Post-fire effects of soil heating intensity and pyrogenic organic matter on microbial anabolism

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Abstract Wildfires cause direct and indirect CO_2 emissions due to combustion and post-fire decomposition. Approximately half of temperate forest carbon (C) is stored in soil, so post-fire soil C cycling likely impacts forest C sink strength. Soil C sink strength is partly determined by soil microbial anabolism versus catabolism, which dictates the amount of C respired versus stored in microbial biomass. Fires affect soil C availability and composition, changes that could alter carbon use efficiency (CUE) and microbial biomass production, potentially influencing C sink recovery. Wildfire intensity is forecast to increase in forests of the western United States, and understanding the impacts of fire intensity on microbial anabolism is necessary for predicting fire-climate feedbacks. Our objective was to determine the influence of soil heating intensity and pyrogenic organic matter (PyOM) on microbial anabolism. We simulated the effects of fire intensity by heating soils to 100 or 200 °C for 30 min in a muffle furnace, and we amended the soils with charred or uncharred organic matter. Higher intensity soil heating (200 °C) led to lower microbial biomass carbon (MBC) accumulation, greater C respiration, and lower CUE proxies compared to unheated soils. Conversely, lower intensity heating (100 °C) yielded MBC accumulation and estimated CUE that was similar to unheated soils. Soils amended with PyOM exhibited similar MBC accumulation compared to uncharred organic matter, but lower CO_2 emissions. These results indicate that high intensity soil heating decreases soil C-sink strength over the short-term by decreasing the amount of microbial anabolism relative to catabolism.

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Introduction

Wildfire disturbances are common in mixed-conifer forests of the western United States, resulting in direct CO_2 emissions during biomass combustion (Flannigan et al. 2009; Chen et al. 2017). Over the long-term, these emissions can have a neutral impact on atmospheric carbon (C) concentrations if an equal amount of CO_2-C is assimilated by plant regrowth during forest recovery, but, over the short-term, CO_2 emissions represent a decrease in the size of forest C sink (Bowman et al. 2009; Loehman et al. 2014). Temperate forests store $\sim$ 50% of ecosystem C in soils (Pan et al. 2011), so losses to soil C due to combustion (Campbell et al. 2007), and post-fire necromass decomposition could make substantial contributions to fire-induced C emissions (Meigs et al. 2009; Zhao et al. 2012; Campbell et al. 2016). Understanding the amount of soil C retained versus respired in the aftermath of fire is thus necessary for accurate representation of the influence of fire on the strength of the forest C sink.

The relative amount of microbial anabolism (biomass production) versus catabolism (C mineralization) determines the fate of soil C over both short and long timescales (Schimel and Schaeffer 2012; Liang et al. 2017). Microbial carbon use efficiency (CUE) represents the balance between anabolic and catabolic processes over short timeframes (Manzoni et al. 2012; Sinsabaugh et al. 2013; Spohn et al. 2016a). An index of the amount of total C uptake that is assimilated into microbial biomass during decomposition (Geyer et al. 2016), CUE is the proximal driver determining whether C is lost to atmosphere versus retained in soil (Cotrufo et al. 2013). Microbial anabolism also influences C storage over longer time scales because microbial necromass is more efficiently stabilized in soils compared to other types of organic matter and may account for > 50% of total soil C (Liang et al. 2019; Ni et al. 2020). The physiological responses of soil microbes to fire may therefore dictate the magnitude of post-fire soil emissions in the short term, and affect the size of soil C stocks and soil-climate feedbacks over the long-term (Allison et al. 2010; Frey et al. 2013; Wieder et al. 2013; Liang et al. 2019). Identifying the mechanistic processes that impact C sequestration following disturbance has been identified as a key component of managing forests for C storage in the future (Birdsey et al. 2006).

Determining post-fire patterns in microbial anabolism and catabolism will thus contribute to understanding of C source/sink strength of burned ecosystems.

CUE and microbial biomass production are influenced by substrate quality and complexity, microbial community structure, nutrient availability, and environmental factors such as soil temperature and moisture (Frey et al. 2013; Geyer et al. 2016; Spohn et al. 2016b; Liang et al. 2019; Domeignoz-Horta et al. 2020). For example, CUE is lower for structurally complex/stable molecules (e.g. lignin, aromatic molecules) that require a larger enzyme investment compared to simpler, labile molecules (e.g. sugars) (Manzoni et al. 2012; Frey et al. 2013; Sinsabaugh et al. 2013). Relatedly, CUE decreases when nutrient availability is low due to microbial direction of metabolism toward catabolic processes that support nutrient acquisition, for example mining of nutrients from complex organic molecules (Geyer et al. 2016; Spohn et al. 2016b; Chen et al. 2020; Soong et al. 2020). In some cases, CUE increases with microbial diversity (Domeignoz-Horta et al. 2020), and may differ for fungi versus bacteria as consequence of differences in microbial biomass stoichiometry (Six et al. 2006; Keiblinger et al. 2010; Manzoni et al. 2012; Sinsabaugh et al. 2013).

Fire alters soil C chemistry, nutrient availability, and microbial community structure, with potentially contrasting impacts on microbial biomass production. Fire induces temporary pulses in soil extractable organic carbon (EOC) (Wang et al. 2012) and inorganic nitrogen (N) (Wan et al. 2001), potentially increasing microbial biomass production and CUE via positive effects on labile C and N availability. However, fire also generates pyrogenic organic matter (PyOM), which is more chemically stable and aromatic than its precursor material (Preston and Schmidt 2006; Bird et al. 2015), and thus may be used less efficiently. The conversion of organic matter to PyOM may therefore lead to less bioavailable C and lower microbial biomass production. There is a dearth of information on the influence of PyOM on microbial anabolism in burned systems, but biochar amendment studies can provide some insight. Biochar is a form of PyOM produced under controlled pyrolysis conditions and applied as a soil amendment in agricultural systems. Biochar additions to soil have frequently been found to increase microbial biomass, potentially via beneficial effects on soil nutrient retention, pH, soil moisture,

and by providing microhabitat (Lehmann et al. 2011). A study in temperate pastures found that biochar-CUE was lower than values typically reported for non-biochar feedstock (Fang et al. 2018), but other studies have found that biochar increases overall CUE via beneficial effects on soil bio-physiochemical properties (Jiang et al. 2016; Guo et al. 2020). In addition to influencing C and N chemistry, fire increases the predominance of bacteria relative to fungi (Dooley and Treseder 2012; Pressler et al. 2018), in part due lower sensitivity of bacteria to soil heating (Neary and DeBano 2005). Bacteria may use simple C substrates more efficiently than fungi when N is abundant, exhibit rapid growth rates, and act as the primary decomposers of the labile fraction of PyOM; in contrast, fungi may more efficiently decompose the recalcitrant fraction of PyOM (Steinbeiss et al. 2009; Lehmann et al. 2011; Yu et al. 2018; Pérez-Guzmán et al. 2020).

Fire intensity is predicted to increase in ecosystems across the globe (Flannigan et al. 2009), and there are reasons to suspect that effects of fire on microbial anabolic and catabolic processes vary with fire intensity and/or severity. For example, the amount of PyOM generated has been shown to increase with fire intensity (Czimczik et al. 2003; Sawyer et al. 2018) and severity (Miesel et al. 2015), which could have negative impacts on microbial anabolism by decreasing C bio-availability, or positive impacts via beneficial effects on soil properties. Changes to labile C and nutrient availability could also influence microbial anabolism, but there is a dearth of information regarding the immediate impacts of fire intensity on EOC and inorganic-N pools. Used as an alternative to field-based research, lab-based soil heating studies have indicated that EOC increases with soil heating temperature up to at least ∼ 300 °C (Bárcenas-Moreno and Bååth 2009). Various experiments have found that inorganic-N concentrations exhibit no changes to moderate increases in soils heated from 150–210 °C (Serrasolsas and Khanna 1995; Choromanska and DeLuca 2002; Prieto-Fernández et al. 2004), with larger increases beginning to occur at ∼ 400 °C (Raison 1979; Choromanska and DeLuca 2002). Laboratory-based heating studies have also assessed the effects of heating intensity on microbial biomass carbon (MBC), and microbial community structure (Serrasolsas and Khanna 1995; Díaz-Raviña et al. 1996; Fernández et al. 1997; Prieto-

Fernández et al. 1998; Guerrero et al. 2005; Bárcenas-Moreno and Bååth 2009), but whether these characteristics lead to post-heating changes in the balance between microbial anabolism and catabolism is not well studied.

Small changes in the balance between microbial anabolism and catabolism can have substantial impacts on soil C emissions and stocks (Allison et al. 2010; Schimel and Schaeffer 2012; Wieder et al. 2013; Liang et al. 2017), potentially exacerbating or modulating the effects of fire intensity on ecosystem C loss. Here, our objectives are to determine (1) how a proxy for CUE and the balance of MBC accumulation versus C mineralization vary with soil heating intensity and (2) whether PyOM influences these processes differently in soil subjected to contrasting soil heating intensities. We hypothesized that (1) estimated CUE and MBC accumulation will increase with soil heating intensity due to greater EOC availability, and (2) estimated CUE and MBC accumulation will be higher in soils amended with uncharred organic matter compared to charred organic matter regardless of soil heating intensity. We used factorial incubation experiments in which we subjected a forest soil to two levels of heating intensity and applied two C substrates in charred and uncharred form and multiple types of charred and uncharred plant litter to determine a proxy for CUE and the level of MBC accumulation versus C mineralization.

Materials and methods

Site description and sample collection

We collected soil and litter samples from the Plumas National Forest in the Sierra Nevada Mountain Range, California, USA. The ecosystem is a dry mixed-conifer forest dominated by *Pinus ponderosa, P. lambertiana, P. jeffreyi, Abies concolor, Pseudotsuga menziesii,* and *Calocedrus decurrens,* with lesser cover by *Quercus kelloggii*. Dry mixed-conifer forests of the region are fire-adapted, having exhibited a mean fire rotation of 23 years prior to Euro-American settlement (1500–1850 C.E.) (Mallek et al. 2013). Soils in our plots are from the Skalan series, a loamy-skeletal, isotic, mesic Vitrandic Haploxerlaf with slightly acidic pH (Soil Survey Staff 2018). The

30 year mean annual precipitation is 1080 mm and mean annual temperature is 10.6 °C.

From four sites within the forest, we collected mineral soil at two sampling points located 40 m apart. At each sampling location, we removed the litter (Oi) and duff (Oe + Oa) layers overlying the mineral soil, and then collected mineral soil to 10 cm depth using a 10 cm diameter soil auger. We collected three types of litter from the forest, including *Q. kelloggii* (black oak), *P. ponderosa* (ponderosa pine), and mixed litter. We collected black oak and ponderosa pine litter samples from the litter surface directly under a tree of each species, and we collected only leaf material that was recognizable as belonging to the target tree species. The mixed litter sample included litter recognizable as deriving from *P. ponderosa* and litter that was derived from either *A. concolor*, *P. menziesii* or both. Mineral soils and litter samples were stored on ice until transported to the lab, after which mineral soils were stored at -20 °C, and litter samples were air-dried and stored at ambient temperature until the commencement of the lab experiments.

Generation of pyrogenic organic matter

We generated PyOM from two simple C substrates (glucose, ascorbic acid) and from the three litter types collected in the field. Glucose is commonly used in CUE and substrate induced respiration experiments, and ascorbic acid has been demonstrated to effectively induce differential respiration responses among soil types (Degens and Harris 1997). We charred glucose (210 °C) and ascorbic acid (200 °C) at temperatures at the lower end of their thermal degradation ranges (Örsi 1973; Jingyan et al. 2013). We weighed ~ 5 g of each substrate into a small aluminum dish, covered the dish with aluminum foil, and heated in a muffle furnace by ramping to the target temperature over 30 min and then holding at temperature for another 30 min.

We sterilized the litter via autoclave (121 °C) for 30 min and then oven-dried overnight at 65 °C. We charred the litter by placing aluminum-foil wrapped litter in a muffle furnace that had been pre-heated to 200 °C and held at temperature for one hour. We then ramped the temperature to 300 °C and held at temperature for another hour. The litter was then homogenized using a mortar and pestle to pass a 500-micron sieve. We measured charred substrate and

litter C and N concentrations using a dry-combustion elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA).

Experimental design

We conducted two experiments to assess the amount microbial anabolism versus catabolism after a soil heating disturbance. In one experiment, we measured a proxy for community-scale CUE, which quantifies gross production efficiency of the microbial community over short timescales (up to 48 h) before biomass turnover occurs (Geyer et al. 2016). In the second experiment, we measured net MBC accumulation and net C mineralization over 14 days. To account for potential heterogeneity of soil across our sampling sites, we composited equal masses of sieved (2 mm) mineral soil from the plots prior to applying soil heating treatments.

Estimated carbon use efficiency experiment

We determined impacts of soil heating and substrate pyrolysis on a CUE proxy using a factorial experiment in which we applied two levels of soil heating (plus unheated controls) and two labile substrate types (glucose, ascorbic acid) in charred and uncharred form. We also included substrate controls in which only DI water was added. Each treatment was replicated 18 times, equally divided between two incubation blocks that were performed consecutively. For each incubation block, we pre-incubated nine soil replicates for seven days. For each replicate, we weighed 130 g (dry mass equivalent (dme)) of soil into 0.473 L mason jars, added water to bring soil moisture to 40% water-holding capacity (WHC), and incubated in the dark at ambient temperature (~ 23 °C).

After pre-incubation, we applied heat treatments of 100 °C or 200 °C by covering the mason jars with aluminum foil and heating the jars in a muffle furnace for 45 min. Following heating, we weighed 5 g (dme) subsamples into 50 mL centrifuge tubes, adjusted soil moisture to 50% WHC, capped the tubes with septa-fitted lids, and incubated in the dark at ambient temperature. Before adding substrates, we measured CO_2-C respiration daily until respiration rates differed by < 10% among soil heating treatments, which occurred after five days. We waited until respiration

rates were similar so that CUE estimates would not be influenced by differences in basal respiration. We measured soil respiration rates by flushing the incubation tubes with ambient air, tightly capping, and measuring CO_2-C concentrations of 1 mL gas aliquots after $\sim$ 30 min and again after $\sim$ 6 h using an infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA).

On the sixth day after heating, we added charred and uncharred substrates to the incubation tubes at 1 mg substrate-C g^{-1} soil in 0.5 mL DI water. We monitored respiration rates over 24 h by measuring CO_2-C concentrations after $\sim$ 1.5, 4, 8, 12, and 24 h. We extracted three replicates per treatment in each block for determination of EOC immediately after substrate addition, and we extracted the remaining six replicates after 24 h. EOC extractions were performed by adding 25 mL K_2SO_4 directly to the incubation tubes, agitating on a reciprocating shaker for 1 h, and filtering through 11 μm pore-size filters (Whatman Grade 1). We determined EOC concentrations in the extracts spectrophotometrically after potassium-dichromate oxidation (Cai et al. 2011). We calculated a proxy for CUE over the 24 h after substrate addition as:

$$CUE = \frac{(\Delta EOC - \Sigma CO_2\text{-}C)}{\Delta EOC} \quad (1)$$

where ΔEOC is the change in EOC over the 24 h after substrate addition and is assumed to represent total microbial C uptake; ΣCO_2-C is cumulative C respired over the 24 h (Tiemann and Billings 2011; Geyer et al. 2016). This calculation is a proxy for CUE because we did not apply isotopically labeled substrates and so cannot directly quantify the amount of substrate-C uptake and respiration.

Microbial biomass accumulation experiment

We determined impacts of soil heating and substrate pyrolysis on MBC accumulation and cumulative C mineralization using a factorial experiment in which we applied two levels of soil heating (plus unheated controls) and three litter types (ponderosa pine, black oak, and mixed litter) in charred and uncharred form, plus unamended controls. Each treatment was replicated 18 times, equally divided between two incubation blocks that were performed consecutively, except for mixed litter treatments which were replicated nine times and incubated in a single block.

Soil pre-incubation was performed as described above, except that replicates were 150 g (dme) and pre-incubated for ten days. After pre-incubation, we applied heat treatments of 100 °C or 200 °C as described above. We oven-dried soil subsamples from six replicates of each heat treatment at 65 °C for 24 h, pulverized the soil in a ball mill, and submitted to the University of Georgia Stable Isotope Ecology Laboratory for C and N analysis.

Immediately after applying the heat treatments, we initiated the soil incubation by weighing 10 g (dme) subsamples into 50 mL centrifuge tubes, added 80 ± 5 mg charred or uncharred litter to each tube, mixed by vortexing for 30 s, and added water to bring soil moisture to 50% WHC (n = 6 per treatment combination per block). We incubated the soils for 14 days, measuring respiration on days 1, 2, 3, 4, 5, 7, 9, and 13.

Twenty-four hours after heat treatments, we extracted a subset of unamended samples (n = 6 per heat treatment) for EOC and microbial biomass C (MBC) using direct chloroform fumigation (Witt et al. 2000). We extracted all remaining incubated soils 14 days after applying treatments (n = 12 per treatment combination). We measured MBC as the difference in EOC between fumigated and unfumigated samples. We divided our MBC estimates by a correction factor of 0.33, a published value based on dichromate-oxidation methods performed on a variety of soils, including soils that were similar in pH, total C, and total N to the soils we used for this experiment (Cai et al. 2011). We calculated net accumulation of MBC as the difference in MBC between days 1 and 14 of the incubation.

For one incubation block, we determined fungal and bacterial activity in a subset of soils < 24 h after heating (n = 6 per heat treatment) using selective respiratory inhibition (Anderson and Domsch 1973). Additionally, we incubated an extra set of heated and unheated soils amended with uncharred or charred pine material (n = 6) to determine post-incubation differences in fungal and bacterial activity. For selective respiratory inhibition, we weighed four equal soil masses (2.5 g dme) from each incubation tube into septa-capped 20 mL scintillation vials, applied glucose at 8 mg g^{-1} in 0.2 mL DI water, and agitated on a reciprocating shaker for 1 h. We then applied one of

four biocide treatments to each of the vials: no biocide addition, the bactericide bronopol at 100 μg g^{-1}, the fungicide cycloheximide at 8 mg g^{-1}, or the addition of both biocides at these concentrations. Vials were capped and placed on a reciprocating shaker for 6 h, after which accumulated CO_2-C was measured with an infrared gas analyzer. Fungal and bacterial activity was determined as:

$$Fungal\ (or\ Bacterial)\ Activity = \frac{A - B(or\ C)}{A - D} \quad (2)$$

where A is respiration in the absence of inhibitors, B is respiration in the presence of the fungicide, C is respiration in the presence of bactericide, and D is respiration in the presence of both biocides. The inhibitor additivity ratio (IAR), a measure of non-target or antagonistic effects of the antibiotics was determined as:

$$IAR = \frac{(A - B) + (A - C)}{A - D} \quad (3)$$

The concentrations of glucose and antibiotics for determination of bacterial and fungal activity were selected based on the optimization procedures described by Bailey et al. (2003) using the same soil as was used for the CUE experiments. These preliminary procedures yielded an IAR of 1.01 ± 0.07 (SD). An IAR of 1.0 indicates no non-target or antagonistic effects.

Statistical analysis

We performed all statistical analysis in the R statistical computing environment (v 3.6.1) (R Core Team 2019). We applied linear mixed-models using the nlme package (v 3.1.140) (Pinheiro et al. 2019) to assess the response of C respiration, EOC, MBC, and a CUE proxy to our experimental treatments. All models initially included soil heating level, litter or substrate type, and char status as main effects, all possible two- and three-way interaction effects, and incubation block as a random effect. Interaction effects that exhibited p-values ≥ 0.15 were sequentially removed from the models. For main effects that were significant at α = 0.05, we compared marginal means using Tukey-adjusted p-values using the emmeans package (v.1.4.4) (Lenth 2020). For the MBC accumulation experiment, we also applied general linear models to determine impact of soil heating and uncharred and

charred pine litter on fungal and bacterial activity. We applied one-way ANOVAs to determine the impact of soil heating on C and N concentrations.

Results

Estimated carbon use efficiency experiment

Soil heating caused an immediate increase in soil respiration rate that was positively associated with heating intensity (p < 0.001; Table 1). Compared to unheated soils, heating soils to 100 °C caused a pulse in respiration that lasted two days, and heating soils to 200 °C caused a pulse that lasted four days. By five days after heating, the heated soils did not differ in respiration rates compared to unheated soils. Over five days post-heating, soils heated to 100 °C respired 8.6% more CO_2-C, and soils heated to 200 °C respired 58.9% more CO_2-C compared to unheated soils. Charring of glucose and ascorbic acid resulted in visual changes in appearance (Fig. S1) and slight increases in C concentration (Table 2). We did not have enough soil to measure total C and N in post-heated soils for the estimated CUE experiment, but we used the same soil and heating methods as for the microbial biomass accumulation experiment and so presume soil heating did not affect total C and N (Table 3).

There were significant effects of soil heating (p < 0.001) and a heating × substrate identity interaction (p < 0.001) on the CUE proxy, but no main effects of substrate identity or charring on the estimate (Fig. 1). Soils heated to 200 °C exhibited ∼ 68% lower estimated CUE in response to ascorbic acid application than the other soil heating treatments. The CUE proxy is driven by the level of EOC uptake and CO_2-C respired, so we separately assessed the response of these metabolic functions to the treatments. There were significant effects of soil heating (p < 0.001) and a charring × substrate interaction (p < 0.001) on EOC uptake. Additionally, there were marginally significant differences of substrate identity (p = 0.070) and a heating × substrate interaction (p = 0.067). For soils that received no substrate additions (i.e. water only), unheated and soils heated to 100 °C exhibited similar EOC uptake, whereas soils heated to 200 °C exhibited net negative uptake (i.e. EOC concentrations increased over the incubation)

Table 1 Respiration rate by day and cumulative CO_2-C respired over five days after soil heating and prior to adding substrates for the carbon use efficiency experiment (mean $\pm$ SE)

Respiration Rate (mg CO_2-C kg^{-1} d^{-1})	Unheated	100 °C	200 °C
Day 1	22.74 $\pm$ 0.37a	31.41 $\pm$ 0.50b	43.69 $\pm$ 3.05c
Day 2	25.53 $\pm$ 0.45a	30.15 $\pm$ 1.17b	56.42 $\pm$ 1.22c
Day 3	22.53 $\pm$ 0.43a	23.49 $\pm$ 0.56a	40.41 $\pm$ 0.36b
Day 4	27.34 $\pm$ 0.56a	27.65 $\pm$ 0.57a	36.55 $\pm$ 0.58b
Day 5	31.59 $\pm$ 1.88a	28.18 $\pm$ 1.88a	28.67 $\pm$ 1.82a
Cumulative Respired (mg CO_2-C kg^{-1})	129.73 $\pm$ 2.58a	140.88 $\pm$ 2.68b	206.20 $\pm$ 3.38c

Lower-case letters indicate Tukey-adjusted significant differences in respiration among soil heating treatments ($\alpha = 0.05$)

Table 2 Carbon and nitrogen concentrations for uncharred and charred carbon substrates and litters used in the carbon use efficiency and microbial biomass carbon accumulation experiments

	C (%)		N (%)	
	Uncharred	Charred	Uncharred	Charred
Glucose	40.00	42.61	–	–
Ascorbic Acid	40.92	41.09	–	–
P. Ponderosa Litter	50.62	63.59	0.25	0.43
Q. Kellogii Litter	47.23	65.79	2.54	3.31
Mixed Litter	34.99	52.91	0.59	0.75

Table 3 Total soil carbon and nitrogen concentrations, extractable organic carbon (EOC), and microbial biomass carbon (MBC) 24 h after soil heating and prior to adding litter for the microbial biomass accumulation experiment

	Unheated	100 °C	200 °C
Total C (g kg^{-1})	45.86 $\pm$ 5.18a	45.07 $\pm$ 7.59a	46.60 $\pm$ 15.63a
Total N (g kg^{-1})	1.74 $\pm$ 0.004a	1.72 $\pm$ 0.02a	1.75 $\pm$ 0.04a
EOC (mg kg^{-1})	40.41 $\pm$ 3.14a	60.61 $\pm$ 2.16b	120.94 $\pm$ 2.01c
MBC (mg kg^{-1})	138.08 $\pm$ 13.49b	85.06 $\pm$ 12.24a	134.31 $\pm$ 7.98b

(Fig. 2). For soils receiving ascorbic acid, differences in EOC uptake were driven by heating effects: soils heated to 200 °C exhibited $\sim$ 42% less EOC uptake than unheated soils. In contrast, EOC uptake in soils that received glucose was driven by both charring and soil heating: within heating treatments, EOC uptake was $\sim$ 32–56% lower for charred glucose than uncharred glucose. Within charring treatments, EOC uptake was $\sim$ 27–30% lower for soils heated to 100 °C compared to unheated soils. For CO_2-C respired, there were significant effects of soil heating ($p < 0.001$) and a charring × substrate interaction

($p < 0.001$). For soils that received no substrate additions, soils heated to 200 °C exhibited $\sim$ 13% lower respiration compared to unheated soils (Fig. 2). Similar to EOC uptake, drivers of differences in CO_2-C respiration varied among substrate type. For soils receiving ascorbic acid, differences in respiration were driven by heating, with soils heated to 100 °C respiring $\sim$ 28% less CO_2-C than the other treatments. Soils that received glucose exhibited differences in respiration due to both substrate charring and soil heating. Within heating treatments, soils respired $\sim$ 28–60% less CO_2-C in response to
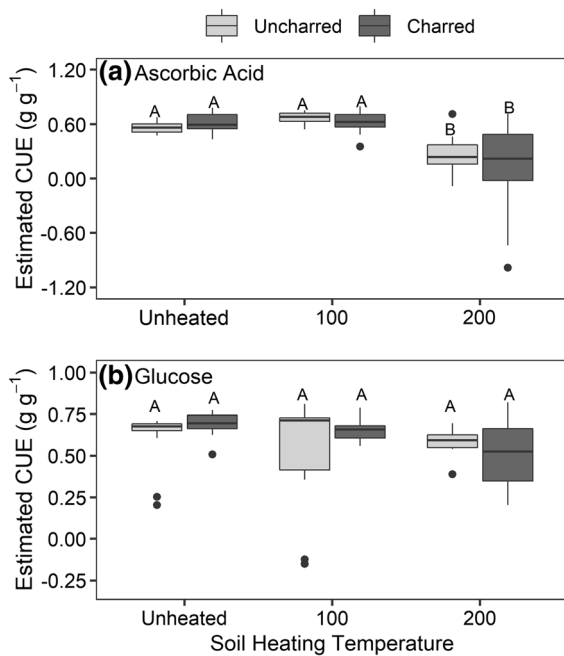
Fig. 1 A proxy for carbon use efficiency (CUE) for unheated soils and soils heated to 100 °C and 200 °C that received ascorbic acid (**a**) or glucose (**b**) in uncharred or charred form. Shading of boxplots indicates whether soils received uncharred or charred substrates. Each boxplot represents the distribution of 12 replicates. Upper-case letters indicate Tukey-adjusted significant differences among the heating × charring treatment combinations within substrate types ($\alpha = 0.05$)

charred glucose application, and, within charring treatments, soils heated to 100 °C respired $\sim$ 6–40% less CO_2-C.

Microbial biomass accumulation experiment

Soil heating led to increases in EOC in soils extracted 24 h after heating: soils heated to 200 °C had approximately two times more EOC than soils heated to 100 °C and three times more EOC than unheated soils (Table 3). Microbial biomass was lower only in soils heated to 100 °C, where MBC was 37–38% lower than for the other treatments 24 h after heating. Soil heating did not alter total C and N concentrations ($p = 0.55$ and $p = 0.68$, respectively). Litter charring led to increases in C and N concentrations compared to uncharred litter (Table 2).

MBC decreased over the incubation period in soils heated to 200 °C for all litter types (Fig. 3), while CO_2-C respiration was elevated by $\sim$ 6–105% compared to unheated soils ($p = 0.001$). CO_2-C respiration

was also elevated in soils heated to 100 °C for soils that received no litter, uncharred oak, and uncharred pine by $\sim$ 32–59%, but these soils also accumulated 28–390% more MBC over the incubation. Within soils that received oak or pine litter, the charred version induced 33–61% less CO_2-C respiration compared to the uncharred counterpart and exhibited similar MBC accumulation. Within soils that received mixed litter, CO_2-C respiration and MBC accumulation were similar between charred and uncharred forms. After the 14-day incubation, soils heated to 200 °C had 28–76% less MBC than unheated soils, and soils heated to 100 °C had similar MBC to unheated soils. MBC was similar between charred and uncharred litter among all soil heat treatments.

EOC uptake over the 14-day incubation increased with soil heating intensity for soils that did not receive litter inputs ($p < 0.001$). EOC uptake was 3.90 ± 1.15 mg kg^{-1} for unheated soils, 21.36 ± 1.47 mg kg^{-1} for soils heated to 100 °C, and 40.53 ± 1.25 mg kg^{-1} for soils heated to 200 °C. When considered on a relative basis, EOC uptake was similar for soils heated to 100 °C ($34.67 \pm 2.38\%$ of initial EOC) and 200 °C ($33.52 \pm 1.03\%$), both of which exhibited greater EOC uptake than unheated soils ($9.66 \pm 2.88\%$). Cumulative 14-day respiration was positively correlated with EOC uptake only for soils heated to 100 °C ($r = 0.61$, $p = 0.037$), and MBC accumulation was positively correlated with EOC uptake only for soils heated to 200 °C ($r = 0.71$, $p = 0.014$).

Despite IAR values being very near the target value of 1.0 during preliminary optimization procedures (see methods), the IAR values we observed during the experiment were substantially higher. Immediately post heating, IAR was 1.42 ± 0.03, and after the 14-day incubation IAR was 1.27 ± 0.04. These values indicate non-target effects of one of the antibiotics (Bailey et al. 2003; Rousk et al. 2009). The bactericide was very likely responsible for the non-target effects because the inhibition resulting from bactericide application alone was similar to the inhibition that resulted from application of both biocides in conjunction. Thus, we limit the dissemination of our results to the impacts of treatments on fungal activity only. Immediately after heating, fungal activity did not differ among the soil heating treatments. After the 14-day incubation, there were significant main effects of soil heating ($p = 0.017$) and litter charring
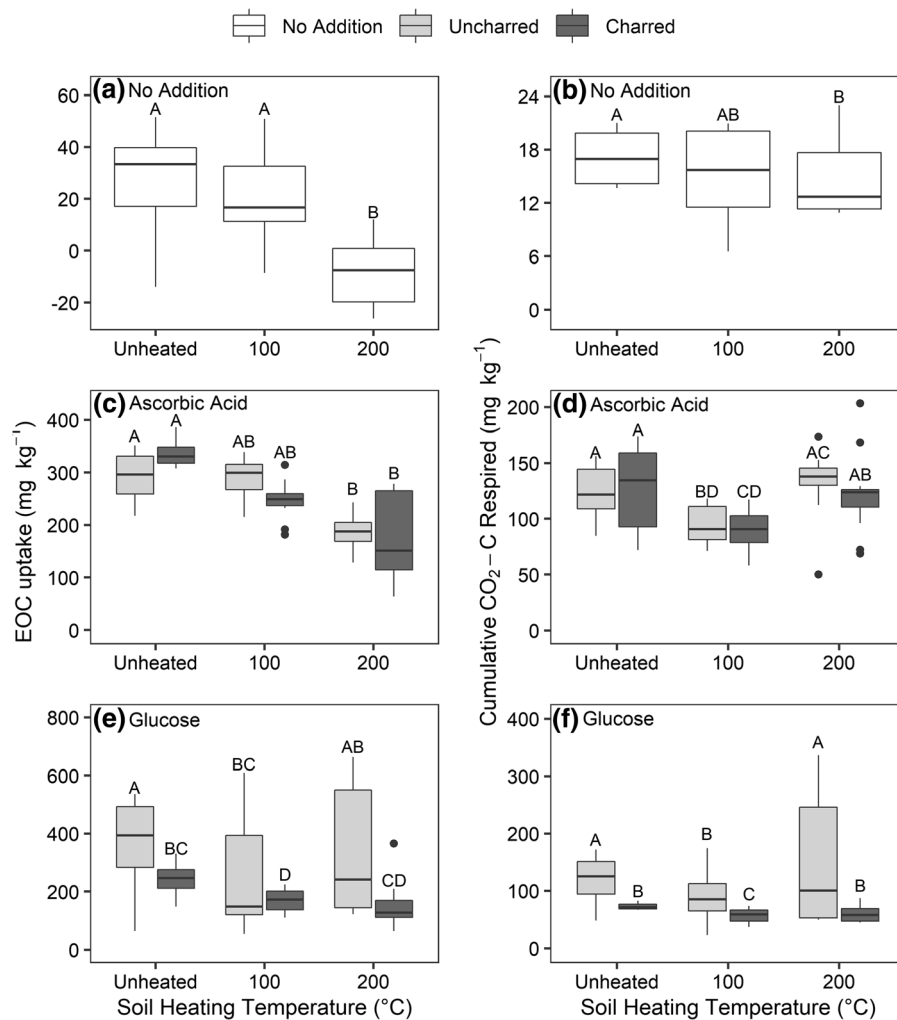
Fig. 2 Uptake of extractable organic carbon (**a** and **c**) and 24-h cumulative respired CO_2-C (column and **d**) for unheated soils and soils heated to 100 °C and 200 °C that received ascorbic acid or glucose in uncharred or charred form. Shading of boxplots indicates whether soils received uncharred or charred substrates. Each boxplot represents the distribution of 12 replicates. Upper-case letters indicate Tukey-adjusted significant differences among the heating × charring treatment combinations within substrate types ($\alpha = 0.05$)

(p = 0.047) on fungal activity, but no interaction effects (p = 0.12). Fungi contributed to 31.40 ± 3.49% of respiration in soils heated to 200 °C, which was significantly less than 47.69 ± 5.49% in unheated soils. At 40.40 ± 5.17%, fungal respiration in soils heated to 100 °C did not differ from the other treatments. Although, the results of our general linear model indicated that soils amended with charred versus uncharred pine litter exhibited higher fungal activity, pairwise means comparisons did not uncover differences in fungal activity between these treatments.

Fungal activity was not significantly correlated with cumulative C respired or change in MBC.

Discussion

Soil heating intensity underlies estimated carbon use efficiency and microbial biomass accumulation

We did not find support for our hypothesis that the CUE proxy and MBC accumulation would be
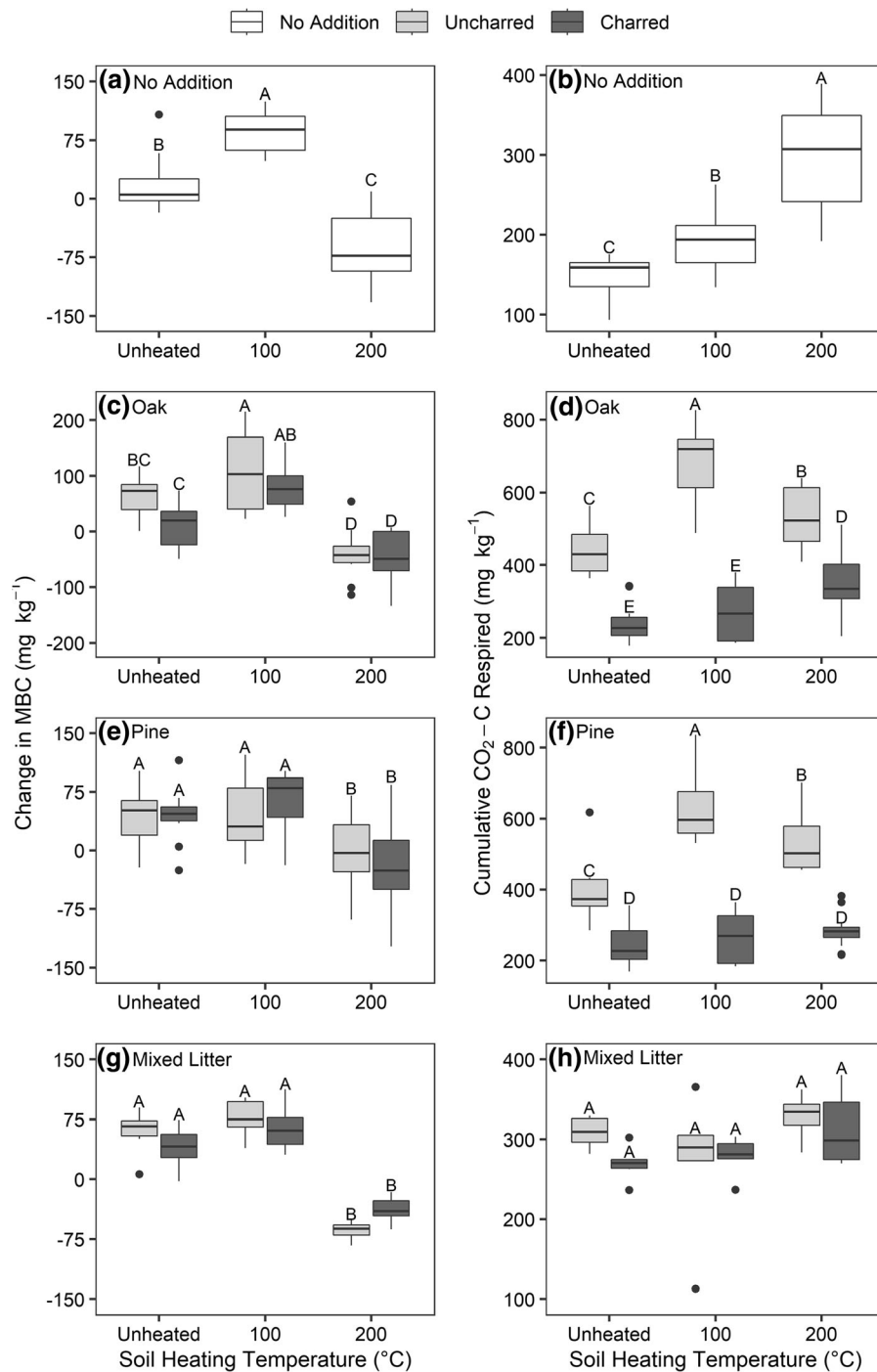
Fig. 3 Change in microbial biomass carbon (a, c, e, g) and 14-day cumulative respired CO_2-C (column, d, f, h) for unheated soils and soils heated to 100 °C and 200 °C that no litter, *Q. kelloggii* (Oak), *P. ponderosa* (Pine), or mixed leaf litter in uncharred or charred form. Shading of boxplots indicates whether soils received uncharred or charred litter. Each boxplot represents the distribution of 12 replicates, except for mixed litter treatments, which had 6 replicates. Upper-case letters indicate Tukey-adjusted significant differences in among the heating × charring treatment combinations within litter treatments ($\alpha = 0.05$)

positively correlated with soil heating intensity. Rather, heating soils to 200 °C decreased the CUE proxy and led to net negative MBC accumulation. Interestingly, in the MBC accumulation experiment, MBC content measured 24 h after heating was lower in soils heated to 100 °C than those heated to 200 °C. This suggests rapid microbial growth in the 24 h after heating. EOC content increased with heating intensity, and the assimilation of this flushed EOC by the surviving microbial community could have fueled the rapid growth in the soils heated to 200 °C. Indeed, a laboratory heating study performed on soils collected from *Pinus halepensis* forest in Spain found that bacterial growth was several times higher in soils heated between 80 and 400 °C than unheated controls within 2–4 days of soil heating (Bárcenas-Moreno and Bååth 2009). Furthermore, Bárcenas-Moreno and Bååth (2009) found that peak growth rate was greater and occurred earlier in soils heated to higher temperatures, an effect that correlated with greater initial increases in EOC.

Differences in the balance between anabolism versus catabolism in heated soils are likely due to the combined effects of heating on labile C availability, nutrient availability, and microbial biomass stoichiometry. For example, the greater MBC accumulation in soils heated to 100 °C versus 200 °C could be related to the combined effects of microbial cell lysis and threshold effects of heating on organic molecules impacting the quality of available C. Microbial lysis during heating results in the release of labile organic molecules, for example carbohydrates, proteins, and amino acids (González-Pérez et al. 2004). Such molecules are likely to be efficiently utilized by the surviving microbial populations in the absence of nutrient limitation (Cotrufo et al. 2013), potentially explaining why MBC accumulation sometimes increased in soils heated to 100 °C compared to unheated soils. Additionally, protein and amino acids represent labile forms of organic N (Jones et al. 2004; Schimel and Bennett 2004; Kielland et al. 2007), and 100 °C is below the threshold of N volatilization (Bodí et al. 2014). Thus, low intensity soil heating could potentially increase labile organic N availability and allow fast-growing microorganisms with high nutrient demands to rapidly increase in biomass. Concurrently, increases in bioavailable N could suppress less efficient decomposition of complex and recalcitrant organic matter (Janssens et al. 2010) and decrease the

need for N-mining (Moorhead and Sinsabaugh 2006; Craine et al. 2007).

In contrast, heating soils to 200 °C likely caused greater mortality to the microbial community (Pingree and Kobziar 2019) and thus greater input of organic molecules, but 200 °C is above or near the threshold at which these labile organic molecules are destructively distilled, volatized, or pyrolyzed (González-Pérez et al. 2004; Massman et al. 2010). Thus, although there was an overall increase in EOC after 200 °C heating, this C may be in a less bioavailable form. Studies assessing chemical changes of plant biomass during pyrolysis indicate that loss of thermally labile compounds and aromatization reactions occur at 200 °C (Hatton et al. 2016), and carbohydrate loss and transformation is one of the first processes to occur (Chatterjee et al. 2012). Moreover, soil carbohydrate content derived from both plants and microbes has been found to decrease immediately following wildfire, and remain depressed for at least 15 months (Martín et al. 2009). Greater carbohydrate loss and increased dominance of lignin and pyrogenic compounds appears to occur at higher fire severity (Miesel et al. 2015), and water-extractable organic matter is more aromatic after both prescribed fires and wildfires (Vergnoux et al. 2011; Hobley et al. 2019). Thus, at higher soil heating intensity, low MBC accumulation could be due to low availability of labile C that can be efficiently transformed into MBC.

In addition to the potential effects on C quality, 200 °C represents the lower threshold for N-volatilization and loss of soil proteins (Russell et al. 1974; Bodí et al. 2014; Lozano et al. 2016), potentially leading to decreased N availability and increasing the need for N-mining and investment in extracellular enzymes. Although total N did not change in response to heating (Table 3), it is possible that the N became less bioavailable. For example, Prieto-Fernández et al. (2004) found that chemically labile (acid hydrolysable) organic N was not impacted when soils were heated to 150 °C but decreased by > 50% when heated to 210 °C, despite total N remaining unchanged. Similarly, the same study found labile organic N was lower in soils collected immediately after a wildfire, and this decrease was independent of changes to total soil N (Prieto-Fernández et al. 2004). Some labile organic N will be converted to inorganic N and retained in soil as available N, but substantial increases in inorganic N might not occur until soil is heated at much higher

temperatures ($\sim$ 400 °C) than we employed (Raison 1979; Choromanska and DeLuca 2002; Guerrero et al. 2005).

In concert with changing N availability, altered microbial community composition in response to soil heating could affect CUE and MBC accumulation by inducing a disconnect between microbial biomass stoichiometry and nutrient availability. If soil heating induces a shift in the microbial community from fungi and oligotrophic bacteria with high C:N biomass ratios—and thus lower nutrient requirements—towards copiotrophic bacteria with lower C:N ratios and higher nutrient requirements (Fierer et al. 2007; Sinsabaugh et al. 2013), dissimilatory decomposition of labile C could be coupled to ex vivo catabolic processes in order to obtain limiting nutrients from complex organic matter (Moorhead and Sinsabaugh 2006; Craine et al. 2007; Manzoni et al. 2017). In fact, we found that fungal activity was significantly decreased in soils heated to 200 °C. Additionally, the relative abundance of copiotrophic bacterial phyla typically increase within one day to one week post-fire (Pérez-Valera et al. 2017; Prendergast-Miller et al. 2017), suggesting shifts to microbial communities with higher nutrient requirements. Indeed, our observed high respiration rates and lack of associated MBC accumulation (Fig. 3) supports the explanation that catabolic, rather than anabolic, processes are dominant in soils heated to 200 °C. Similarly, the lower CUE proxy in 200 °C soils that received ascorbic acid could be due to the decomposition of this labile C substrate being coupled to nutrient acquisition.

Importantly, higher MBC accumulation in soils heated to 100 °C does not necessarily reflect increased C sequestration compared to unheated soils. In fact, greater C respiration in the absence of new C inputs reflects a net loss of C, regardless of an associated accumulation of MBC. Thus, the increased C respiration in soils heated to 100 °C compared to unheated soils indicates a net loss of soil C during the immediate post-heating period (Table 1 and Fig. 3). However, the high MBC accumulation does represent efficient C recycling, limiting the contribution of microbial mortality to post-fire emissions and suggesting that microbial anabolism is high following low-intensity soil heating. In contrast, soils heated to 200 °C exhibited high post-heating respiration rates, continuing decreases in microbial biomass, and lower estimated CUE even in soils amended with a relatively labile C source (Figs. 1 and 2). The CUE proxy we employed may overestimate CUE because microbes are able to utilize the labile substrates more efficiently than native organic matter (Geyer et al. 2019). It is possible that such an overestimation is greater for unheated and low intensity heated soils because these soils have lower EOC than soils heated at high intensity (Table 2), and substrates therefore represent larger labile C additions on a relative basis. Additionally, soils heated to 200 °C exhibited C exudation over 24 h after water-only additions (Fig. 2a), perhaps leading to artificially low estimates of EOC uptake and therefore CUE. However, our MBC accumulation experiment did not rely on labile substrate additions and results consistently indicated that soils heated to 200 °C had lower MBC accumulation and high respiration compared to other treatments (Fig. 3). This suggests that high-intensity soil heating results in high microbial catabolism relative to anabolism, which exacerbates C emissions after heating over the short term.

Pyrogenic organic matter decreases soil respiration

We did not find support for our hypothesis that the proxy for CUE and MBC accumulation would be higher in soils amended with uncharred organic matter compared to charred organic matter. In most cases, regardless of soil heating intensity, application of PyOM led to decreases in C respiration without negatively affecting EOC uptake or MBC accumulation compared to the uncharred counterpart (Figs. 2 and 3). These findings indicate that PyOM addition decreases microbial catabolism without negatively impacting anabolism. The decrease in catabolism was likely not due to pyrolysis releasing labile C that could be used more efficiently, because EOC extractions of charred and uncharred litter indicated that there was $\sim$ 90% less extractable C in charred litter. One possible explanation for these patterns is that PyOM additions positively affected soil properties in ways that increased the efficiency of decomposition. For example, PyOM has been shown to increase CUE and microbial abundance by increasing nutrient availability, soil oxygen concentrations, pH, and by providing sorption sites for bacteria (Lehmann et al. 2011; Jiang et al. 2016; Fang et al. 2018). Alternatively, low-intensity soil heating could select for microbial groups that are adapted to efficient use of PyOM (see Whitman et al. 2019). The lower amounts of

catabolism in response to PyOM addition could also be due to low bio-availability of the charred biomass. However, low microbial use of PyOM may depend on the availability of an alternative labile C pool, and EOC can remain depressed for decades following wildfire (Prieto-Fernández et al. 1998). In the case of high intensity fires, aboveground plant mortality and biomass combustion may be high, removing a source of future labile soil C inputs from litter deposition and root inputs (González-Pérez et al. 2004; Grady and Hart 2006; Kavanagh et al. 2010). In these cases, the microbial community may switch to use of PyOM after the residual labile C pool is depleted, leading to less efficient C use over the intermediate or long term. An analogous substrate-limitation mechanism has been found to explain lignin degradation when soil carbohydrate pools becomes depleted (Hall et al. 2020).

There was a clear negative impact of soil heating on fungal activity, supporting other research finding that fungal biomass is more negatively affected by fire than bacterial biomass (Dooley and Treseder 2012). Although we did not observe a relationship between fungal respiration and MBC accumulation, decreased fungal biomass could negatively impact MBC accumulation in soils with high PyOM concentrations because fungi may be better able to utilize organic matter characterized by high aromaticity and C:N (Jastrow et al. 2007; Keiblinger et al. 2010; Dungait et al. 2012).

Conclusions

High intensity soil heating decreased the strength of soil C sink over the short term by decreasing microbial anabolism and increasing catabolism. Higher soil heating intensity likely increases the amount of C lost to combustion during fire events, and our results suggest that this effect may be exacerbated by low CUE, low accumulation of MBC, and high soil respiration rates over the short-term in soils that experience high heating intensity. In addition to exacerbating short-term C losses, decreased anabolism relative to catabolism could lead to long-term reductions in soil C storage via less microbial necromass, which is a persistent stock of C. Fire intensity is predicted to increase in many fire-prone ecosystems, and a negative relationship between intensity and

microbial anabolism could lead to positive fire-climate feedbacks. Disrupted fire regimes are already being observed in temperate coniferous forests of the western United States, and if these altered fire regimes lead to lower anabolism and decreased CUE, the C sequestration ability of these forests could be permanently reduced. We found some beneficial impacts of PyOM in ameliorating C losses via decreased respiration, but over longer timeframes, PyOM could either negatively influence CUE due to its chemical recalcitrance, or positively influence CUE via beneficial effects on soil physicochemical properties. Our study used lab-based soil heating to estimate direct effects of fire on a CUE proxy and MBC accumulation, providing conceptual insight important for informing future investigations to determine whether the influences of soil heating and PyOM on CUE and MBC accumulation persist over the long-term.

Data Availability Data available upon request.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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