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REVIEW

How interactions between microbial resource demands, soil organic matter stoichiometry, and substrate reactivity determine the direction and magnitude of soil respiratory responses to warming

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Abstract

Recent empirical and theoretical advances inform us about multiple drivers of soil organic matter (SOM) decomposition and microbial responses to warming. Absent from our conceptual framework of how soil respiration will respond to warming are adequate links between microbial resource demands, kinetic theory, and substrate stoichiometry. Here, we describe two important concepts either insufficiently explored in current investigations of SOM responses to temperature, or not yet addressed. First, we describe the complete range of responses for how warming may change microbial resource demands, physiology, community structure, and total biomass. Second, we describe how any relationship between SOM activation energy of decay and carbon (C) and nitrogen (N) stoichiometry can alter the relative availability of C and N as temperature changes. Changing availabilities of C and N liberated from their organic precursors can feedback to microbial resource demands, which in turn influence the aggregated respiratory response to temperature we observe. An unsuspecting biogeochemist focused primarily on temperature sensitivity of substrate decay thus cannot make accurate projections of heterotrophic CO_2 losses from diverse organic matter reservoirs in a warming world. We establish the linkages between enzyme kinetics, SOM characteristics, and potential for microbial adaptation critical for making such projections. By examining how changing microbial needs interact with inherent SOM structure and composition, and thus reactivity, we demonstrate the means by which increasing temperature could result in increasing, unchanging, or even decreasing respiration rates observed in soils. We use this exercise to highlight ideas for future research that will develop our abilities to predict SOM feedbacks to climate.

Keywords: Arrhenius function, heterotrophic respiration, microbial adaptation, nitrogen mineralization, organic matter decomposition, soil organic carbon, soil respiration, soil warming, stoichiometry, substrate quality

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Introduction

Given the large reservoir of organic carbon contained in Earth's soils (soil organic carbon, SOC), understanding the mechanisms governing responses of SOC mineralization to increasing temperature is critical for predicting future atmospheric CO_2 concentrations. A large proportion of SOC is composed of compounds possessing slow turnover rates (Trumbore, 2000), requiring significant amounts of energy to decompose (Ågren and Bosatta 2002; Ågren & Wetterstedt, 2007). Enzyme kinetics provide a framework for predicting the temperature sensitivity of SOC decomposition and predict that the decay of such compounds, with their typically high activation energies (E_a), is relatively more sensitive to warming than more labile SOC pools (Davidson & Janssens, 2006; Sierra, 2012). Rates of CO_2 release from slow-turnover pools with rising temperatures may still be smaller in absolute terms than rates of CO_2 release from more labile pools, but because a large proportion of SOC is composed of slow-turnover material, even a small change in the C dynamics of these pools could have a significant effect on the release of microbially derived CO_2 with warming.

Many studies explore the temperature sensitivity of soil organic matter (SOM) decay (the change in decay rate per unit change in temperature, often assessed via changes in microbial CO_2 release). However, interpretation of empirically realized temperature sensitivities of SOM pools of varying degrees of recalcitrance remains difficult. Some of the observed temperature sensitivities of decay – termed here apparent temperature sensitivities (Davidson & Janssens, 2006) – qualitatively support

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predictions from enzyme kinetics (Biasi *et al.*, 2005; Knorr *et al.*, 2005; Conant *et al.*, 2008a,b; Feng & Simpson, 2008; Feng *et al.*, 2008; Hakkenberg *et al.*, 2008; Hartley & Ineson, 2008; Craine *et al.*, 2010). For example, in a recent review, Conant *et al.* (2011) highlight how incubation studies assess responses to temperature of only relatively decomposable compounds, and tend to report temperature sensitivities of decay consistent with enzyme kinetics. However, longer term warming experiments indicate that the apparent temperature sensitivity of soil respiration to warming is not always uniform, and can decline over time (Peterjohn *et al.*, 1994; Oechel *et al.*, 2000; Luo *et al.*, 2001; Rustad *et al.*, 2001; Melillo *et al.*, 2002; Eliasson *et al.*, 2005). Furthermore, recent work highlights the importance of apparent variation in microbial metabolism with temperature as a driver of heterotrophic respiratory responses to warming (Bradford *et al.*, 2008, 2010), although the mechanisms driving metabolic changes remain unclear. These conflicting responses and our challenges interpreting them highlight our lack of process-based understanding of SOM decay responses to temperature.

The challenges of projecting SOM feedbacks to warming result from the multitude of drivers of heterotrophic CO_2 release: the presence of mineralizable substrates, substrate availability at exo-enzymatic reaction sites, the resource requirements of soil microorganisms, the stoichiometry of SOM compounds, and the E_a of SOM compounds and thus their architectural complexity. Several studies highlight how declining responses of SOM decay with increasing temperature can be influenced by decreasing substrate availability (Davidson & Janssens, 2006; Kirschbaum, 2006; Larionova *et al.*, 2007; Gershenson *et al.*, 2009). Although exo-enzyme activities prompt inferences about microbial resource requirements, and measuring SOM stoichiometry is feasible, it remains unclear how microbial metabolism and associated C and nutrient utilization of compounds with different E_a vary with temperature, particularly *in situ*. Only through linking microbial resource requirements, SOM stoichiometry, and microbial use of compounds with different E_a can we approach the goal of quantitatively predicting, with confidence, the decay response of any given SOM pool to shifts in temperature relevant to anthropogenic climate change.

Here, we briefly outline our current understanding of the drivers of temperature sensitivity of SOM decomposition, and then describe two important concepts insufficiently explored in current investigations of SOM responses to temperature change. First, we highlight how warming may change microbial resource demands, and the potential consequences of such changes for community structure, total biomass, and physiology. Next, we describe how a link between SOM C:N and reactivity or E_a, which defines the temperature sensitivity of decay, can alter the relative availability of C and N as temperature changes. By examining how changing microbial resource needs interact with inherent SOM structure and composition – and thus reactivity – we demonstrate the means by which increasing temperature could result in increasing, unchanging, or even decreasing respiration rates observed in soils. Finally, we highlight ideas for future research that will develop our abilities to predict heterotrophic CO_2 losses from soil in a warming world.

Current theoretical constructs

Whereas apparent temperature sensitivity can be estimated from the slope of an Arrhenius plot derived from the decay of aggregated SOM by a microbial community, intrinsic temperature sensitivity is realized when only one substrate is being degraded by one exo-enzyme, and reaction rate is limited by reaction site structure, not substrate availability. Under such conditions, the intrinsic temperature sensitivity is characterized by the Arrhenius function:

$$V_{\max} = A \cdot e^{\frac{-E_a}{RT}} \quad (1)$$

where $V_{\max}$ is the maximum decomposition rate of a specific pool of SOM, A characterizes molecular collision frequency and orientation (the pre-exponential or A factor), E_a is the activation energy of decay, R is the gas constant, and T is temperature.

Quantification of intrinsic temperature sensitivities of decay is critical for understanding the mechanisms driving discrepancies between apparent and intrinsic temperature sensitivities, but is difficult to accomplish empirically. Each organic compound within a soil matrix exhibits a unique molecular structure and thus intrinsic E_a for a given exo-enzyme, multiple exo-enzymes can induce decay of a compound, and conditions within soil profiles can impose varying restrictions on compounds' availability to exo-enzymatic reaction sites. Craine *et al.* (2010) demonstrate that the Arrhenius function captures the general relationship between E_a and rate of soil respiration in many soils. However, significant residual variation and the nonlinear nature of temperature sensitivity necessitate detailed knowledge of intrinsic temperature sensitivities of decay and microbial responses to temperature regime if we hope to predict respiration for particular soils.

Further challenging investigators is the issue of substrate availability. If substrate availability decreases due to microbial substrate depletion or reduced rates of diffusion, Michaelis–Menten kinetics can become a

significant driver of decay rates. Under such conditions, V_{max} will be governed primarily by the half-saturation constant (K_m) and that term's temperature sensitivity (Davidson *et al*., 2012), and apparent temperature sensitivity will be lower than that predicted from enzyme kinetics (Davidson *et al*., 2006; Kirschbaum, 2006; Larionova *et al*., 2007; Gershenson *et al*., 2009). Acknowledging the combined influence of Arrhenius and Michaelis–Menten approaches (Davidson & Janssens, 2006; Davidson *et al*., 2006, 2012) represents a key advance in our efforts to predict SOM breakdown with changing temperatures, but does not address two key features likely influencing apparent temperature sensitivities of decay. Potential changes with temperature in (1) microbial C and nutrient needs, and (2) relative flows of C and N liberated during decay may induce additional discrepancies between apparent and intrinsic temperature sensitivities of decay, distinct from those prompted by limited substrate availability. We discuss each of these features below, establishing linkages between them that help reconcile discrepancies between apparent temperature sensitivities of decay and those predicted by enzyme kinetics. Furthermore, these two features represent feasible and sometimes counteracting mechanisms driving observed respiratory responses to warming. Although not all of the mechanisms we illuminate may be viable in all soils, examining the possibility of their occurrence is important for gaining predictive power of soil feedbacks to climate.

Drivers of microbial C and nutrient economies with changing temperature

Despite the many empirical and theoretical studies of microbially mediated C fluxes, we do not understand what governs apparent variability in patterns of microbial C acquisition and allocation – a cell's C economy. The C economy of a soil microorganism is determined by the availability of C in the surrounding soil matrix, the C required for microbial functioning, and the ability of the microbe to obtain C from soil via an investment in exo-enzymes. Microbes require C for the manufacture and repair of cellular structure, exo-enzyme production, and respiration, and microbial C acquisition depends in large part on C liberated by extracellular enzyme activity. Carbon taken up by microbes can ultimately contribute to either biomass generation (anabolism) or respiration (catabolism), determined in part by the organism's basal metabolic C requirements (del Giorgio & Cole, 1998; Franklin *et al*., 2011). It is difficult to quantify C fate after microbial uptake, but culture experiments suggest that bacteria use excess C with a high degree of plasticity, sustaining maximum rates of catabolism regardless of whether this leads to growth (Russell, 1991; Russell & Cook, 1995). Presumably, maintaining the high flux rates associated with this bacterial 'energy spilling' (Russell & Cook, 1995) is advantageous even when growth does not result, allowing cells to maintain membrane potentials and active transport mechanisms for growth when conditions become favorable (Dawes, 1985). Thus, microbes appear likely to maximize growth potential (Franklin *et al*., 2011), even at the expense of C use efficiency (Tempest *et al*., 1985). Certainly, C use efficiency can decline when nutrients are limited and C is relatively plentiful (Manzoni *et al*., 2008a), and C use strategies appear to vary between microbial groups (del Giorgio & Cole, 1998; Thiet *et al*., 2006; Ziegler & Billings, 2011), exemplifying the complex interactions between C availability and microbial C demand and allocation. As a result, an unsuspecting biogeochemist focused primarily on substrate availability and reactivity and unaware of potential microbial adaptation risks incorrect interpretation of respiratory responses to warming.

Recent discussion of microbial C economies and soil warming has centered on two features of microbial communities: their mass specific respiration (MSR), and their composition and biomass. Evidence from many taxa suggests that MSR increases with temperature (Gillooly *et al*., 2001). However, some soil studies suggest that warming reduces the respiration rate per unit of microbial biomass (Bradford *et al*., 2008, 2009), although this conclusion has spurred controversy (Hartley *et al*., 2007, 2008, 2009). Bradford *et al.* (2010) suggest that microbial adaptation to warming might occur via production of exo-enzymes that are more stable in a warmer environment, but that the increased stability of such exo-enzymes typically comes with a cost of lower catalytic rates of substrate transformation. The lower catalytic rates of more stable enzymes thus could result in apparently lower MSR (Bradford *et al*., 2010). However, microbes adapting to warming by producing exo-enzymes with lower catalytic rates is not a reflection of changing C requirements *per se* (and therefore not of MSR either). Instead, the production of exo-enzymes with lower catalytic rates decreases the rate at which cleaved substrates become available for microbial uptake, relative to the rate that would have been realized at that temperature with less stable exo-enzymes. Therefore, a warming-induced need for exo-enzymes with increased stability, and the resulting lower catalytic rates, may mask any increases in metabolic rates.

The conditions determining the relative importance with warming of changes in metabolic rates (Gillooly *et al*., 2001) or enzyme catalytic rates (Bradford *et al*., 2010), likely competing processes, have significant implications for respiratory losses from soil and remain

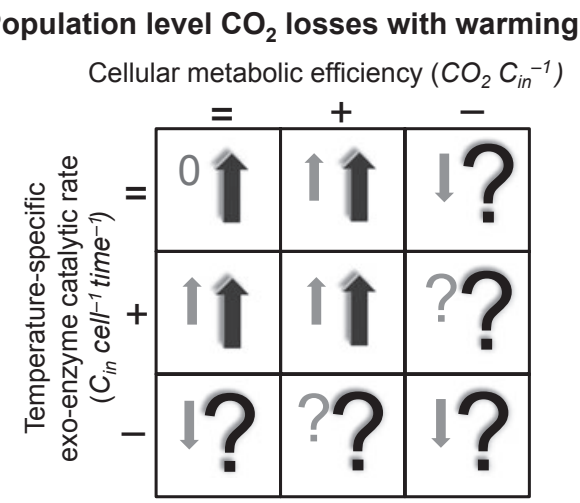

Fig. 1 Possible responses of a microbial population's CO_2 losses to warming, which are the net result of three factors (assuming saturated enzyme kinetics). Two factors are depicted on the axes: cellular metabolic efficiency (CO_2 C_{in}^{-1}) and temperature-specific exo-enzyme catalytic rate (C_{in} $cell^{-1}$ $time^{-1}$; no change =, increase +, or decrease −). The small gray symbols in each box (0, ↑, ?) represent the net result of changes on both axes with warming on the population's microbial CO_2 losses (no change, increase, or unknown due to counteracting changes on both axes). The product of CO_2 C_{in}^{-1} and C_{in} $cell^{-1}$ $time^{-1}$ yields population level CO_2 losses with warming. The third factor, the general increase in process rates with temperature, overlays these responses. The large black symbols in each box (↑, ?) represent the net effect of increasing process rates overlaid on changes in both axes' variables (increased or unknown net effects, respectively). For example, boxes with two '?' indicate counteracting adaptations to warming on both axes with unknown effects on cellular respiration (small, gray '?'), overlaid on an increase in process rates with temperature mitigating declines or exacerbating increases in respiration induced by adaptation (large, black '?'). The bottom row corresponds to a decline in temperature-specific exo-enzyme catalytic rates as proposed by Bradford *et al.* (2010). In the absence of any shift in C allocation, the middle column corresponds to a decline in metabolic efficiency with warming, consistent with enhanced metabolic costs for most taxa as temperature increases (Gillooly *et al.*, 2001).

unclear (Fig. 1). For example, a decrease in cellular metabolic efficiency with warming, paired with a counteracting decline in the exo-enzyme catalytic rate, would result in a change in cellular respiratory losses of CO_2. The direction of that change is difficult to predict because we do not know which change would have the dominant effect. However, both of these changes would be overlaid on a warming-induced increase in process rates that would mitigate respiratory declines or exacerbate respiratory increases (bottom center box in Fig. 1).

In addition to potential changes with temperature in the catalytic rate of exo-enzymes and microbial metabolic rates, microbial community structure may change with temperature, with consequences for observed respiration responses. Shifts in biomass or the relative abundances of functional groups may occur as a result of varying C utilization patterns across taxa or as a result of environmental conditions linked to the new temperature regime. There is some empirical evidence that total microbial biomass varies with temperature, although this is not a universal response (Rinnan *et al.*, 2007; Feng & Simpson, 2008; Frey *et al.*, 2008), but the mechanisms driving these changes remain unclear. Changing relative abundances of functional groups may occur, for example, if some populations mitigate their C demand more so than others as temperature rises by shifting C allocation more toward maintenance and away from exo-enzyme production, reducing their ability to acquire resources from SOM. Variation in ability to compete for SOC, as well ability to turn SOC into new biomass, likely results in changes in community structure and in the activity of community constituents, both of which will influence whole community respiratory responses to warming. Furthermore, more indirect changes in the environment prompted by rising temperatures may also result in altered community structure. For example, if rising temperature induces a generally drier soil profile, Gram + bacteria, with their relatively thick cell walls, may be better able to persist than Gram- bacteria. Indeed, changes in community structure with temperature have been observed in multiple studies (Zogg *et al.*, 1997; Andrews *et al.*, 2000; Biasi *et al.*, 2005; Zhang *et al.*, 2005; Frey *et al.*, 2008), although we are uncertain why such changes occur.

Changes in microbial community structure or total biomass could induce measurable variation in soil respiratory responses to temperature (Fig. 2). For example, even if total biomass remains constant, an increase over time in the relative abundance of a population exhibiting lower MSR may result in an eventual decline in soil respiratory response to increasing temperature, as is sometimes observed (Peterjohn *et al.*, 1994; Oechel *et al.*, 2000; Luo *et al.*, 2001; Rustad *et al.*, 2001; Melillo *et al.*, 2002; Eliasson *et al.*, 2005). Alternatively, if total microbial biomass changes simultaneously with relative abundance, the C mineralization rate could increase, perhaps transiently as observed in some studies (Peterjohn *et al.*, 1994; Oechel *et al.*, 2000; Luo *et al.*, 2001; Rustad *et al.*, 2001; Melillo *et al.*, 2002; Eliasson *et al.*, 2005), decrease, or even remain constant. Furthermore, if total biomass increases sufficiently with warming, C mineralization may increase even if the relative abundance of microorganisms exhibiting lower MSR increases. All of these potential respiratory responses to warming are again overlaid on the most fundamental consequences of increasing temperature – faster reaction rates induce increased material fluxes through

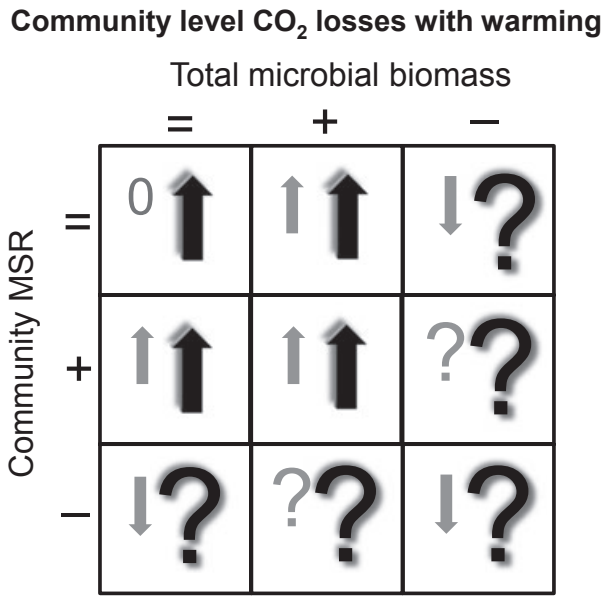

Fig. 2 Possible responses of a microbial community's CO_2 losses to warming, which is the net result of three factors. Two factors are depicted on the axes: total microbial biomass and community level mass specific respiration (MSR, no change =, increase +, or decrease –). An increase in community MSR would occur, for example, if warming induces an increase in the relative abundance of microbes exhibiting high MSR. The small gray symbols in each box (0, ↑, ?) represent the net result of changes on both axes, due to adaptation of the community to temperature, on microbial community CO_2 losses (none, increased, or unknown due to counteracting changes on both axes). The product of the two axes' values yields community level CO_2 losses with warming. The large, black symbols in each box (↑, ?) represent the net effect of increasing process rates overlaid on changes in both axes' variables (increased or unknown net effects, respectively).

the soil's microbial funnel. Some of these responses will clearly result in an increase in microbial community respiration, but because we do not yet know which of these responses will dominate with warming, responses often combine to generate an unknown net effect (Fig. 2).

Further complicating microbial responses to warming, evidence suggests that the flows of *both* C and other resources through microbial communities shift with temperature regime (Sinsabaugh *et al.*, 2008, 2009). Here, we focus on nitrogen (N) as a key nutrient of interest, given its importance as a driver of ecosystem processes, but one could just as easily focus on any other essential nutrient. If microbial responses to warming differ among populations, there could be consequences for the relative rates at which C and N are liberated in response to temperature increases, and for feedbacks to the microbial community. Carbon and N economies appear to vary among microbial populations (Guggenberger *et al.*, 1999; Simpson *et al.*, 2004; Glaser *et al.*, 2006; Liang *et al.*, 2007; Rinnan & Bääth, 2009; Ziegler & Billings, 2011), likely linked to microbial selection of substrates with varying quality (del Giorgio & Cole, 1998; Berggren *et al.*, 2007; Manzoni *et al.*, 2008a), and to temperature variability (Biddanda & Cotner, 2002; Allison *et al.*, 2010). Furthermore, microbial C and N economies are important drivers of climate-carbon cycle feedbacks, as indicated by general circulation models (Thornton *et al.*, 2009). These observations underscore the importance of accurately characterizing the mechanisms driving both C and nutrient requirements of microorganisms for predicting future decomposition rates. A recent review of biogeochemical models indicates how our assumptions about the degree of plasticity in microbial biomass C:N have direct consequences for how we interpret SOM decomposition, N immobilization, and CO_2 losses (Manzoni & Porporato, 2009). In spite of this work and other recent empirical and theoretical advances linking microscale or cellular processes and stoichiometry (Manzoni *et al.*, 2008b; Manzoni & Porporato, 2009; Doi *et al.*, 2010; Franklin *et al.*, 2011; Loladze & Elser, 2011), it remains unclear what physiological or stoichiometric properties govern these economies, particularly with changing temperature. To further explore microbial C and N economies and substrate choice in the context of temperature change, we must depict the linkages between SOM C:N ratios, and the ease with which microorganisms can access those elements in compounds of varying reactivity. By integrating these linkages with the features of C and nutrient economies presented above, we develop new concepts to help explain the sometimes contradictory responses of SOM decay to warming.

SOM attributes as governors of relative C and N flows with warming

In the literature describing the reactivity and composition of decaying OM, degree of decomposition and age are often inversely related to C:N ratios, and associated with increases in the relative abundance of structurally complex molecules. C:N ratios of decaying OM decline over experimental time frames (Rice & Tenore, 1981; Melillo *et al.*, 1984; Nadelhoffer & Fry, 1988; Tremblay & Benner, 2006), and observations of C:N ratios in SOM fractions suggest that this trend continues across time scales of decades to centuries (Six *et al.*, 2002; Billings, 2006). Furthermore, SOM fractions reveal declines in C:N ratios with average SOC radiocarbon age and associated degree of decomposition (Ewing *et al.*, 2006; Sollins *et al.*, 2006). Increasingly

slower turnover rates of SOM fractions as decomposition proceeds over time, relative to fresher material, are reflected in a multitude of studies isolating coarsely defined stages of decay using incubation time (Townsend *et al*., 1997; Hartley & Ineson, 2008; Craine *et al*., 2010), depth in profile (Leavitt *et al*., 1996; Paul *et al*., 1997, 2001; Gaudinski *et al*., 2000; Ewing *et al*., 2006; Trumbore, 2009), and size, density, and chemical fraction of origin (Leavitt *et al*., 1996; Trumbore & Zheng, 1996; Paul *et al*., 1997, 2001; Gaudinski *et al*., 2000; Sollins *et al*., 2006; Castanha *et al*., 2008).

Concurrent with a decline in C:N ratio as organic matter decays, we observe a general increase in relative abundances of slow-turnover organic matter (Baldock & Preston, 1995), typically comprised of more complex and decay-resistant molecular architectures. Variation in turnover time can reflect many SOM properties (Thornley & Cannell, 2001; reviewed in Kleber, 2010), such as physical protection of substrates associated with clays (Sollins *et al*., 2006; Grandy & Neff, 2008) and aggregate formation (Six *et al*., 2002). However, we emphasize here that variation in substrate reactivity with degree of decomposition can reflect meaningful changes in *inherent* substrate reactivity over time (Lorenz *et al*., 2007), i.e., reactivity determined by properties of the substrate itself, in isolation from potential environmental constraints such as protection. Indeed, inherent substrate reactivity tends to decline as decomposition proceeds, even in the absence of phenomena such as mineral-associated protection (Benner *et al*., 1986; Hedges *et al*., 1994; Opsahl & Benner, 1995; McTiernan *et al*., 2003; Tremblay & Benner, 2006, 2009). As a result, concurrent declines in SOM fractions' C:N ratios and inherent reactivity as they decompose suggest a negative relationship between C:N of SOM fractions and the E_a required to initiate their decay (Fig. 3).

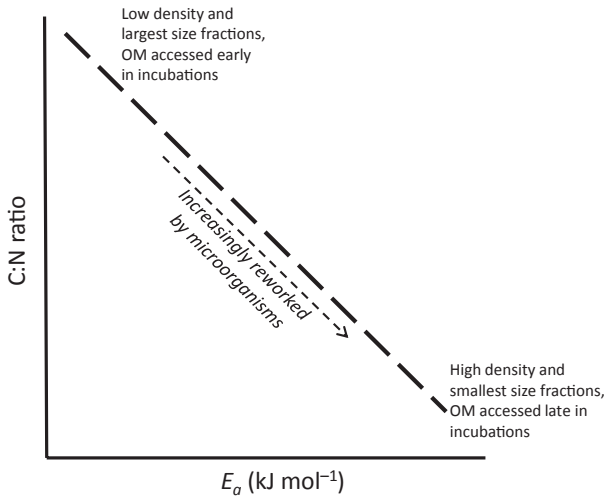

Fig. 3 Hypothesized relationship between the activation energy (E_a) required to initiate decay of soil organic matter (SOM) fractions, denoted using examples of separation techniques, and those fractions' C:N ratios. This relationship is not universal given compounds like lignin, which contains no N and has a relatively slow turnover time and, presumably, a relatively high E_a compared with many other plant compounds.

Using organic compounds commonly found in soil, we can illustrate how any variation in substrate C:N with its E_a of decomposition, regardless of its direction, can influence the availability of C and N liberated from organic macromolecules and feedback to microbial resource economies. For example, pyrimidine, an aromatic compound containing two N atoms substituted for C and derived from plant phenolics (Schulten & Schnitzer, 1997) has a relatively low C:N ratio (C:N = 2). In contrast, chitin, a key component of fungal cell walls (Killham, 1994), has a slightly higher C:N ratio (C:N = 8). Chitin contains no aromatic rings, is composed of a repeating chain of N-acetyl glucosamine, and features N on a single-bonded side chain. Although we do not know the absolute values of E_a of decay for chitin and pyrimidine and the multiple isozymes that can induce their breakdown, it is reasonable to assume that pyrimidine, the lower C:N substrate, possesses a higher E_a of decay than chitin due to the relative stability of aromatic rings. The varying E_a of decomposition for pyrimidine and chitin suggests that their decay rates respond differentially with warming, and we demonstrate how this feature combined with a negative relationship between substrate E_a and C:N (Fig. 3) can result in a decline in the liberation of C relative to that of N.

We can use the representative substrates of chitin and pyrimidine to explore how warming could alter exo-enzyme-driven C and N release, invoking a relationship between substrate C:N and E_a of decay. To focus solely on the potential effects of such a relationship, we assume that exo-enzyme concentrations and substrate pool sizes remain constant with warming, that pyrimidine and chitin are the only two substrates available for C and N (all other essential nutrients are nonlimiting), and that all C and N in each compound becomes available for microbial uptake upon decay. For the enzyme catalyzed decomposition of substrate S, we write an equation for the rate of element liberation, using C as an example. For a single substrate S,

$$\frac{dC_s}{dt} = \left(\frac{d[S']}{dt}\left[\frac{C}{S'}\right] + \frac{d[S'']}{dt}\left[\frac{C}{S''}\right]\right) S_{\text{total}} \qquad (2)$$

in which $\left[\frac{C}{S'}\right]$ and $\left[\frac{C}{S''}\right]$ are the C contents of the products of S decay. Because we assume that the products can be

entirely assimilated by microbes and that decomposition is not substrate limited, we can write the rate of C liberated from two substrates as:

$$\frac{dC_{S12}}{dt} = V_{\max S1}(T)\left[\frac{C}{S1}\right]S1_{\text{total}} + V_{\max S2}(T)\left[\frac{C}{S2}\right]S2_{\text{total}} \tag{3}$$

where V_{maxS1} and V_{maxS2} exhibit Arrhenius temperature dependence ($V_{\max S}(T) = A \cdot e^{\frac{-E_a}{RT}}$). Writing an analogous equation for N and dividing the C liberation equation by the N liberation equation yields the ratio of C and N liberated during decay from two specified substrates for which we know the C and N content, which we term the C:N flow ratio

$$\frac{dC_{S12}}{dN_{S12}} = \frac{\frac{A_1}{A_2}e^{\frac{E_{a2}-E_{a1}}{RT}}\frac{S1_{\text{total}}}{S2_{\text{total}}}\left[\frac{C}{S1}\right] + \left[\frac{C}{S2}\right]}{\frac{A_1}{A_2}e^{\frac{E_{a2}-E_{a1}}{RT}}\frac{S1_{\text{total}}}{S2_{\text{total}}}\left[\frac{N}{S1}\right] + \left[\frac{N}{S2}\right]} \tag{4}$$

where A_1 and A_2 are the A factors for the two substrates. Empirical measurement of absolute values for A factors is difficult, but the ratio of these values is more readily obtained (Pilling & Seakins, 2005).

Equation (4) demonstrates how the C:N flow ratio is determined by the C and N content of the substrates, the slope of the E_a–C:N relationship, the ratio of substrate pool sizes, the ratio of pre-exponential factors, and temperature. We select chitin as *S1* and pyrimidine as *S2* ($\left[\frac{C}{S1}\right] = 8$, $\left[\frac{C}{S2}\right] = 4, \left[\frac{N}{S1}\right] = 1, \left[\frac{N}{S2}\right] = 2$). To characterize the influence of a negative E_a–C:N relationship on the C:N flow ratio, we used 50 KJ mol^{-1} as a midpoint for the absolute range of E_a values in conjunction with two plausible slopes for the relationship between E_a and C:N (Davidson & Janssens, 2006; Lehmeier *et al*., in press). Because little is known about the ratio of SOM substrate pool sizes and virtually nothing is known about the relative magnitude of substrate A factors, we computed the influence of a negative E_a–C:N relationship over a range of values for both. In Fig. 4a, we plot the C:N flow ratio as a function of the pool size ratio for a fixed A factor ratio. We see that a steeper slope of the E_a–C:N relationship results in a greater overall temperature effect (black lines compared with red lines). We also observe that the temperature effect is most pronounced over a particular range of chitin to pyrimidine pool size ratios, and that for any given ratio of substrate pool sizes, warming results in a decline in the C:N flow ratio. The influence of the E_a–C:N relationship on C:N flow in response to warming is further mediated by the ratio of A factors (Fig. 4b). If the A factor ratio is relatively small [i.e., chitin (*S1*) exhibits an A factor substantially smaller than that of pyrimidine (*S2*)] and the ratio of chitin : pyrimidine pool sizes is relatively large, temperature can have a meaningful influence on the C:N flow ratio (Fig. 4b).

Any reduction in C:N flow ratio resulting from a temperature increase could exacerbate any extant C limitation, or shift microbes from being N- to C limited. Such changes in relative resource availability, derived solely from substrate characteristics, could have meaningful consequences on microbial losses of CO_2 with warming even in the absence of microbial adaptation to the new temperature regime. In theory, we could observe a negative respiratory response to warming as microbes experience greater relative C limitation, although such a response may be mediated by shifts in microbial community functioning, as discussed in the following section. Enhanced microbial C limitation with warming due to substrate characteristics offers an alternative explanation to the decline in respiratory responses to rising temperature sometimes observed in long-term experiments and frequently attributed to declining substrate availability (Peterjohn *et al*., 1994; Oechel *et al*., 2000; Luo *et al*., 2001; Rustad *et al*., 2001; Melillo *et al*., 2002; Eliasson *et al*., 2005).

Of course, focusing on only two SOM compounds does not reflect the complexities of real soil profiles, but the structural differences between chitin and pyrimidine illustrate important functional consequences, even if they are a caricature. Soils contain a complex suite of SOM compounds concurrently undergoing decay, not all of which require breakdown for uptake (Schulten and Schnitzer 1997; Geisseler *et al*., 2010), and exhibit great variation in diffusion of substrates to enzymatic reaction sites. Furthermore, despite the evidence suggesting a negative relationship between fraction C:N and its E_a of decay, it is difficult to assess the strength of this relationship for the individual compounds comprising those fractions, and not all compounds will adhere to the relationship. Certainly lignin, with its aromatic structure, relatively low reactivity (Opsahl & Benner, 1995, 1999; Kögel-Knabner, 2002), and lack of N does not adhere to this concept, nor do some labile, N-rich proteins (Brzostek & Finzi, 2012). However, lignin is not an important component of low C:N SOM (Thevenot *et al*., 2010), and an abundance of N-rich organic compounds exhibit relatively complex, frequently aromatic structures (Schulten and Schnitzer 1997). Basic chemical principles inform us that bond energies are substantially higher for the double C=C bonds (612 kJ mol^{-1}) in pyrimidine than for the single C–C bonds of chitin (347 kJ mol^{-1}; Weast, 1983; Masterston *et al*., 1985), and thus these two substrates effectively capture important functional differences among SOM compounds. Indeed, published values of E_a of decay for isolated enzyme-substrate pairings, at optimal pH and temperature, suggest that aromatic rings require greater energy to decay: the aromatic compounds catechol and dopamine, when paired with

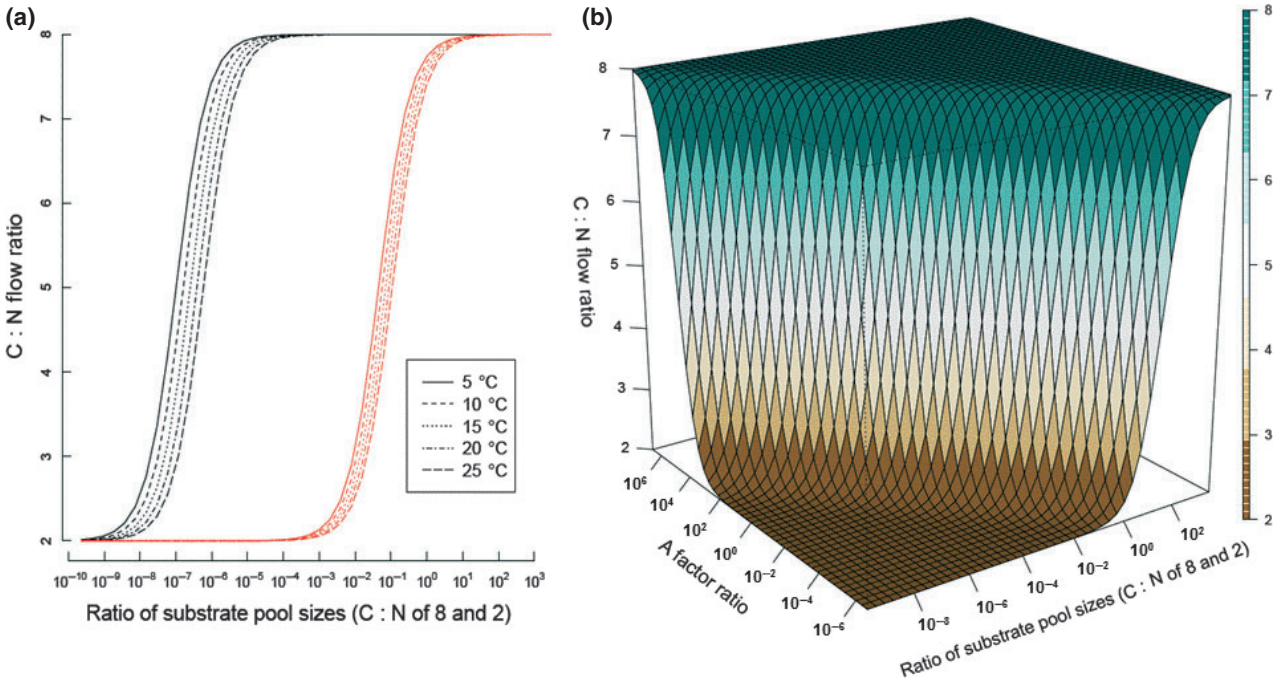

Fig. 4 The influence of a negative relationship between substrate E_a of decay and substrate C:N on the C:N flow ratio, as mediated by ratio of substrate pool sizes and *A* factors. We define the C:N flow ratio as the ratio of C and N liberated during decay from two substrates (see text and Equation 5 for details). For a fixed *A* ratio of 0.0001 (a), different slopes of the E_a–C:N relationship (black lines represent a slope of −10; red lines −5) shift the range of substrate pool size ratios over which a temperature effect on C:N flow ratio is prominent. Warming results in a decline in the C:N flow ratio, and this effect is more pronounced with a steeper slope. For a fixed E_a–C:N slope of −5 (b), varying the *A* ratio further shifts the range of substrate pool size ratios over which the temperature effect is prominent.

polyphenol oxidase, exhibit E_a of decay of approximately 14 and 12 kJ mol^{-1}, respectively (Sakiroglu *et al.*, 2008). E_a of decay of the more simply structured aldouronic acids are approximately half these values (6 kJ mol^{-1}) when paired with α-D-glucuronidase (Mierzwa *et al.*, 2005).

Our simplified scenario captures an important feature of the aggregate reactivity and stoichiometry of bulk SOM fractions and illustrates how a functional relationship between C:N and E_a of decay may arise. The above exercise illustrates that (1) a relationship between SOM substrate E_a and C:N, even when relatively shallow, dictates variation in the ratio of C and N liberated from SOM with changes in temperature, and (2) the magnitude of this effect is governed by the relative size of substrate pools and the ratio of substrate *A* factors. The ratio of SOM substrate pool sizes for which E_a of decay and C:N have been quantified is rarely if ever available, and *A* factors of such compounds are not known. To our knowledge, only one study reports *A* factors for biogeochemically relevant substrate-enzyme pairings (Lehmeier *et al.*, in press). To understand how soil warming may alter microbial resource availability, microbial community structure and function, and ultimately the associated respiratory CO_2 losses, we must expand our knowledge of absolute pool sizes of SOM substrates with varying composition, the temperature sensitivity of decay of those substrates, and their *A* factors.

Linking SOM reactivity and stoichiometry to changing microbial economies with warming

The logic presented above dictates that any relationship between substrate E_a and C:N ratio, regardless of form, will influence SOM decomposition dynamics in response to warming. If C:N varies negatively with E_a and microorganisms do not change their decomposition strategies, we would expect microbes to become progressively more C limited, as the ratio of liberated C and N becomes closer to the C:N of high E_a substrates. (Note that we assume a soil system in which enzyme kinetics are saturated.) There are multiple potential outcomes of such a change in the relative availability of C and N (Fig. 5). We first consider outcomes given a constant microbial decomposition strategy – i.e., microorganisms maintain production of the same exo-enzymes, at the same rate, as in the previous, cooler temperature regime. We then consider potential outcomes of soil warming if microbes

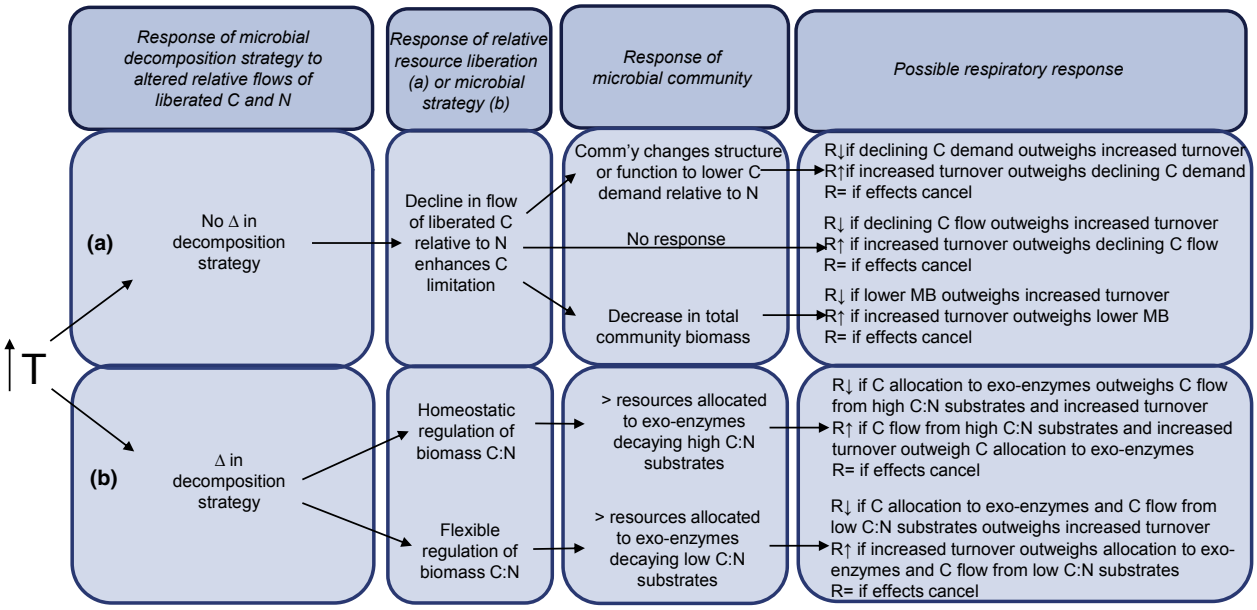

Fig. 5 Potential responses of microbial decomposition strategies to altered relative flows of C and N upon substrate decay with warming, and subsequent logical outcomes of respiratory CO_2 losses (R), with no change in (a) and changing (b) microbial decomposition strategies. Scenarios assume that SOM fraction C:N ratios vary negatively with E_a of decay, such that lower C:N fractions experience a greater relative temperature sensitivity of decay, and higher C:N fractions a greater absolute temperature sensitivity of decay, consistent with enzyme kinetics. All responses may occur simultaneously among different microbial populations.

alter their decomposition strategy in response to altered C:N flow ratios.

First, we might expect microbial communities to exhibit changes in structure or function in response to altered availability of C and N, particularly if we assume they undergo no change in decomposition strategy (Fig. 5a). For example, microorganisms might exhibit phenotypic changes to lower their C requirements, relative to N, if C becomes more limiting with warming. A microorganism may enhance the uptake of 'luxury' nutrients into vacuoles (Malmgren-Hansen *et al*., 1991; Schmidt *et al*., 1997), for example, for eventual repair of N-rich structural components. There are multiple studies suggesting that microorganisms can vary their resource allocation as availability changes. For example, evidence suggests that enhanced organic N availability can result in increased C use efficiency of some microbial groups (Ziegler & Billings, 2011) and greater production of exo-enzymes needed to decompose relatively labile, C-rich substrates (Tiemann & Billings, 2011). Other work suggests that C use efficiency may decline with warming (Steinweg *et al*., 2008), although such results are not universal (Dijkstra *et al*., 2011). The physiological mechanisms underlying such responses remain unclear, but it seems feasible that the degree to which the C and N demands of the microbes can be adjusted to match the new, enzymatically determined flow regime will influence rates of SOM decay. A second potential outcome of increasing C limitation with warming is an increase in the efficiency with which microorganisms use C, potentially mitigating any increase in MSR due to enhanced metabolic rates with warming (Gillooly *et al*., 2001). Third, if C limitations are sufficiently severe with warming, we also might see a decline in microbial biomass. If microbial populations experience differences in any of these potential responses, all else equal, we likely would see changes in their relative abundances. All of these potential shifts in microbial structure and function, induced by a change in the relative flows of liberated C and N with warming, overlie the respiratory increases resulting from the relatively simpler increased process rates induced by warming (Fig. 5a).

These potential responses to altered flows of C and N from decaying substrates, all independent of increases in microbial metabolic rates and production of exo-enzymes exhibiting slower catalytic rates with warming, reflect constant decomposition strategies. However, more microbially mediated consequences of altered soil temperature become evident if we allow decomposition strategy to vary, as it might if microbes sense a change in the relative liberation of C and N upon decay (Fig. 5b). Microorganisms may employ plastic decomposition strategies to achieve homeostatic regulation of their stoichiometry. As such, communities exhibiting no change in population relative abundances with warming may be reflective of changing decomposition strategies. Indeed, plasticity in decomposition

strategies, presumably related to the regulation of C uptake relative to N, is consistent with apparent shifts in exo-enzymatic activity rates as relative substrate availabilities vary (Sinsabaugh *et al.*, 2008; Tiemann & Billings, 2011). Homeostatic regulation of C:N stoichiometry might induce microbes to invest more in exo-enzymes that access relatively high C:N substrates, permitting them to maintain the relative flow rates of liberated C and N experienced in the cooler temperature regime. Alternatively, if microorganisms simultaneously change their decomposition strategies and biomass stoichiometry, they may preferentially generate exo-enzymes to decompose substrates offering the greatest relative yield with warming, which we hypothesize to exhibit lower C:N (Fig. 3).

Further challenges for predicting microbial responses to warming and associated changes in relative C and N flow rates arise when we consider changes in microbial resource demands associated with varying costs of exo-enzyme production. This feature may become important particularly if microbial decomposition strategies shift with altered flow rates of liberated C and N resources. If microorganisms use different substrates with warming that require shifting production of exo-enzymes, each with a distinct resource cost, such a scenario would feedback to resource demand and, in turn, substrate choice. The relative costs of exo-enzyme production to microbes remain unknown, but the varying size and composition of exo-enzymes (Mierzwa *et al.*, 2005; Kocabas *et al.*, 2008) suggests that each is associated with a distinct resource cost as well as the energetic costs required for its generation. Changes in decomposition strategy, then, can feedback to resource demand and patterns of substrate decay.

Exploiting these ideas for better predictions of future soil CO_2 flux

We have outlined a diverse range of potential microbial responses to warming, formalized an apparent relationship between SOM fraction E_a of decay and C:N, and established linkages between microbial responses and SOM characteristics. In so doing, we have revealed how microbial respiratory responses to soil warming may result in an increase, no change, or even a decrease in CO_2 efflux, highlighting the challenges of mechanistically interpreting such data. However, the concepts we present also highlight ways forward for predicting soil feedbacks to warming. Experiments explicitly addressing several important questions will represent significant steps toward a mechanistic understanding of SOM decay responses to warming:

1 With warming, to what extent is observed MSR reduced by microbial production of more stable exo-enzymes with lower catalytic rates vs. increased by enhanced metabolic rates (Fig. 1)?
2 To what extent do shifts in community structure with warming, when present, reflect changed competitive abilities of some populations due to altered MSR, vs. responses to other environmental variables affected by increased temperature like soil moisture (Fig. 2)?
3 To what extent does E_a of decay, and hence inherent temperature sensitivity of decay, vary with substrate C:N (Fig. 3)? Linked to this question,
   (a) are substrate pool sizes and A factors of the appropriate magnitudes for temperature to have a meaningful influence on C:N flow ratios (Fig. 4)?
   (b) does a relationship between substrate E_a of decay and C:N result in altered relative flows of C and N with warming distinct from plastic microbial decomposition strategies (Fig. 5a)?
   (c) if microorganisms retain their decomposition strategy with warming even with altered flows of C relative to N, does community structure change such that resource demands match the new flow regime (Fig. 5a)?
   (d) if microorganisms alter their decomposition strategy in response to an altered C and N flow regime, do they exhibit homeostatic regulation of their biomass stoichiometry (Fig. 5b)?
4 What are the production costs of different exo-enzymes (Fig. 5b)?
5 To what extent do the respiratory consequences of any of these processes mitigate or accentuate the predicted increase in respiratory CO_2 losses resulting solely from increased process rates with warming?

Although phenomena like SOM protection, varying substrate availability, and diffusion constraints can dampen apparent temperature sensitivities of substrate decay, it is important to clarify all potential drivers of decay responses to temperature when Arrhenius kinetics are dominant. Only under such conditions can ideas about intrinsic temperature sensitivities of decay be fully explored, and the relative importance of enzyme kinetics, microbial substrate choices, and abiotic influences on substrate decay rates discerned. Given that real soils present a complex suite of substrates to largely uncharacterized microbial communities, often under conditions in which Michaelis–Menten dynamics appear more dominant than Arrhenius kinetics, addressing these questions likely will require a reductionist experimental approach. Many of these questions may be best answered employing simplified microbial

communities offered isolated or paired substrates of known structure and stoichiometries, where substrate and exo-enzyme diffusion to reaction sites are promoted. Under such conditions, the Arrhenius relationship is a relevant descriptor of temperature sensitivity of decay for the system. As with any reductionist approach to complex systems, a key difficulty will be assessing the extent to which results of these efforts are applicable to real soil profiles. However, by linking the potential roles of substrate E_a of decay, stoichiometry, pool sizes, and A factors with microbial physiological responses to and substrate choices with warming, we can begin to develop a truly mechanistic understanding of SOM decay with warming.

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