

PRIMARY RESEARCH ARTICLE

Low-intensity frequent fires in coniferous forests transform soil organic matter in ways that may offset ecosystem carbon losses

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Abstract

The impact of shifting disturbance regimes on soil carbon (C) storage is a key uncertainty in global change research. Wildfires in coniferous forests are becoming more frequent in many regions, potentially causing large C emissions. Repeated low-intensity prescribed fires can mitigate wildfire severity, but repeated combustion may decrease soil C unless compensatory responses stabilize soil organic matter. Here, we tested how 30 years of decadal prescribed burning affected C and nitrogen (N) in plants, detritus, and soils in coniferous forests in the Sierra Nevada mountains, USA. Tree basal area and litter stocks were resilient to fire, but fire reduced forest floor C by 77% (−36.4 Mg C/ha). In mineral soils, fire reduced C that was free from minerals by 41% (−4.4 Mg C/ha) but not C associated with minerals, and only in depths ≤ 5 cm. Fire also transformed the properties of remaining mineral soil organic matter by increasing the proportion of C in a pyrogenic form (from 3.2% to 7.5%) and associated with minerals (from 46% to 58%), suggesting the remaining soil C is more resistant to decomposition. Laboratory assays illustrated that fire reduced microbial CO₂ respiration rates by 55% and the activity of eight extracellular enzymes that degrade cellulosic and aromatic compounds by 40–66%. Lower decomposition was correlated with lower inorganic N (−49%), especially ammonium, suggesting N availability is coupled with decomposition. The relative increase in forms of soil organic matter that are resistant to decay or stabilized onto mineral surfaces, and the associated decline in decomposition suggest that low-intensity fires may promote mineral soil C storage in pools with long mean residence times in coniferous forests.

KEYWORDS

carbon, coniferous forest, extracellular enzymes, fire frequency, nitrogen, Sierra Nevada, soil organic matter, soil respiration

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1 | INTRODUCTION

The response of soil organic matter to global change is a large source of uncertainty in climate change predictions (Jackson et al., 2017; Paustian et al., 2016; Schlesinger & Amundson, 2019). Disturbances have the potential to trigger large losses of soil organic matter (SOM) by promoting physical losses, combustion, enhanced decomposition, or changing the type and amount of biomass inputs (James et al., 2021; Lal, 2005; Pellegrini et al., 2018; Prichard et al., 2017; Walker et al., 2019). Disturbances do not always lead to net declines in SOM, however, because compensatory responses such as changes in SOM decomposition or plant productivity may offset the losses (Harden et al., 1999; Johnson & Curtis, 2001). Understanding how disturbances trigger compensatory responses could help reduce potential SOM losses due to intensifying disturbance regimes.

Fire is an important global disturbance, emitting an average of 2.2 PgC annually via biomass combustion (van der Werf et al., 2017). Although combustion of aboveground biomass releases large amounts of carbon (C) to the atmosphere, changes in SOM can also contribute significantly to the total effects of fire on ecosystem C storage (Hatten et al., 2008; Johnson & Curtis, 2001; Pellegrini et al., 2015; Turetsky et al., 2015; Wardle et al., 2003). The vulnerability of SOM to fire varies across ecosystems, however, due to factors such as biomass inputs, combustion of SOM, and decomposition (Aaltonen, Köster, et al., 2019; Boerner et al., 2009; Pellegrini et al., 2020; Turetsky et al., 2015; Walker et al., 2019); for example, SOM may increase, decrease, or not change over multiple years following a fire (Butler et al., 2019; Haase & Sackett, 1998; McKee, 1982; Pellegrini et al., 2020; Raison et al., 1985). Consequently, the effect of fire on the turnover of SOM on decadal timescales may influence the effect of changing fire frequencies on C storage.

Fire can either speed up or slow down soil C losses by changing the physicochemical properties of SOM and decomposition activity (Aaltonen, Palviainen, et al., 2019; Arocena & Opio, 2003; Flanagan et al., 2020; Jian et al., 2018; Ketterings et al., 2000). For example, fire can increase decomposition by increasing soil pH and mobilizing dissolved C that stimulates microbial growth (Wang et al., 2012; Wardle et al., 2008). In other cases, fire can decrease decomposition through heat-induced microbial mortality, loss of fungal communities capable of breaking down recalcitrant organic matter, and lower organic matter inputs (Aaltonen, Palviainen, et al., 2019; Dooley & Treseder, 2012; Flanagan et al., 2020; Pellegrini et al., 2020).

Fire-induced shifts in the properties of SOM may indicate whether fire increases or decreases decomposition because different forms of SOM vary in their decomposability (Cotrufo et al., 2013; Sollins et al., 1996; Von Lützow et al., 2008). First, SOM associated with minerals tends to be less accessible to microbes (Cotrufo et al., 2013; Jackson et al., 2017; Kögel-Knabner et al., 2008; Sollins et al., 1996; Von Lützow et al., 2008); if fire increases the proportion associated with minerals, further SOM losses may be reduced (e.g., Aaltonen, Köster, et al., 2019). Second, the chemical composition of organic matter is important; pyrogenic C (PyC) tends to persist in soils

over millennial timescales (i.e., exhibits long mean residence times) (Czimeczik et al., 2005; Lehmann et al., 2008; Swift et al., 1979). In turn, fire-driven increases in these compounds may also contribute to soil C being less prone to losses via decomposition (Flanagan et al., 2020; Neff et al., 2005). Given that microbial groups with distinct capacities to degrade certain forms of SOM (e.g., amount of aromatics) (Pellitier & Zak, 2018) can differ in their sensitivity to fire (Whitman et al., 2019), assays of extracellular enzymes can provide insight into how fire may shift the forms of decomposing SOM (Boerner et al., 2005; Butler et al., 2019; Holden et al., 2013).

In coniferous forests, which contribute ~10% to global fire C emissions, wildfire-driven C emissions will likely rise because high-severity wildfires are becoming more frequent due to longer wildfire seasons and drier conditions (Abatzoglou & Williams, 2016; Moritz et al., 2012; Westerling et al., 2006). These wildfires tend to be intense partly due to historical fire exclusion, and are expected to become more intense in the future as higher temperatures and dryness increase fuel flammability, as well as insect- and pathogen-driven tree mortality increase fuel load (Millar & Stephenson, 2015). More severe fires can trigger large C emissions from soil C stocks that have accumulated for centuries to millennia (Bormann et al., 2008; Walker et al., 2019). These interacting factors coupled with severe fires can result in large-scale forest community conversion (Coop et al., 2020). Prescribed burning is one management technique to reduce fire severity because prescribed fires are generally lower intensity and reduce the severity of future fires by combusting accumulated fuel (Stephens et al., 2013), which may also lower soil C losses. Prescribed burning incurs C costs by reducing aboveground biomass stocks (fire reduces biomass in trees, litter, and the forest floor; Schwilk et al., 2009). But whether prescribed fire changes soil C storage in coniferous forests is less clear (Boerner et al., 2008, 2009; Hatten et al., 2005; Moghaddas & Stephens, 2007) and could be one management avenue to promote soil C sequestration in forests (Jandl et al., 2007).

To test how decades of prescribed burning impact the storage of C in plants and soils as well as the turnover of SOM, we sampled a long-term prescribed burning manipulation experiment in a mountainous coniferous forest in the western United States in Sequoia and Kings Canyon National Parks. We first tested the hypothesis that fire reduces the inputs of plant biomass into the soils by measuring tree population sizes and the C stocks in litter and the forest floor and mineral soils. Next, we tested whether changes in SOM were accompanied by shifts in the form of SOM based on the hypotheses that (a) fire-driven reductions in fresh inputs from plant biomass should result in a more pronounced reduction in SOM free from minerals than SOM associated with minerals and (b) thermal transformations of SOM result in more recalcitrant forms of SOM reflected by lignin and PyC content. The hypothesis that fire reduces decomposition activity was tested using laboratory incubations to quantify soil CO₂ respiration as well as extracellular enzymes, which provide more insight into changes in the decomposition of particular forms of SOM. Finally, we tested the hypothesis that lower decomposition is coupled with more recalcitrant SOM and lower availability of nutrients using ordination analyses.

2 | METHODS

2.1 | Site description and experimental design

We sampled a fire experiment in Sequoia and Kings Canyon National Parks in California, USA. The experiment was established in the National Parks in the 1980s, on plots 0.10 ha in size with a variety of vegetation types. We sampled six plots where fire had been excluded for >150 years and where prescribed burns had been conducted every 10–15 years ($n = 3$ true replicates in each fire treatment distributed across the landscape, plots located in Giant Forest, latitude: 36.60, longitude: -118.73; sample photographs in Figure S1), starting in 1982–1986 for these plots. The decadal burns are considered to be within the natural fire return interval (Swetnam et al., 2009). Burn treatments were conducted under conditions to promote low fire intensities (May–July or September–October).

The study area has a Mediterranean climate, receiving on average 1092 mm in precipitation (1920–2017), with ~50% of the precipitation occurring as snow followed by dry summers with a period of water deficit. The plots are at ~2100 m elevation and have mean annual air temperatures ranging from 1.9 to 13.6°C. The texture in the top 15 cm of mineral soil analyzed was 62% clay, 29% silt, and 9% sand on average. The plots have varying underlying geologies, but the dominant underlying rock type in the study area is Mesozoic granite. In Giant Forest, where many of our plots are located, the granodiorite is medium-grained hypidiomorphic-granular hornblende biotite and dated at 97–102 million years (Sisson & Moore, 1984). Soil types vary across the area but Xeric Haplohumults is one of the dominant types in the Giant Forest area (Huntington & Akeson, 1987). We do not expect any directional bias in our results arising from underlying geologies given there was no strong spatial clustering of the plots. We did not measure soil pH in our study, but other studies in Giant Forest found a pH of around 6, with no significant effect of a prescribed fire after 2 years (Hamman et al., 2008).

These plots were sampled in September 2017. The last fires to occur prior to our sampling took place in June–July of 2012 for two plots and July 2013 for one plot; thus, time since last fire was 4–5 years at the time of sampling. Within each plot, we sampled underneath trees as well as in “open” areas outside of tree canopies, which were dominated by herbaceous and shrubby species in the burned plots and thick litter and forest floor layers in the unburned plots.

2.2 | Vegetation and fuel surveys

Present-day tree communities were dominated by *Abies concolor* and *Sequoiadendron giganteum*. There was little understory vegetation in plots where fire had been excluded, which had deep accumulations of litter, forest floor, and coarse wood debris. In the burned plots, understory plant species such as *Ceanothus* and *Lupinus*, both of which fix atmospheric N_2 , and various grasses were prevalent where fire had removed the forest floor and litter layer.

To quantify changes in trees and aboveground detritus, we used data collected by the National Park Service following Fire Monitoring Handbook protocols (USDA National Park Service, 2003) on tree basal area, stem density, litter (Oi layer), and forest floor (Oe layer) to evaluate changes in potential fresh plant biomass inputs and detritus inputs into the mineral soil. We measured C and N content in the litter and forest floor in the same way as in mineral soil (described below). Tree communities were surveyed inside each replicate plot, with the first survey in the burned plots taking place the year before the first burn and subsequent measurements immediately after the burn and again at 1-, 5-, and 10-year intervals. In the unburned plots, surveys started in 2007.

2.3 | Soil sampling

We sampled mineral soils at nine locations within each plot (six under tree canopies and three away from canopies), where we aggregated five soil cores partitioned into the top 0–5 cm and 5–15 cm layers of the mineral horizon. We measured total soil C and N and inorganic N on both depth layers, but only fractionated (described below) and measured microbial activity and pyrogenic C content in the top layer. We focused on the upper soil profile because on average it tends to be the most responsive to fire (Pellegrini et al., 2018). Because repeated burning can also affect SOM in deeper soil layers, we tested whether fire effects were minimal past 5 cm depth. Soils were passed through a 2 mm sieve and partitioned into three subsamples: (a) fresh soil for soil moisture and inorganic N and phosphorus (P) analyses, (b) frozen soil for enzyme and respiration analyses (-20°C), and (c) dried soil for total and fractionated C and nutrient analyses and pyrogenic C. Soil moisture content, determined by drying soils at 105°C for 48 hours, was used to adjust all analytical chemistry values to grams per dry weight of soil.

We sampled the forest floor and litter directly above the samples taken from the mineral horizon. We characterized the compound composition of the forest floor layer using an ANKOM Fiber Analyzer (ANKOM Technology). Specifically, we measured cellulose, hemicelluloses and bound protein, and the acid-nonhydrolyzable compounds (e.g., lignin), and N. All C fractions were determined on an ash-free dry mass basis.

2.4 | Soil nutrients

Total soil C and N were measured by combusting dried and ground soil on a Costech coupled Elemental Analyzer (Costech Analytical Technologies, Inc.) at Stanford University. Soil was ground for 15 minutes on a ball mill until it was pulverized into a homogeneous fine powder. Total P was measured by extracting 2 g of ground soil in 10 ml of 12 M HNO_3 using a CEM MarsXpress Microwave Digestor (CEM Corporation Matthews) for 4 hours. The concentrations were then analyzed on Inductively Coupled Plasma Mass Spectrometer Thermo XSeries II (Thermo Fisher Scientific).

We measured soluble inorganic N and P (IN and IP, respectively) on soils within 48 hours of collection. Inorganic N was measured by extracting ~5 g of freshly sampled soil via shaking for 2 hours in 50 ml of 1 M KCl. Inorganic P was measured by extracting ~5 g of freshly sampled soil via shaking in 50 ml of 0.5 M NaHCO₃ for 2 hours. Following shaking, the samples were centrifuged and filtered through Grade 41 ashless Whatman filters. Inorganic N (ammonium, nitrate and nitrite) and IP were analyzed on an automated spectrophotometer WestCo SmartChem 200 discrete analyzer (Unity Scientific) at Stanford University.

2.5 | Pyrogenic carbon

We measured pyrogenic C (PyC) concentration and structure using the benzene polycarboxylic acid (BPCA) method (Glaser et al., 1998) with modifications (Matosziuk et al., 2019). Briefly, we digested soils containing ~3 gC in concentrated HNO₃ at 170°C and analyzed digests using high-performance liquid chromatography (Matosziuk et al., 2019; Schneider et al., 2011). BPCA estimates of PyC are conservative given that during cleavage of condensed structures some of the carbon molecules are oxidized into molecules smaller than a benzene carboxylic acid (e.g., oxalic acid) or a benzene carboxylic acid with fewer than 3 carboxylic groups (Glaser et al., 1998). We did not measure PyC on all the sub-samples within each plot, but rather aggregated sub-samples into two replicate samples per-plot, spanning all the replicate plots.

2.6 | Density fractionations

We performed density fractionations to quantify mineral-associated organic matter by suspending soil in a solution with a density that partitions material bound or free from minerals (Crow et al., 2007). We separated the light fraction (<1.6 g/cm³) from the medium + heavy fractions (>1.6 g/cm³) to estimate the amount of non-mineral-associated organic C (non-MAOC) and mineral-associated organic C (MAOC), respectively; we did the same for N. We added ~2 g of sieved and dried soils to a 1.6 g/ml or cm³ sodium polytungstate solution. Soils were shaken for 1 hour at 160 revolutions per-minute, centrifuged at 4000 revolutions per-minute for 30 minutes, and aspirated to isolate the supernatant containing the floating light fraction onto a previously baked quartz filter. We repeated the shaking, centrifuging, and aspiration two more times. The supernatant was then removed and the pellet of remaining soil was dried and removed. Samples were analyzed for total C and N.

2.7 | Enzyme activity

To assess potential turnover of C, N, and P, we measured hydrolytic and oxidative extracellular enzyme activity in a subset of samples at each plot according to the methods of Sinsabaugh et al. (1992)

and Saiya-Cork et al. (2002). The enzyme analyses were performed using protocols presented in Hobbie et al. (2012) at the University of Minnesota. Hydrolytic enzymes were measured fluorometrically using substrates labelled with methylumbelliferone (excitation at 365 nm and emission at 450 nm). Oxidative enzymes were measured via absorbance on a microplate spectrophotometer (460 nm) with the substrates of L-3,4-dihydroxyphenylalanine (phenol oxidase) and L-3,4-dihydroxyphenylalanine and hydrogen peroxide (peroxidase). We homogenized ~0.5 g of samples that had been brought to room temperature in 125 ml of acetate buffer (pH 5.0, 50 mmol/L). Substrate additions and incubations were conducted in 96-well plates: 16 replicate sample wells (sample solution and substrate); eight replicate blank wells (sample solution and buffer); eight negative control wells (substrate and buffer); and eight quench standard wells (standard and sample solution). This allowed us to correct for negative controls, blanks, and quenching. We incubated plates in the dark at 20°C for 2 and 22 hours for hydrolytic and oxidative enzymes, respectively.

Hydrolytic enzymes measured were as follows: cellobiohydrolase (CBH, degrades cellulose, EC 3.2.1.91), β -glucosidase (BG, degrades cellulose, EC 3.2.1.21), α -glucosidase (AG, degrades starch, EC 3.2.1.20), β -xylosidase (BX, degrades hemicellulose, EC 3.2.1.37), N-acetyl- β -D-glucosaminidase (NAG, degrades chitin, EC 3.2.1.14), and acid phosphatase (AP, degrades phosphomonoesters, EC 3.1.3.2). The oxidative enzymes were as follows: phenol oxidase (PO, degrades polyphenols, EC 1.10.3.2) and peroxidase (PX, degrades polyphenols/lignin, EC 1.11.1.7).

Enzyme activities were analyzed both individually and by taking the sum of all hydrolytic C-acquisition enzymes and the oxidative C-acquisition enzymes separately. We refer to the group of hydrolytic enzymes as degrading "labile" substrates and the oxidative enzymes as degrading "recalcitrant" substrates because of the higher energy required to breakdown the polyphenols and lignin targeted by oxidative enzymes. NAG and AP were analyzed separately to test for changes in potential N and P turnover, respectively.

2.8 | Microbial respiration

We measured short-term microbial respiration as an indicator of soil organic C decomposition using 35-day laboratory incubation assays. Soils were placed in 487 ml glass mason jars allowing for 1:5 ratio of soil:headspace (100–150 g soil), brought to 50% soil water saturation, and left with a loosely placed lid to equilibrate for 48 hours. For each measurement at weekly intervals, we capped the jars, took an initial sample of the headspace from all the blanks, let the jars sit at room temperature (20°C) for 24 hours, and then sampled the headspace of the jars with soils with a syringe through the septum. We did this for 5 weeks. In between measurements, the lids were loosely placed on top of the jars and the soil was kept at 50% soil water saturation. We never performed incubations within 48 hours of adding water to avoid measuring pulsed responses. Gas samples were analyzed on a Shimadzu Gas Chromatograph (Shimadzu Corporation)

at Stanford University. We did not analyze respiration on density-fractionated soils given the potential for sodium polytungstate to suppress microbial activity (Crow et al., 2007).

2.9 | Data analysis

To test for the effect of fire on tree communities, we analyzed tree population sizes after the first fire and then throughout the entire time series. We first compared tree population sizes between pre-fire and the 5 years following the first fire using a mixed-effects model with a fixed effect of fire and replicate plot as a random intercept. To test the overall effect of fire on tree communities over the entire experimental period, and how it changed through time, we incorporated year as a fixed effect and analyzed all tree surveys. Data (both here and for subsequent analyses) were log transformed to fulfill the assumptions of normally distributed residuals. Analyses were performed in R, using package *lme4* for fitting mixed-effects models and *lmerTest* for testing significance based on Satterthwaite's approximation for degrees of freedom and a Type III ANOVA (Bates et al., 2015; Kuznetsova et al., 2017; R Development Core Team, 2010).

We tested the effect of fire on aboveground litter and the forest floor using surveys following the third burn carried out from 2011 to 2013 and compared these to measurements in the unburned plots over the course of the same time period. We analyzed the data using linear mixed-effects models with replicate plot as a random intercept and year as a fixed effect. We also compared the surveys prior to the first burn (in 1982–1986) and the following year to evaluate the immediate loss from a burn.

To test for the effect of fire on soil C and N (total and different fractions) and total P, we also used mixed-effects models to nest the replicate soil cores within the replicate fire treatment plots.

Inorganic N had zero values (nitrate was below detection in some samples), so we averaged the values across the replicate soil cores within each replicate plots and analyzed the effects of fire using linear models.

We analyzed the effect of fire on soil respiration by averaging values within replicate plots because of high variability among samples, and included both fire and sampling time ($n = 5$ extraction periods) and their interaction in a linear model. We also tested how the responses were related to SOM by incorporating measurements of soil C (total and different fractions) into the models in a separate series of tests. The effects of fire on enzymes were analyzed using mixed-effects models with replicate plot as a random effect and fire treatment as a fixed effect, and a separate series of tests including soil C as a covariate. We also correlated enzyme activity with mineral soil N and organic matter lignin and lignin/N content.

To evaluate the multivariate coupling across mineral soil variables, we performed a principal components analysis (*prcomp*) on scaled and zero-centered log-transformed data. We did not analyze the concentration of PyC because of the lower sample size.

3 | RESULTS

3.1 | Initial decline but long-term resilience of tree populations

Prescribed burning initially reduced tree stem abundance. The abundance of trees declined over the 5 years following the first fire ($F_{1,9} = 7.2, p = 0.025$) from 443 (± 127) individuals/ha prior to the fire to 225 (± 50) individuals/ha 5 years after the fire (Figure 1a). Over the course of the experiment, however, tree densities stopped declining and even increased in one of the plots (Figure 1a). Basal area tended to be lower after the first fire, although the changes were

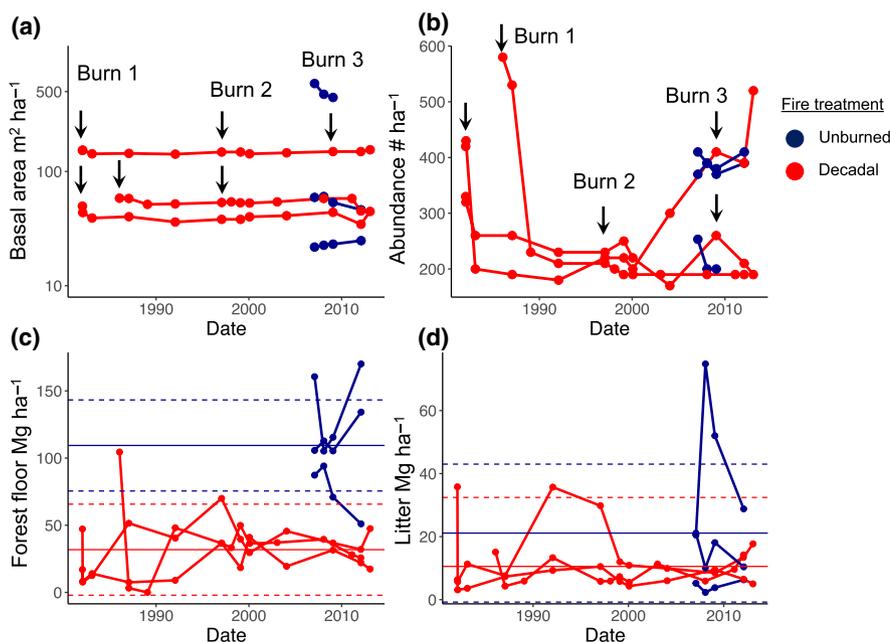


FIGURE 1 Changes in tree populations and stocks of litter and forest floor through time through the different plots (individual lines). The arrows in (a, b) indicate the dates of fires in the burn plots. The timing of fires sometimes differed across plots. Panels (c, d) are total stocks in the forest floor and litter layers in total mass. The solid lines in (c, d) illustrate the mean values over all sampling periods with the dash lines illustrating \pm standard deviation. The solid lines are used to visualize the time-independent means of the treatments and do not represent a rolling average through time. Statistics are in the text

only marginally significant ($F_{1,7} = 4.5$, $p = 0.073$). After 5 years following the first fire, basal area declined by 6.8–9.5 m²/ha on average (4–19% of pre-fire basal area). Over time, the growth of the surviving trees offset the initial declines such that there were no significant fire effects on basal area over the entire 30-year period ($F_{1,32} = 0.8$, $p = 0.36$). Taken together, the reintroduction of low-intensity fires killed small trees but left large trees (which dominated basal area) relatively unchanged.

3.2 | Reduction of C and N stocks in the forest floor but not the litter layer

Repeated burning reduced stocks of C and N in the forest floor but not the litter layer, but most of these losses occurred in the forest

floor while leaf litter was more resilient (Figure 1c,d, Table 1). When we calculated C stocks using the C content of the litter and forest floor, plots burned three times had 77% less C in the forest floor but no significant difference in the litter layer (forest floor: 47.5 ± 15.4 vs. 11.1 ± 3.9 Mg C/ha, $F_{1,6.5} = 13.9$, $p = 0.008$; litter: 9.95 ± 10.3 vs. 5.1 ± 2.3 Mg C/ha, $F_{1,6.6} = 0.26$, $p > 0.50$; ±SD). Nitrogen stocks displayed similar trends, with burned plots having 80% lower forest floor stocks but no significant change in litter stocks (forest floor, 1.12 ± 0.30 vs. 0.23 ± 0.09 Mg N/ha, $F_{1,8.2} = 22.1$, $p = 0.001$; litter: 0.15 ± 0.16 vs. 0.08 ± 0.03 Mg N/ha, $F_{1,6.6} = 0.27$, $p > 0.50$). The forest floor in the burned plots also lower per-mass concentrations of C and N ($F_{1,15} = 8.0$, $p = 0.013$ and $F_{1,15} = 15.7$, $p = 0.001$, respectively), illustrating both mass combustion and direct volatilization contributed to losses of C and N stocks.

TABLE 1 Descriptive statistics of litter and soil C and soil N (units in parentheses) in the different fire treatments and their associated standard deviations

Pool	Variable	Decadal	SD	Unburned	SD
Litter	Litter C (Mg C/ha)	5.09	2.26	9.95	10.26
Litter	Litter N (Mg N/ha)	0.08	0.03	0.15	0.16
Forest floor	Forest floor C (Mg C/ha)	11.10	3.92	47.51	15.35
Forest floor	Forest floor (Mg N/ha)	0.23	0.09	1.12	0.30
Organic	%Lignin	33.02	6.52	29.17	3.55
Organic	%HemiCell	6.64	0.89	6.96	0.85
Organic	%Cell	15.78	2.96	13.58	1.91
Organic	%Lignin N ⁻¹	43.84	16.73	29.68	17.87
Mineral (0–5 cm)	Prop C in PyC (g PyC/g-C)	0.075	0.014	0.032	0.017
Mineral (0–5 cm)	PyC (g PyC/kg-soil)	3.10	1.10	1.56	0.74
Mineral (0–5 cm)	Stock total C (Mg C/ha)	13.88	3.01	17.79	3.61
Mineral (0–5 cm)	Stock light C (Mg C/ha)	6.32	1.65	10.69	2.47
Mineral (0–5 cm)	Stock heavy C (Mg C/ha)	7.55	1.65	7.10	1.47
Mineral (0–5 cm)	Prop soil C light	0.46	0.05	0.58	0.05
Mineral (0–5 cm)	Prop soil mass light	0.07	0.02	0.12	0.03
Mineral (0–5 cm)	C content of light (%)	34.19	4.29	33.08	1.11
Mineral (0–5 cm)	C content of heavy (%)	3.09	0.72	3.11	0.75
Mineral (0–5 cm)	Stock total N (Mg N/ha)	0.57	0.24	0.60	0.27
Mineral (0–5 cm)	Stock light N (Mg N/ha)	0.20	0.12	0.32	0.20
Mineral (0–5 cm)	Stock heavy N (Mg N/ha)	0.36	0.16	0.27	0.14
Mineral (0–5 cm)	Prop soil N light	0.36	0.11	0.53	0.16
Mineral (0–5 cm)	N content of light (%)	1.08	0.14	1.02	0.14
Mineral (0–5 cm)	N content of heavy (%)	0.15	0.07	0.12	0.06
Mineral (0–5 cm)	Total inorg. N (mg N/g-soil)	8.97	2.47	17.35	9.71
Mineral (0–5 cm)	NH ₄ (mg N/g-soil)	6.66	2.80	6.66	2.80
Mineral (0–5 cm)	NO ₃ (mg N/g-soil)	2.32	1.05	1.47	1.09
Mineral (5–15 cm)	Stock total C (Mg C/ha)	27.94	6.78	35.94	10.51
Mineral (5–15 cm)	Stock total N (Mg N/ha)	1.12	0.37	1.42	0.50
Mineral (5–15 cm)	Total inorg. N (mg N/g-soil)	6.28	1.54	9.81	4.88
Mineral (5–15 cm)	NH ₄ (mg N/g-soil)	4.86	1.97	8.56	5.20
Mineral (5–15 cm)	NO ₃ (mg N/g-soil)	1.42	0.76	1.25	1.19

3.3 | Fire effects on mineral soil C depended on mineral association and depth

In the mineral soils, fire effects depended on soil density fractionation group. Repeatedly burned plots had lower C, but only in the fraction not associated with minerals (non-MAOC). Across all the plots, half of total soil C was in the non-MAOC fraction. Fire reduced the amount of non-MAOC by 41% (6.3 vs. 10.7 Mg C/ha, in burned vs. unburned plots, respectively, $F_{1,39} = 5.5$, $p = 0.025$, Figure 2a, Table 1). Mineral-associated organic C (MAOC) remained unchanged ($p > 0.50$, Figure 2b, Table 1). Thus, fire reduced the proportion of total C not associated with minerals (46% vs. 58% in burned vs. unburned, respectively, $F_{1,3.7} = 7.0$, $p = 0.06$, Figure 2c). The decline in non-MAOC was due to a lower mass of soil not associated with minerals (7% vs. 12% in burned vs. unburned, Figure 2d) rather than a change in C concentration of the non-MAOC (mean of 340 mg C/g-fraction). Consequently, repeated burning reduced the pool of soil C free from minerals, resulting in the remaining total soil C being in a form less accessible for microbial decomposition.

C:N ratios of mineral-associated organic matter were 21% lower in the burned relative to unburned plots (20 vs. 26, $F_{1,39} = 13.9$, $p < 0.001$), while C:N remained unchanged in the non-MAOC pool (33, $p > 0.50$).

Analyses of total C and C:N in the 5–15 cm layer illustrated that fire did not change either the pool or C:N ratio ($F_{1,4.0} = 1.2$, $p = 0.33$ and $F_{1,3.5} = 1.0$, $p = 0.38$).

3.4 | Fire reduced nitrogen availability but only in the upper soil layer

Although total mineral soil N stocks were unaffected by fire treatment ($p > 0.50$), the trend toward fire reducing N in the light fraction (−1.2 Mg N/ha, −37%, $F_{1,3.4} = 5.9$, $p = 0.083$) resulted in a greater proportion of N being associated with minerals in burned plots in the top 0–5 cm (Table S2, $F_{1,3.6} = 14.5$, $p = 0.023$). Despite little change in total N, fire reduced total inorganic N by 49% (−8.6 mg N/kg-soil,

$F_{1,10} = 6.2$, $p = 0.032$). The lower IN was due to a 59% reduction in ammonium (−9.5 mg N/kg-soil) but not nitrate ($F_{1,10} = 7.8$, $p = 0.019$, $F_{1,10} = 2.1$, $p = 0.18$, respectively). Fire effects on total N and inorganic N pools were absent in the 5–15 cm layer ($p > 0.20$ for all tests). Consequently, fire changed the bioavailability of N but only in the top layer of the soil.

3.5 | Fire increased chemical recalcitrance of organic matter

We next measured potential chemical transformations relevant to decomposition in the forest floor and mineral soil. First, we evaluated how fire changed C fractions in the forest floor because of their relationship with mass loss and thus inputs into the mineral soil (cellulose, hemicellulose, starch, and lignin). Fire increased the concentration of cellulose by a small amount (~2%) but did not change either hemicellulose or lignin content (Table 1, Table S2, Figure S2). Fire increased lignin:N ratios, which increased from 30 to 44 in the unburned vs. decadal burned plots, respectively ($F_{1,4.4} = 6.9$, $p = 0.05$, Figure 3a). Changes in lignin:N were likely due to thermal effects and not shifts in plant communities because we (a) controlled for tree species identity by sampling under the same species and (b) found no difference between sampling locations underneath tree canopies lacking any understory vegetation vs. away from trees under herbaceous and shrubby plants (Figure 3b).

In the mineral soil, fire altered the amount of pyrogenic C (PyC), increasing the concentration of PyC relative to bulk soil as well as the proportion of soil C in a PyC form. On average, PyC made up twice as much C in the burned relative to unburned treatments, but the proportion was relatively low (7.5% vs. 3.2%, respectively, $F_{1,4} = 18.0$, $p = 0.014$, Figure 3c). These estimates are likely on the lower end, with conversion factors from BPCA estimates to total PyC ranging from 2.1 to 4.3 (Glaser et al., 1998). Similarly, the absolute concentration relative to bulk soil mass was double in the burned plots (3.1 vs. 1.6 g PyC/g-soil, gain of 98%), resulting in stocks of PyC doubling from 4.1 to 8.2 kg PyC/ha (Figure 3d). Thus, repeated burning increased the remaining C in a

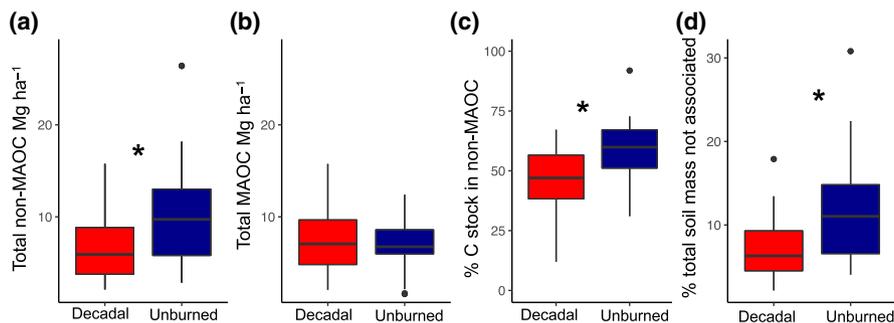


FIGURE 2 Stocks of mineral soil organic carbon (C) in the top 0–5 cm in burned vs. unburned treatments partitioned by carbon (a) free from minerals (non-MAOC) and (b) associated with minerals (MAOC). (c) Percent of total C that is non-MAOC. (d) Percent of total soil mass that is non-MAOC. All panels are box and whiskers plots and asterisks indicate an effect of fire ($p < 0.10$) using linear mixed-effects models

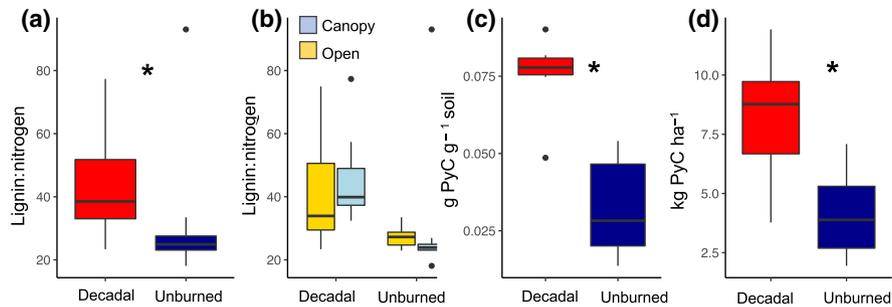


FIGURE 3 Fire effects on potential indicators of chemical recalcitrance. (a) Lignin:nitrogen ratio in the forest floor. (b) Lignin:nitrogen ratio in the forest floor partitioned by samples collected under trees or in areas away from trees. (c) Pyrogenic carbon (PyC) relative to total soil carbon. (d) Stocks of PyC. All panels are box and whiskers plots and the asterisk indicates significant effects ($p < 0.05$; no asterisk in (b) signifies a lack of an effect of tree canopy)

pyrogenic form, but the contribution to total stocks was relatively small.

3.6 | Fire reduced CO_2 respiration and extracellular enzyme activity

To test how fire impacted soil organic C decomposition, we measured both total CO_2 production via respiration and the potential decomposition of different forms of organic matter via extracellular enzyme assays. Repeatedly burned plots had $55 \pm 5\%$ lower CO_2 respiration rates on average across the 35-day incubation period ($F_{1,28} = 16.5$, $p < 0.001$, Table 2). The respiration rates declined through time, but the difference between the treatments persisted (59% lower after 7 days, 60% lower after 35 days, Figure 4a; fire: $F_{1,24} = 26.6$, $p < 0.001$, time: $F_{4,24} = 5.3$, $p = 0.003$, Table 2). Incorporating soil C as a covariate illustrated that even for a given amount of soil C, respiration rates were lower in burned plots, demonstrating that lower respiration was not just due to lower SOM concentrations (fire: $F_{1,20} = 17.1$, $p < 0.001$, total soil C: $F_{1,122} = 136$, $p < 0.001$, Figure 4b; mixed-effects models on individual samples nested within replicate plots). Consequently, fire reduced soil C losses as CO_2 , consistent with the hypothesis that greater recalcitrance of SOM is coupled with lower decomposition-based soil C losses.

Extracellular enzyme assays revealed that fire likely reduced decomposition of a wide variety of soil organic compounds such as cellulose, hemicellulose, starch, chitin, organic phosphorus, and polyphenols, as indicated by lower soil enzyme activity with burning ($p \leq 0.05$, Figure 4c, Table 2, Table S2). The only enzyme that was higher in the burned treatment was peroxidase, which degrades polyphenols, including PyC, and lignin (Figure 4c, Table 2, Table S2). Enzyme activity was significantly and positively related to soil C for all enzymes except peroxidase (Figure S3, Table S2). Similar to respiration, accounting for C did not remove the effect of fire on enzyme activity (Figure S3). Thus, fire reduced the potential activity of decomposers that could not be explained by losses of SOM alone, which occurred for a wide variety of organic compounds.

TABLE 2 Descriptive statistics of microbial activity (means and standard errors) between fire treatments. Respiration rates are reported for each extraction period (number indicating day) and units are $\text{ppm-CO}_2 \text{ g-soil}^{-1} \text{ hr}^{-1}$. Enzyme notations are as follows: CBH = cellobiohydrolase, BG = β -glucosidase, AG = α -glucosidase, BX = β -xylosidase, NAG = N-acetyl- β -D-glucosaminidase, AP = acid phosphatase, PO = phenol oxidase, PX = peroxidase. C-hydro = sum of hydrolytic C-acquisition enzymes, C-oxi = sum of oxidative C-acquisition enzymes. Units for enzymes are $\text{nmol g-soil}^{-1} \text{ hr}^{-1}$

Variable	Decadal	SEM	Unburned	SEM
Resp-7	69.8	16.5	169.7	48.6
Resp-14	57.1	8.0	127.4	34.8
Resp-21	48.1	8.9	91.9	20.2
Resp-28	37.6	4.3	85.7	23.3
Resp-35	25.5	4.0	63.0	17.8
AG	22	6.0	41	14
BG	537	90	890	274
BX	61	13	157	33
CBH	90	27	177	65
C-Hydro	710	135	1282	379
PO	165	13	375	83
PX	857	83	753	270
C-oxi	1022	90	1127	349
NAG	286	92	834	331
AP	523	108	1241	321

3.7 | Factors influencing enzyme and respiration activity

We next evaluated whether changes in the potential turnover of SOM were associated with nutrient content and forms of SOM. Lower IN concentrations were correlated with lower potential NAG activity ($r^2 = 0.49$), the enzyme that breaks down chitin, both in absolute amounts and when accounting for total SOM ($F_{1,38} = 22.7$, $p < 0.001$ and $F_{1,30} = 6.7$, $p = 0.015$, respectively). In contrast to lower N, total P and inorganic P were not affected by fire ($p > 0.50$ and $F_{1,10} = 2.3$, $p = 0.16$, respectively) nor was inorganic P related to

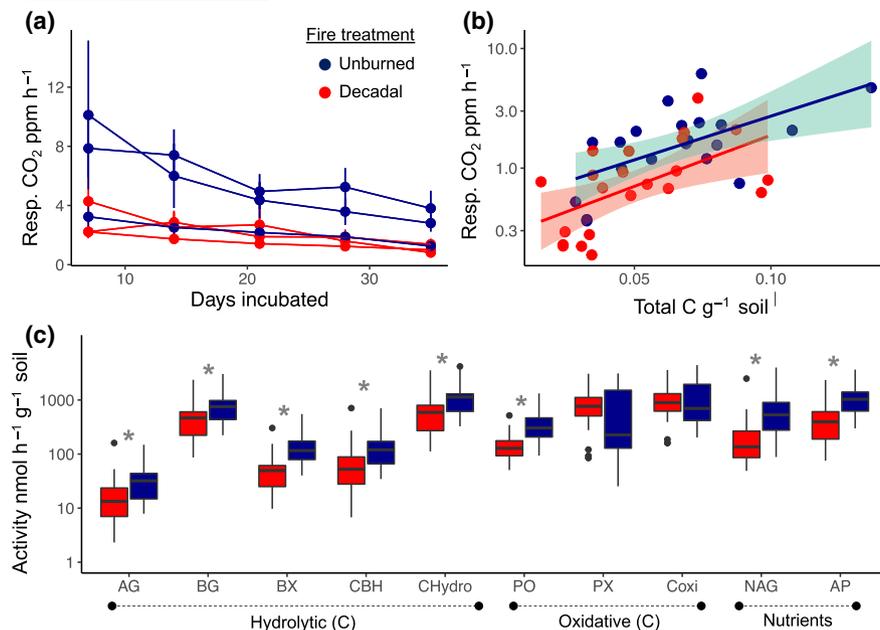


FIGURE 4 Microbial activity in the different fire treatments (unburned vs. one burn per decade). (a) Respiration rates of the incubations with each line representing a replicate plot, and the standard error reflecting the variability in the sub-samples within a plot. (b) Regression between initial total soil carbon concentration and the respiration rates of those samples. Shading illustrates the standard error of the regression model. Rates of CO₂ production are expressed as the concentration in the headspace of the incubation jar per-hour per-gram soil. (c) Box and whiskers plots of potential extracellular enzyme activity partitioned into hydrolytic (carbon acquisition) and oxidative (carbon acquisition) as well as nitrogen and phosphorus acquisition (abbreviations defined in Table 2). NAG is also used to acquire carbon. Units express the rates of substrate reacted upon per-hour per-gram soil. Asterisks indicate significant effect of fire using mixed-effects models ($p < 0.05$)

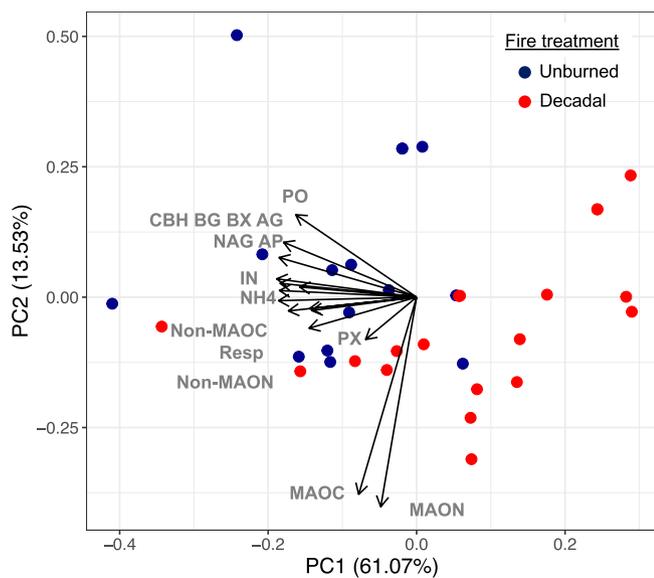


FIGURE 5 Principal components analysis of microbial activity and mineral soil carbon (C) and nitrogen (N) content across soil samples taken from burned and unburned plots. The arrows indicate the loadings of the variables (Resp = respiration, MAO = mineral-associated organic). The colors of the points correspond with fire treatments. Enzyme labels are given in Table 2 and the components are given in Table S3

AP enzyme activity (enzyme involved in mineralizing organic P to PO₄, Figure S4). Thus, fire reduced the bioavailability and potential turnover of N, both in terms of the proportion of total N free from

minerals and the concentration of ammonium, illustrating a decline in N availability.

A principal components analysis of enzyme activity, respiration, soil C and N partitioned by fractions, and inorganic N revealed a strong coupling between the different properties of SOM and decomposition activity. The first two principal axes explained 78% of the variance (Figure 5), with the first axis partitioning most of variability between the two fire treatments. The first axis depended on non-mineral-associated organic matter, inorganic N, respiration rates, and most enzyme activity rates. The second axis depended on mineral-associated organic matter. All enzymes were positively coupled with one another except for peroxidase, which was orthogonal (Figure 5). Inorganic N was the most closely correlated with the enzymes and CO₂ respiration was most strongly coupled with non-MAOC and non-MAON (Figure 5). Both C and N associated with minerals were orthogonal to the other variables and not along the axis separating the fire treatments (Figure 5). Consequently, prescribed burning dampened the amount and turnover of soil organic matter primarily within the non-mineral associated pool. Nitrogen dynamics were coupled with C, suggesting there is a concurrent decline in N availability and turnover that likely interacts with C decomposition.

4 | DISCUSSION

The reintroduction of fire after a century of fire suppression altered the ecosystem C and N cycles, but in ways that suggest resilience in

the capacity of these coniferous forests to store soil C. Although fire reduced potential inputs into the mineral horizon, SOM became less accessible and more recalcitrant, which was coupled with declines in heterotrophic respiration and activity of extracellular enzymes that decompose both labile and recalcitrant organic matter compounds (Figure 6).

Changes in tree basal area and abundances were primarily due to a loss of smaller trees, with the large fire-resistant trees persisting. The sustained production of leaf litter resulting from the stable tree population allowed for production to compensate for losses of the litter layer 4–5 years after a fire (although litter was combusted in each fire event, Figure 6). Repeated fire decreased C and N in the forest floor over time, consistent with past studies in coniferous forests comparing plots with sequential burning (Binkley et al., 1992; Burns, 1952; Hatten et al., 2008; McKee, 1982). The change in forest floor stocks in our study was larger than average responses of many other coniferous forests (Nave et al., 2011), likely a result of historical fire exclusion and the presence of giant sequoias that create large detritus stocks. The drop in forest floor C and N concentrations suggests a disproportionate reduction during volatilization, consistent with the thermal thresholds for C and N to be volatilized being lower than other elements (Butler et al., 2019; Raison et al., 1985). Combined with the rise in the lignin:N ratio of the detritus, it is likely that there are lower inputs from the forest floor into the mineral soil in the burned plots.

Repeated burning reduced soil C in the fraction of organic matter not associated with minerals resulting in SOM on average being less accessible to microbes (Cotrufo et al., 2013; Sollins et al., 1996). Our observed loss of only non-MAOC is consistent with the hypothesis that a reduction in new inputs results in a depletion of organic

matter available to microbes. We cannot rule out that the difference in loss susceptibility may arise due to greater propensity for non-MAOM to be directly volatilized, but are not aware of empirical studies demonstrating this phenomenon exists. Lower C:N ratios are unlikely to arise due solely to higher inputs from the N-fixing plants that colonized the burned plots because there was no change in the C:N of non-MAOM, which is more reflective of fresh plant inputs. In turn, the decline in C:N ratios of MAOM suggests a greater recycling of SOM from microbial biomass (lower C:N ratio) rather than fresh inputs (higher C:N ratio) (Cotrufo et al., 2019; Kögel-Knabner et al., 2008), although further analysis is needed to verify the exact mechanism leading to shifting C:N ratios. Taken together, repeated burning reduced potential fresh biomass inputs into mineral soil but caused a transition toward a greater fraction of the SOM inaccessible for decomposition.

In addition to lower accessibility, fire caused several changes in the chemical properties of the forest floor and mineral horizon that can result in higher SOM recalcitrance (Figure 6). First, increases in the lignin:N ratio in the forest floor suggest lower decomposition and N release (Melillo et al., 1982; Swift et al., 1979) from the forest floor that presumably moves down into the mineral profile. Second, fire increased PyC, both in terms of absolute stocks and the proportion of the soil C, consistent with previous studies (Matosziuk et al., 2019; Preston & Schmidt, 2006; Santín et al., 2015). Pyrogenic C is thought to have some of the longest mean residence times in soils (although see Zimmermann et al., 2012), suggesting that an increase in this pool may be especially important for reducing long-term C losses. Our estimates of PyC are likely to be conservative because our method to quantify PyC, BPCA, is known to underestimate the amount PyC in the soil, in some cases by as much as twofold

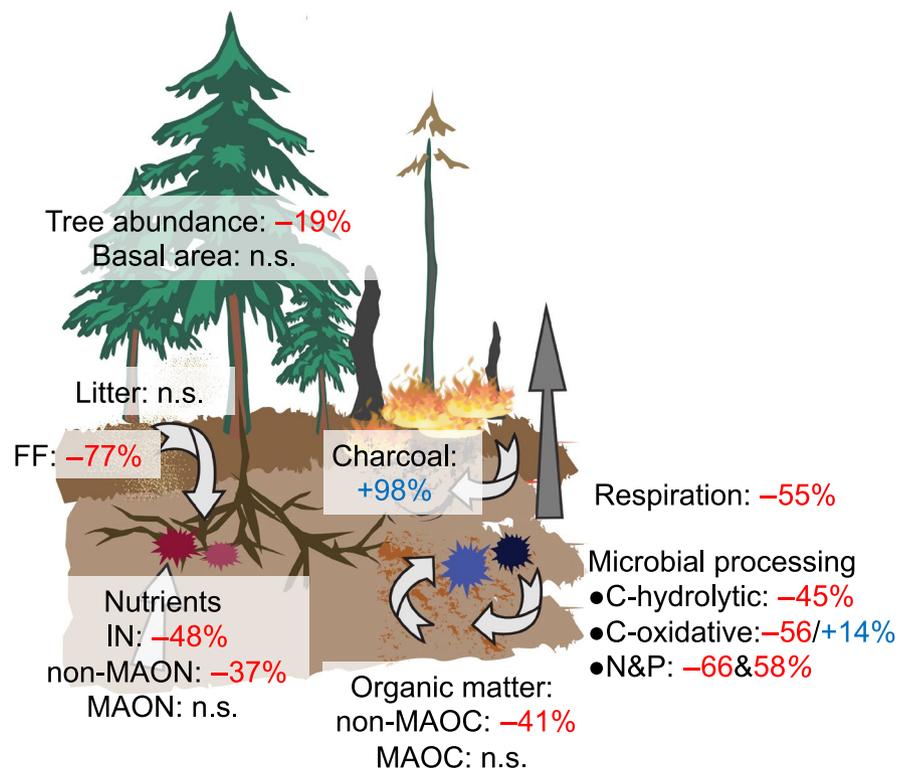


FIGURE 6 Fire reduced the stock of carbon (C) in the ecosystem but triggered several compensatory responses that may lead to a reduction in long-term losses of soil organic matter (Tables 1 and 2). The values are percentage differences (burned – unburned)/unburned, with the red and blue illustrating losses and gains, respectively. MAO refers to the C and nitrogen (N) associated with minerals. Microbial processing refers to extracellular enzyme activities. The C-oxidative enzymes have two values because phenol oxidase and peroxidase responded differently. IN = inorganic N. Tree abundance is for basal area. FF = forest floor. “n.s.” refers to not significant

to fourfold (Glaser et al., 1998). Additionally, we are unable to determine how combustion of PyC might have changed stocks (Doerr et al., 2018), but the significant differences even after three fires illustrate that PyC accumulates even when fires recur every decade.

The absolute magnitude of change in PyC was lower than MAOM: fire increased the relative contribution of the two stabilizing pools to C stocks from 3.2% to 7.5% for PyC and 46% to 58% for MAOM in the unburned vs. burned plots, respectively. Consequently, the stabilization of SOM by minerals accounted for the largest shift in stability and may help to explain the variable response of SOM in coniferous forests to repeated burning (Burns, 1952; Hart et al., 2005; Hatten et al., 2008; McKee, 1982; Pellegrini et al., 2018). Pyrogenic C formation could be more important in other systems that have different edaphic properties that favor PyC formation (e.g., lower soil oxygen) (Flanagan et al., 2020), but we propose that in aerobic soils with sufficient matrix stabilization potential, fire-driven changes in mineral associations may be a more important stabilization mechanism.

Fire also reduced the activity of decomposers, consistent with the observed increases in the recalcitrance of the residual SOM (Figure 6). First, respiration incubations illustrated that CO_2 production both per-soil mass and per-soil C was significantly lower in the burned plots. Thus, fire-driven reductions in decomposition activity cannot be explained by a reduction in total organic matter alone. Rather, decomposition shifts are likely a result of changes in the physicochemical properties of SOM that we observed, as well as changes in the biomass, composition and life-history characteristics of the microbial community known to occur in coniferous forests (Hart et al., 2005; Pressler et al., 2018; Whitman et al., 2019). The relative sensitivity of some extracellular enzymes over others points to important shifts in the potential substrate being decomposed from more cellulose-based compounds in the unburned plots to more phenol-based compounds in the burned plots.

Although our decomposition assays were laboratory incubations, they agree with observed fire-driven declines in field measurements of soil CO_2 fluxes taken from other sites (Dooley & Treseder, 2012; Pellegrini et al., 2020). Further work monitoring changes throughout the season will give insight into how decomposition dynamics change through time (Boerner et al., 2005).

Inorganic N was strongly positively coupled with the changes in extracellular enzymes. Part of the correlation between C-acquisition and P-acquisition enzymes may be a result of IN limiting their activity while the correlation with the N-acquisition enzyme, NAG, may be caused by NAG limiting the production of IN. NAG is also used to acquire C, limiting our ability to infer that its production is reflective of greater N demand. We did find a decline in AP, suggesting the potential turnover of P is lower with fire, but inorganic P did not change, pointing to fire promoting N limitation relative to P limitation. The fire-driven promotion of N limitation is consistent with hypotheses that fire recycles P but volatilizes N because P has a much higher temperature thresholds for volatilization (Neary et al., 2005; Raison, 1979).

Other factors that we did not measure could also explain changes in SOM. First, the stabilization of organic matter by minerals may be enhanced by fire-driven changes in the properties of minerals or physical structure in the soil that could further promote the formation of MAOM (Ketterings et al., 2000), albeit this tends to require very high temperatures. Second, fire could reduce decomposition by shifting the functional groups of microbes by filtering for taxa with different rates of growth and decomposition, not necessarily because of changes in the properties of SOM (Day et al., 2019). Third, measuring microbial biomass, which fire can change in temperate coniferous forests (Choromanska & DeLuca, 2001), will improve our understanding of how shifts in microbial communities contribute to the observed differences in decomposition activity. Fourth, nutrient losses could limit microbial biomass and activity (Hartman & Richardson, 2013), which may explain the positive correlation between IN and both respiration and extracellular enzyme activity. Finally, changes in plant communities with different symbiotic strategies such as N-fixing shrubs or trees with different mycorrhizal strategies may also contribute to changes in soil C and nutrient turnover (Pellegrini et al., 2021). These are just a few of the potential processes that may contribute to fire-driven changes in organic matter decomposition, but all deserve future study.

Although our study was conducted in one ecosystem and compared one frequency treatment, the findings are relevant to other contexts. Our site gives insight into the reintroduction of a fire frequency thought to capture the historical ("natural") fire frequency in this particular coniferous forest. The exact natural fire frequencies in other ecosystems differ, and as a consequence so do the frequency of fires in manipulation experiments (Hatten et al., 2005). To control or account for this, perhaps the restoration of fire back to its historical interval should be the main treatment effect used to compare results across sites to evaluate whether there are generalizations in how fire restoration impacts SOM.

5 | CONCLUSIONS

Our results demonstrate that repeated prescribed burning caused multi-faceted changes in the storage and turnover of SOM, which are transformations that point to fire promoting more stable mineral C stocks in coniferous forests. Consequently, while fire may reduce C storage over the short term, there are multiple transformations that increase the recalcitrance of the residual SOM pool and potentially buffer against C emissions due to heterotrophic respiration over the long term. The relevance of fire-driven reductions in heterotrophic respiration for the total ecosystem C flux is unclear and needs to be compared to the magnitude of combustion fluxes. Given that C emissions from soil C respiration globally are ~40-fold higher than those from biomass combustion, the impact of fire on this flux may play an important role in the C balance of ecosystems, especially those with large pools of SOM. Hence, fire-driven changes in the stability of SOM may offer an important management opportunity to promote

long-term C storage in soil, especially if it reduces potential vulnerability to other global change drivers.

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DATA AVAILABILITY STATEMENT

Data are available on Figshare <https://doi.org/10.6084/m9.figshare.14402129>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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