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The soil priming effect: Consistent across ecosystems, elusive mechanisms

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ABSTRACT

Organic matter input to soils can accelerate the decomposition of native soil carbon (C), a process called the priming effect. Priming is ubiquitous and exhibits some consistent patterns, but a general explanation remains elusive, in part because of variation in the response across different ecosystems, and because of a diversity of proposed mechanisms, including microbial activation, stoichiometry, and community shifts. Here, we conducted five-week incubations of four soils (grassland, piñon-juniper, ponderosa pine, mixed conifer), varying the amount of substrate added (as ^{13}C-glucose, either 350 or 1000 μg C g^{-1} $week^{-1}$) and either with no added nitrogen (N), or with sufficient N (as NH_4NO_3) to bring the C-to-N ratio of the added substrate to 10. Using four different ecosystems enabled testing the generality of mechanisms underlying the priming effect. The responses of priming to the amount and C-to-N ratio of the added substrate were consistent across ecosystems: priming increased with the rate of substrate addition and declined when the C-to-N ratio of the substrate was reduced. However, structural equation models failed to confirm intermediate responses postulated to mediate the priming effect, including responses postulated to be mediated by stoichiometry and microbial activation. Specifically, priming was not clearly associated with changes in microbial biomass or turnover, nor with extracellular enzyme activities or the microbial C-to-N ratio. The strongest explanatory pathways in the structural equation models were the substrate, soil, and C-to-N ratio treatments themselves, with no intermediates, suggesting that either these measurements lacked sufficient sensitivity to reveal causal relationships, or the actual drivers for priming were not included in the ancillary measurements. While we observed consistent changes in priming caused by the amount and C-to-N ratio of the added substrate across a wide array of soils, our findings did not clearly conform to common models offered for the priming effect. Because priming is a residual flux involving diverse substrates of varying chemical composition, a simple and generalizable explanation of the phenomenon may be elusive.

1. Introduction

Organic matter inputs to soils occur from growing plants as root exudates and senesced plant tissues, substrates to the decomposer soil community. These inputs vary over time and space, and they often occur in pulses eliciting responses in the decomposer community, both direct responses as the community uses the new organic matter as a substrate for metabolism and growth, and indirect responses, for example, priming (Kuzyakov, 2002). Priming is "extra decomposition of native soil organic matter in a soil receiving an organic amendment" (Bingeman et al., 1953), first documented in soils over 90 years ago (Löhnis, 1926; Broadbent, 1947; Broadbent and Norman, 1947). In priming, inputs of carbon (C) to soil enhance the microbial degradation of the C in soil that was already present, C that would have, otherwise, remained soil organic matter (SOM). The opposite can also occur, where inputs of C to soil reduce the microbial degradation of the SOM present before the addition, sometimes referred to as "negative priming" (Guenet et al., 2010a) or "preferential substrate use" (Blagodatskaya et al., 2007; Liu et al., 2017). Mechanisms driving priming are unclear – although many have been invoked, such as microbial activation, stoichiometry, and N limitation (Kuzyakov et al., 2000; Fontaine et al., 2003; Blagodatskaya et al., 2007; Wild et al., 2019; Perveen et al., 2019). One of the problems with testing the mechanisms involved with the priming effect is that they are typically postulated to be chain reactions, with an initial

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stimulus causing a response, which in turn elicits further responses. It may not be feasible to test each step in such chain reactions with independent inference tests. Nevertheless, some effort – beyond speculation – to evaluate the proposed mechanisms may illumine the underpinnings of this widespread biogeochemical phenomenon.

One proposed model for priming is called "microbial activation" (Kuzyakov et al., 2000), in which the addition of new substrate to soil stimulates microbial metabolism and biomass growth (Helal and Sauerbeck, 1984; Sallih and Bottner, 1988; Cheng and Coleman, 1990; De Nobili et al., 2001). According to the microbial activation model, initially, microbial activity and growth may be supported by the added substrate, but subsequently microorganisms shift to native SOM once the new substrate is depleted. Support exists for the "microbial activation" model. Microbial biomass turnover increased with root exudation and had a positive relationship with rhizosphere priming (Cheng, 1999). Substrate additions in plant-free incubations can also stimulate microbial biomass and priming (Liu et al., 2017), and such responses can be associated with increased microbial turnover (Perelo and Munch, 2005).

Carbon-nitrogen stoichiometry may also drive priming of native SOM, induced by the added C substrate, as microorganisms meet demands for balanced growth by accessing N contained in SOM. According to this stoichiometry model, the magnitude of priming depends strongly on the balance of C and N limitation of microbial growth (Kuzyakov et al., 2000). Much SOM consists of organic compounds that, for their large size or lack of suitable transporters in microbial cell membranes, are not directly imported into microbial cells. Furthermore, N within SOM occurs in complex forms, including heteroaromatic polymers and peptides complexed with other polymers (Knicker et al., 2002; Knicker, 2011). Nitrogen release from chitin or proteins may be less important during priming, despite increased N limitation of microbial growth in response to C addition (Wild et al., 2019). Enzymes like phenol oxidases and peroxidases, which are capable of degrading complex C compounds, are thought to be expressed for the acquisition not just of C but also of N (Talbot et al., 2008; Courty et al., 2009; Sinsabaugh, 2010). For these reasons, the stoichiometry model posits increased enzyme activity in concert with priming, and in particular an increase in the production of extracellular enzymes capable of degrading complex soil C compounds (Fontaine et al., 2003).

Components of the stoichiometry model are supported by observations in the literature. Labile substrate inputs can increase extracellular enzyme activity (Blagodatskaya et al., 2014; Chen et al., 2014), and the response appears to be mediated by stoichiometry: β-glucosidase and cellobiohydrolase activity increased with soil C content (Sinsabaugh et al., 2008), whereas increased N availability reduced production of enzymes that break down SOM (Sinsabaugh et al., 2005). Consistent with this, N addition with labile C can decrease priming compared to C input alone (Blagodatskaya et al., 2007; Guenet et al., 2010b), although these findings are not universal (Chen et al., 2014; Wu et al., 1993). Natural variation in nutrient availability might be expected to affect priming: but observations are inconsistent. For example, soils higher in nutrient content have been reported to induce a smaller priming effect than low nutrient soils in some cases (Dimassi et al., 2014), whereas others have found the opposite (Perveen et al., 2019), and still others have found priming to be consistent in soils of different nutrient contents (Qiao et al., 2014).

Regardless of the mechanisms, priming has been invoked as a potential mediator of soil C balance large enough to consider in global-scale models of the C cycle (Heimann and Reichstein, 2008). Substrate inputs, such as glucose and cellulose, have been reported to cause priming (Blagodatskaya et al., 2014, 2011) that could result in a negative C balance (Fontaine et al., 2004a) and reduce soil C content (Fontaine et al., 2004b; Fontaine and Barot, 2005). However, glucose input can also increase soil C content even with priming (Qiao et al., 2014), a finding confirmed through meta-analysis where C gain through retention of the added C substrate was found to exceed priming-induced C loss (Liang et al., 2018; Finley et al., 2018; Liu et al., 2017). Thus, it is critical to evaluate priming and its relationship to retention of added C as a mechanism of soil C accumulation.

Here, we evaluated priming and its relationship with microbial characteristics by conducting 5-week incubation experiments using soils from four different ecosystems varying in climate, organic matter content, microbial biomass, and enzyme activity (Table 1). We hypothesized that 1) Priming declines when N is added along with C, compared to C alone; 2) priming increases with increased microbial growth and biomass turnover; 3) priming is associated with increased extracellular enzyme production; and 4) priming is positively associated with soil C accumulation. Hypotheses 1–3 include concepts addressed by the microbial "activation" and "stoichiometry" models for the priming effect. In both cases, these models involve sequential responses difficult to test individually. Thus, we combined these ideas using structural equation modeling to search for general drivers of the priming effect consistent with these widely invoked explanations.

2. Materials and methods

2.1. Field sampling and experimental design

This work occurred in soils from four ecosystems (cool desert grassland, piñon-juniper woodland, ponderosa pine forest, and mixed conifer forest) located along an elevation gradient near Flagstaff, Arizona, USA (Blankinship et al., 2010, Table 1). We conducted two experiments, one in July and one in October 2014. The designs of the two experiments were identical, crossing soil by the C-to-N ratio of the substrate added to induce priming, with the exception that the October experiment utilized a higher rate of substrate addition compared to the July experiment. We selected the rates of substrate addition based on previous measurements in these same soils assessing their priming responses to C addition alone. This work showed that the magnitude of priming increases with the rate of substrate addition, and that positive priming appears to occur consistently when substrate is added at rates above ~250 μg C g soil^{-1} week^{-1} (Liu et al., 2017). The two rates we selected were slightly (350 μg C g soil^{-1} week^{-1}, July experiment) and considerably (1000 μg C g soil^{-1} week^{-1}, October experiment) higher than that threshold, with the idea that this would provide a range of priming responses over which putative drivers could be explored.

For each experiment, we collected four replicate surface samples (0–10 cm) from each site. Each replicate sample was a pooled composite of multiple cores sampled from a single location. Visible roots and rocks were removed. Soils were passed through a 2-mm sieve and stored at 4 °C. Total soil C and N and potential activities of β-glucosidase, cellobiohydrolase, and phenol oxidase did not differ between the two collection times (Table S1). Baseline microbial biomass C (MBC) and N, potential peroxidase activity, and bulk soil $\delta^{13}C$ were significantly higher for the July experiment. Inferences about the role of the rate of substrate addition are weakened by temporal dissociation between levels and by these observed differences in soil characteristics. On the other hand, responses of priming to substrate addition have already been thoroughly documented for these soils (Liu et al., 2017), and, the responses we observed to the rates of substrate addition used in July and October conform well to those observed previously (Liu et al., 2017) (see Results). Therefore, we treat the differences in the rate of substrate addition in the July and October experiments as a factor in the linear models used to explore drivers of the priming effect. Our main focus was on exploring priming over a broad range of soil types and soil conditions and evaluating associations with proposed mechanisms that might provide a general mechanistic explanation of the priming effect.

2.2. Laboratory incubations

For each sample, we added 40 g (dry weight) soil to specimen cups (120 ml), and adjusted soil moisture content to at 70% of water holding capacity. Specimen cups were placed in mason jars (473 ml) for one

Table 1
Characteristics of the soils used in the priming experiments, including the carbon, nitrogen, and $\delta^{13}C$ composition of the soil microbial biomass, activities of the soil enzymes β-glucosidase (BG), cellobiohydrolase (CBH), phenol oxidase (POX) and peroxidase (PER), and total soil carbon, nitrogen, and $\delta^{13}C$ composition.

	Soil Microbial Biomass			Soil Enzyme Activities			
	Carbon	Nitrogen	$\delta^{13}C$	BG	CBH	POX	PER
	μg C g soil^{-1}	μg N g soil^{-1}	‰, VPDB	nmol g^{-1} h^{-1}	nmol g^{-1} h^{-1}	nmol g^{-1} h^{-1}	nmol g^{-1} h^{-1}
Grassland	151.83 ± 40.92	10.46 ± 3.94	−13.58 ± 1.31	36.62 ± 7.19	2.91 ± 1.09	0.69 ± 0.22	1.19 ± 0.37
Piñon-Juniper	142.31 ± 28.83	10.75 ± 5.65	−16.06 ± 1.62	26.56 ± 10.1	2.23 ± 0.93	0.76 ± 0.26	1.39 ± 0.36
Ponderosa	128.58 ± 31.06	9.06 ± 6.38	−19.58 ± 1.39	43.17 ± 24.23	3.25 ± 1.52	0.65 ± 0.29	1.45 ± 0.45
Mixed Conifer	253.66 ± 52.65	38.84 ± 14.54	−19.95 ± 0.88	60.71 ± 23.65	2.69 ± 1.35	0.64 ± 0.16	1.00 ± 0.45

	Bulk Soil		
	Carbon	Nitrogen	$\delta^{13}C$
	%	%	‰, VPDB
Grassland	1.59 ± 0.36	0.13 ± 0.02	−15.91 ± 0.45
Piñon-Juniper	1.55 ± 0.3	0.13 ± 0.02	−16.81 ± 0.88
Ponderosa	1.72 ± 0.49	0.1 ± 0.03	−21.35 ± 1.14
Mixed Conifer	4.35 ± 0.28	0.33 ± 0.02	−21.44 ± 0.41

week of preincubation (22 °C) to allow for recovery from physical disturbance of soils prior to the incubation. After preincubation, weekly substrate addition treatments began. One set of soils received only deionized water (control), and treatment samples received either glucose alone (which we refer to as the "no N" treatment) or with sufficient N as ammonium nitrate (NH_4NO_3) such that the C-to-N ratio of the added substrate was equal to 10 (which we refer to as the "C:N = 10" treatment). We determined priming using isotope mass balance, introducing an artificially high ^{13}C tracer with the substrate additions. To achieve the ^{13}C signal, universally labeled ^{13}C-glucose (D-Glucose-^{13}C, 97 atom%; Cambridge Isotope Laboratories, MA, USA) was added to natural abundance glucose, such that the added substrate had a $\delta^{13}C$ signature of 1357‰ for the July experiment and 1159‰ for the October experiment.

Most priming studies apply substrates in a single addition, yet repeated substrate additions have been proposed to better represent substrate inputs through root exudation in the field (Hamer and Marschner, 2005; Liu et al., 2017; Qiao et al., 2014). A single substrate addition has also been reported to induce stronger priming than multiple additions, possibly overestimating the priming effect (Qiao et al., 2014; Wang et al., 2019). For these reasons, we elected to use multiple substrate additions (one per week) over the 5-week periods.

Each treatment had four replicates, and all soils were incubated under the same conditions as the preincubation. Each week, we sealed the jars to measure CO_2 fluxes during three measurement periods: 0–2 days, 2–5 days, and 5–7 days after the weekly substrate addition (Liu et al., 2017). These periods were short enough that jars did not become anoxic. After each sampling, jars were open to room air before resealing for the next measurement interval. A 10 ml sample was taken for CO_2 concentration using a flow through system attached to a Licor 6262 infra-red gas analyzer. For $^{13}CO_2$ values, a 60 mL gas sample was injected to a Picarro CO_2 and CH_4 isotope spectrometer (Picarro G2202-*i*). CO_2-free air was used to dilute gas samples when CO_2 concentrations were greater than 2000 ppm. $\delta^{13}CO_2$ values were read from the Picarro approximately 4–5 min after injection. In October 2014, the same procedures were repeated with substrate C and N added at a higher rate. Glucose was added at 1000 μg C g^{-1} week^{-1} without or with N added at 100 μg N g^{-1} week^{-1}, along with a control where only deionized water was added.

2.3. Microbial biomass and extracellular enzyme activity

Soil samples were taken before and at the end of the incubations to analyze microbial biomass and enzyme activity. Soil MBC and microbial biomass N (MBN) were measured using the chloroform-fumigation-extraction method (Vance et al., 1987) with minor modifications as described in Liu et al. (2017): 20 g of soil after chloroform fumigation (five days) and unfumigated soil samples were extracted with 50 ml K_2SO_4 solution (0.05 M), shaken for 1 h at 200 rpm using an orbital shaker (VWR S-500), and the filtrate collected (filtered through Whatman #3). The filtrate was oven-dried at 60 °C to constant weight (~72 h) and ground to fine powder with a pestle and mortar. The ground extracts were analyzed for C and N with an elemental analyzer coupled to a mass spectrometer (Thermo Fisher Scientific, Florida, USA). MBC and MBN were calculated as the difference between fumigated and unfumigated soil samples in C or N content, divided by corrections for extraction efficiencies: 0.45 for MBC and 0.54 for MBN (Vance et al., 1987).

Activity of β-glucosidase, cellobiohydrolase, phenol oxidase, and peroxidase was analyzed using standard fluorescence and colorimetric techniques (German et al., 2011). Briefly, 1.0 g soil (dry weight equivalent) was homogenized using a Waring Commercial Blender with 125 ml of sodium acetate buffer (50 mM; pH = 6.0) for 2 min. The solution was stirred at 350 rpm on a magnetic plate for 30 min, and 200 μl aliquots were transferred to a 96-well plate.

β-glucosidase and cellobiohydrolase assays were conducted with eight replicates per sample. A 50 μl of substrate solution (200 mM 4-Methylumbelliferyl β-D- glucopyranoside and cellobioside) was added to plate wells, where blank wells received 250 μl sodium acetate buffer, and reference wells received 200 μl sodium acetate buffer and 50 μl of 100 μM 4-methylumbelliferone standard. Negative control wells received 200 μl sodium acetate buffer and 50 μl substrate solution. After transferring 200 μl soil slurry to sample wells, homogenate controls received 50 μl sodium acetate buffer, quench controls received 50 μl 4-methylumbelliferone standard, and assay wells received 50 μl substrate solution. Plates of both assays were incubated (1.5–2.5 h) in the dark at room temperature (Finley et al., 2018). Fluorescence was measured for each well on a plate reader at excitation of 365 nm and emission of 450 nm. Calculations of fluorescent enzyme activity were performed as described in German et al. (2011):

$$\text{Activity}\left(\text{nmol g}^{-1}\text{h}^{-1}\right) = \frac{\text{Net fluorescence} \times \text{Buffer volume (ml)}}{\text{Emission coeff.} \times \text{Homogenate volume (ml)} \times \text{Time (h)} \times \text{Soil (g)}} \quad (1)$$

where,

$$\text{Net fluorescence} = \left(\frac{\text{Assay} - \text{Homogenate control}}{\text{Quench coefficient}}\right) - \text{Substrate control} \quad (2)$$

$$\text{Emission coefficient}\left(\text{Fluorescence nmol}^{-1}\right) = \frac{\text{Standard fluorescence}}{\text{Standard concentration}\left(\text{nmol ml}^{-1}\right) \times \text{Standard volume (ml)}} \quad (3)$$

$$\text{Quench coefficient}\left(\text{Fluorescence nmol}^{-1}\right) = \frac{\text{Quench control} - \text{Homogenate control}}{\text{Standard fluorescence}} \quad (4)$$

Phenol oxidase and peroxidase colorimetric assays had 16 replicate wells per sample. Each well received substrate solution (25 mM L-dihydroxyphenylalanine; L-DOPA), and peroxidase wells received additional 10 μL of 0.3% H_2O_2. Peroxidase activity was calculated as the difference in oxidation between the H_2O_2-amended plates and the phenol oxidase plates. Both were incubated for 24 h in the dark at room temperature. Calculations of enzyme activity were performed as described in German et al. (2011):

$$\text{Net Absorbance} = \text{Assay} - \text{Homogenate control} - \text{Substrate control} \quad (5)$$

$$\text{Activity}\left(\mu\text{molg}^{-1}\,\text{h}^{-1}\right) = \frac{\text{Net absorbance x Buffer volume (mL)}}{\text{Extinction coeff.} \times \text{Homogenate volume (mL)} \times \text{Time (h)} \times \text{Soil (g)}} \quad (6)$$

2.4. Microbial biomass C turnover

We calculated microbial biomass turnover using soil respiration, biochemical efficiency (BE), and changes in MBC during incubation following Hagerty et al. (2014). We used metabolic flux analysis to estimate biochemical efficiency. Related to C use efficiency (Geyer et al., 2016), biochemical efficiency specifically refers to the proportion of C flow through the central metabolic network that is allocated to biosynthesis (Dijkstra et al., 2011a; Hagerty et al., 2014). Biochemical efficiency was measured using position-specific ^{13}C-labeled isotopologues of glucose (U–^{13}C and 1–^{13}C) and pyruvate (1–^{13}C and 2, 3–^{13}C) as metabolic tracers as part of the October 2014 experiment. $^{13}CO_2$ accumulation was measured after 0, 20, 40 and 60 min (four replicates per time point) in the first and last weeks of incubation for the grassland soil without substrate amendment, and for the high substrate rate treatments at low and high C-to-N ratios. The ratios between $^{13}CO_2$ production rates from glucose and pyruvate isotopologues were calculated and used to model metabolic pathway activity and biochemical efficiency (Dijkstra et al., 2011b). We found no significant effects of substrate or C-to-N ratio treatments on biochemical efficiency, and little variation. Therefore, to estimate microbial turnover for all soils, we assumed that biochemical efficiency observed for the grassland soil (mean = 0.72) was representative for the other soils and treatments in our experiment. Similar values of biochemical efficiency have been observed in other studies using the same technique, and in response to varying substrate inputs and soils from different ecosystems (Dijkstra et al., 2011a, Dijkstra et al., 2011b; Dijkstra et al., 2011c; Hagerty et al., 2014; van Groenigen et al., 2013), evidence supporting our assumption that biochemical efficiency is consistent. We calculated microbial biomass turnover using soil respiration, BE, and changes in MBC during incubation following Hagerty et al. (2014). Total respiration (R_{tot}) is associated with two processes: CO_2 released to produce new microbial biomass (R_g) and turnover of biomass to CO_2 (R_τ). We assume that incorporation of microbial biomass in the SOM pool is negligible during the incubation. The total (gross) growth of microorganisms during the incubation is calculated as:

$$\Delta MBC_g = \frac{BE}{1 - BE} \times R_g \quad (7)$$

The net increase or decrease of MBC is measured as ΔMBC_n (μg g^{-1} soil). If there are no changes in MBC ($\Delta MBC_n = 0$; stationary assumption; Hagerty et al., 2014), respiration associated with turnover is calculated as:

$$R_T = \frac{BE}{1 - BE} \times R_g \quad (8)$$

However, when MBC increases or decreases over time, then the turnover is calculated as:

$$R_T = \frac{BE}{1 - BE} \times R_g - \Delta MBC_n \quad (9)$$

Since $R_{tot} = R_g + R_\tau$, then respiration associated with biomass turnover is:

$$R_T = BE \times R_{tot} - (1 - BE) \times \Delta MBC_n \quad (10)$$

Turnover time was estimated as R_τ/μ_{MBC}, where μ_{MBC} is the mean value of MBC over time.

2.5. Soil ^{13}C accumulation

To assess the relationship between priming and retention of the added C, we measured net retention of the ^{13}C tracer at the end of the incubation. We chose this approach, because priming, itself, already captures the effect of substrate addition on the loss of non-labeled (i.e., "old") C. Net ^{13}C retention reflects the other side of the soil C balance equation. At the end of the incubation, all soils were analyzed for C and ^{13}C at the Colorado Plateau Stable Isotope Laboratory www.isotope.nau.edu. Soils were dried for 48 h at 105 °C, and a ~3 g subsample was ground and milled to a fine powder. Subsamples were weighed into tin capsules for Dumas combustion using a Costech ECS 4010 Elemental Analyzer coupled to a Thermo DeltaPLUS Delta V Advantage isotope-ratio mass spectrometer via a Thermo ConFlo III device. δ^{13}C values were converted to atom percent ^{13}C, and tracer ^{13}C accumulation was

calculated using mass a mixing model, where the proportion of soil C at the end of the incubation that was derived from the ^{13}C-labeled glucose (p_G) is calculated as:

$$p_G = (A_T - A_C)/(A_G - A_C) \quad (11)$$

Where A indicates the atom percent ^{13}C of the amended soil at the end of the incubation (A_T), the control soil receiving no glucose amendments (A_C), and the added glucose (A_G). The mass of accumulated tracer ^{13}C, expressed as μg tracer ^{13}C per g soil, was calculated as the product of $pSOC_G$ and the total soil C at the end of the experiment.

2.6. Priming calculation

The priming effect was calculated as the difference between respiration derived from SOM in substrate-amended and control samples. Soil respiration derived from SOM and from the added substrate in substrate-amended soils were estimated using isotopic mass balance:

$$C_{SOM} = C_{total} \times \frac{A_{CS} - A_G}{A_{CN} - A_G} \quad (12)$$

where C_{SOM} and C_{total} are CO_2–C (μg g^{-1}) derived from native SOM and substrate-amended samples, and A indicates atom percent ^{13}C signatures of CO_2 measured from the substrate-amended sample (A_{CS}), of the added glucose (A_G), and of CO_2 released from native SOM in the non-amended control samples (A_{CN}).

2.7. Statistical analyses

We tested for homogeneity of variances using Levene's test. Variances of weekly priming data were homogenous after log transformation ($P > 0.05$). Because priming can be negative, this was log transformation after adding the minimum observed value plus 1. Significance of terms in the linear models was insensitive to log-transformation.

We used a linear mixed effects model to determine responses of priming to substrate rate (two levels, low and high), C-to-N ratio treatment (two levels, where the C-to-N ratio is undefined (i.e., no N) or equal to 10), soil (4 levels, one for each of the 4 ecosystems included), and week (5 weeks), with subject (incubation jar) as a repeated measure. The inclusion of substrate rate in our analysis is confounded by the temporal separation of the low (April 2014) and high (October 2014) substrate treatments. While incubation conditions, sample collection, and methods were consistent between the two measurement periods, significant effects of substrate rate in our analysis will include any influence of collection period. Given the resources available to conduct each experiment, this was a compromise between the scale of the experiments involved and the desire to equalize the time interval between field sampling and the laboratory experiments.

Models were constructed using the *lmer* function in the lmerTest package in R. We used model selection, choosing the linear model with the lowest value of the Bayesian Information Criterion, an approach taking into account likelihood, but penalizing complexity. We also used this model selection approach with analysis of variance to analyze response variables such as microbial biomass, turnover, and enzyme activities. For each response variable, we assessed the response to substrate treatment, such that each observation was a paired difference between the treatment with the added substrate, and the control with no added substrate. Because each response variable was evaluated as a paired difference, the dataset included positive and negative values. We used this approach to match priming as the main response variable of interest, which is also calculated as a paired difference, reasoning that the greatest sensitivity in understanding responses of priming would be in comparably calculated changes in other response variables thought to be involved. Postulated driver variables for priming (MBC, N, and turnover, and enzyme activity) are presented in figures showing significant effects only. The full dataset is archived on Mendeley Data.

We used structural equation modeling (SEM) to assess possible causal relationships among variables, in order to evaluate two model constructs that reflect common conceptual explanations of the priming effect: activation and stoichiometry. The specific models postulated here are only iterations of these ideas, as no single explicit formulation has been universally accepted for either model explaining priming. We therefore refer to the models tested here as "microbial biomass and turnover" for the scheme representing the concept of microbial activation of priming, and as "microbial C:N and enzyme activities" for the scheme representing the concept as stoichiometric control over priming. We use these labels to acknowledge that different formulations of these models are possible. Furthermore, microbial activation and stoichiometry are not mutually exclusive. Therefore, we also tested a combined model that included all postulated causal pathways. We refer to this as the "combined model".

Because there is no single universally accepted best test of absolute goodness of fit, we conducted three: the χ^2 goodness of fit test, the RMSEA test, and the Bollen-Stine goodness of fit test. We compared the relative fit of the three models using the Bayesian Information Criterion (Akaike's Information Criterion gave qualitatively identical answers). All models included substrate rate, substrate C-to-N ratio, and ecosystem as potential direct drivers of the priming effect, to capture pathways caused by the treatments and not represented by the postulated causal intermediates. The ecosystem factor was modeled as a composite variable. For the microbial biomass/turnover SEM, we postulated that priming would be positively related to changes in microbial biomass and turnover, both of which would be directly affected by the rate of substrate input, its C-to-N ratio, and the ecosystem. For the microbial stoichiometry/enzyme activity SEM, we postulated that priming would be positively associated with the total potential extracellular enzyme activity. In order to weight the enzymes tested equally, we performed a z-transformation for activity values for each enzyme, and then used the sum of z-transformed values for each replicate as the estimate of total extracellular enzyme activity. We also postulated that priming would be positively associated with the relative allocation to enzymes responsible for degrading more complex substrates compared to enzymes degrading relatively simple substrates. This "enzyme ratio" variable was calculated as: (phenol oxidase + peroxidase activity)/(β-glucosidase + cellobiohydrolase activity), again using z-transformed values to weight activity of each enzyme equally. We further postulated that the potential enzyme activity would be positively related to the C-to-N ratio of the soil microbial biomass, reflecting the idea that enzyme production is a strategy to acquire N. Finally, each of these drivers was, in turn, postulated to be influenced by the rate of substrate addition, its C-to-N ratio, and the ecosystem from which the soil sample was collected.

3. Results

3.1. Priming

The priming effect was most parsimoniously described by a linear model including the C-to-N ratio of the added substrate, the rate of substrate addition (or month of experiment), their interaction, the soil of origin, and time as significant factors (Table 2). Among all models

Table 2
Best model for repeated measures analysis of variance for priming. The best model included main effects of substrate, C:N, soil type, and week, with an interaction between substrate and C:N.

	Sum Sq	Mean Sq	DF	F-value	P-value
Substrate	4.87	4.87	1,57	475.272	<0.001
C-to-N ratio	2.02	2.02	1,57	197.688	<0.001
Soil	0.59	0.20	3,57	19.334	<0.001
Week	0.30	0.30	1,255	29.014	<0.001
Substrate × C-to-N ratio	0.55	0.55	1,57	53.923	<0.001

Table 3
Variance of response variables explained by model terms, calculated as eta-squared.

	Substrate	C:N	Soil	Substrate × C:N	Substrate × Soil	C:N × Soil	Substrate × C:N × Soil
Cumulative Priming	55%	25%	6%	10%	0%	1%	1%
Enzyme Activities							
β-glucosidase	22%	1%	13%	2%	13%	3%	3%
Cellobiohydrolase	20%	2%	8%	3%	9%	2%	2%
Phenol Oxidase	2%	8%	2%	0%	11%	2%	5%
Peroxidase	1%	2%	22%	3%	27%	3%	3%
Microbial Biomass							
Carbon	11%	0%	32%	11%	16%	0%	2%
Nitrogen	7%	37%	11%	6%	15%	2%	1%
C-to-N ratio	0%	12%	37%	0%	14%	3%	1%
Turnover	75%	0%	9%	1%	3%	0%	0%

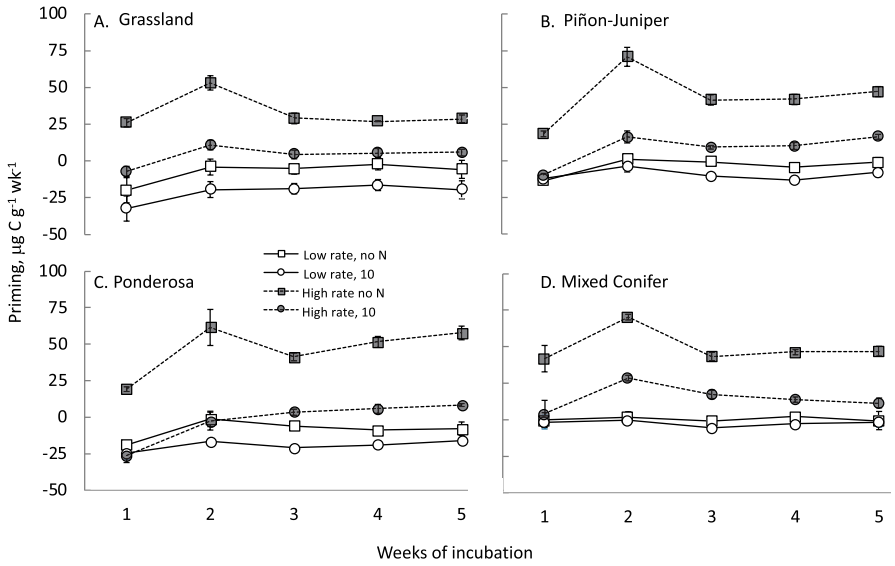

Fig. 1. Temporal dynamics of priming over five weeks of incubation in four soils: grassland (A), piñon-juniper (B), ponderosa pine (C), and mixed conifer (D). Each soil was subject to a factorial design of low and high rate of substrate addition (350 or 1000 µg C g^{-1} $week^{-1}$) and a low or high C-to-N ratio of the added substrate (10 or no N).

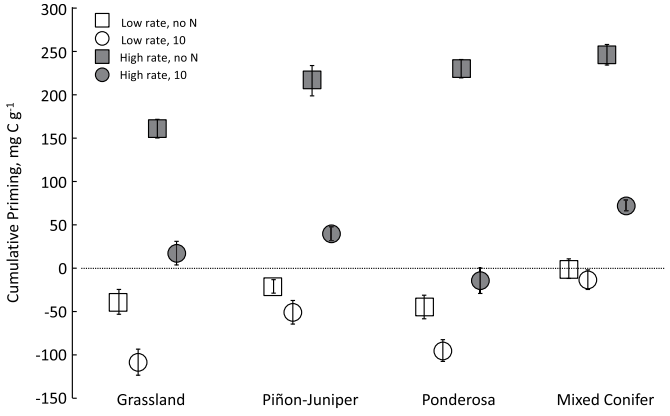

Fig. 2. Cumulative priming for the four soils and treatments described in Fig. 1.

assessed, this model had the lowest Bayesian Information Criterion value; additional terms included made only minor contributions to explaining the variance (Table 3).

Priming increased over time ($P < 0.001$), lowest during week 1, and peaking in a number of cases during week 2 (Fig. 1). Added substrate as C alone consistently increased priming, and priming was always higher in the experiment with the higher rate of C addition (Fig. 1); these findings were quantitatively consistent over time during the 5-week incubations. Thus, although the priming effect increased over the 5-week incubations, the effects of treatments on priming were consistent over this time period, and no interactions with time were significant in the most parsimonious model (Table 2).

Table 4
The response of priming to substrate addition, calculated as the change in priming in response to the difference in substrate addition between the experiments conducted in July (350 µg C/g/week) and October (1000 µg C/g/week).

	C:N = 10	no N
Grassland	0.194 ± 0.036	0.309 ± 0.037
Piñon-Juniper	0.139 ± 0.020	0.367 ± 0.034
Ponderosa Pine	0.132 ± 0.026	0.422 ± 0.028
Mixed Conifer	0.134 ± 0.025	0.380 ± 0.023

Cumulative priming was highest for the mixed conifer soil, intermediate for the piñon-juniper soil, and lowest for (and indistinguishable between) the grassland and ponderosa pine soils. Priming was higher in the October experiment with the higher rate of substrate addition compared to the July experiment with the lower rate of substrate addition (Fig. 2). Priming increased with the C-to-N ratio of the added substrate, higher in the no N substrate treatment compared to the treatment where C:N = 10 (Fig. 2). There were no interactions between soil type and the substrate or C-to-N treatments (Table 2), indicating that

Table 5
Summary of significance terms from linear mixed effects model analysis of soil activity measures in response to substrate and CN treatments. As described in the Methods, each model selected for a response variable was that with the lowest Bayesian Information Criterion score. The first column shows the response variables considered. Numbers are P-values from Analysis of Variance models. Empty cells indicate terms that were excluded in the best model for that response variable.

	Substrate	C:N	Soil	Substrate × Soil
Enzyme Activities				
Cellobiohydrolase	<0.001			
Phenol oxidase		<0.050		
β-glucosidase	<0.001		<0.010	<0.010
Peroxidase	0.303		<0.001	<0.001
Microbial Biomass				
Turnover	<0.001		<0.001	
Carbon	<0.001		<0.001	<0.001
Nitrogen	<0.001	<0.001	<0.001	<0.001
C-to-N ratio	0.981	<0.001	<0.001	<0.010

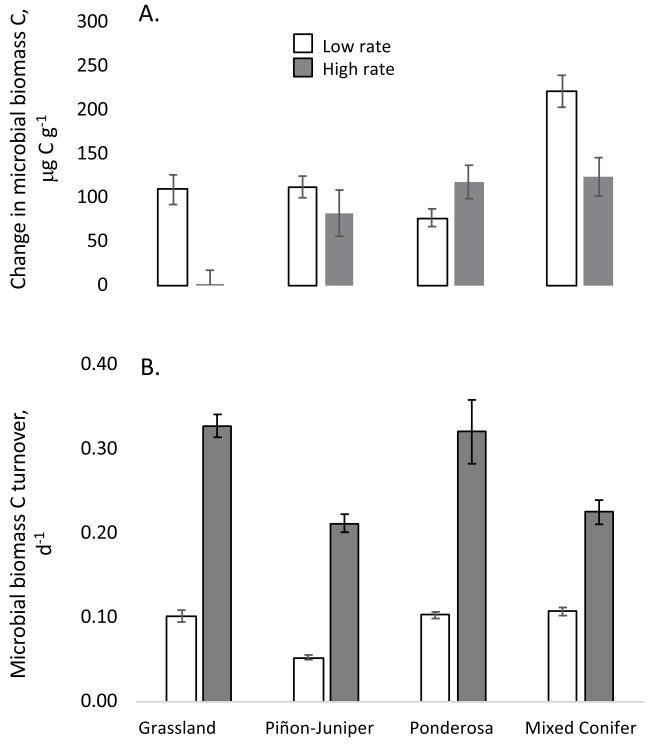

Fig. 3. Responses of microbial biomass carbon (A), and microbial biomass turnover (B) to the rate of substrate addition across the four ecosystems examined. Values shown are means standard errors of the mean response to substrate addition (n = 8); means are averaged across C-to-N ratio treatments, for which main effects and interactions were not included in the best models.

treatments elicited similar responses of priming across the four ecosystems considered. During the October experiment with the higher rate of substrate addition, the no N treatment caused an especially large increase in priming (substrate × C:N ratio interaction, Table 2).

The sensitivity of priming to the rate of substrate input ranged between 0.30 and 0.42 for the treatment without added N (Table 4), consistent with a prior study which found the sensitivity of priming to range in these same soils from 0.25 to 0.50, calculated across six levels of substrate addition (Liu et al., 2017). The consistency in sensitivity values between this and the previous study indicates that much of the difference between the July and October experiments can be explained by their different rates of substrate addition. Adding N to bring the C-to-N ratio of the added substrate to 10 substantially reduced the sensitivity of

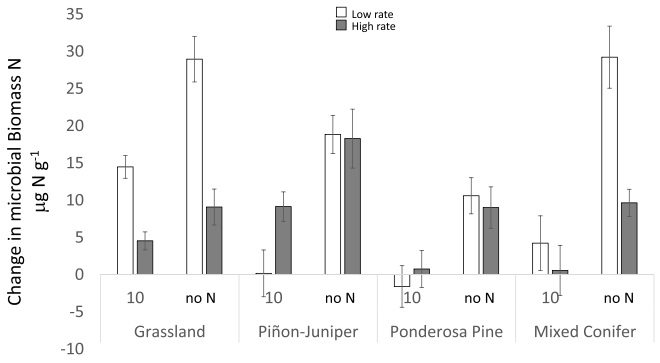

Fig. 4. Response of microbial biomass nitrogen to the rate of substrate addition and C-to-N ratio treatments across the four soils. Values are means plus or minus standard error of the mean (n = 4).

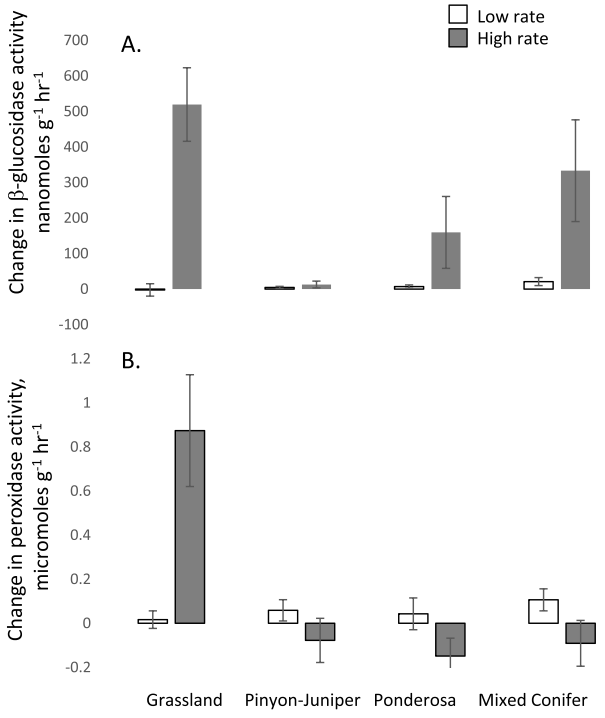

Fig. 5. Responses of β-glucosidase (A) and peroxidase (B) potential enzyme activities to the rate of substrate addition across the four ecosystems examined. Values shown are means standard errors of the mean response to substrate addition (n = 8); means are averaged across C-to-N ratio treatments, for which main effects and interactions were not included in the best models.

priming to the rate of substrate addition (Table 4).

3.2. Microbial biomass and turnover

Soil MBC increased strongly when the rate of substrate addition was low in the mixed conifer soil, less so in the other three soils, and exhibited no response in the grassland soil at the high rate of substrate addition (soil × substrate interaction, Table 5, Fig. 3A). The response of biomass turnover was highest in the experiment with the high rate of substrate addition, across soils and C-to-N ratio treatments, causing an average of 4-fold stimulation (Table 5, Fig. 3A). Rates of microbial turnover were lower for the piñon-juniper and mixed conifer soils compared to the grassland and ponderosa (Fig. 3B). Microbial biomass N increased more strongly in the experiment where the rate of substrate addition was low, especially in the high C-to-N ratio treatment, and in the mixed conifer and grassland soils (Table 5; Fig. 4).

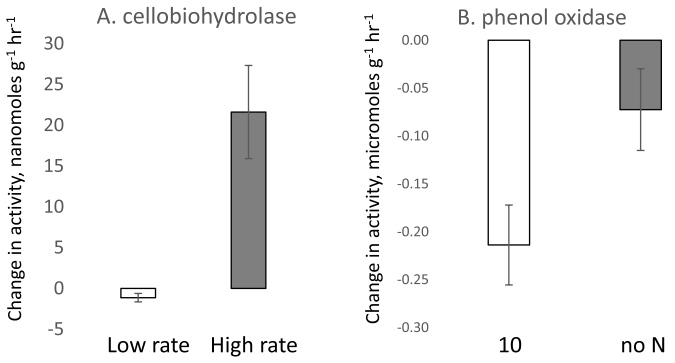

Fig. 6. Response of potential cellobiohydrolase enzyme activity to the substrate treatment (A) and of phenol oxidase to the C-to-N ratio treatment (B). Values are means plus or minus standard error of the mean (n = 32), averaged across treatments and soils not included in the best models.

Table 6
Best model for analysis of variance for ^{13}C accumulation. The best model included all terms: main effects of substrate, C:N, and soil type, and all interactions.

	Sum Sq	Mean Sq	DF	F-value	P-value
Substrate	1949863	1949863	1	79.456	<0.001
C-to-N ratio	415541	415541	1	16.933	<0.001
Soil	1166236	398745	3	15.841	<0.001
Substrate × C-to-N ratio	352094	352094	1	14.348	<0.001
Substrate × soil	372695	124232	3	5.062	0.004
C-to-N ratio × soil	320066	104232	3	4.348	0.009
Substrate × C-to-N ratio × soil	437930	24540	3	5.948	0.002

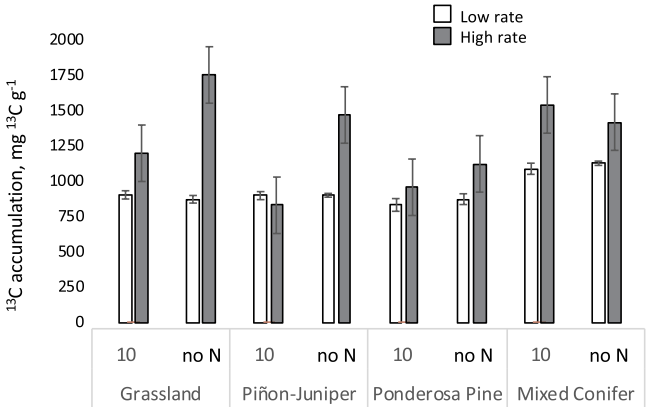

Fig. 7. Response of ^{13}C accumulation in soil to the rate of substrate addition and C-to-N ratio treatments across the four soils. Values are means plus or minus standard error of the mean (n = 4).

3.3. Enzyme activities

Responses of potential activities of β-glucosidase were higher in the experiment with high-substrate addition (Fig. 5), and the magnitude of the stimulation varied among soils. For example, β-glucosidase activity in the piñon-juniper soil did not respond to the rate of substrate input, whereas all other sites exhibited increased activity in response to the high rate of substrate input (soil × substrate interaction, Table 5). Response of peroxidase to substrate input varied across soils (Fig. 4, Table 5). In the grassland and mixed confier soils, peroxidase activity increased markedly with the high rate of substrate addition (Fig. 3). In contrast, in the other two soils, responses of peroxidase activity tended

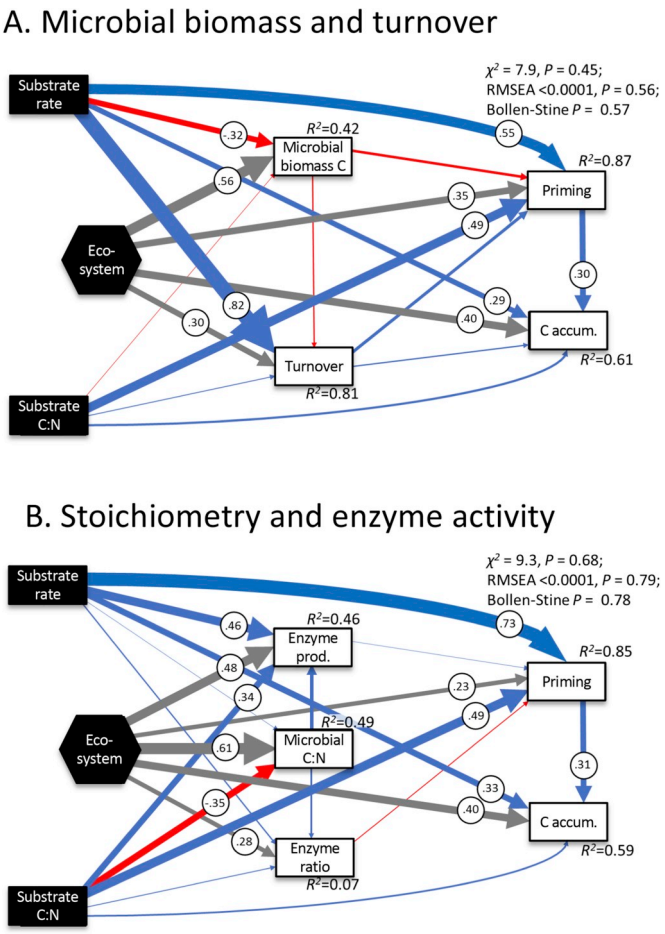

Fig. 8. Fitted structural equation models for the microbial biomass/turnover model (A), and the stoichiometry/enzyme activity model (B). Open rectangles represent measured response variables. Filled rectangles represent binary experimental treatments. Filled hexagons represent composite variables used to model multi-level categorical experimental treatments. Arrows represent hypothesized causal influences proposed in the models. Arrow thickness represents the estimated strength of these influences, also indicated by path coefficients (in circles; omitted when <0.2 for visual clarity). Arrow color indicates the direction of estimated causal influences (red = negative, blue = positive, gray = sign cannot be ascribed). R^2 is interpreted as proportion of variance explained for a given response variable. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

to diminish with the higher rate of substrate addition (soil × substrate interaction; Table 5). The response of cellobiohydrolase activity to C addition increased in the experiment with the high rate of substrate input, but cellobiohydrolase was non-responsive in the experiment with the low rate of substrate input (Fig. 6A). The response of cellobiohydrolase activity was insensitive to the C-to-N ratio treatment and did not differ among soils (Table 5). Carbon addition reduced potential activity of phenol oxidase. The mean response of phenol oxidase to C addition across all treatments was negative (−0.14 ± 0.03), and the reduction was larger when combined with added N (C:N = 10) compared to the no N treatment (Fig. 6).

3.4. Soil ^{13}C accumulation

The best model for ^{13}C accumulation in soil included significant main effects and all possible interactions (Table 6). Overall, more soil ^{13}C accumulated with the high rate of substrate addition compared to the low (Fig. 7), and in the no N treatment compared to that where the C-to-

N ratio was 10. These patterns were especially evident for the grassland and piñon-juniper sites (Fig. 7), explaining the significant 3-way interaction term in the model (Table 6).

3.5. Structural equation modeling

The microbial biomass/turnover model (BIC = 161.7) had the best relative fit of the three models (stoichiometry/enzyme activity model BIC = 188.1, combined model BIC = 278.5). Whereas both the microbial biomass/turnover and the stoichiometry/enzyme activity models fit the data well according to three absolute fit criteria (Fig. 8), the tests of the combined model produced mixed results ($\chi^2 = 33.1$, $P = 0.02$; RMSEA = 0.1, $P = 0.07$; Bollen-Stine $P = 0.21$. Note that in all of these tests, the P-value indicates the probability of good fit; commonly, $P > 0.05$ is considered adequate, though this is only convention). Thus, we removed the combined model from consideration and turned to the microbial biomass/turnover and stoichiometry/enzyme activity models to interpret specific paths and mechanisms within the models. The microbial biomass/turnover model explained 87% of the variation in priming and 59% of the variation in ^{13}C accumulation (Fig. 8A).

Cumulative priming was primarily associated with the substrate, C-to-N ratio, and soil source treatments directly (Fig. 8A). Change in MBC influenced priming ($P = 0.02$), but the effect was weak (Fig. 8A). The effect of microbial turnover on priming was detectable, but minor ($P = 0.09$). ^{13}C accumulation was largely and directly determined by soil. Microbial turnover ($P = 0.73$), and priming ($P = 0.17$) were negligible influences on ^{13}C accumulation. Microbial turnover and MBC were both strongly influenced directly by the substrate treatments. The stoichiometry/enzyme activity model (Fig. 8B) also explained well over half of the observed variation in priming ($R^2 = 0.85$) and ^{13}C accumulation ($R^2 = 0.59$). As with the microbial biomass/turnover model, the direct effects of the experimental factors had by far the most influence on priming and ^{13}C accumulation for the stoichiometry/enzyme activity model, and the inclusion of the responses of microbial C-to-N ratio, enzyme ratio, and total enzyme production did little to explain these focal variables or each other (Fig. 8B).

4. Discussion

4.1. Priming

Results presented here support the hypothesis that the magnitude of priming declines as N availability increases (Kuzyakov, 2002; Sinsabaugh et al., 2005; Blagodatskaya et al., 2007; Guenet et al., 2010a). The two experiments reported here used soils collected at different times of year, each with a different rate of substrate addition, making it challenging to infer causal relationships about the effect of substrate addition rate. Nevertheless, the higher rate of priming observed in the experiment with higher substrate additions was quantitatively consistent with past experiments using these same soils: here, as in the previous work (Liu et al., 2017), priming increased consistently with the rate of substrate addition (Figs. 1 and 2). The higher rate of priming with higher substrate addition is also consistent with previous findings (De Nobili et al., 2001; Zhang et al., 2013; Liu et al., 2017), and with the idea that a threshold quantity of substrate addition is necessary to elicit priming (Chen et al., 2014; Liu et al., 2017; Reischke et al., 2015).

Even though cumulative priming ranged widely, from negative to positive across the four ecosystems examined (Fig. 2), the patterns observed were broadly consistent with priming responses reported in the literature: priming declined when the C-to-N ratio of the substrate was reduced, and increased with the rate of substrate addition (Liu et al., 2017). These observations provide a range of priming responses to different substrate addition rates, stoichiometry, and ecosystem type, a range over which general conceptual models of controls on priming can be evaluated.

4.2. Microbial biomass and turnover

Microbial biomass C turnover was higher in the experiment with the higher level of substrate addition (Fig. 3), a pattern that has been observed previously (Kuikman et al., 1990; Bremer and van Kessel, 1990). There are several possible explanations for increased turnover with higher rates of substrate input, including increased activity of predators and grazers over time (Kaiser et al., 2014), a community shift toward certain microbial groups with more rapid growth and turnover rates (Bernard et al., 2007; Cleveland et al., 2007; Eilers et al., 2010; Morrissey et al., 2019), or an increase in growth and mortality even without a major shift in composition (Hungate et al., 2015).

Even at the low rate of substrate input, microorganisms responded to the added substrate, as indicated by increased MBC (Fig. 3), suggesting that microorganisms utilized the added glucose as an energy source for growth (Hungate et al., 2015; Morrissey et al., 2017). These findings indicate that low levels of substrate addition replaced existing metabolic or cellular constituents, an idea known as “apparent priming” which may be common (Blagodatskaya and Kuzyakov, 2008; Fontaine et al., 2011). Some pool substitution should occur with any input of labile C adequate to cause microorganisms to use that added C for energy or growth, as observed here. Yet, we observed increased microbial biomass in the absence of a priming response, suggesting that “apparent priming” was not an important source of priming in the four soils we tested.

4.3. Enzyme activities

The stimulation of β-glucosidase and cellobiohydrolase activity by high substrate inputs (Figs. 5 and 6) suggests a relationship with the priming effect. Consistent with this, sucrose inputs have been found to increase β-glucosidase and cellobiohydrolase activities and to induce priming (Chen et al., 2014). Our findings that phenol oxidase was most strongly suppressed by added N (i.e., in the C:N = 10 treatment) is consistent with the idea that nutrient addition decreases enzyme activity (Nottingham et al., 2012, 2015), which could explain a reduction in priming when N is available. We focused on enzymes that degrade C substrates, because priming is a phenomenon in which changes in C degradation ensue from C substrate addition. Nevertheless, documenting responses of enzymes that specialize in N acquisition would provide a more complete picture of how changes in resource allocation to address stoichiometric imbalances are associated with the priming effect.

4.4. Soil ^{13}C accumulation

Soil ^{13}C accumulated under both rates of substrate input, but the increase was 37% higher at the high rate of input, consistent with the idea that C input stimulates both priming and SOM formation (Chen et al., 2014; Cheng et al., 2014; Liang et al., 2018; Finley et al., 2018). Substrate inputs at the lower C-to-N ratio treatment reduced ^{13}C accumulation by 16%, which contrasts with the expectation that added N should increase soil C accumulation (Zhang et al., 2014). Consistent with prior studies that substrate additions enhanced soil C accumulation (Chen et al., 2014; Dalenberg and Jager, 1989), our study shows that at least 30% of the C added to the soil was retained by the end of experiments (data not shown). The structural equation models suggest positive effects of both priming and turnover on ^{13}C accumulation in soil (Fig. 6). These findings are consistent with microbial byproducts of metabolism as sources of soil C accumulation (Dalenberg and Jager, 1981; De Nobili et al., 2001; Wu et al., 1993; Cotrufo et al., 2013; Joergensen and Scheu, 1999).

4.5. Drivers of the priming effect

The structural equation models we evaluated were unable to discern a major influence of key postulated intermediates in priming, including

enzyme activity, microbial biomass growth, microbial turnover, and indices of microbial stoichiometry (C-to-N ratio). These are commonly invoked in the literature to explain the priming effect (Kuzyakov et al., 2000; Fontaine et al., 2003). There are three possible explanations for the absence of strong relationships between postulated intermediates and the priming effect in the structural equation models: 1) the postulated models are correct, but the causal relationships were obscured by high measurement error; 2) the postulated models are correct, but the measurement techniques used were poor proxies for the processes involved; or 3) the postulated models are incorrect.

Measurement error could mask relationships by introducing between-sample differences in a driving process without the associated response in priming. The result would be non-significant and weak associations between variables that were in fact causally related. Our use of the 5-week incubation period may have obscured relationships between the temporal dynamics of priming and the causal mechanisms behind it. Future work with more detailed temporal resolution of putative drivers may reveal relationships we were unable to detect. Still, the coefficients of variation in the driving variables we assessed were not unusual: calculated across four replicate samples for each treatment combination, the median coefficients of variation were 0.20 for microbial biomass, 0.23 for potential enzyme activities, 0.13 for ^{13}C accumulation, and 0.19 for turnover, which are not unusually high for soil processes. These typical coefficients of variation caution against dismissing the absence of strong relationships in the structural equation models as a result of measurement error.

There may be merit in the idea that the techniques used are indirect and poor proxies for the processes of interest. For example, potential enzyme assays are thought to detect the amount of enzyme present in a sample, not the actual rate of activity (Tiedje, 1982). Similarly, methods for estimating microbial biomass, like chloroform fumigation, involve calibrations that are difficult to validate (Joergensen, 1996), problems that extend to estimates of microbial turnover when the same biomass measurements are used in the calculation. Despite these potential challenges, enzyme activity, microbial biomass, and microbial turnover were sensitive to substrate addition, to the C-to-N ratio of the added substrate, and to differences among soils, indicating responsiveness to the same factors that elicited strong differences in priming. Therefore, dismissing the absence of relationships as a consequence of insensitive measurement techniques may not be warranted. On the other hand, the processes we evaluated are not the only processes invoked in the "stoichiometry" and "activation" explanations for the priming effect. For example, we did not evaluate enzymes catalyzing N mineralization (deamination), even though these enzymes may be important for priming. There may be dynamics in the physiological responses to pulsed substrate additions that could not be detected given the time scales of our measurements, and more temporal detail might illuminate clearer relationships.

Nevertheless, the possibility that the specific models we tested are incorrect cannot be dismissed. While we found that the rate of substrate addition and its C-to-N ratio influence priming, we also found that frequently postulated drivers of this response, like enzyme production and biomass turnover (Kuzyakov, 2002; Fontaine et al., 2003), were unrelated to priming. Changes in extracellular enzyme activity may not have been involved in the priming responses we observed. The simplest alternative explanation is that priming occurred through increased utilization of small molecular weight compounds that could be assimilated without extracellular breakdown. There are explanations for a priming response that do not involve extracellular enzyme production: for example, the substrate addition may have increased the availability of other low-molecular-weight substrates through abiotic exchange (Keiluweit et al., 2015), or it may have supported growth into uninhabited microsites where low-molecular weight compounds were available, e.g., by hyphae, consistent with the postulated role of fungi in mediating the priming effect (Carney et al., 2007; Talbot et al., 2008).

Differential responses of microorganisms varying in ecological strategies or traits are frequently speculated to be involved in the priming effect (Bell et al., 2003; Blagodatskaya et al., 2007; Kramer and Gleixner, 2006; Landi et al., 2006; Blagodatskaya and Kuzyakov, 2008). In an experiment with a design nearly identical to that presented here, we found that glucose addition caused many soil bacteria to increase their use of SOM as a growth substrate, even while they continued to use glucose as well (Morrissey et al., 2017). There was a strong phylogenetic signal linking bacteria that specialized in glucose use, but there was no phylogenetic coherence to the group of organisms involved with priming, suggesting that priming is not a distinct bacterial strategy. Nevertheless, increased activity and abundance of organisms capable of using both substrates may help explain priming.

Priming can be important: as a part of the C economy of the rhizosphere (Zhu et al., 2014), because of its possible role in modulating soil C accumulation and loss (Carney et al., 2007; van Groenigen et al., 2014), because its very occurrence challenges assumptions of models of the soil C cycle (Heimann and Reichstein, 2008), and because in some cases there is evidence that priming involves the breakdown of long-lived SOM (Fontaine et al., 2007). It is also scientifically intriguing, as evidenced by the volume of publications measuring, describing, and attempting to decipher the phenomenon. Priming can be easily measured, especially in the lab in experiments such as that presented here, because of reduced variance in homogenized soil incubations compared to the field, and because of precise gas exchange and isotope techniques. In part because of this sensitivity, priming effects can exhibit statistically significant differences that invite interpretation and a search for mechanistic explanations.

Nevertheless, simple explanations for priming may prove elusive, because in priming experiments like ours, there is no evidence that the additional substrates utilized during priming are a chemically coherent group. Often accurately referred to as "native SOM" (e.g., Carney et al., 2007), the substrates utilized in priming share only two sure commonalities: that they were assimilated and utilized in response to labile substrate addition, and that they lack the isotopic label of the added substrate that elicited the priming response. The substrates may in fact vary widely in chemical structure, elemental composition, and interaction with soil minerals, such that their utilization increases without any coherent — or measurable — physiological or ecological shift. Because of this possible range of variation in the nature of the substrate subject to priming — including its chemical composition, age, and distribution within the soil matrix — there may not be any singly consistent or generalizable mechanism.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.107617.

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