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Soil mineral assemblage and substrate quality effects on microbial priming

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1. Introduction

Native soil organic carbon (SOC) decomposition rates may be altered through increased carbon (C) input, a phenomenon known as SOC priming (Blagodatskaya et al., 2011). Quantifying priming is important because it may modulate long-term SOC storage in ecosystems and therefore C biogeochemical cycling. Priming is positive when more SOC is decomposed or, conversely, negative when less native SOC is decomposed after C amendment (Kuzyakov et al., 2000; Kuzyakov, 2002; Bader and Cheng, 2007). Yet, controls over the direction and magnitude of the priming effect and the consequences for soil C balance remain uncertain (Dijkstra et al., 2013; Liu et al., 2017).

The quality of plant-derived organic compounds entering the soil influences microbial activity and may subsequently impact the priming effect (De Nobili et al., 2001; Hamer and Marschner, 2005a). Microorganisms can assimilate simple (low-molecular weight) substrates more readily than chemically complex (e.g. cellulose or lignin) compounds, which require extracellular enzyme production for breakdown and depolymerization (Fontaine et al., 2003). Additions of simple substrates, such as those exuded from root tips, can result in positive or negative priming, the latter possibly because microorganisms utilize new C in preference to native soil organic matter (Cheng, 1999; Guenet et al., 2010). In some cases, complex substrate additions have elicited larger positive priming responses than simple substrate additions (Fontaine et al., 2003). One possibility is that more extracellular enzymes are produced in response to complex substrates than in response to simple substrates (Schimel and Weintraub, 2003), accelerating decomposition of native SOC (Allison and Vitousek, 2005). However, considerable uncertainty remains in how substrates of different quality may impact soil microorganisms, and ultimately mineralization of otherwise stable SOC.

There is currently a paradigm shift in what constitutes "stable" SOC. The view that SOC comprises humic substances that are resistant to microbial decomposition is being discarded in favor of SOC that could be labile, but prevented from microbial access via protective associations with minerals (Lehmann and Kleber, 2015). Therefore, investigating SOC priming from a mineral assemblage framework is needed to better understand the priming phenomenon.

Soil mineral assemblages, especially those enriched in short-range order (SRO) materials, can strongly impact SOC cycling through various mineral-organic associations (Kleber et al., 2015). Prevalent in soils derived from volcanic parent materials, SRO materials are amorphous mineraloids that include aluminosilicates (e.g. allophane and imogolite), Fe-oxyhydroxides (e.g. ferrihydrite), and Al-oxyhydroxides (Shoji et al., 1993). Soils abundant in SRO materials generally contain large, slow-cycling SOC pools (Zunino et al., 1982; Matus et al., 2014) that are largely composed of easily degradable organic compounds protected by SRO materials (Saggar et al., 1994; Torn et al., 1997; Parfitt et al., 2002). In contrast, soils dominated by 2:1 and 1:1 phyllosilicate clays typically have comparatively smaller yet faster-cycling C pools (Harsh et al., 2002; Fontaine et al., 2007). Soils rich in SRO materials are thought to stabilize SOC by (1) SRO materials adsorbing and rendering organic compounds unavailable for microbial utilization (Torn et al., 1997); (2) SRO materials adsorbing and deactivating extracellular enzymes (Saggar et al., 1994; Miltner and Zech, 1998); (3) inducing Al toxicity on the microbial biomass (Illmer et al., 2003); or (4) forming organo-metal complexes (Tate and Theng, 1980; Heckman et al., 2009; Matus et al., 2014). Thus, SRO materials can exert a major influence on SOC priming because of their interactions with microbial substrates and enzymes.

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Recent studies have investigated priming effects from various fresh C substrate inputs in soils abundant in SRO materials (Rasmussen et al., 2008; Crow et al., 2009; Khan et al., 2012; Herath et al., 2015; Keiluweit et al., 2015), with varying results. Some studies have found weak priming responses in high SRO soils after litter additions (Rasmussen et al., 2008; Khan et al., 2012; Herath et al., 2015). A single input of pine litter (a relatively complex C substrate) elicited strong positive priming in soils low in SRO materials (< 5 g kg^{-1} allophane), but only weak priming in soil high in SRO materials (50–78 g kg^{-1} allophane, Rasmussen et al., 2008). In this case, extracellular enzyme production may have increased from complex C input, thereby stimulating priming in low SRO soils (Schimel and Weintraub, 2003). If so, then perhaps increased enzyme production may have been rendered ineffective in high SRO soils by adsorption to SRO material surfaces (Saggar et al., 1994). Weaker priming responses associated with fresh corn litter input occurred in an Andisol, a high SRO soil, compared to stronger priming responses from an Alfisol, a low SRO soil (Herath et al., 2015). In an allophanic Andisol derived from basalt parent material, the application of easily decomposable pea residues led to a small positive priming effect but a 50% increase in SOM-derived microbial biomass, which was attributed to a possible stimulation of extracellular enzyme production or through increased microbial growth on organic matter (Khan et al., 2012). In contrast, priming effects can be large in high SRO soils (Crow et al., 2009; Keiluweit et al., 2015), and thus, there are inconsistent patterns of priming effects in soils high in SRO materials. Our study aims to fill a large gap in our understanding of how priming is affected by the interactions between SRO materials, microorganisms, quality of fresh C inputs, and enzyme activities.

We conducted a laboratory experiment testing mineral and microbial controls on priming. This incubation was conducted with soils naturally varying in SRO content to observe priming responses to repeated additions of simple or complex substrates. Priming responses were measured by monitoring respiration rates, microbial biomass C, and enzyme activities throughout the incubation. This work expands upon past research from the same natural soil systems investigating mineral control of SOC dynamics including priming (Rasmussen et al., 2005, 2006, 2007, 2008).

In this study, we explored the following questions: Is extracellular enzyme activity greater in soil with lower SRO content and does this result in greater priming compared to soils with higher SRO content? Do complex substrate additions elicit stronger extracellular enzyme activity compared to simple substrates and therefore elicit stronger priming responses? We hypothesized that priming is influenced by an interaction between substrate quality and soil mineral assemblage, specifically soil SRO content.

2. Materials and methods

2.1. Study system

Soil samples were collected from a lithosequence along the western slope of the Sierra Nevada and the southwestern slope of the Cascade Range in California from three different parent materials. The three lithologies – granite, basalt, and andesite – represent distinct mineral assemblages. Vegetation at these sites was white fir (*Abies concolor*) dominated mixed conifer forest. Climate (mean annual precipitation of 115 ± 10 cm yr^{-1} and mean annual surface temperature of 9.1 ± 0.9 °C), slope (< 10%), aspect (southwest and west-facing), and canopy position (outside of tree canopy) were similar at each sampling location (Rasmussen et al., 2006). Soil samples were collected from the A horizon from 0 to 11 cm depth after carefully removing the litter layer, and were sieved to < 2 mm prior to incubation. The soil mineral assemblages (Dahlgren et al., 1997; Rasmussen et al., 2007, 2010) and surface SOC dynamics (Rasmussen et al., 2006, 2007, 2008) have been well characterized at these sites (Table 1). The granite soil contains negligible amounts of allophane, an SRO aluminosilicate, whereas the

Table 1
Soil characterization data (± SE) for granite, basalt, and andesite soil material collected from sample sites and used for the laboratory incubation.

	Basic soil properties				Organic C variables			Mineralogy variables (g kg^{-1})*							
Parent material	pH 1:1 H_2O	Clay (g kg^{-1})	CEC (cmol kg^{-1})	Base saturation (%)	C (g kg^{-1})	C: N	$\delta^{13}C$ (‰)	MBC (μg C g^{-1})	Fe_d	Fe_o	Al_o	Si_o	Al_p	Allophane	Clay mineralogy	Soil classification
Granite	6	77	18	46	31.12 (1.7)	27.98 (0.3)	−26.08 (0.01)	570.8 (16.2)	4.4 (0.1)	2.8 (0.3)	6.4 (1.0)	1.1 (0.1)	2.7 (0.8)	-	HIV > K > > G	Coarse-loamy, mixed, superactive, mesic Humic Dystroxerept
Basalt	6.5	63	30	51	59.95 (2.1)	19.60 (0.2)	−25.56 (0.03)	170.5 (6.8)	7.0 (0.4)	2.7 (0.2)	17.9 (2.1)	9.9 (1.7)	7.6 (0.6)	50	SRO > > HIS > K = G	Loamy-skeletal, mixed, superactive, mesic Typic Haploxerept
Andesite	5.8	94	40	51	98.46 (9.4)	24.4 (0.82)	−25.87 (0.02)	786.8 (24.1)	18.6 (1.3)	6.8 (0.4)	31.7 (0.7)	11.2 (0.5)	10.5 (0.1)	78	SRO > > G = K	Medial-skeletal, amorphic, mesic Humic Haploxerand

* Data is a subset of soil data presented in Rasmussen et al. (2006). Clay values represent one data point collected for composite soil samples. Mineralogy variables represent average values of the three pedons sampled at each field site. Abbreviations are as follows: CEC, cation exchange capacity; C, carbon; MBC, microbial biomass carbon; Fe_d, sodium dithionite extractable Fe (crystalline Fe); Fe_o, SRO Fe-oxyhydroxide (oxalate-extracted Fe); Si_o, oxalate-extractable Si; Al_o, oxalate-extractable Al; Al_p, pyrophosphate-extractable Al; G, gibbsite; HIS, hydroxy interlayered smectite; HIV, hydroxy interlayered vermiculite; K, kaolinite/halloysite; SRO, short range order. Allophane content estimated from the Al_o-Al_p/Si_o molar ratio based on Dahlgren (1994). Clay mineralogy was determined by X-ray diffraction and minerals are listed in order of relative abundance based on relative peak intensity in X-ray diffractograms.

basalt soil contains 50 g kg^{-1} and the andesite soil 78 g kg^{-1} of allophane (Rasmussen et al., 2006). As a sum of allophane and direct measurements of SRO Fe-oxyhydroxides, the SRO content of the granite soil was 2.8 g kg^{-1}, the basalt soil was 52.7 g kg^{-1}, and the andesite soil was 84.8 g kg^{-1} (Table 1; Rasmussen et al., 2006). From this, we characterized the granite, basalt, and andesite soils as having low, intermediate, and high SRO contents, respectively. About 60% of the total SOC in the andesite soil is associated with the mineral fraction, while < 25% of the total SOC in the granite soil is in the mineral fraction, with almost 70% in the more bioavailable free-light fraction (C. Rasmussen, *personal communication*). These soils vary in SOC content, cation exchange capacity, phosphorus fixation capacity, and pH, but these differences are largely driven by and co-vary with the presence of SRO materials (Dahlgren et al., 2004). Thus, these soils provide a convenient study system to examine how natural variation in the soil mineral assemblage affects soil C cycling.

2.2. Soil incubation

Substrate amendment treatments (n = 4) included weekly additions of a simple substrate, a complex substrate, and a control that received no C input. We used repeated substrate additions because these are thought to be more characteristic of repeated or continuous C inputs received in natural terrestrial ecosystems compared to a single pulse of C (Hamer and Marschner, 2005b; Qiao et al., 2014). The simple substrate consisted of a soluble mixture of 48% carbohydrates, 40% organic acids and 12% amino acids (Hütsch et al., 2002), simulating rhizosphere exudate composition (Klein et al., 1988; Griffiths et al., 1999; Jones et al., 2002). Expressed as relative molar abundances, we used 4 parts fructose, glucose, sucrose, and lactate, 2 parts succinate, malate, and citrate, and 1 part serine, cysteine, and alanine (Sigma-Aldrich Co. LLC). The uniformly ^{13}C–enriched compounds used in the exudate mixture (99 atom percent) were equally diluted with unlabeled compounds to have an overall enrichment of 1.991 atom percent ^{13}C.

The litter (complex C) was derived from *Pinus ponderosa* seedlings cultivated under ^{13}C enrichment conditions as described in Rasmussen et al. (2008), with once-weekly exposures over an entire photoperiod to 375 mg ^{13}C–CO_2 L^{-1} for five months, yielding forcibly-senesced litter with a ^{13}C enrichment of 1.423 atom percent ^{13}C. Weekly exposures over the life of the seedlings were used to promote uniform isotopic label distribution.

For the soil incubation, 40 g of dry soil was weighed into specimen cups in sealed 473 mL volume Mason jars with septa in the lid to allow for headspace gas extraction. De-ionized water was added to bring soil moisture content up to 60% of field capacity each week. Soils were incubated for one week before substrate addition to equilibrate microbial activity. Before being added to soil, the litter was ground using a ball-mill to achieve greater homogeneity, then added to soils as a dry powder once weekly. The exudate mixture was added in solution. De-ionized water was added separately to soils to maintain moisture at 60% field capacity and the control treatment received a similar amount of de-ionized water each week. Each week for six weeks, 350 μg substrate-C g^{-1} soil was added to each soil sample after which soil was thoroughly mixed. This substrate-C amount was appropriate because it elicited priming in past experiments (Blagodatskaya and Kuzyakov, 2008; Liu et al., 2017), and was in line with a broad range of estimated exudation rates under field conditions (Trofymow et al., 1987; Grayston et al., 1996).

During incubation, CO_2 concentration and excess ^{13}C–CO_2 were measured. Ten mL of headspace atmosphere was taken with a syringe for CO_2 concentration measurements and 60 mL for excess ^{13}C measurements. Within 5 min after closing the jars, a time-zero gas sample was taken for CO_2 concentration and jars were left to incubate in the dark at room temperature (23 °C). CO_2 was allowed to accumulate in the jar headspace between days 0 to 2, days 2 to 5, and days 5 to 7 after substrate addition each week. At the end of each period, gas samples were extracted for CO_2 concentration and ^{13}C measurements from the sealed headspace. After each measurement period, jars were opened, excess CO_2 accumulation was flushed for 0.5 h, jars were re-sealed and the time zero sampling was repeated for the next measurement period. From this, we calculated total CO_2 respired from each sampling period (see Section 2.5). CO_2 concentration was measured using a LI-COR CO_2/H_2O Analyzer Model LI-6262 and ^{13}C excess was measured using a Picarro Cavity Ring-down Spectrometer model G2201-*i* Isotopic CO_2/CH_4. Picarro samples were measured through dilution of the 60 mL of gas with CO_2-free air to a range of 350–1500 ppm CO_2 have enough gas to obtain accurate measurements of atom percent ^{13}C.

2.3. Microbial biomass

Microbial biomass C (MBC) was determined using a modified chloroform fumigation extraction method (Vance et al., 1987) after pre-incubation, and one week after the first and last additions of substrate. From each replicate, a 20 g subsample of soil was mixed with 50 mL of 0.05 M K_2SO_4 to quantify extractable C. Samples were thoroughly mixed on a shaker table at 200 rpm for 1 h, and the supernatant was filtered through a Whatman no. 1 filter paper. A separate 20 g subsample of soil from each replicate was fumigated with chloroform in desiccators for five days and then extracted and filtered under the same conditions as the non-fumigated treatment. The filtered extracts were oven-dried at 60 °C for 48 h, ground with a mortar and pestle, and analyzed for C content and atom percent ^{13}C. We calculated MBC as the difference in extractable C between fumigated and non-fumigated samples and divided by 0.45 as a correction for extraction efficiency (Vance et al., 1987).

2.4. Enzyme assays

Each soil was assayed for cellulose-degrading enzymes, β-glucosidase (βG) and cellobiohydrolase (CBH), and the oxidative enzymes phenol oxidase (PPO) and peroxidase (PER), to measure potential extracellular enzyme activity. Samples were taken two and seven days after the first and last additions of substrate. Immediately after sampling, soils were stored at −20 °C until 30 min before assay preparation. Freeze/thaw effects can potentially influence enzyme activity (Lee et al., 2007), so all samples were subject to the same freeze storage regime, justifying comparisons across the different treatments (Allison et al., 2014). Enzyme assays were conducted using standard colorimetric and fluorometric microplate techniques at room temperature (Saiya-Cork et al., 2002; German et al., 2011). For each sample, 1.0–1.3 g soil (wet weight) was homogenized in a Waring commercial laboratory blender with 125 mL 50 mM sodium acetate buffer (pH 6.0) for 2 min. The resulting homogenate was then stirred continuously at 350 rpm on a magnetic stir plate and 200 μL aliquots were transferred to a 96-well microplate within 30 min of homogenization.

The βG and CBH fluorometric assays were done with 8 replicates per sample. Fifty microliters of substrate solution were added to each plate well (200 mM 4-Methylumbelliferyl-β-D-glucopyranoside for βG and 200 mM 4-Methylumbelliferyl-β-D-cellobioside for CBH). Blanks received 250 μL of 50 mM sodium acetate buffer. Reference standard wells received 200 μL acetate buffer and 50 μL of a 100 μM 4-Methylumbelliferone (MUB) standard. Negative control wells received 200 μL acetate buffer and 50 μL substrate solution. In addition to the 200 μL soil slurry pipetted to each sample well, homogenate controls also received 50 μL acetate buffer, while quench controls received 50 μL MUB standard, and assay wells received 50 μL substrate solution. The final volume for each well was 250 μL and each plate was incubated in the dark between 1.5 and 2.5 h at room temperature. Hydrolase activity was expressed as nmol g^{-1} soil h^{-1}.

The PER and PPO colorimetric assays were done with 16 replicate wells per sample. Oxidative assays had blank, negative control, and homogenate control wells as in the fluorometric assays. The substrate

solution used for both PER and PPO plates was 25 mM L-dihydroxyphenylalanine (L-DOPA), and each well in PER plates also received 10 μL 0.3% hydrogen peroxide solution. PER activity was calculated as the difference between the hydrogen peroxide-amended plates and the PPO plates. The oxidase microplates were incubated in the dark at room temperature for 24 h. Oxidase activity was expressed as $\mu mol\ g^{-1}$ soil h^{-1}. For both fluorometric and colorimetric enzyme assays, extracellular enzyme activity was calculated as described in German et al. (2011).

2.5. Data and statistical analyses

Total respired CO_2, expressed as μg CO_2-C g^{-1} soil, was calculated for each time interval during the incubation period and summed to quantify cumulative respiration. Substrate-derived CO_2-C ($C_{substrate}$) was calculated as:

$$C_{substrate} = C_{total}\,(x(^{13}C)_{total} - x(^{13}C)_{SOC})/(x(^{13}C)_{substrate} - x(^{13}C)_{SOC}) \quad (1)$$

where C_{total} is the total CO_2-C respired from substrate-amended samples, $x(^{13}C)_{total}$ is the ^{13}C atom percent of CO_2-C from the substrate-amended samples, $x(^{13}C)_{substrate}$ is the ^{13}C atom percent of the added substrate, and $x(^{13}C)_{SOC}$ is the natural abundance ^{13}C atom percent of CO_2-C produced in the control samples. SOC-derived CO_2-C of the substrate treatment was calculated as the difference between C_{total} and $C_{substrate}$. These calculations were the same to partition substrate- and SOC-derived microbial biomass for weeks 1 and 6.

The priming effect, expressed as μg CO_2-C g^{-1} soil, was calculated as:

$$Priming = C_{SOC(treatment)} - C_{SOC(control)} \quad (2)$$

where $C_{SOC(treatment)}$ is the CO_2-C derived from SOC of the exudate or litter treatment and $C_{SOC(control)}$ is the CO_2-C derived from SOC of the control.

The cumulative C balance was calculated as:

$$C\ balance = C_{added} - C_{total} \quad (3)$$

where C_{added} is the total amount of substrate C added for either litter or exudate addition over the course of the incubation.

Additionally, we calculated a cumulative “substrate effect” as:

$$Substrate\ Effect = Substrate\ C\ remaining\ in\ soil - Priming \quad (4)$$

While some other priming studies call “C balance” or “net C balance” as the difference in substrate C remaining in soil minus priming (Eq. (4)), we considered this to be a misleading definition, as that calculation does not take into account the baseline C_{SOC}. Therefore, we defined Eq. (3) as “C balance.”

Analysis of variance (ANOVA) was used to determine the effect of parent material, substrate type, and their interactions on cumulative respiration (total, substrate- and SOC-derived), priming, microbial biomass, and enzyme activity. Additionally, we used two-way repeated measures ANOVA to assess effects of time, parent material, substrate type, and their interactions on respiration, priming, MBC, and enzyme activity. Tukey's HSD was used to compare the difference in means of cumulative priming, microbial biomass, and enzyme activity for each substrate and parent material treatment ($\alpha = 0.05$).

We used bootstrapping (resampling 10,000 times with replacement) to calculate 95% confidence limits for the effect sizes for enzyme activity. The effect sizes were calculated as the difference between each treatment and control for each enzyme activity for weeks one and six. If the 95% confidence interval did not overlap zero, we inferred an effect of substrate on enzyme activity. The “plyr” package in R Studio (version 3.1.2) was used for all bootstrapping analyses and JMP pro 11 (SAS Institute Inc., Cary, NC, USA) was used for ANOVA.

2.6. Uniformity of litter labelling

Numerous priming studies use ^{13}C–enriched litter as a substrate, yet they assume that the ^{13}C labelling was homogenous in the plant's tissues. To assess uniformity of ^{13}C label distribution among the plant compounds, we analyzed a subset of the litter samples using the Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, NY, USA) to separate leaf litter into non-polar extract (fats, oils, and soluble cell contents), neutral detergent (cellulose, hemicellulose, lignin), acid detergent (cellulose, lignin) and lignin-only fractions. Total C and ^{13}C atom percent were measured for bulk litter and each isolated fraction on a Carlo Erba NC2100 elemental analyzer at the Colorado Plateau Stable Isotope Laboratory (http://www.isotope.nau.edu) and incorporated these values into possible error in our priming estimates.

We used a single end-member for the mixing model calculations for the litter material (Eq. (1)), assuming the litter was uniformly labeled. However, we assessed the potential for non-uniform labeling of the litter material to influence the outcome of the priming calculation. We compared our single-end-member approach (Eq. (1)) to two alternate scenarios: 1) assuming that each litter fraction contributes equally to CO_2 production from the litter source, despite differences in decomposition rates, or 2) assuming that overall contribution is the weighted average of the concentration of each fraction and its estimated relative decomposition rate. Under scenario 2, relative decomposition rates increased in order from lignin (slowest) to acid detergent fraction, neutral detergent fraction, and to the nonpolar extract (fastest) fraction (with an assumed decomposition rate of 0.5), and that adjacent fractions in this rank order differed in decomposition by a factor of two. These two scenarios span a wide range of assumptions about the consequences of uneven ^{13}C–label distribution in the litter. Additional information on this assessment of uneven litter labelling can be found in the Supplemental Materials.

3. Results

3.1. Respiration and priming

Total soil respiration rates from the granite soil (the lowest SRO soil) declined over time for each treatment including the control, while respiration rates in andesite and basalt remained fairly constant over time (Table S2; Fig. S1). The granite soil treatments had the highest cumulative respiration, followed by basalt and andesite (Fig. 1A). CO_2 respired from native SOC was lowest in the basalt soil and highest in the granite soil across treatments. CO_2 derived from the substrate added was greater for the exudate addition compared to the litter addition and within each substrate addition treatment, the granite soil had the most substrate-derived CO_2-C and the andesite had the least (Fig. 1A). Cumulatively, 36%, 30%, and 21% of the exudate C added was respired for the granite, basalt, and andesite soils, respectively. 16%, 14%, and 8% of the litter C added was respired for the granite, basalt and andesite soils, respectively.

Both exudate and litter additions caused positive priming in all three soil types (Fig. 1B; Table S2). Cumulative priming was greater in the soils with greater SRO content (Fig. 1B). From a linear regression between SRO content (Feo + allophane) of each soil with cumulative priming, litter addition had an R^2 of 0.88 and $p < 0.001$, and exudate addition had an R^2 of 0.83 and $p < 0.001$. Priming in the granite soil was significantly lower compared to andesite and basalt soils under exudate and litter additions (Table S2; Fig. 1B). However, the andesite and basalt soils did not significantly differ in mean priming for either exudate or litter addition (Table S2; Fig. 1B).

The magnitude of priming depended on soil, substrate type and time (Table S2; Fig. 2). The largest priming response occurred in the first week in the andesite and basalt soils due to exudate addition and declined in subsequent weeks (Fig. 2). Priming remained largely constant in the litter treatment, except for the granite soil, in which most

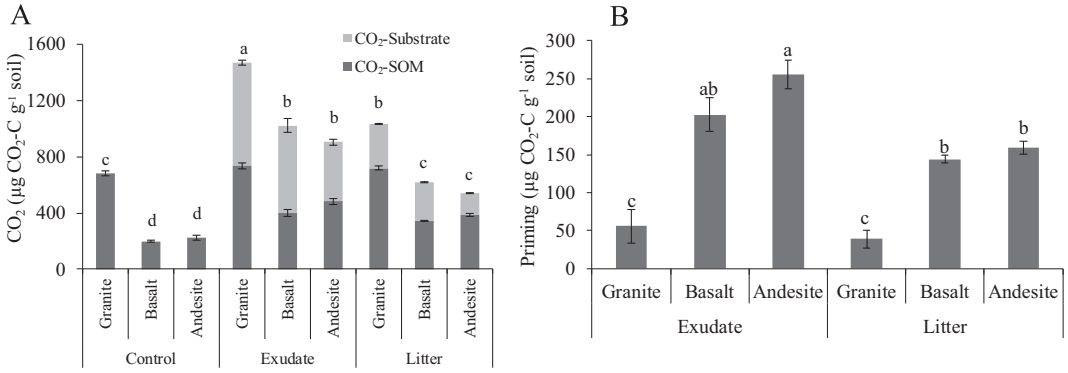

Fig. 1. (A) Cumulative respiration (µg CO_2-C g^{-1} soil) over a 6-week incubation under control (no C), exudate and litter C substrate additions for andesite (highest SRO content), basalt (intermediate SRO), and granite (negligible SRO material content). Stacked bars show respiration derived from soil organic matter (dark bars) and added substrate (light bars). (B) Mean cumulative priming effect with exudate and litter substrate additions. Values indicated by different letters are significantly different (Tukey's HSD post hoc test, α = 0.05). Error bars represent standard error of the mean (n = 4).

(59.3%) of the priming occurred during the last week of the incubation (Fig. 2).

P. ponderosa litter fractions varied in ^{13}C atom percent, with the nonpolar extract fraction having the highest ^{13}C atom percent and the acid detergent fraction having the lowest ^{13}C atom percent (Fig. S3a). The nonpolar extract fraction made up 62% of the total bulk litter C. However, despite the significant differences in litter fraction ^{13}C atom percent, our adjusted priming calculations based on scenarios with different assumptions were not statistically different from our original priming calculations (Fig. S3b). Therefore, the calculated priming effects were unlikely to be appreciably affected by the uneven distribution of the ^{13}C label among different chemical fractions in the litter.

3.2. Microbial biomass carbon

Before the incubation, the andesite soil had the greatest MBC at 787 µg C g^{-1} soil, the granite soil had 571 µg C g^{-1} soil and the basalt soil had 171 µg C g^{-1} soil (Table 1). After the first week of the incubation, MBC decreased compared to pre-incubation MBC, except for the Litter-Basalt soil (Table 1; Fig. 3). In week 1, MBC in the andesite and basalt soils were unresponsive to substrate addition (Table S2; Fig. 3). By the end of week 6, both litter and exudate addition significantly increased microbial biomass for all soils (Table S2; Fig. 3). The basalt soil had the lowest MBC across all treatments, while the andesite soil had the highest MBC at both time points. In the control treatment, MBC was lower in the final week of the incubation compared to the first week for all soil types. At the end of the incubation, substrate addition increased MBC in all soils compared to the control. In the granite and andesite soils, litter addition increased MBC more than exudate addition; in contrast, litter and exudate addition caused similar changes in MBC in the basalt soil (Fig. 3).

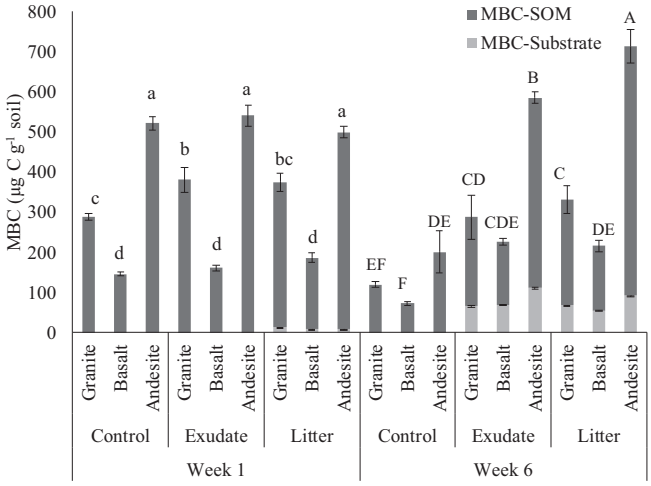

Fig. 3. Microbial biomass C (MBC) for weeks 1 and 6 expressed as µg MBC g^{-1} soil. Stacked bars show total MBC partitioned into SOM (dark grey) and Substrate-derived (light grey) MBC. Bars are means ± SE of the control and treatment (exudate and litter) MBC (n = 4), and values connected by different lowercase letters for week 1 and uppercase letters for week 6 are considered significantly different total MBC (Tukey's HSD post hoc test, α = 0.05).

In week 1, there was a larger fraction of ^{13}C–labeled litter incorporated into MBC, while almost no incorporation from the exudate mixture. However, 15% (litter-andesite) to 30% (exudate-basalt) of

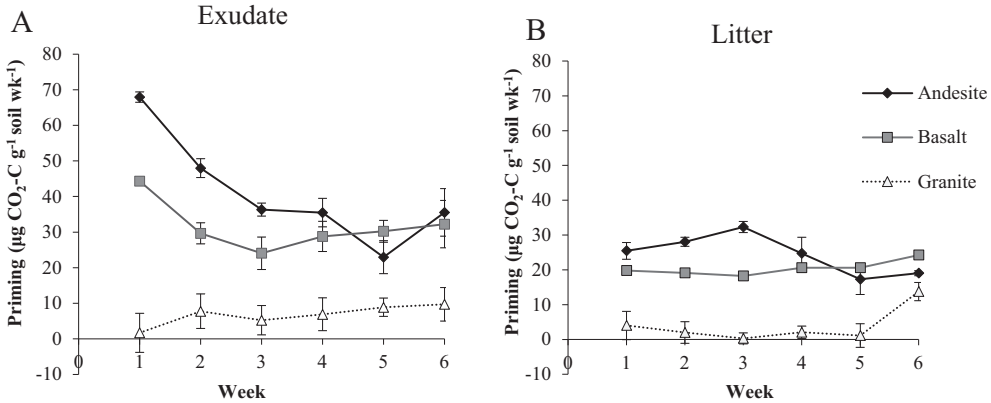

Fig. 2. Weekly priming (µg CO_2-C g^{-1} soil $wk.^{-1}$) among the different soil types and substrate additions. Exudate addition represents a "simple" C substrate and litter addition represents a relatively "complex" C substrate.

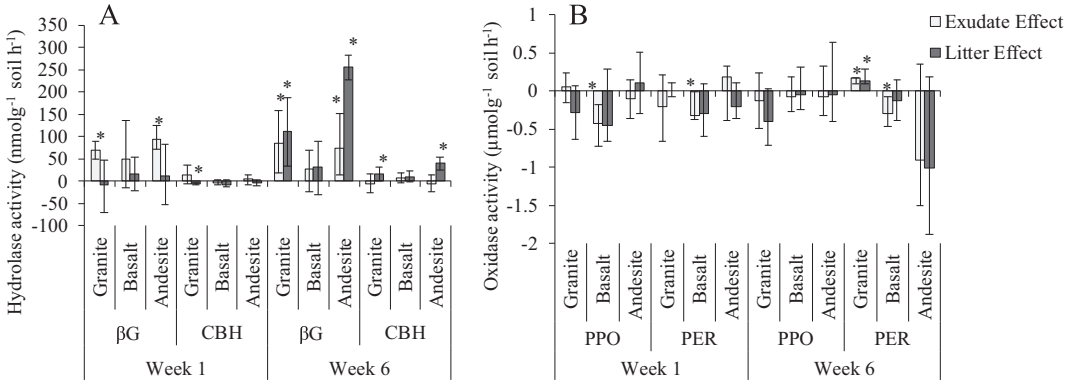

Fig. 4. The effect (difference between the substrate treatment and control) of substrate addition on extracellular enzyme potential activity for week 1 and week 6 for βG, CBH, PPO and PER. Bars are the mean effect size and error bars are bootstrapped 95% confidence limits (n = 4). Units are in nmol g^{-1} soil h^{-1} for βG and CBH (A) and μmol g^{-1} soil $h^{-1}$for PPO and PER (B). Bars with an asterisk indicate 95% confidence limits that do not overlap zero.

MBC was substrate-derived across the treatments by week 6 (Fig. 3; Fig. S2). As a percentage, the basalt soil with exudate addition had the greatest incorporation of substrate into MBC (Fig. S2).

3.3. Extracellular enzyme activity

By the end of the incubation, litter addition increased βG and CBH activities in the andesite and granite soils, but not in the basalt soil (Fig. 4A). Addition of exudates increased βG activity in the granite and andesite soils relative to the control for weeks 1 and 6, but did not affect CBH activity (Table S2; Fig. 4A). By week 6, βG and CBH activities increased most in the andesite soil amended with litter relative to the control (Fig. 4A). Substrate quality did not have a significant effect on activity of the oxidases PPO or PER.

3.4. Carbon balance

Net C balance during the incubation period was positively associated with SRO content (andesite and basalt > granite) for each substrate treatment (Table 2). The andesite and basalt soils with litter addition accumulated the most C, on average 260 ± 8.1 μg C g^{-1} wk.$^{-1}$, and the granite soil with exudate addition accumulated the least, on average 105 ± 36.7 1 μg C g^{-1} wk.$^{-1}$. Despite a positive priming effect for all soils, the C addition from the substrates resulted in more C remaining in the soil than C mineralized (Table 2). For each substrate addition treatment, the andesite soil had the most substrate C remaining in the soil at the end of the incubation (Table 2). In terms of a substrate effect (remaining substrate C in soil minus priming) substrate had more of an effect than soil type, with a greater substrate effect from litter C in all three soils than exudate C (Table 2).

4. Discussion

4.1. Priming dynamics

Our results suggest that differences among soils rather than substrate quality largely drove priming responses. Both exudate and litter additions caused a larger priming response in the soils with greater SRO content – the andesitic and basaltic soils. Granitic soils are often dominated by fast-cycling C pools (Harsh et al., 2002), consistent with our finding that the granite soil had the largest amount of CO_2 respired across all treatments, despite having the lowest SOC content. Despite large cumulative SOC mineralization from the granite soil across all treatments, there was little change in native SOC mineralization in the granite soil from litter or exudate addition. Adding new C to these soils may not have affected the availability of native SOC because the bulk of SOC was already readily available to microorganisms.

Contrary to our hypothesis that extracellular enzymes are inhibited in soils with high SRO content, the relationship between substrate addition, SRO content, and enzyme activity remains unclear. While greater βG and CBH activities were observed under litter addition in the andesite soil, priming in the andesite soil was greater from the simple substrate addition than the litter. This indicates that litter addition induces microorganisms to produce extracellular enzymes to break down cellulose; however, mechanisms other than enzyme production alone must be contributing factors to the priming effect, due to the larger priming response in the high SRO soils from the simple substrate addition. Moreover, if adsorption of enzymes to SRO materials occurred, it remains unknown if adsorbed enzymes would be inhibited (Saggar et al., 1994; Miltner and Zech, 1998). It is possible that the hydrolases remained functional, reacting with complex organic matter and increasing the mineralization of native SOC (Allison, 2006). Another possibility is that hydrolase extracellular enzyme production by

Table 2
The C balance at the end of the incubation for the three soils and two substrate treatments. C balance as calculated by input-output is the amount of substrate C added minus the total CO_2-C respired. C balance under the conventional consideration of priming C balance is calculated as the amount of substrate C remaining in the soil minus CO_2-C from the priming effect, which was called 'substrate effect' here. All values are in μg C g^{-1} soil ± standard error of the mean. Values within each column not sharing the same lower-case letter are considered statistically different (Tukey's HSD post hoc test, α = 0.05).

		Substrate C added	Total CO_2-C respired	C balance (input-output)	Substrate C remaining in soil	Substrate C in MBC	Substrate effect (substrate C remaining - priming)
Substrate	Soil	(μg C g^{-1} soil)					
Exudate	Granite	2100	1467.6^{a} ± 32.6	632.4^{c} ± 32.6	1350.1^{e} ± 20.3	68.2^{b} ± 13.8	1312.8^{b} ± 32.6
	Basalt	2100	1020.8^{b} ± 69.2	1079.2^{b} ± 69.2	1464.5^{d} ± 48.6	69.5^{ab} ± 3.1	1277.2^{b} ± 69.2
	Andesite	2100	904.4^{b} ± 37.9	1195.6^{b} ± 37.9	1666.6^{c} ± 20.6	112.3^{a} ± 10.6	1422.2^{b} ± 37.9
Litter	Granite	2100	1034.7^{b} ± 12.1	1065.3^{b} ± 12.1	1759.9^{bc} ± 2.7	66.8^{b} ± 4.8	1745.7^{a} ± 12.1
	Basalt	2100	618.5^{c} ± 5.4	1481.5^{a} ± 5.4	1801.7^{b} ± 1.9	53.2^{b} ± 2.2	1679.6^{a} ± 5.4
	Andesite	2100	539.7^{c} ± 13.3	1560.3^{a} ± 13.3	1933.7^{a} ± 5.6	91.5^{ab} ± 3.1	1786.8^{a} ± 13.3

microbes was significantly accelerated to overcome the sink of SRO-adsorbed enzymes. On the other hand, oxidation products may have been adsorbed to SRO material surfaces. Allison (2006) found potential PPO activities increased in the presence of allophane, an important SRO material, although PPO oxidation products were found to be easily adsorbed onto allophane (Allison, 2006). Different extracellular enzymes may be differentially influenced by allophane and other SRO materials. However, more research is needed in order to better understand the relationship of the functioning of different enzymes with different minerals.

The basalt soil had no significant increase in extracellular enzyme production for either litter or exudate addition, despite having significant priming responses for both substrates. This suggests another mechanism besides extracellular enzyme production stimulates priming in soils containing SRO materials. However, because we only measured extracellular enzymes involved in C cycling, it is quite likely that other relevant enzymes were not measured. Other important extracellular enzymes, such as those involved in nitrogen or phosphorous cycling, were not measured in this study (Kandeler et al., 1999; Olander and Vitousek, 2000). If microbial mining for nutrients were occurring (Dijkstra et al., 2013), it was not captured in this study.

Another possibility for the greater priming observed in high SRO soils may have occurred because the addition of organic acid induced chemical dissolution or displacement of SRO-bound organic compounds, which consequently increased microbial access to otherwise-protected SOC. Two important processes leading to SOC associations with minerals are adsorption and formation of metal-containing (Fe and Al) organic coprecipitates (Kleber et al., 2015). Root exudates and low-molecular weight organic molecules released by microorganisms facilitate the adsorption or desorption of compounds at the mineral-water interface (Essington, 2004), which can displace native adsorbed SOC from mineral surfaces (Kaiser and Kalbitz, 2012; Kleber et al., 2015). Metal (Fe and Al) organic coprecipitates - formed by processes such as complexation of hydrolyzed Fe and Al species by SOC or through precipitation of insoluble organic-metal complexes - can undergo mineral dissolution induced by various root exudates, particularly organic acids (Kleber et al., 2015). A recently proposed abiotic mechanism hypothesizes that addition of a common root exudate, oxalic acid, promotes dissolution of SOC from SRO phases, mobilizing mineral-bound C to become biologically accessible (Keiluweit et al., 2015). Priming was greater after addition of oxalic acid relative to the more energetically-favorable glucose, suggesting an abiotic mechanism influencing increased mineralization of SOC compared to a microbial-growth driven mechanism (Keiluweit et al., 2015). The exudate mixture used in this study was composed of energetically-favorable compounds of sugars, organic acids and amino acids, with various dissolution and displacement properties (Hütsch et al., 2002). This exudate mixture may have abiotically released SRO-bound SOC through either mineral dissolution of co-precipitates (induced by chelators such as malate) or displacement of SOC adsorbed to mineral surfaces by younger sorbing compounds (Kaiser and Kalbitz, 2012; Kleber et al., 2015). This is supported from the observation that the andesite (highest SRO) soil had greater cumulative priming with exudate addition compared to the litter addition. Root exudates may have caused mineral-associated SOC of various bonding mechanisms to become newly accessible for microbial utilization.

Organic acids may have also played a role, albeit smaller, in releasing native SOC during priming in the litter-amended soil treatments. In the litter incubations, priming was less pronounced than in soils to which exudate C was added. Other studies have found around 3–30% of decomposing litter to be released as DOC (Don and Kalbitz, 2005; Wallace et al., 2008; Wymore et al., 2015). The needle litter used in this study consisted of about 30% water-soluble compounds, of which only about 33% were sugars and phenols (Rasmussen et al., 2008). Up to 75% of pine needle litter can be organic acids (Feng et al., 2010), which means that as much as 20% of the needle litter mass used in this study could consist of organic acids, although the exact composition remains unknown. This is comparable to the organic acid content of the exudate mixture, which consisted of about 18% organic acids. It is possible that due to the weekly wetting of the soil, a portion of these organic acids could have been solubilized throughout the incubation, although likely in lower quantities than solubilized organic acids in the exudate mixture. These released acids could then induce dissolution of native SOC adsorbed to SRO materials (Kaiser and Kalbitz, 2012). Further experimentation is needed to test this possible mechanism.

Another possible explanation for greater priming in high SRO soils is that increased microbial growth and activity due to simple C input alleviated spatial limitations and inaccessibility of SRO-bound SOC. Microorganisms can increase activity in response to addition of labile substrates, because the addition of easily metabolized C substrates provides the energy that is required for C-limited microorganisms to grow (De Nobili et al., 2001). Microbial growth due to substrate input may also alleviate the spatial inaccessibility to organic matter in soil (Schmidt et al., 2011), in turn providing new access to SOM. This may be achieved through increasing biomass so that microbes expand to new areas in the soil mineral matrix. However, during the first week of the incubation, exudate addition in the andesite and basalt soils had minimal effects on microbial biomass and on microbial incorporation of substrate C, even though the largest increase in priming occurred then. This suggests that the net increases in microbial biomass observed after the first input of new C into high SRO soil did not exert a large influence on the relatively large initial priming response. Alternatively, there could have been a large turnover of microbial biomass, with dead microorganisms incorporated into SOM—C.

Another explanation, not mutually-exclusive, is that the large priming effect observed in high SRO soils may not have been derived from mineral-bound SOC, but rather from the free-light, non-mineral associated, fraction of SOC. Another study found that cellulose addition did little to stimulate mineralization of mineral-bound soil organic matter, organic nitrogen in particular (Turner et al., 2017). Understanding the extent of decomposition of the mineral-associated versus the free-light fraction, and which was susceptible to priming from these soils, will be an important next step to understanding the specific mechanisms driving priming in the presence of SRO materials. In terms of both coprecipitation and adsorption, more decomposed SOC is generally mineral-associated compared to fresh organic matter (Kleber et al., 2015). Using the C:N ratios of each soil in the study as a proxy, we can estimate the proportion of SOC that is microbially-processed. Assuming that fresh litter has a C:N ratio of 40:1 and microbially-processed SOM has a C:N ratio of 10:1, we estimate that the andesite SOC was about 53%, the basalt was 68%, and granite was 40% microbially-processed SOC. These are in line with other studies finding that soils with an abundance of SRO materials often have a large fraction of microbial-derived organic matter compared to other soil types (Buurman et al., 2007). However, this does not exclude the possible contribution of the free-light fraction to priming.

Along a similar line, because the SRO content correlates with SOC content in our study soils, the total SOC content itself may also play an important role in priming. Soils rich in SOC often display larger positive priming responses compared to soils poor in SOC (Hart et al., 1986; Kuzyakov et al., 2000; Zhang et al., 2013). The andesite soil had more than three times as much native SOC as the granite soil, allowing for a larger total SOC pool to be primed. SRO materials promote SOC accumulation (Saggar et al., 1994; Torn et al., 1997; Parfitt et al., 2002; Matus et al., 2014), explaining the higher SOC contents in the andesite and basalt soils. We can conclude that soils with greater abundances of SRO materials may result in greater priming responses, but whether this is a direct or indirect influence remains to be fully determined. While our soil gradient is not a clean manipulation of SRO materials, these soils represent a range in natural variation in soil mineral assemblage. Therefore, this study may better reflect the possible responses to simple

and complex substrate input in natural soils along the spectrum of mineral-protected SOC.

The possibility of apparent priming must be considered. Apparent priming can be defined as the replacement of C in existing microbial biomass with ^{13}C without affecting SOM decomposition; in other words, microbial turnover (Blagodatskaya and Kuzyakov, 2008). Apparent priming can be estimated by measuring SOM-derived MBC of the control and substrate-amended treatments (Fontaine et al., 2011). During the first week, the SOM-derived MBC did not change from the control for the andesite and basalt soils, and increased for the granite due to substrate input (Fig. 3), which indicates real priming responses. Additionally, during the last week, SOM-derived MBC increased due to substrate input for all soils which also suggests real priming. We can therefore conclude that real priming occurred throughout this incubation for all soils.

4.2. Temporal priming dynamics

Priming may vary significantly depending on whether fresh C is added as a single addition or via multiple pulses of C (Hamer and Marschner, 2005b). We found cumulative priming was greatest in the andesite soil with repeated additions of litter and exudates compared to the granite and basalt soils. From a 90-day incubation with a single input of ponderosa pine litter, Rasmussen et al. (2007) found that granite and basalt soils in white-fir dominated forests substantially increased native soil C mineralization, whereas limited cumulative priming was found for the andesite soil. However, shortly after the addition of substrate, Rasmussen et al. (2007) also observed larger priming in the andesite soil than in the granite soil. Repeated litter or exudate substrate additions in our study possibly kept priming rates at a high level in the andesite soil, similar to the initial response observed by Rasmussen et al. (2007). This suggests that a consistent input of substrate is required to prime the andesite SOC pool. Repeated additions are more representative of forest ecosystems where regular or continuous root exudation and litterfall occur (Qiao et al., 2014), suggesting priming may be an important control on C dynamics in soils with high SRO abundance in natural systems.

Stimulation of extracellular enzyme production can occur in as little as a few weeks (Rinkes et al., 2014), but can take longer as slower-growing fungi are the primary microorganisms producing various hydrolytic and oxidative enzymes (Burns et al., 2013). During the last week of the incubation, the granite soil's priming response increased substantially from litter input. This corresponded with the significant increase in enzyme activity from both hydrolases as well as PER, which degrade cellulose and lignin, respectively. It is possible that if the incubation continued for a longer duration, there would be greater priming induced for the granite soil due to increased enzyme production or activity.

4.3. Carbon balance

In terms of the net change in SOC content, all soils accumulated C over the course of the experiment despite exhibiting positive priming. These incubations do not predict long-term C stocks, but may provide insight into C turnover and residence time. For example, higher SRO content soil generally exhibits greater C stocks that turn over more slowly compared to soil with low SRO content (Zunino et al., 1982; Torn et al., 1997; Rasmussen et al., 2005). In particular, allophanic soils can have up to 10 times larger SOC contents compared to other mineral soils (Zunino et al., 1982). This is consistent with our findings that high SRO soils mineralized less substrate C despite increased native C mineralization. Other studies have also observed an increase in C balance despite positive priming (Qiao et al., 2014). On the other hand, Herath et al. (2015) found weak priming in the presence of SRO materials yet a lower C balance compared to an Alfisol in a 510-day experiment. This coupling of greater priming with C accumulation may be a factor of SOC cycling in high SRO soils, but long-term C balance effects remain unclear.

If the increased SOC mineralized had previously been SRO-associated, the stability of C associated with high SRO content may involve more exchange between old and new C than previously considered. Additionally, C accumulation was proportional to priming so that, despite greater stimulation of native SOC mineralization in the high SRO soil, C accumulation was greater in the high SRO soil as well. The substantial capacity of soils with greater SRO contents to sequester and store C may be explained by a displacement of the native, more microbially-processed SOC with the new, stronger adsorbing organic compounds on the surface of SRO materials (Kaiser and Kalbitz, 2012). This may lead to increased older, stable C becoming physically accessible for microbial attack (Conant et al., 2011), but the net effect is that more organic C ends up adsorbed or coprecipitated in mineral associations (Kleber et al., 2015). New organo-mineral formation under new litter input was preferentially associated with existing organo-mineral complexes with rough surfaces (Vogel et al., 2014), which may help explain greater C accumulation despite greater priming in the high SRO soils. Conversely, in soils with few SRO materials, relatively less C is adsorbed to the mineral phase and therefore new C cannot release old C to promote high rates of priming. However, it is still unclear whether the primed SOC is mineral or non-mineral associated SOC in this study, and further investigation is required.

4.4. Litter labelling

In litter decomposition studies, differences in $\delta^{13}C$ between different plant compounds can occur. Lipids and alkanes can be 4–10‰ depleted compared to bulk litter (Hobbie and Werner, 2004), and carbohydrates are usually higher in $\delta^{13}C$ compared to lignin (Benner et al., 1987). Pulse-labelling of litter introduces yet another potential complication, as the timing of ^{13}C label application with respect to the timing of synthesis of different carbon compounds may cause uneven ^{13}C enrichment in different plant tissue compounds (Sangster et al., 2010; Haddix et al., 2016). Although many studies have utilized similar repeat pulse-labelling methods to generate labeled litter to use in priming experiments (Loya et al., 2004; Rasmussen et al., 2008; Frøseth and Bleken, 2014; Wang et al., 2014), testing the isotopic composition of different compounds is rarely done. We found that the pine needle litter, used to elicit priming in this study, was heterogeneously ^{13}C–labeled, despite being subjected to pulses of ^{13}C–CO_2-enriched air at regular intervals proportional to the rate of photosynthesis over the entire life of the *P. ponderosa* saplings. This heterogeneity in enrichment within litter could lead to erroneous estimates of litter-derived respiration due to uneven decomposition rates. However, after estimating possible error in priming under different assumptions of litter decomposition, we found significant differences in priming between the two scenarios, but did not find significant differences between each scenario and our priming estimates (Fig. S3B). We therefore conclude that our priming estimates using the repeat-pulse-labeled litter in this study were not affected by the uneven ^{13}C distribution in the litter. We suggest that label distribution be assessed in decomposition studies using plant tissues labeled through repeated pulses, as the quality of labelling may affect priming estimations (Sangster et al., 2010).

5. Conclusions

Our results show that addition of simple and complex C substrates to soils may enhance decomposition of native SOC to a greater degree in soils with a high abundance of SRO mineral phases, indicating that mineral-bound C may be susceptible to microbial decomposition. The activity of hydrolases (βG and CBH) increased with the addition of complex substrates in the high SRO soil that also exhibited the greatest priming. Our findings are inconsistent with the hypothesis that complex C addition induces large priming effects in low SRO soils due to

stimulated extracellular enzyme production and low priming in high SRO soils owing to low enzyme activity. We also found that priming increased with greater net C accumulation. Our research suggests that understanding mineral protection of native SOC and mineral interactions with microorganisms and different types of fresh organic carbon inputs are critical to understand the priming effect, which could have a long-term effect on soil C storage and global C cycling.

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Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2018.01.039.

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