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Reduced substrate supply limits the temperature response of soil organic carbon decomposition

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ABSTRACT

Controls on the decomposition rate of soil organic carbon (SOC), especially the more stable fraction of SOC, remain poorly understood, with implications for confidence in efforts to model terrestrial C balance under future climate. We investigated the role of substrate supply in the temperature sensitivity of SOC decomposition in laboratory incubations of coarse-textured North American soils sampled from paired native pine and hardwood forests located across a 20 °C gradient in mean annual temperature (MAT). In this study we show that for this wide range of forest soils, the supply of labile substrate, controlled through extended incubation and glucose additions, exerts a strong influence on the magnitude of SOC decomposition response to warming. When substrate supply was high, either in non-depleted soils or in soils first depleted of labile C through extended incubation but then amended with glucose, SOC decomposition rates responded to increased temperature with a mean Q_{10} of 2.5. In contrast, for the depleted soils with no substrate added, SOC responded to varying temperature with a mean Q_{10} of 1.4. Our laboratory study shows for upland forest soils that substrate supply can play a strong role in determining the temperature response of decomposing SOC. Previous studies have described the effect of substrate availability of temperature responses on soil respiration, but few have described the effect on decomposition of more stable SOC. Because substrate supply is likely to vary strongly – both spatially and temporally, these findings have important implications for SOC processing in natural systems.

1. Introduction

The Earth's mineral soils represent a large terrestrial reservoir of organic carbon (C) derived from the accumulation of detrital residues and by-products of microbial decomposition processes (Paul and Clark, 1996; Schmidt et al., 2011). Most of this C is resistant to decomposition because of chemical recalcitrance and protection through association with soil minerals (Melillo et al., 1989; Schimel et al., 1994; Torn et al., 1997; Six et al., 2002) and because of the effect of abiotic and biotic conditions, including substrate supply and quality, on microbial communities (Fontaine et al., 2007; Schmidt et al., 2011). While the age of most soil organic C (SOC), including that found in the top 20 cm of the mineral soil, extends into centuries (Trumbore et al., 1996; Fissore et al., 2009), the very large size of this reservoir means that even small increases in the turnover rate of global SOC stocks in response to warming will exert a large influence on atmospheric [CO_2]. Global temperatures are rising (IPCC, 2007), and so if SOC decomposition is strongly temperature sensitive, warming should cause a net transfer of C from soils to the atmosphere, resulting in an increase in atmospheric [CO_2] and a positive feedback to future warming (Holland et al., 2000).

Despite the importance of understanding temperature responses to accurately modeling the global C cycle, the realized – as observed in the field – temperature sensitivity of SOC decomposition remains poorly quantified, and so projected future rates of SOC decomposition in a warmer world are uncertain (Cox et al., 2000; Giardina and Ryan, 2000; Davidson and Jannssens, 2006; Bradford et al., 2008; Conant et al., 2011). Several studies have described the effect of substrate availability on temperature responses of soil respiration (Gu et al., 2004; Bengtson and Bengtsson, 2007), but few have described the effect on decomposition of more stable SOC. Studies have shown that the apparent – as observed through lab studies – temperature sensitivity of SOC decomposition (Davidson and Jannssens, 2006) can vary from

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ephemeral or minor temperature responses (Giardina and Ryan, 2000; Fissore et al., 2009), to strong persistent responses (Conant et al., 2008). Kinetic theory predicts both strong responses to increased temperatures, and increasing temperature sensitivity with increasing C recalcitrance (Ågren, 2000; Ågren and Bosatta, 2002; Davidson and Jannssens, 2006). However, in biochemical systems, enzyme activity can respond to increases in temperature only when substrate supply, even that of recalcitrant materials, exceeds reaction rate (Giardina and Ryan, 2000; Davidson and Jannssens, 2006). Overall, the mechanisms driving either weak or strong, ephemeral or persistent responses of SOC decomposition to temperature remain poorly understood and have been rarely examined (Karhu et al., 2009; Conant et al., 2011).

Where temperature responses appear to be either weak or ephemeral, two competing hypotheses have emerged to explain patterns of declining temperature sensitivity. The first postulates that substrate supply regulates enzyme activity, which can increase in response to temperature only in the presence of excess substrate (Giardina and Ryan, 2000). Therefore, apparent insensitivity to warming results from substrate limitations to microbial decomposition rather than from temperature limitations to enzyme activity (Davidson and Jannssens, 2006). An alternative hypothesis postulates that apparent declines in temperature sensitivity result from the masking of a strong temperature response of recalcitrant SOC decomposition by large losses of labile SOC (Knorr et al., 2005). In either case, current models for terrestrial C cycling and SOC responses to warming have for the most part ignored the effect of substrate supply on microbial enzymatic activity and its implications for SOC losses (Allison et al., 2010; Miltner et al., 2011). To date, microbial activity has been only implicitly accounted for in first order-kinetic equations used to describe SOC decomposition (e.g. Knorr et al., 2005). To this extent, reduced C use efficiency in response to substrate deficiencies may counteract model-based responses of SOC decomposition to warming (Allison et al., 2010). Positive microbial response to sugar addition has been observed to be related to enhanced stable soil organic matter formation (Bradford et al., 2012), further emphasizing the important role of microbial activity in the formation and stabilization of C in soil as a function of substrate supply (Miltner et al., 2011).

North American forests are considered net sinks of C (Ryan et al., 2010) and large climate benefits are derived from this large-scale uptake of atmospheric CO_2. However, if SOC decomposition is strongly temperature sensitive, especially that of stable, long-lived C that has accumulated in forest soils over centuries, then there is potential for the sink strength of these forest soils to diminish and even reverse. There is increasing evidence that more accurate predictions of the potential climate feedback of terrestrial C depend on accurately characterizing this response to warming (Frank et al. 2010). Yet to date, investigations of the drivers of SOC response to temperature has been inconclusive.

We developed an experimental design that combines laboratory-based warming with soil incubation and labeled substrate addition to discriminate between competing hypotheses about SOC decomposition response to warming. Specifically, we tested the hypothesis that for upland forest soils the supply of labile substrate regulates to the sensitivity of SOC decomposition responses to warming. According to this view, we predict that substrate supply to soil heterotrophic microbes exerts a larger influence over SOC decomposition responses to warming than temperature.

2. Methods

2.1. Study area and soil sampling

We collected the top 20 cm of mineral soil using a 10 cm diameter soil auger at eight forested sites across five bioclimatic regions in North America, as listed in Table 1. The selected sites for this study spanned a 20 °C MAT range from Colorado to South Carolina. At each site we sampled one hardwood and one pine stand, with the only exception of Kentucky where only hardwoods were sampled. Sampled stands were mature and included mixed broadleaf deciduous and monotypic pine forest types with native understory plant species (Fissore et al., 2008, 2009). At each of the eight sites, we sampled three cores within a few meters of each other that were then composited into a single soil sample. Soils were immediately (<2 d) shipped in coolers with blue ice to the USDA Forest Service Forestry Laboratory in Houghton, MI where they were fresh sieved (2 mm) to separate rocks and roots. Bulk soil samples were then dried at 30 °C in a forced-air oven to constant moisture, and stored in an air-conditioned lab (15 °C). From bulk soil samples, one set of sub-sampled soils for each composite soil sample was taken to determine soil texture following Carter (1993) and pH with a pH meter for a solution of 20 g soil, 20 ml H_2O, and 200 μl of 1 M $CaCl_2$.

Detailed description of site characteristics and sampling procedures can be found in Fissore et al. (2008). Briefly, soil textures ranged from sands to sandy loams to loams, corresponding to clay content between 3.5% (a sand soil) to 20.0% (a loam soil) as reported in Table 1 (Fissore et al., 2008). Soil pH did not vary significantly across sites and was for the most part sub-acid to sub-neutral. Soil C concentrations ranged from 1% to 2%, with the exception of a hardwood site in Colorado, where we measured 8.1% C (Fissore et al., 2008).

2.2. Incubation

For our incubation experiment we used a second and third set of sub-sampled soils from the oven dried composite samples as described above. One set (hereafter 'depleted') was depleted of labile C during 300 d of incubation at constant temperature (30 °C) and moisture (60% water holding capacity, WHC). The other set (hereafter 'non-depleted') was stored dry as above in an air-conditioned lab at a nearly constant room temperature of 15 °C for 300 days. Following 300 days of incubation for the depleted soils, non-depleted soil samples were brought to 60% WHC and

Table 1
Site and soil characteristics of the samples used in the incubation.

Location	MAT °C	MAP mm/yr	Forest type	Soil texture	Soil type	Clay %	pH	C %
Colorado	−2	474	*Pinus contorta*	Sandy-loam	Entic Haplocryods	8.3	5.8	2.20
Colorado	−2	474	*Populus tremuloides*	Sandy-loam	Entic Haplocryods	10.0	6.1	8.07
Minnesota	4	702	*Populus tremuloides*	Sandy-loam	Mixed frigid Typic Udipsamment	8.0	5.8	1.93
Minnesota	4	702	*Pinus resinosa*	Sandy-loam	Mixed frigid Typic Udipsamment	4.5	5.6	1.45
Kentucky	12	850	*Acer* spp.	Loam	Mesic Typic Hapludult	16.0	5.7	2.10
Kentucky	12	850	*Acer* spp.	Sandy-loam	Mesic Typic Hapludult	8.0	4.6	1.22
South Carolina	18	1332	*Acer* spp.	Loam	Thermic Aeric Endoaquults	20.0	4.4	2.06
South Carolina	18	1332	*Pinus virginiana*	Sand	Thermic Glossaquic Hapludalf	3.5	4.9	0.88

incubated for three days at 30 °C, with the goal of mirroring the incubation conditions of the depleted soils incubation. Preparation of each incubation consisted in placing 30 g of each soil previously oven-dried at 30 °C into 120 ml specimen cups. Each soil sample was brought to moisture corresponding to 60% of WHC, and then each specimen cup was placed into a 1-L Mason jar for incubation, for a total of 16 jars. At time of measurement, each jar was sealed with a lid and septum for gas collection. To guarantee air tightness each lid and septum was greased with silicon-based Dow Corning Silicon High Vacuum Grease. To prevent anaerobiosis, jars were kept partly open between gas sampling events and sealed 24 h before headspace gas sampling.

Immediately following soil sample preparation and rewetting, non-depleted samples were set-aside for three days prior to commencement of measurements so that the experiment would not include the large spike in CO_2 evolution that immediately follows rewetting. We felt that this spike would complicate efforts to answer questions about substrate supply and temperature sensitivity of SOC decomposition. Based on our previous work with these soils, we are confident that the soils were still essentially non-depleted after three days relative to the soils depleted of labile substrate following 300 days of incubation (Fissore et al., 2009). During this three-day post-wetting but pre-experiment period, head gas samples were not collected.

Following the three day post-wetting period, the Mason jars with the non-depleted soils were joined with the Mason jars with depleted soils in a Precision 815 Low Temperature Incubator (Winchester, VA, USA) for the substrate manipulation experiments. Throughout these incubations, samples were maintained at 60 ± 5% of WHC by periodically checking for weight loss and amending with water. Rates of CO_2 efflux were measured by sampling 50 ml of headspace gas from each sealed Mason jar at the end of each interval after subtracting for baseline (time 0) headspace CO_2 concentration, as described in Fissore et al. (2008).

To examine how altered temperature influences SOC decomposition in non-depleted soils, and in soils depleted of labile C after 300 d of incubation, we cycled lab incubation temperature (LIT) for four 11 h periods: from 30 °C to 35 °C; from 35 °C to 30 °C; from 30 °C to 25 °C; and, from 25 °C back to 30 °C. Following this cycle of temperatures, samples were maintained at 30 °C and 60 ± 5% of WHC. Headspace gas samples were collected and analyzed for C concentration at the end of each 11-h interval, before each temperature switch. The reason for choosing lab incubation temperatures between 25 and 35 °C were twofold: first, many incubation experiments have been conducted at or near 30 °C (Paul and Clark, 1996), which is considered near-optimal for promoting microbial activity; second, all of the soils selected for this study experience *in situ* temperatures at or close to 30 °C at some point during the growing season, although with some degree of variability due to the wide MAT range at which these soils formed (Table 1). For instance, for our coolest Colorado sites, July maximum 10-yr mean daily summer air temperatures for nearby Rand, Colorado (station 056820) regularly reach above 27 °C (Western Regional Climate Center), with higher temperatures for single dates. We quantified CO_2 efflux using an Agilent 6890 Gas Chromatograph (Agilent, Inc. Palo Alto, CA) and at each sampling event we took baseline measurement of headspace CO_2 concentration.

To examine the possible role of substrate supply in determining the magnitude of SOC decomposition temperature response, we augmented the supply of labile C to soil microbes by amending the paired sets of non-depleted and depleted soils with sugarcane-derived glucose (Fisher Scientific, Inc. Glucose is a C4 source of C with $^{13}\delta$ of −10.2‰) added as solution in micro-drops coinciding with a moisture addition to maintain soils at 60% WHC. Enough glucose was added in one single application to each soil sample to match twice the SOC loss observed during 300 d of incubation (as reported in Fissore et al., 2009), and therefore additions were specific for each soil sample (Table 2). Specifically, the amount of C lost during 300 d incubation was calculated as cumulative respired CO_2–C equivalent as derived from headspace gas sampling measurements taken throughout the 300 d incubation at constant WHC and temperature (Fissore et al., 2009). All soils were then incubated under constant conditions of 60% WHC and 30 °C for two months prior to starting the temperature manipulations, as described above. We selected 2 months because we did not want to capture the initial very large pulse of CO_2 derived from initial additions of labile substrate (Bradford et al., 2012), but rather after significant portion of the substrate had been taken up by microbial biomass. Hence tests of our hypotheses may be somewhat conservative. Values of Q_{10} were calculated according to the equation:

$$Q_{10} = (R1/R2)\hat{1}0/(LIT1 - LIT2) \quad (1)$$

where R1 and R2 refer to SOC decomposition rates (mg C g^{-1} soil C d^{-1}) and LIT1 and LIT2 are the lab incubation temperatures during 11 h-periods. Because we amended soils with glucose from sugarcane, a C4 grass, whereas forest trees rely on a C3-C photosynthetic pathway, we were able to investigate the fraction of the added glucose that remained in soils by measuring changes in $\delta^{13}C$ signature according to the equation:

$$C\ gain(\%) = (\delta_f - \delta_i)\Big/(\delta_{gl} - \delta_i)\cdot 100 \quad (2)$$

where δ_f is the $\delta^{13}C$ at the end of the incubation, δ_i is the initial $\delta^{13}C$, and δ_{gl} is the glucose $\delta^{13}C$. Our number of samples was reduced from 8 to 7 in this lab analysis because of instrument error during analysis of one of the samples, and there was not enough material left to repeat the analysis (Table 3). We conducted analyses of initial

Table 2
Carbon content in fresh and after depleted (through 300 d incubation) soil sample.

Location	Site ID	Forest type	Initial soil C gC 30 g soil^{-1}[a]	Final soil C[b] gC 30 g soil^{-1}	C added[c] gC 30 g soil^{-1}
Colorado	CO1P1	*Pinus contorta*	0.66	0.52	0.70
Colorado	CO1A1	*Populus tremuloides*	2.42	2.28	0.73
Minnesota	MN1H1	*Populus tremuloides*	0.58	0.52	0.31
Minnesota	MN1P1	*Pinus resinosa*	0.44	0.26	0.27
Kentucky	KY1H1	*Acer* spp.	0.63	0.55	0.38
Kentucky	KY1H3	*Acer* spp.	0.37	0.30	0.31
South Carolina	SC1H1	*Acer* spp.	0.62	0.58	0.20
South Carolina	SC1P1	*Pinus virginiana*	0.26	0.21	0.28

[a] Thirty grams refer to the amount of soil that was used for the incubation experiment.
[b] After 300-d incubation at 30 °C.
[c] As glucose.

Table 3
Initial and post-glucose addition delta ^{13}C values for depleted and non-depleted soils samples.

Location	Site ID	Initial $\delta^{13}C$ ‰	Post-glucose $\delta^{13}C$ depleted[a] ‰	Post-glucose $\delta^{13}C$ non-depleted[b] ‰
Colorado	CO1P1	−25.2	−23.5	−24.5
Minnesota	MN1H1	−26.6	−25.5	−25.8
Minnesota	MN1P1	−26.0	−24.3	−25.3
Kentucky	KY1H1	−26.8	−26.0	−25.9
Kentucky	KY1H3	−27.3	−26.2	−26.5
South Carolina	SC1H1	−26.9	−26.3	−26.2
South Carolina	SC1P1	−26.6	−24.8	−25.8

[a] Soils that have been depleted of labile C during 300-d incubation.
[b] Soils that have not been previously incubated to deplete labile forms of C.

and final %C and $\delta^{13}C$ on a Costech Elemental Combustion System 4010 connected to a ThermoFinnigan ConfloIII Interface Deltaplus Continuous Flow Stable Isotope Ratio Mass Spectrometer (IRMS). Difference in incorporation of glucose-derived C and in Q_{10} between soils depleted and non-depleted in labile C was assessed using *t*-test at $\alpha = 0.05\%$. We do not anticipate that the lack of data from the lost incubated soil sample will compromise the study.

3. Results and discussion

Our laboratory experiment was designed to test the hypothesis that substrate supply regulates the magnitude of the SOC decomposition response to warming. Depleting soils of labile forms of SOC over 300 days of incubation allowed us to examine the temperature responses of SOC decomposition for more stable forms of SOC. Since we did not observe significant differences in Q_{10} between forest types (deciduous versus conifer), all samples were combined for the statistical analyses presented here.

Soil C decomposition in the non-depleted soils responded to step changes in LIT with an estimated Q_{10} values of 2.6 (S.E. = 0.3; $n = 8$), while soil C decomposition in soils depleted of labile C responded to the temperature changes with Q_{10} values of 1.4 (S.E. = 0.1, $n = 8$) indicating that SOC decomposition in non-depleted soils was significantly more responsive to temperature SOC decomposition in depleted soils (Fig. 1a). This finding supports the hypothesis that substrate supply, and by extension substrate quality, exerts an important influence on the sensitivity of SOC decomposition rates to warming. Following glucose additions to the depleted soils and after repeating the 11-h step changes in LIT, the response of SOC decomposition to LIT manipulation did not differ significantly between depleted and non-depleted soils (Fig. 1b), with Q_{10} values also averaging 2.6 (S.E. = 0.4, $n = 8$) for both amended and depleted soils. Carbon decomposition rates two months after glucose addition, and for each of the gas sampling events, were only slightly higher in non-depleted than depleted soils – for example, on the first sampling event after first 11 h period at 30 °C soil C decomposition rate averaged 0.45 and 0.42 mg C/g C for non-depleted and depleted soils, respectively.

Our findings suggest that the reduced temperature sensitivity ($Q_{10} = 1.0–1.4$) previously identified by studies relying on *in situ* warming (Melillo et al., 1989; Luo et al., 2005), stand manipulation (Lavigne et al., 2003; Pypker and Freeden, 2003), and inverse modeling (Ise and Moorcroft, 2006) may derive from substrate limitations to SOC decomposition, such that substrate supply limits responses to temperature. Criticism has been expressed towards the use of Q_{10} approaches, especially when investigating more stable fractions of SOC (Sierra, 2012). While criticisms have been directed at simple Q_{10}-based descriptions of soil C responses to warming, Q_{10} is a convenient and robust way to characterize temperature response in a laboratory setting where moisture and environment are constant.

Bradford et al. (2008) identified both reductions in labile C and thermal adaptation of microbial decomposers as drivers of reduced temperature sensitivity of SOC decomposition. Given the short-term nature of our temperature manipulations (11 h cycles), we suspect that thermal adaptation does not explain our results. In a similar controlled incubation experiment, in which glucose was added to soil samples throughout a 77-day incubation period, thermal adaptation was observed only after over 77 days (Bradford et al., 2010). Nevertheless, thermal adaptation of microbial decomposers to elevated temperatures may reflect a physiological response to long-term declines in substrate supply under higher temperatures (Kirschbaum, 2004).

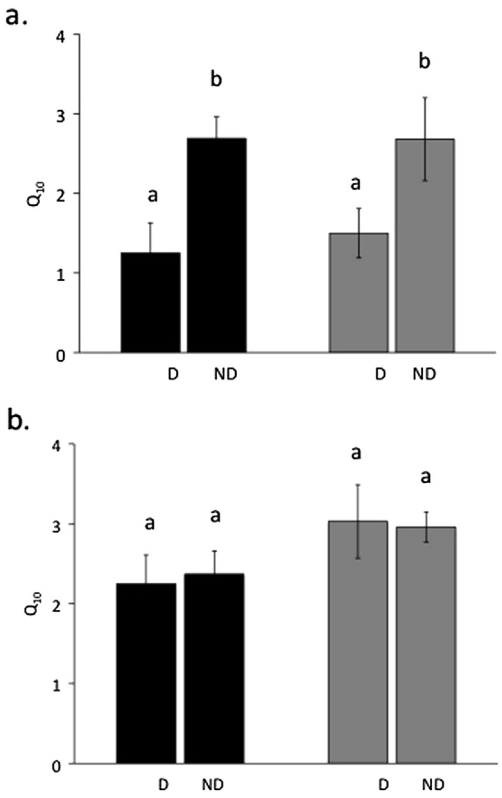

Fig. 1. Laboratory incubation results showing the temperature dependence of SOC on substrate supply. **a**. Temperature sensitivity (Q_{10}) of soil depleted (D) and non-depleted (ND) in labile forms of C in response to changes in lab incubation temperature (LIT) of *minus* 5 °C (indicated in black for both panels) and *plus* 5 °C (indicated in gray for both panels) from the baseline LIT of 30 °C. Bars represent average ± one S.E., $n = 8$. **b**. Temperature responses (Q_{10}) in soils depleted (D) and non-depleted (ND) in labile C after the addition of labile C substrate (glucose). Bars represent average ± one S.E., $n = 8$. Values of Q_{10} were obtained from the analysis of SOC decomposition rates (mg C g^{-1} soil C d^{-1}) as calculated from data of CO_2 efflux. Different letters represent significant difference.

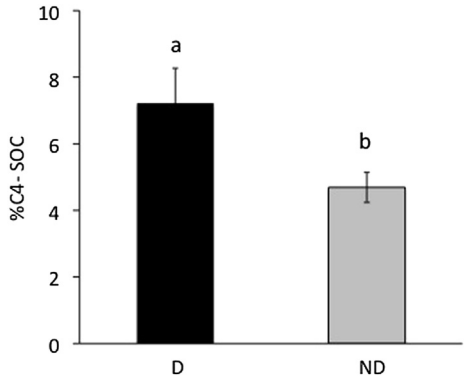

Fig. 2. Glucose-derived C (C4-C) stabilized in depleted (D) versus non-depleted (ND) soils during the lab incubation. Bars represents average ± S.E., $n = 7$. Different letters represent significant difference.

Our results show that the turnover of more stable forms of SOC are less sensitive to warming than the turnover of more labile SOC, and so conflicts with the hypothesis that the decomposition of recalcitrant soil C will be more sensitive to warming than labile soil C (Knorr et al., 2005). The greater sensitivity of recalcitrant SOC to temperature presented by Knorr et al. (2005) in their modeling study based on tropical soils may reflect important, but not yet validated assumptions about the distributions of labile and recalcitrant SOC forms and activation energies for SOC turnover. Our results also conflict with the incubation study of Fang et al. (2005), which relied on a 100 d soil incubation to show that SOC decomposition rates both early and late in the incubation were equally sensitive to altered temperatures. Based on our substrate addition experiment, we suggest that 100 d is insufficient to deplete the organic-rich soils used in that study of labile C. Further, the assumed 20–50 yr residence time used to calculate the contribution of recalcitrant SOC to soil CO_2 efflux likely does not accurately describe residence time for recalcitrant C in soils incubated by Fang et al. (2005). In British mineral soils similar to the one sampled in Sitka spruce plantation by Fang et al. (2005) and sampled from just the top 3 cm of the surface mineral soil (Podzols and Gleysols), residence times for total SOC ranged from 500 to 1000 yr (Bol et al., 1999). Residence time for deeper soils or for recalcitrant C fractions likely would be substantially longer than 20–50 yr, and so the single pool model of exponential decay used by Fang et al. (2005) would yield greatly reduced contributions of recalcitrant C to efflux over their 100 d incubation.

It is unclear what factors (e.g., vegetation type, soil texture, warming incubation temperature) could have led to the discrepancy observed between our results and those by Conant et al. (2008), who used a similar experimental approach consisting of warming soils that were depleted of labile SOC at different degrees to examine temperature sensitivity of SOC decomposition. In their case samples were taken from two grassland soils at one location, both with clay content >29%. It is possible that lower initial incubation temperature for some sets of soils (4 °C and 15 °C) in the study by Conant et al. (2008) may have played a role in affecting total SOC responses to subsequent changes in temperature, possibly related to microbial activity and composition in soils with higher labile C content that those examined in our study. Further investigation is needed to understand if SOC formed under forests responds differently to changes in temperature than SOC formed under grasslands. Overall, the lack of studies with comparable methods, soil types and vegetation types limits our ability to understand these differences across studies.

Forecasting SOC decomposition response to warming at the ecosystem level is difficult, as laboratory incubations cannot account for the large number of biotic and abiotic factors affecting *in situ* SOC cycling. Nevertheless, one can anticipate that in soils more depleted in SOC (our soils had lost <15% of initial SOC in 300 d; Fissore et al., 2008) or with high clay content, reductions in temperature sensitivity may be more pronounced. In line with this expectation, radiocarbon evidence for SOC residence times from our sites indicates that across this extensive gradient in MAT, the acid insoluble SOC fraction is insensitive to changes in MAT and that mineral associated SOC is strongly protected (Fissore et al., 2009), in line with earlier radiocarbon work for stable SOC (Trumbore et al., 1996). Further, our results describe soils that were maintained at moisture levels optimal for microbial activity (Fissore et al., 2008). In those areas of the world where warming will result in increased moisture losses that drive moisture levels below those optimum for microbial activity, realized SOC sensitivity to temperature should also be reduced. Conversely, where warming results in the thawing of soils and extends the period when SOC can be decomposed, realized rates may increase as substrate supply is enhanced under warmer and wetter conditions (Giardina and Ryan, 2000; Davidson and Jannssens, 2006).

Because forest-derived SOC was isotopically distinct from the added sugarcane-derived glucose (C3 versus C4 photosynthetic pathways, respectively), we were able to quantify the proportion of C4-derived glucose incorporated into each soil at the end of the experiment. Analyses of ^{13}C:^{12}C of amended soils before and after the incubation, in combination with standard mixing equations (Balesdent et al., 1987), showed that soils depleted in labile SOC stabilized 66% more C4-C (derived from the added glucose) than non-depleted soils (7.8 ± 1.2%, $n = 7$ versus 4.7 ± 0.1%, $n = 7$, $P < 0.001$; Table 3, Fig. 2), indicating a higher retention rate for soils depleted of labile SOC. Greater stabilization of glucose-derived C in turn indicates more efficient use of substrate by the microbial community, likely relating to greater substrate limitations to microbial biomass and activity in depleted soils. This is supported by the observation that rates of total CO_2 efflux and SOC decomposition for amended-depleted and non-depleted soils were similar, with non-depleted soils showing slightly higher SOC decomposition rates during the second LIT manipulation experiment. We cannot estimate whether substrate additions and the higher retention rate of glucose-derived C4-C for depleted soils stimulated priming of the older C3-derived SOC, as the required $^{13}CO_2$ measurements were not made.

The finding that soils depleted in labile SOC stabilized more C4-derived C than non-depleted soil provides strong evidence that substrate supply limited microbial activity more strongly in depleted than non-depleted soils. Nevertheless, this increase in the retention of added labile C is surprising and may have been due to incubation-related changes in microbial community composition, and perhaps increased C use efficiency, or some combination of the two. Others have speculated that mineralization and retention of substrate-derived C depends on the reactivity and quantity of substrate being added (Yang and Janssen, 2002), with the quality of the residue positively affecting initial CO_2 efflux rates (as observed in our depleted soils following glucose addition), but resulting in greater accumulation in the long term. Others have also found similar findings of rapid initial mineralization of glucose-derived C compared to more recalcitrant substrates (Voroney et al., 1989). Because of the strong seasonality of detrital inputs to soils across our gradient, the enhanced retention of labile C in depleted soils may provide insights into the dynamics of soil C processing in responses to the periodicity of labile C input and retention in field environments.

We conclude that for upland forest soils, substrate supply appears to exert a strong control on the response of SOC

decomposition rate to warming, and that for these ecosystems, substrate supply may limit responses of SOC decomposition to warming. However, these results do not contradict the view that a decrease in SOC storage may occur as a result of global warming, either because of reduced productivity where moisture limits plant growth or because of accelerated decomposition of fresh detritus, which is clearly temperature sensitive. Further, as previously highlighted (Giardina and Ryan, 2000), if decomposition rates across ecosystems are limited by substrate supply more than by temperature, then ecosystems that currently store large quantities of labile C, but are anaerobic or frozen for extended periods each year, may release C to the atmosphere following warming and drying at much higher rates than expected under standard Q_{10} assumptions (Hick-Pries et al., 2013). Clearly, robust, *in situ* based estimates of Q_{10} response to warming are needed across a wide diversity of ecosystem types and soils to accurately model terrestrial C cycling.

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