b	0.28±0.05 a	7.46±0.65 b	2.11±0.12 a	6.87±0.07 b
	Pine	0.24±0.09 ab	4.69±0.42 b	72.0±6.1 b	532.2±47.1 b	73.5±4.9 b	1.72±0.23 a	0.26±0.07 a	9.34±0.78 a	2.97±0.42 a	6.79±0.08 b
	Grassland	0.30±0.06 a	10.38±2.34 a	187.4±20.4 a	1179±137.5 a	163.8±19.9 a	0.22±0.12 c	0.27±0.06 a	9.25±0.52 a	2.84±0.57 a	7.25±0.07 a

Same letters indicate no significant differences within each soil depth at the 0.05 level

Table 2 The percentage of soil organic carbon (SOC) stocks at depths relative to the whole soil column (0–300 cm) under three vegetation cover types: cedar (*Juniperus virginiana*), pine (*Pinus ponderosa*), and native semi-arid C₄ grasslands

Soil depth (cm)	SOC stocks (%)			SOC stocks (kg m ⁻²)		
	Cedar	Pine	Grassland	Cedar	Pine	Grassland
0–300	100±0 a	100±0 a	100±0 a	6.80±0.35 b	5.87±0.54 b	7.39±0.58 a
10–300	77±3 b	74±5 b	88±1 a	5.19±0.18 b	4.35±0.68 b	6.52±0.57 a
30–300	57±4 b	60±7 b	70±2 a	3.87±0.27 b	3.59±0.71 b	5.20±0.49 a
100–300	30±3 b	41±11 ab	44±3 a	1.99±0.21 b	2.48±0.89 ab	3.32±0.45 a
170–300	19±3 b	24±7 ab	25±2 a	1.29±0.20 b	1.46±0.52 b	1.91±0.25 a
240–300	9±3 a	12±5 a	8±1 a	0.62±0.17 b	0.70±0.33 a	0.59±0.10 b

Same letters indicate no significant differences among vegetation cover types within each soil depth at the 0.05 level

Fig. 1 Stocks of SOC based on equivalent soil mass (ESM) at different soil depths under three vegetation cover types: cedar (*Juniperus virginiana*), pine (*Pinus ponderosa*), and native semi-arid C₄ grasslands. Black bars represent standard errors ($n=12$). Same letters indicate no significant differences among vegetation cover types within each soil depth at the 0.05 level

Subsoil SOC storage declines on conversion of native grassland to cedar or pine forest

Cedar and pine have lower belowground allocation of C than grass

Despite more litter input to the topsoil (0–10 cm), cedar or pine forests did not increase SOC storage in the subsoil (10–300 cm) compared to grasslands (Fig. 1). Grasslands contained $26 \pm 3\%$ and $50 \pm 6\%$ more SOC than cedar and pine forests in the subsoil (Table 2), in part because of higher allocation of C belowground (February et al. 2020; Jackson et al. 2002; Wigley et al. 2020) and greater abundance

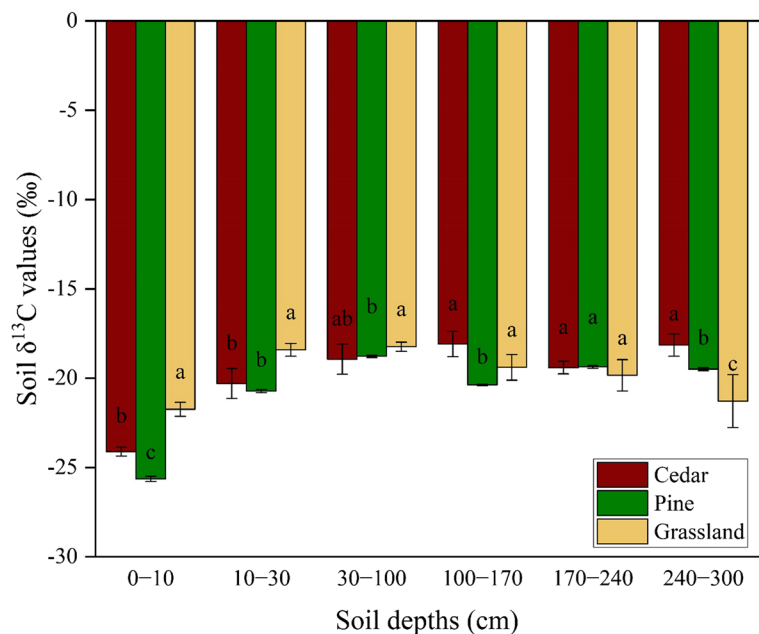
of fine roots with higher root tip attrition and faster turnover (Upson et al. 2016). The SOC losses will continue until the new C input from cedar and pine can accumulate in the subsoils and compensate for the losses of the original SOC, maybe over several centuries or even millennia. The new C input to the topsoil can move to the subsoils through bioturbation and downward leaching and might accumulate over time (Cagnarini et al. 2019; Vormstein et al. 2020). The accumulation rate of the C input from new plants can increase with stand age as the roots and rhizome network continue to establish and develop, providing more C input (Leifeld et al. 2021; Richards et al. 2017). The evaluations of the effects of cedar and

Table 3 The percentage of original grasslands soil organic carbon (SOC) replaced by tree C and the mean residence time (MRT) of the original grassland SOC under stands of cedar (*Juniperus virginiana*) and pine (*Pinus ponderosa*)

Vegetation type	Soil depth (cm)	Replaced by tree C (%)	Replacing rate (% per year)	MRT
Cedar	0–10	40 ± 4 bc	0.62 ± 0.06 b	149 ± 30 d
	10–30	30 ± 7 cde	0.47 ± 0.11 cd	245 ± 47 abc
	30–100	32 ± 10 cde	0.49 ± 0.14 bcd	234 ± 112 abcd
	100–170	18 ± 3 e	0.27 ± 0.04 d	352 ± 67 ab
	170–240	24 ± 4 de	0.37 ± 0.06 cd	264 ± 55 abcd
	240–300	18 ± 2 e	0.27 ± 0.04 d	349 ± 50 a
Pine	0–10	70 ± 4 a	0.85 ± 0.04 a	73 ± 9 e
	10–30	45 ± 4 b	0.55 ± 0.05 bc	158 ± 26 cd
	30–100	29 ± 3 cde	0.35 ± 0.04 d	273 ± 43 ab
	100–170	30 ± 6 bcd	0.36 ± 0.07 d	300 ± 64 ab
	170–240	44 ± 5 bcd	0.54 ± 0.07 bcd	150 ± 24 bcde
	240–300	26 ± 5 cde	0.32 ± 0.07 cd	306 ± 58 a

Same letters indicate no significant differences across all treatments at the 0.05 level

Fig. 2 Soil $\delta^{13}\text{C}$ values at different soil depths under three vegetation cover types: cedar (*Juniperus virginiana*), pine (*Pinus ponderosa*), and native semi-arid C_4 grasslands. Black bars represent standard errors ($n = 12$). Same letters indicate no significant differences among vegetation cover types within each soil depth at the 0.05 level



pine invasion into native grassland on C storage need to be continued for long-term.

Changes in subsoil SOC storage mirror soil microbial biomass

Greater subsoil SOC storage under grasslands may be also associated with the higher microbial biomass (Fig. 3A). This may be fostered by greater herbaceous plant diversity and productivity in native grasslands compared to cedar and pine forests at this

field site (Mazis et al. 2021). Higher plant diversity and productivity may favor faster microbial growth and turnover leading to greater production of microbial products and necromass (Prommer et al. 2020) and ultimately SOC formation (Camenzind et al. 2023). However, accumulation of SOC and associated microbial biomass depend on stabilization mechanisms. The higher clay and silt contents under grassland, especially in the subsoil (Tables S1), may facilitate SOC accumulation through clay-associated microbial necromass and products (Churchman

Table 4 The percentage of soil microbial biomass (MB) stocks at depths relative to the whole soil column (0–300 cm) under three vegetation cover types: cedar (*Juniperus virginiana*), pine (*Pinus ponderosa*), and native semi-arid C₄ grasslands

	Soil depth (cm)	Stocks (%)			Stocks (mmol FAMES m ⁻²)		
		Cedar	Pine	Grassland	Cedar	Pine	Grassland
Total	0–300	100±0 a	100±0 a	100±0 a	38.4±8.8 b	39.9±9.6 b	65.7±10.5 a
	10–300	88±7 a	90±7 a	85±9 a	34.0±7.0 b	36.1±6.7 b	55.8±9.0 a
	30–300	55±5 b	58±5 a	62±8 a	21.0±4.7 b	23.3±5.3 b	41.0±8.1 a
	100–300	18±2 b	32±4 a	32±5 a	6.9±1.8 c	12.6±4.0 b	20.7±5.2 a
	170–300	8±1 b	19±3 a	14±2 a	3.3±0.9 c	7.4±3.0 a	9.30±2.1 a
	240–300	3±0.3 b	10±1 a	1±0.2 a	1.1±0.3 b	3.8±2.0 a	0.4±0.1 c
Bacterial	0–300	100±0 a	100±0 a	100±0 a	10.1±2.3 b	12.4±2.7 b	23.7±3.7 a
	10–300	87±9 a	93±10 a	86±8 a	8.8±1.8 b	11.5±2.0 b	20.3±3.2 a
	30–300	53±4 a	57±4 a	61±6 a	5.4±1.2 b	7.1±1.5 b	14.4±2.9 a
	100–300	10±2 b	26±3 a	29±3 a	1.0±0.3 c	3.2±1.0 b	7.0±2.1 a
	170–300	3±0.2 b	13±1 a	12±1 a	0.3±0.1 c	1.7±0.7 b	2.9±0.8 a
	240–300	1±0.1 b	6±0.5 a	0.1±0.01 b	0.1±0.03 a	0.8±0.4 a	0.03±0.02 a
AMF	0–300	100±0 a	100±0 a	100±0 a	1.5±0.4 b	0.6±0.1 c	2.5±0.4 a
	10–300	88±8 a	95±10 a	83±8 a	1.4±0.3 c	0.5±0.1 c	2.1 0.4 a
	30–300	53±5 a	54±5 a	59±6 a	0.8±0.2 b	0.3±0.08 c	1.5±0.3 a
	100–300	22±3 ab	21±2 b	29±4 a	0.3±0.1 b	0.1±0.05 c	0.7±0.2 a
	170–300	11±2 a	12±1 a	12±2 a	0.2±0.06 a	0.07±0.03 b	0.3±0.09 a
	240–300	5±1 a	5±1 a	0.2±0.01 b	0.07±0.03 a	0.03±0.02 b	0.01±0.01 b
SF	0–300	100±0 a	100±0 a	100±0 a	2.4±0.6 a	2.1±0.5 a	2.9±0.5 a
	10–300	91±9 a	96±11 a	83±9 a	2.2±0.5 a	2.0±0.5 a	2.5±0.4 a
	30–300	53±6 a	66±7 a	64±6 a	1.3±0.3 a	1.4±0.4 a	1.9±0.3 a
	100–300	21±3 b	39±5 a	34±4 a	0.5±0.1 b	0.8±0.3 a	1.0±0.2 a
	170–300	11±2 b	24±3 a	18±3 a	0.3±0.08 a	0.5±0.2 a	0.5±0.1 a
	240–300	4±1 b	13±2 a	1.6±0.5 c	0.1±0.03 a	0.3±0.1 a	0.05±0.02 a

AMF, arbuscular mycorrhizal fungi; SF, saprophytic fungi; ECM, ectomycorrhizal fungi. Same letters indicate no significant differences among vegetation cover types within each soil depth at the 0.05 level

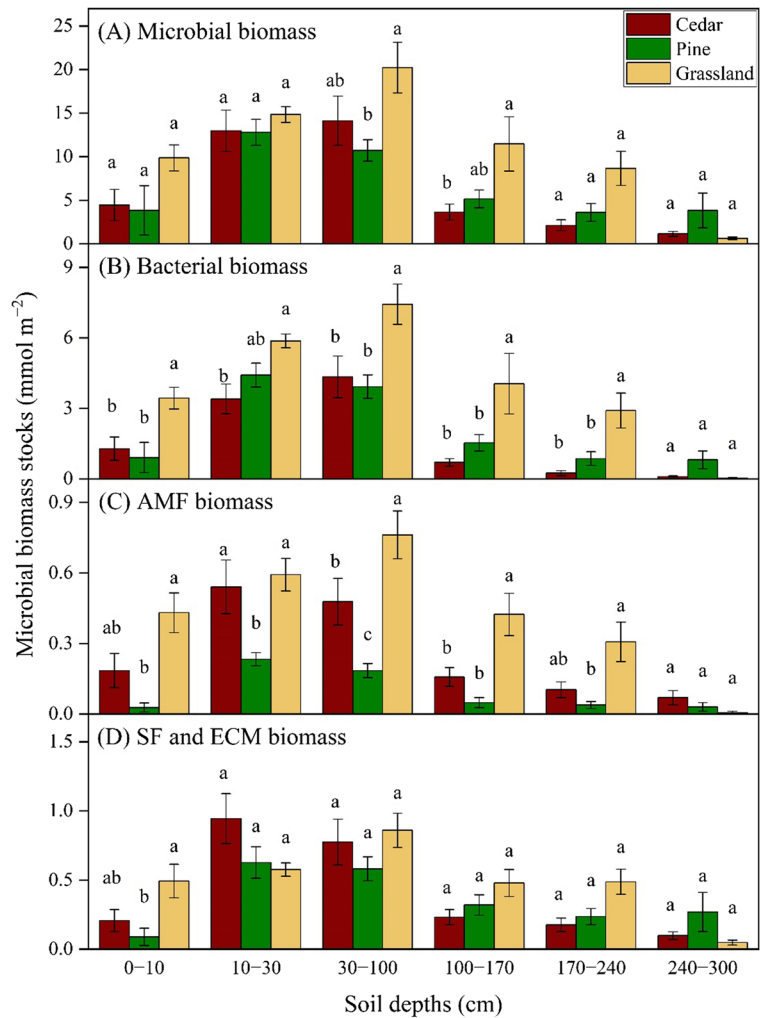
et al. 2020; Georgiou et al. 2022; Matus 2021). Clay and clay lenses can retain more water and nutrients (Pepper and Gentry 2015), providing better habitats for soil microbes. Overall, the greater soil moisture (Mazis et al. 2021), pH, and nutrients under grassland (Table 1) would be more favorable to microbial processes central to SOC formation and stabilization.

Conversion of native grassland to cedar or pine forest leads to changes in soil microbial communities

Besides microbial biomass, changes in microbial community structure might also lead to SOC losses under cedar and pine forests. We observed a shift in soil microbial community composition from AMF and bacteria to SF and ECM when converting

native grassland to cedar or pine forests (Fig. 3). Although SF and EMC did not differ in amount with vegetation (Fig. 3D), losses of bacterial and AMF biomass under cedar and pine (Fig. 3B and C) make SF and ECM a larger proportion of the total microbial biomass, especially under pine. This shift in microbial community composition is likely to alter microbial community function. Many SF and ECM are capable of decomposing SOC to obtain nutrients such as N (Pellitier et al. 2021; Shah et al. 2016; Zak et al. 2019). Soil nutrients are likely limited in our experimental sites as reflected by the low nutrient and high sand contents (Table 1 and S1) and no anthropogenic input. Thus, decomposition may be highly dependent on nutrient scavenging in these low nutrient soils. Despite the

Fig. 3 Fatty acid methyl ester (FAME) stocks at different soil depths under three vegetation cover types: cedar (*Juniperus virginiana*), pine (*Pinus ponderosa*), and native semi-arid C₄ grasslands. AMF, arbuscular mycorrhizal fungi; SF, saprophytic fungi; ECM, ectomycorrhizal fungi. Black bars represent standard errors ($n=12$). Same letters indicate no significant differences among vegetation cover types within each soil depth at the 0.05 level



losses of AMF in both cedar and pine soils, cedar supports a higher biomass of AMF compared to pine (Fig. 3C). AMF can allocate 4–25% of photosynthetic C from their plant host to fungal mycelia (Parihar et al. 2020) that contributes to the pool of microbial products and necromass (Agnihotri et al. 2022; Jørgensen et al. 2022). Although soil bacteria carry out many of the same functions as SF, their biomass and activities are highly dependent on microsite conditions given the small pores they tend to inhabit. Evidence for a change in soil properties on conversion of grassland to cedar or pine forest is clearly shown for the 0–100 cm depth by a shift in bacterial community structure (Fig. 4).

SOC turnover rate and storage by soil depth

The original grassland SOC had shorter mean residence time (MRT) in topsoil (0–10 cm) than subsoil (10–300 cm, Table 3), suggesting more stabilized SOC in the subsoil. Usually, SOC stabilized in subsoil is horizontally stratified and its MRT increases with soil depths (Rumpel and Kögel-Knabner 2011). However, our MRT from 10 to 300 cm showed no significant differences (Table 3). This unexpected homogeneity of MRT in the subsoil might result from the weakly developed soil structure with little horizonation and a more homogenous distribution of decomposition-relevant resources such as water and

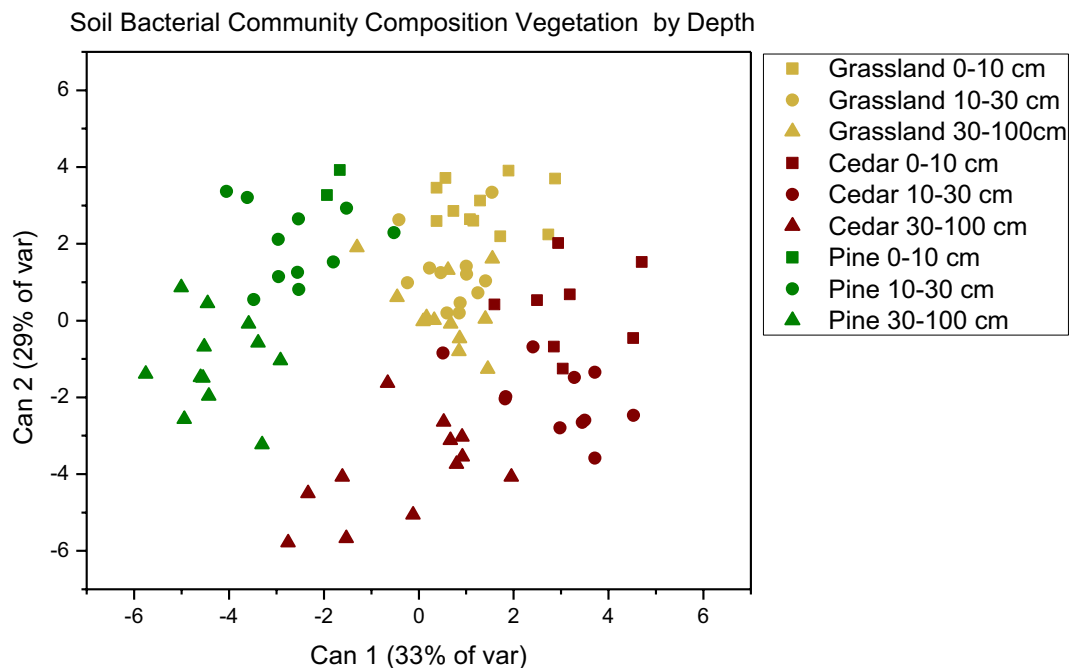


Fig. 4 Canonical correlation analysis of soil bacterial fatty acids normalized (nmol%) to soil microbial biomass by soil depth interval to 100 cm on three vegetation cover types: cedar

(*Juniperus virginiana*), pine (*Pinus ponderosa*), and native semi-arid C_4 grasslands

nutrients across the soil profile. Since the majority of the SOC in the 0–300 cm depth ($74 \pm 5\%$ to $88 \pm 1\%$) was stored in the subsoil (Table 2), focusing solely on topsoil can lead to misinterpretations. Subsoils may play a more critical role in terrestrial C budget than previously assumed.

Conclusions

We evaluated the SOC storage and turnover as affected by vegetation change from native semi-arid C_4 dominated grasslands to eastern red cedar or ponderosa pine forest. Conversion of native grasslands to cedar or pine forest increased topsoil SOC storage but decreased subsoil SOC storage. The decrease in SOC storage was associated with reduced microbial biomass and altered microbial community structure under forests relative to the grasslands. This decrease may also be mediated by the declines in clay content, soil moisture, pH, and nutrients. Our results demonstrated the critical role of subsoil in SOC storage. Subsoil should be considered for more

accurate evaluation of global SOC storage. In the scenario of vegetation cover change from native grasslands to cedar or pine forest, the potential above-ground C storage in woody biomass might partially offset the belowground C loss, which needs further investigation.

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Data Availability Data are archived at GitHub (<https://github.com/lilidong1111/SOC>).

Declarations

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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