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The Michaelis–Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study

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Abstract

Decomposition of soil organic matter (SOM) is mediated by microbial extracellular hydrolytic enzymes (EHEs). Thus, given the large amount of carbon (C) stored as SOM, it is imperative to understand how microbial EHEs will respond to global change (and warming in particular) to better predict the links between SOM and the global C cycle. Here, we measured the Michaelis–Menten kinetics [maximal rate of velocity (V_{max}) and half-saturation constant (K_m)] of five hydrolytic enzymes involved in SOM degradation (cellobiohydrolase, β-glucosidase, β-xylosidase, α-glucosidase, and *N*-acetyl-β-D-glucosaminidase) in five sites spanning a boreal forest to a tropical rainforest. We tested the specific hypothesis that enzymes from higher latitudes would show greater temperature sensitivities than those from lower latitudes. We then used our data to parameterize a mathematical model to test the relative roles of V_{max} and K_m temperature sensitivities in SOM decomposition. We found that both V_{max} and K_m were temperature sensitive, with Q_{10} values ranging from 1.53 to 2.27 for V_{max} and 0.90 to 1.57 for K_m. The Q_{10} values for the K_m of the cellulose-degrading enzyme β-glucosidase showed a significant ($P = 0.004$) negative relationship with mean annual temperature, indicating that enzymes from cooler climates can indeed be more sensitive to temperature. Our model showed that K_m temperature sensitivity can offset SOM losses due to V_{max} temperature sensitivity, but the offset depends on the size of the SOM pool and the magnitude of V_{max}. Overall, our results suggest that there is a local adaptation of microbial EHE kinetics to temperature and that this should be taken into account when making predictions about the responses of C cycling to global change.

Keywords: carbon cycle, decomposition, global change, microbe, respiration, warming carbon use efficiency

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Introduction

The world is warming as a result of anthropogenic activities that increase atmospheric CO_2 concentrations (IPCC, 2007). However, the largest source of CO_2 emission is soil respiration, primarily from microbial decomposition of soil organic matter (SOM; Davidson *et al.*, 2006; Prentice *et al.*, 2001). Global stocks of organic carbon (C) existing as SOM are at least four times greater than that of the global stocks of C in the atmosphere and living plants (Jobbágy & Jackson, 2000), and the decomposition of this SOM is predicted to increase with increasing temperature (Davidson & Janssens, 2006), thereby causing a positive feedback loop between atmospheric CO_2 concentrations, temperature, and microbial decomposition (Cox *et al.*, 2000; Prentice *et al.*, 2001).

Most of the predictions of a positive feedback between temperature and microbial decomposition do not take into account the kinetics of extracellular hydrolytic enzymes (EHEs) secreted by microbial decomposers into the environment (Allison *et al.*, 2010). As long as SOM is accessible for microbial decomposers (Ekschmitt *et al.*, 2005; Plante *et al.*, 2009), the actions of EHEs represent the rate limiting step of decomposition, marking the conversion of SOM into dissolved organic C, which is then metabolized by microbial decomposers (Nannipieri *et al.*, 2002; Allison *et al.*, 2007). Indeed, EHE activity is temperature sensitive (Koch *et al.*, 2007; Wallenstein *et al.*, 2009; Stone *et al.*, 2012), which has led to predictions of increased respiration of soil CO_2 with increasing temperature (Davidson & Janssens, 2006). However, it is unclear whether microbial EHEs are locally adapted to different temperature regimes,

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and it is, therefore, challenging to predict enzymatic responses to warming.

Latitudinal gradients in enzyme temperature sensitivity have been observed for metabolic enzymes in insects (Huestis *et al*., 2009), intertidal invertebrates (Dong & Somero, 2009), and fishes (Johns & Somero, 2004), and for the digestive enzyme trypsin in fishes (Hofer *et al*., 1975). One unifying theme of the temperature adaptation of enzymes is that cold-adapted enzymes tend to be more responsive to increasing temperature than warm-adapted enzymes (Somero, 2004; Koch *et al*., 2007; Dong & Somero, 2009), primarily due to differences in protein structure that cause cold-adapted proteins to lose function more readily as temperatures increase (Hochachka & Somero, 2002). This loss of function is most evident when examining the Michaelis–Menten kinetics of the enzymes at different temperatures. For instance, the Michaelis–Menten constant (K_m) increases more strongly with increasing temperature in cold-adapted enzymes than in warm-adapted enzymes (Hochachka & Somero, 2002; Johns & Somero, 2004; Somero, 2004; Dong & Somero, 2009; Huestis *et al*., 2009). K_m is the substrate concentration at half-maximal enzymatic velocity (V_{max}), and is indicative of the affinity an enzyme has for its substrate (German *et al*., 2011b). Therefore, an increase in K_m indicates a decrease in overall enzyme function. In fact, an increase in K_m can counteract an increase in enzyme V_{max} under warming conditions, thereby reducing the temperature sensitivity of decomposition in soils (Davidson *et al*., 2006).

The temperature sensitivity of enzymes may be a function of 'local adaptation' (Williams, 1966; Belotte *et al*., 2003) of organisms to a prevailing temperature regime across season or latitude. Although tests of local adaptation have been difficult to design for soil microbial communities (Belotte *et al*., 2003; Reed & Martiny, 2007), a 'natural experiment' using a latitudinal gradient may be one way to test for local adaptation of microbial physiology. Such an experiment could be particularly valuable for understanding the Michaelis–Menten kinetics of microbial enzymes, as has been done for invertebrate enzymes (Johns & Somero, 2004; Dong & Somero, 2009; Huestis *et al*., 2009). Although the Michaelis–Menten kinetics of some EHEs in soils have been studied in a range of different systems (Tabatabai, 1994; Nannipieri & Gianfreda, 1998; Marx *et al*., 2005; Zhang *et al*., 2010), comparisons of microbial EHE kinetics across a latitudinal gradient, which aim to examine the temperature sensitivity of enzymes as a function of mean annual temperature (MAT), are lacking. The effects of temperature and season on microbial EHE activity have been studied (Koch *et al*., 2007; Wallenstein *et al*., 2009), and indeed, enzymes measured in winter are more sensitive to increases in temperature than enzymes measured in summer (Koch *et al*., 2007).

The goal of this investigation was to examine whether extracellular enzymes display characteristics of local adaptation to different MATs and to assess the combined effect of V_{max} and K_m temperature sensitivities on C decomposition rates. We examined the Michaelis–Menten kinetics of microbial EHEs in response to temperature in soils from five locations ranging from the boreal zone (Alaska) to a tropical rainforest (Costa Rica; Table 1). We chose to measure the kinetics of five EHEs involved in the degradation of common C substrates in soils, and that are known to follow

Table 1 Geographic coordinates and general soil information for the sampling sites used in this study

Location*	Coordinates	Habitat	Soil type	pH†	MAT (°C)	Moisture (%)	OM (%)
Delta Junction, Alaska, USA	63°55′N 145°44′W	Boreal Forest	Inceptisols	5.0	0	39.14 ± 0.32	16.76 ± 0.89
Bear Brook, Maine, USA	44°52′N 68°06′W	Temperate Forest	Spodosols	5.0	5	23.25 ± 3.03	89.00 ± 3.86
Fernow, West Virginia, USA	39°03′N 79°49′W	Temperate Forest	Inceptisols	5.0	9	63.81 ± 3.57	21.16 ± 1.10
Irvine, California, USA	33°44′N 117°42′W	Temperate Grassland	Alfisols	6.8	17	2.43 ± 0.17	3.06 ± 0.08
Golfo Dulce, Costa Rica	8°43′N 83°37′W	Tropical Rain Forest	Ultisols	5.8	26	39.54 ± 0.55	15.06 ± 0.53

Moisture and organic matter (OM) values are mean ± SEM. MAT, mean annual temperature.

*Soils were collected during the growing season for Alaska (May), Maine (November), West Virginia (May), and California (April), whereas they were collected during the dry season in Costa Rica (January).

†To match the bulk soil pH of each site in our enzyme assays, different buffer systems were used as appropriate for each site. For Alaska, Maine, and West Virginia we used 50 mM sodium acetate, pH 5. For California, we used 100 mM maleate buffer pH 6.8, and for Costa Rica we used 25 mM maleate buffer, pH 5.8.

Table 2 Enzymes and substrates used in this study

Enzyme	E.C. number	OM constituent*	Synthetic substrate†	Substrate concentrations‡ (μM)
Cellobiohydrolase	3.2.1.91	Cellulose degradation products	4-MUB-β-D-cellobioside	1–100
β-glucosidase	3.2.1.21	Cellulose degradation products	4-MUB-β-D-glucopyranoside	2–200
α-glucosidase	3.2.1.20	Starch degradation products	4-MUB-α-D-glucopyranoside	2–200
β-xylosidase	3.2.1.37	Hemicellulose degradation products	4-MUB-β-D-xylopyranoside	2–200
N-acetyl-β-D-glucosaminidase	3.2.1.52	Chitin degradation products	4-MUB-*N*-acetyl-β-D-glucosaminide	4–400

*The compounds found in soil organic matter that act as substrates for each enzyme.
†For each synthetic substrate, the fluorescent dye 4-methylumbelliferone is abbreviated as MUB.
‡These concentrations represent the substrate concentrations used in the actual assays, not the stock substrate concentrations. Eight concentrations were used within each substrate concentration range, which were determined to be appropriate for the determination of Michaelis–Menten kinetics for each enzyme.

Michaelis–Menten kinetics (Table 2). Based on previous studies of metabolic enzymes (Hochachka & Somero, 2002; Johns & Somero, 2004; Somero, 2004; Dong & Somero, 2009; Huestis *et al*., 2009), we hypothesized that microbial EHEs from cooler climates would be more sensitive to temperature change, and that K_m in particular would show increasing temperature sensitivity with increasing latitude. We then integrated our enzyme kinetic measurements with a mathematical model to compare the effects of V_{max} and K_m temperature sensitivities on soil C dynamics.

Materials and methods

Soil collection and handling

Soils were collected from five locations representing a latitudinal gradient from boreal to tropical forest (Table 1). At each location, soil cores (2.5 diameter × 5 cm depth) were taken with a soil corer (n = 6–10), individually placed in ziplock bags, and kept cold (~4 °C) for transport back to the laboratory (UC Irvine or Cornell University). Upon arrival at the laboratory, individual cores were mixed by hand and frozen at −20 °C until measurement of extracellular enzyme activities (within 2 months). Freezing is known to affect the activity levels of EHEs (Lee *et al*., 2007), but all samples were frozen similarly, so we do not expect that freezing affected our ability to make comparisons across sites.

Enzyme assays

Five enzymes targeting C- and N-containing substrates found in soils were assayed following a modified version of the method described by German *et al.* (2011b and Table 2). Homogenate was prepared by dispersing 1 g of soil (wet weight) in 125 mL of buffer prepared at the appropriate pH for each sample site (Table 1). Fifty μL of fluorometric substrate solution was combined with 200 μL of soil homogenate in a microplate and incubated for 1 h at 4, 10, 16, 22, 28, 34, or 40 °C. For soils from Maine and West Virginia, 10 °C was replaced by 7 °C and 22 °C was replaced by 23 °C, due to the differential availability of incubators among laboratories (Stone *et al*., 2012). However, these slight changes did not affect the overall analysis because we were able to determine the temperature sensitivity of the enzymes across the same range of temperatures. Each enzyme was assayed at a range of substrate concentrations at each temperature (Table 2), the data from which were used to examine the Michaelis–Menten kinetics of each enzyme at each temperature in the soils from the five sampling locations (Fig. 1).

Following the 1 h incubation, the reaction was stopped by the addition of 10 μL of 1 M NaOH, and after a 10 min development period, the amount of fluorescence was determined in a fluorometer (Biotek Synergy 4, Winooski, VT, USA) at 360 nm excitation and 460 nm emission. The assay of each enzyme at each substrate concentration was replicated twice in each plate, and each plate included a standard curve of the product (4-methylumbelliferone, MUB), substrate controls (for each substrate concentration), and homogenate controls. Enzymatic activity (nmol product released h^{-1} g^{-1} dry soil) was calculated from the MUB standard curve following German *et al.* (2011b). Soil dry mass was determined following German *et al.* (2011a).

Enzyme kinetics and statistical analyses

The Michaelis–Menten constant (K_m) and maximal velocity (V_{max}) were determined for each enzyme at each of the measured temperatures using the Michaelis–Menten equation:

$$V = \frac{V_{max}[S]}{K_m + [S]}, \quad (1)$$

where V is the reaction velocity (a function of enzyme concentration), [S] is the substrate concentration, K_m is the substrate concentration at half-maximal velocity, and V_{max} is the maximal velocity. V_{max} and K_m values were determined using nonlinear regression (Stone *et al*., 2012), and were log-transformed

(natural log, ln). The effects of collection location, temperature, and their interaction were analyzed with ANCOVA, using temperature as a covariate (Stone *et al*., 2012). Regression coefficients (slopes and intercepts) were plotted as linear relationships. Slopes represent the temperature sensitivities of each enzyme kinetic parameter and were also expressed as Q_{10} values according to:

$$Q_{10} = \exp(\text{slope} \times 10), \tag{2}$$

following Wallenstein *et al*. (2009). To examine variation in temperature sensitivity of each enzyme by location, the Q_{10} values for K_m and V_{max} were regressed against the MAT of the collection sites. Although seasonality can affect the temperature response of these enzymes (e.g., Wallenstein *et al*., 2009), we believe that MAT is the best explanatory variable for this study because many enzymes within the soil matrix are stabilized (e.g., Tabatabai, 1994; Marx *et al*., 2005), and hence, the kinetics of these enzymes are more reflective of long-term temperature trends as opposed to seasonal temperature changes. Moreover, we are making broad scale comparisons across 55° of latitude, thus making MAT a better predictor of overall isoform expression than temperature at the time of collection. A significant relationship between Q_{10} and MAT would potentially suggest local adaptation of the enzymes to different temperature regimes. All statistics were run using the statistical program R (R development Core Team 2010).

Mathematical model

To explore the combined effects of V_{max} and K_m temperature responses on decomposition rates, we constructed a simple model that was parameterized with enzyme kinetic data from our field sites. The model is based on Davidson *et al*. (2006) and Allison *et al*. (2010), and represents decomposition rate as a function of C input rate I, microbial turnover τ_M, microbial biomass C_{MIC}, extracellular enzyme V_{max} and K_m, and the pool size of soil organic C (C_{SO}):

$$\frac{dC_{SO}}{dt} = I + \tau_M \cdot C_{MIC} - C_{MIC} \cdot \frac{V_{max} \cdot C_{SO}}{K_m + C_{SO}}. \tag{3}$$

C inputs and dead biomass enter the C_{SO} pool, and C_{SO} is lost through decomposition, which is assumed to be a Michaelis–Menten process represented by the last term in Eqn (3). Microbial biomass change is a function of microbial turnover and assimilation of decomposed soil organic C, which occurs with C use efficiency (CUE) ε:

$$\frac{dC_{MIC}}{dt} = \varepsilon \cdot C_{MIC} \cdot \frac{V_{max} \cdot C_{SO}}{K_m + C_{SO}} - \tau_M \cdot C_{MIC}. \tag{4}$$

The soil respiration rate (R) is then the fraction of decomposition not assimilated by microbial biomass:

$$R = (1 - \varepsilon) \cdot C_{MIC} \cdot \frac{V_{max} \cdot C_{SO}}{K_m + C_{SO}}. \tag{5}$$

We introduce temperature sensitivity for V_{max} and K_m by making them exponential functions of temperature:

$$V_{max} = e^{V_{slope} \cdot T + V_{int}} \cdot a_V, \tag{6}$$

and

$$K_m = e^{K_{slope} \cdot T + K_{int}} \cdot a_K, \tag{7}$$

where V_{slope} and K_{slope} and V_{int} and K_{int} are the fitted slopes and intercepts, respectively, from regressions of ln(kinetic parameter) on temperature (T, in °C) based on our empirical data. a_V and a_K are coefficients that can be tuned to generate stable and reasonable equilibrium C pool sizes in the model.

At steady state, $dC_{SO}/dt = dC_{MIC}/dt = 0$ and we can solve for C_{SO} at equilibrium:

$$\widehat{C}_{SO} = \frac{\tau_M \cdot K_m}{\epsilon \cdot V_{max} - \tau_M}; \frac{\tau_M}{V_{max}} < \epsilon < 1, \tag{8}$$

where ε must be larger than τ_M/V_{max}, otherwise microbes cannot assimilate enough C to compensate for microbial turnover; if $\varepsilon = 1$, then microbes respire no C, all C is assimilated, and biomass grows indefinitely. Solving Eqn (3) for C_{MIC} at steady state and substituting Eqn (8), we obtain the following expression for microbial biomass at equilibrium:

$$\widehat{C}_{MIC} = \frac{I \cdot \epsilon}{\tau_M \cdot (1 - \epsilon)}. \tag{9}$$

Thus, microbial biomass increases with the ratio of inputs to microbial turnover and with CUE.

We used the model along with enzyme V_{max} and K_m temperature sensitivities from our regression analyses with β-glucosidase to assess the effect of a 5 °C temperature increase on calculated soil organic C and microbial biomass pools at each field site. We used the β-glucosidase data because this enzyme targets an abundant C substrate in soil (i.e., cellulose), had V_{max} temperature sensitivities similar to the other enzymes, and showed the strongest latitudinal in K_m temperature sensitivity. The intercepts and absolute values of V_{max} and K_m are determined by many factors aside from temperature that we did not measure, such as substrate availability, nutrient availability, and microbial community composition. Therefore, we set V_{int} and K_{int} equal to their cross-site averages and used the same values for a_V and a_K across sites. Because there is some evidence that microbial CUE declines with increasing temperature (Devêvre & Horwáth, 2000; Steinweg *et al*., 2008), we included a linear temperature sensitivity function for ε with intercept ε_0 and conducted model runs with and without a nonzero slope ε_{slope}:

$$\epsilon = \varepsilon_{slope} \cdot T + \epsilon_0. \tag{10}$$

Other parameter values are given in Table 3.

Results

Enzyme kinetics

V_{max} increased with temperature for all enzymes at all sites (Table 4 and Fig. 2). The magnitude of the temperature response of ln(V_{max}) varied across enzymes, ranging from 0.042 to 0.082 °C^{-1}, corresponding to Q_{10}

Table 3 Model parameter descriptions, values, and units

Parameter	Description	Value	Units
I	Carbon input rate	0.001	mg cm^{-3} h^{-1}
τ_M	Microbial biomass turnover rate	0.0005	h^{-1}
V_{max}	Maximum catalytic rate	0.0019–0.0126	mg^{-1} microbial biomass cm^{-3} h^{-1}
V_{slope}	Regression coefficient	0.046–0.063	ln(nmol g^{-1} h^{-1}) $°C^{-1}$
V_{int}	Regression intercept	5.47	ln(nmol g^{-1} h^{-1})
a_V	Tuning coefficient	8×10^{-6}	mg^{-1} microbial biomass cm^{-3} h^{-1}
K_m	Half-saturation constant	243–360	mg cm^{-3}
K_{slope}	Regression coefficient	0.007–0.034	ln(μmol L^{-1}) $°C^{-1}$
K_{int}	Regression intercept	3.19	ln(μmol L^{-1})
a_K	Tuning coefficient	10	mg cm^{-3}
ε	Carbon use efficiency	0.134–0.630	mg mg^{-1}
ε_{slope}	Carbon use efficiency temperature slope	0 or −0.016	mg mg^{-1} $°C^{-1}$
ε_0	Carbon use efficiency temperature intercept	0.5 or 0.63	mg mg^{-1}
T	Temperature	0–31	°C

values of 1.53 to 2.27 (Table 4). Interactions among collection location and temperature were significant for cellobiohydrolase, β-glucosidase, β-xylosidase, but not for α-glucosidase or *N*-acetyl-β-D-glucosaminidase, indicating that the temperature sensitivity of V_{max} varied across locations (Table 4).

As observed for V_{max}, K_m changed with temperature for all enzymes (Table 5 and Fig. 2). The magnitude of the temperature response of ln(K_m) varied across enzymes, ranging from −0.011 to 0.045 $°C^{-1,}$ corresponding to Q_{10} values of 0.90 to 1.57 (Table 5). The Q_{10} values for K_m were all lower than the Q_{10} values observed for V_{max} (Tables 4 and 5). β-glucosidase, β-xylosidase, and *N*-acetyl-β-D-glucosaminidase K_m values all showed significant interactions between location and temperature, whereas no interactions among location and temperature were detected for cellobiohydrolase and α-glucosidase K_m values (Table 5 and Fig. 2).

Of all of the enzymes tested, only β-glucosidase showed a significant relationship ($P = 0.004$) between the temperature sensitivity (Q_{10}) of K_m and the MAT of the collection locations (Fig. 3). All other regressions of K_m Q_{10} ($P > 0.143$) and V_{max} Q_{10} ($P > 0.620$) against temperature were not significant. All of the V_{max} and K_m values gathered in this study are presented in Tables S1 and S2.

Mathematical model

When CUE was held constant, microbial biomass did not vary with temperature, and simulated soil organic C stocks were markedly lower at the higher temperature sites (Table 6). For the parameters we used, the percent of soil organic C lost with 5 °C warming was similar across the sites, ranging from −20% in California to −29% in Alaska (Fig. 4a, open bars). When K_m was held constant with temperature, there was no offset of V_{max} temperature sensitivity, and soil organic C declined strongly with increasing temperature (Fig. 4a, filled bars). Although the K_m temperature sensitivities that we measured for β-glucosidase were not high enough to completely negate C losses due to increased V_{max}, allowing K_m to vary with temperature offset nearly 30% of the soil C lost in the three coolest sites (Fig. 4a). However, in our tropical site (Costa Rica), allowing for K_m temperature sensitivity reduced soil C losses by <10% due to the low K_m temperature sensitivity for β-glucosidase at this site.

When we allowed microbial CUE to decline with increasing temperature, the model predicted very different C_{MIC} and C_{SO} pool sizes, but changes in K_m temperature sensitivity had a similar effect on the fraction of C_{SO} lost with warming (Fig. 4b). Modeled microbial biomass declined by an order of magnitude from the Alaska site to the Costa Rica site due to a decline in CUE from 0.63 to 0.21 mg mg^{-1}, which has a nonlinear effect on microbial biomass (Eqn 9; Table 6). Due to the negative effect on decomposer biomass, the C_{SO} pool did not decline as much with increasing temperature, and actually increased by 4–11% and 25–30% with 5 °C warming in California and Costa Rica, respectively (Fig. 4b). The effect of K_m temperature sensitivity is more pronounced when CUE is also temperature sensitive, and offsets most of the percentage C lost in Alaska, Maine, and West Virginia (Table 6; Fig. 4b). With temperature-sensitive CUE, the predicted soil C loss is smaller (e.g., −20% vs. −40% for temperature sensitive and non-temperature-sensitive CUE, respectively, in Alaska), thus magnifying the effects of temperature-sensitive K_m values (Fig. 4).

Table 4 Slope, intercept, R^2, and Q_{10} values for regressions of V_{max} against assay temperature of five microbial hydrolytic enzymes measured in soils from five different locations

Location	Intercept	Slope	R^2	Q_{10}
Cellobiohydrolase				
Alaska	3.68 ± 0.25	0.064 ± 0.010	0.51	1.90
Maine	5.69 ± 0.18	0.082 ± 0.007	0.87	2.27
West Virginia	2.97 ± 0.21	0.070 ± 0.008	0.79	2.01
California	2.24 ± 0.15	0.059 ± 0.006	0.67	1.81
Costa Rica	1.07 ± 0.16	0.078 ± 0.006	0.75	2.17
Location: $F_{4,24} = 66.77$; $P < 0.001$; Location × Temperature interaction: $F_{4,153} = 2.59$; $P = 0.039$				
β-Glucosidase				
Alaska	5.85 ± 0.17	0.055 ± 0.007	0.62	1.73
Maine	7.51 ± 0.21	0.063 ± 0.008	0.76	1.88
West Virginia	4.94 ± 0.23	0.059 ± 0.009	0.69	1.80
California	4.93 ± 0.12	0.046 ± 0.005	0.63	1.58
Costa Rica	4.11 ± 0.11	0.061 ± 0.004	0.72	1.84
Location: $F_{4,26} = 49.11$; $P < 0.001$; Location × Temperature interaction: $F_{4,156} = 2.82$; $P = 0.027$				
β-Xylosidase				
Alaska	3.74 ± 0.20	0.042 ± 0.008	0.59	1.53
Maine	5.69 ± 0.25	0.070 ± 0.010	0.71	1.98
West Virginia	2.95 ± 0.13	0.065 ± 0.005	0.93	1.92
California	2.80 ± 0.13	0.053 ± 0.005	0.71	1.71
Costa Rica	3.18 ± 0.08	0.054 ± 0.003	0.80	1.72
Location: $F_{4,20} = 75.71$; $P < 0.001$; Location × Temperature interaction: $F_{4,141} = 3.00$; $P = 0.021$				
α-Glucosidase				
Alaska	2.74 ± 0.21	0.053 ± 0.009	0.57	1.70
Maine	4.75 ± 0.19	0.060 ± 0.008	0.75	1.81
West Virginia	2.54 ± 0.16	0.050 ± 0.006	0.77	1.64
California	2.30 ± 0.13	0.051 ± 0.005	0.69	1.67
Costa Rica	1.86 ± 0.11	0.060 ± 0.004	0.75	1.82
Location: $F_{4,24} = 51.78$; $P < 0.001$; Location × Temperature interaction: $F_{4,153} = 1.01$; $P = 0.407$				
N-Acetyl-β-D-glucosaminidase				
Alaska	5.51 ± 0.30	0.052 ± 0.012	0.37	1.69
Maine	7.60 ± 0.18	0.062 ± 0.007	0.79	1.87
West Virginia	4.58 ± 0.20	0.069 ± 0.008	0.80	2.00
California	3.40 ± 0.12	0.061 ± 0.005	0.77	1.84
Costa Rica	4.20 ± 0.08	0.062 ± 0.003	0.85	1.85
Location: $F_{4,24} = 52.02$; $P < 0.001$; Location × Temperature interaction: $F_{4,159} = 1.59$; $P = 0.181$				

Values are mean ± SEM. Data were analyzed with ANCOVA, with location as a main effect and temperature as a covariate. All temperature effects were significant ($P < 0.001$).

Discussion

The results of this study showed consistent support for the hypothesis that V_{max} and K_m are temperature sensitive for soil microbial EHEs. However, our hypothesis that cold-adapted EHEs would show greater temperature sensitivity for K_m than warm-adapted EHEs was only supported for the cellulose-degrading enzyme β-glucosidase. No clear patterns were observed for the four other EHEs investigated. To our knowledge, this is only the second study to examine the temperature sensitivity of K_m in soil EHEs, and in the other, Stone *et al.* (2012) also observed more variation in the K_m response to temperature than in the V_{max} response.

The temperature adaptation of proteins is primarily driven by changes in key amino acid residues involved in adjusting the K_m and the catalytic rate constant (K_{cat}) of a protein, thereby allowing the protein to be more 'flexible' (and active) under cooler temperatures, and more 'rigid' at warmer temperatures (Fields, 2001; Johns & Somero, 2004). This pattern is mediated through a balance of enthalpy and entropy (Johns & Somero, 2004; Somero, 2004). The overarching effect is that rates of reaction can be held relatively constant

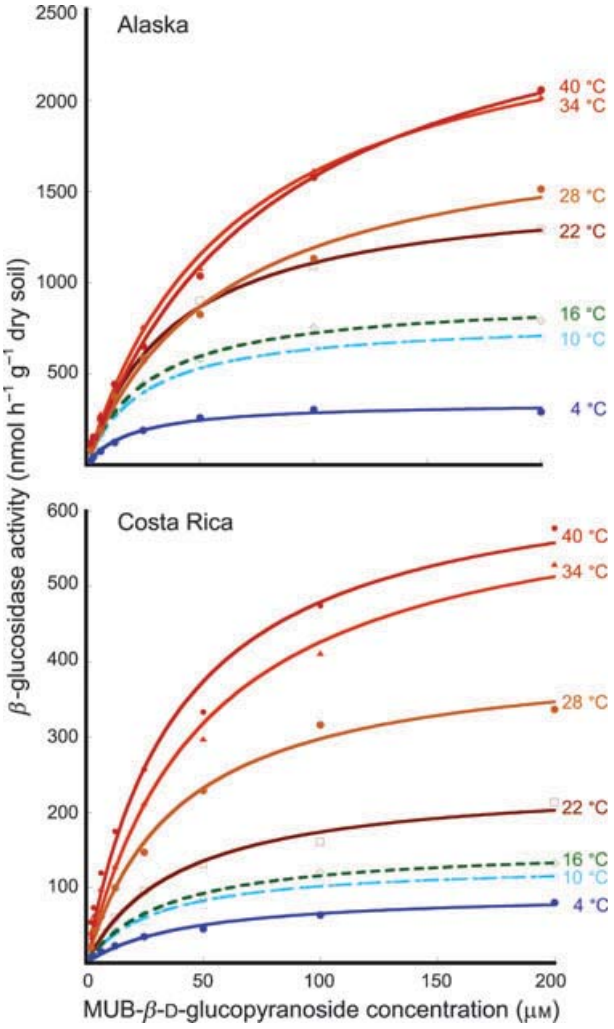

Fig. 1 Michaelis–Menten plots (enzyme activity as a function of substrate concentration) of β-glucosidase measured at seven different temperatures in Alaskan and Costa Rican soils. The line observed at each temperature is labeled. Values are means and errors were deliberately not included for clarity. The full name of the substrate is methylumbelliferyl-β-D-glucopyranoside.

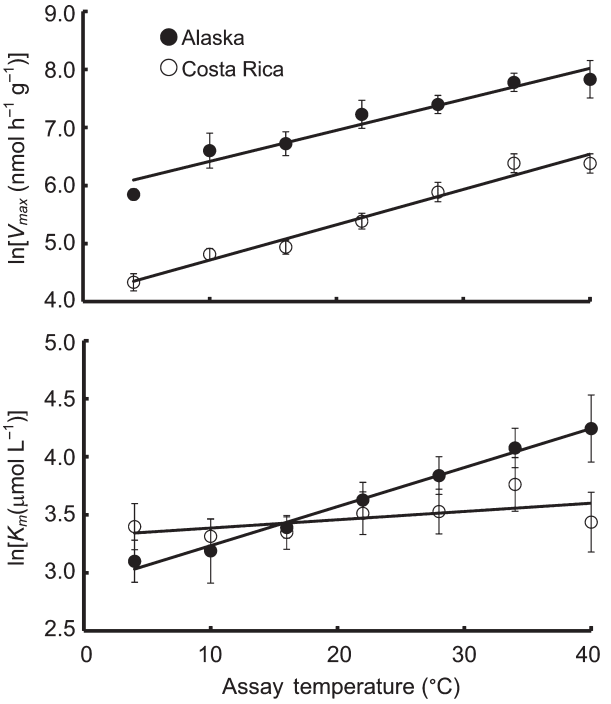

Fig. 2 β-glucosidase V_{max} (top panel) and K_m (bottom panel) plotted as a function of assay temperature in soils from Alaska and Costa Rica. Values are mean ± SEM (n = 6–10). Data from all collection sites (not just Alaska and Costa Rica) were analyzed with ANCOVA, with collection location as the main effect and temperature as the covariate (see Tables 4 and 5).

under different temperatures (Somero, 2004). This local adaptation of enzyme activity to a temperature regime is visible for β-glucosidase in this study: at assay temperatures relevant to ambient temperatures for Alaska (4 °C) and Costa Rica (28 °C), the ln(V_{max}) values of this cellulose-degrading enzyme are equal (Fig. 2). However, the K_m values for β-glucosidase show evidence of a tradeoff between flexibility and function, with greater K_m temperature sensitivity in colder sites than in warmer sites. This finding is consistent with latitudinal trends in the K_m temperature response of metabolic enzymes (Johns & Somero, 2004; Dong & Somero, 2009; Huestis *et al.*, 2009), and the protease trypsin (Hofer *et al.*, 1975). Enzymatic K_m response to temperature is important for decomposition because EHE saturation may be a key limiting factor for enzymatic degradation of SOM (Schimel & Weintraub, 2003).

This study provides further evidence that the K_m of microbial EHEs should be measured, especially in studies of global change (Zhang *et al.*, 2010; German *et al.*, 2011b; Stone *et al.*, 2012). Whereas V_{max} estimations can provide information about rates of decomposition under saturating substrate concentrations, the K_m values of EHEs can elucidate enzymatic responses to varying substrate concentrations under different temperatures, which is likely important in comparisons among sites, or among seasons at a given site. We recognize that we measured 'apparent' K_m in bulk soil samples, as has been done previously (Tabatabai, 1994; Nannipieri & Gianfreda, 1998; Marx *et al.*, 2005; Zhang *et al.*, 2010; Stone *et al.*, 2012), but this investigation provides impetus for more detailed studies of isolated EHEs (e.g., Skálová *et al.*, 2005) across temperature gradients to better inform our understanding of responses of decomposition to global climate change.

The use of isolated enzymes can reveal true patterns of temperature response that might be missed in assays of bulk soil enzyme kinetics, as was employed in this study. For instance, we did not observe the expected

Table 5 Slope, intercept, R^2, and Q_{10} values for regressions of K_m against assay temperature of five microbial hydrolytic enzymes measured in soils from five different locations

Location	Intercept	Slope	R^2	Q_{10}
Cellobiohydrolase				
Alaska	1.37 ± 0.31	0.039 ± 0.012	0.20	1.48
Maine	2.87 ± 0.27	0.043 ± 0.011	0.45	1.54
West Virginia	1.69 ± 0.29	0.045 ± 0.011	0.45	1.57
California	1.57 ± 0.17	0.015 ± 0.007	0.09	1.16
Costa Rica	1.89 ± 0.34	0.023 ± 0.013	0.06	1.26
Location: $F_{4,24} = 7.58$; $P < 0.001$; Location × Temperature interaction: $F_{4,153} = 1.73$; $P = 0.146$				
β-Glucosidase				
Alaska	2.88 ± 0.15	0.034 ± 0.006	0.46	1.41
Maine	3.65 ± 0.25	0.031 ± 0.010	0.35	1.37
West Virginia	2.78 ± 0.27	0.028 ± 0.011	0.27	1.33
California	3.33 ± 0.11	0.013 ± 0.004	0.16	1.14
Costa Rica	3.32 ± 0.15	0.007 ± 0.006	0.02	1.07
Location: $F_{4,26} = 4.62$; $P = 0.006$; Location × Temperature interaction: $F_{4,167} = 4.35$; $P = 0.002$				
β-Xylosidase				
Alaska	3.86 ± 0.36	−0.009 ± 0.014	0.02	0.91
Maine	4.83 ± 0.35	0.004 ± 0.013	0.01	1.04
West Virginia	2.50 ± 0.48	0.044 ± 0.019	0.30	1.55
California	2.79 ± 0.15	0.025 ± 0.006	0.27	1.28
Costa Rica	3.83 ± 0.17	−0.011 ± 0.007	0.04	0.90
Location: $F_{4,20} = 8.47$; $P = 0.002$; Location × Temperature interaction: $F_{4,141} = 5.97$; $P < 0.001$				
α-Glucosidase				
Alaska	3.39 ± 0.33	0.031 ± 0.014	0.15	1.36
Maine	4.58 ± 0.27	0.020 ± 0.011	0.15	1.22
West Virginia	2.96 ± 0.28	0.017 ± 0.011	0.10	1.18
California	2.67 ± 0.14	0.017 ± 0.006	0.17	1.19
Costa Rica	3.67 ± 0.18	0.025 ± 0.007	0.14	1.28
Location: $F_{4,24} = 33.79$; $P < 0.001$; Location × Temperature interaction: $F_{4,153} = 0.35$; $P = 0.845$				
N-Acetyl-β-D-glucosaminidase				
Alaska	3.69 ± 0.21	0.018 ± 0.008	0.12	1.20
Maine	4.33 ± 0.25	0.037 ± 0.010	0.41	1.44
West Virginia	4.16 ± 0.23	0.040 ± 0.009	0.51	1.50
California	2.98 ± 0.11	0.024 ± 0.004	0.42	1.27
Costa Rica	3.38 ± 0.010	0.017 ± 0.004	0.22	1.19
Location: $F_{4,24} = 27.45$; $P < 0.001$; Location × Temperature interaction: $F_{4,159} = 3.05$; $P = 0.019$				

Values are mean ± SEM. Data were analyzed with ANCOVA, with site as a main effect and temperature as a covariate. All temperature effects were significant ($P < 0.001$).

MAT–K_m relationship for any enzyme except β-glucosidase. This result may be due to our focus on bulk soils that contain an array of compounds (e.g., polyphenols, clay) that can interfere with enzyme function (Allison, 2006) and affect kinetic parameters (Marx *et al.*, 2005). Moreover, adsorption and desorption reactions are temperature sensitive (Thornley & Cannell, 2001) and could affect enzymes in a variable manner. Additionally, soils contain endogenous substrates, which may interfere with assays via competitive inhibition. (Stone *et al.*, 2012). Alternatively, different enzymes may show varying temperature responses due to conservation of some crucial K_m value, thereby affecting conformational microstates of the enzymes (Somero, 2004). However, our data showed that Q_{10} values for K_m were generally >1.2, with β-xylosidase providing the only exception (Table 5).

A limitation to our study is that we focused on enzymes with well-resolved assay methods, which follow Michaelis–Menten kinetics in soils, and could be used to parameterize our model. Oxidative enzymes, including phenol oxidase and peroxidase, degrade phenolic compounds using oxygen as an electron acceptor, and can depolymerize lignin (Claus, 2004; Sinsabaugh,

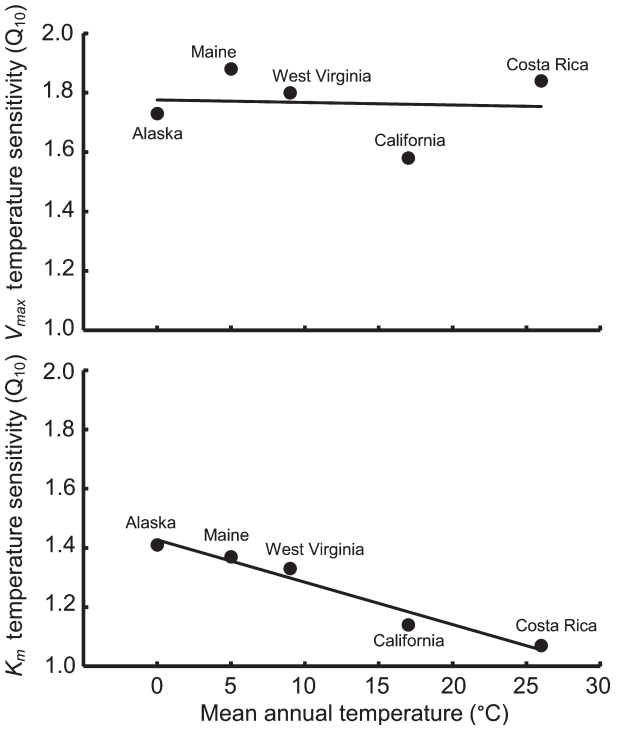

Fig. 3 Temperature sensitivity (Q_{10}) of β-glucosidase V_{max} (top panel) and K_m (bottom panel) plotted as a function of mean annual temperature for each of the collection locations. The V_{max} regression is not significant ($R^2 = 0.01$; $P = 0.905$), whereas the K_m regression is significant ($R^2 = 0.96$; $P = 0.004$).

2010). Given the diversity of C structures in SOM (including phenols and lignin), oxidative enzymes are important in SOM decomposition (Sinsabaugh, 2010). However, the methods of assaying these enzymes are not well resolved (Sinsabaugh, 2010; German *et al*., 2011b), especially in bulk soil samples, and assays of these enzymes do not generally follow Michaelis–Menten kinetics, making their behavior in response to temperature and substrate concentrations difficult to measure or model. Hence, phenol oxidase or peroxidase activities were not measured in this study, or in that of previous studies that examined the effects of temperature on Michaelis–Menten kinetics of microbial EHEs (Zhang *et al*., 2010; Stone *et al*., 2012).

Our model results suggest that variation in K_m temperature sensitivity can influence the amount of SOM lost from soil under increasing temperatures. As K_m temperature sensitivity increases, there is an increasing offset of the C lost due to V_{max} temperature sensitivity. Depending on whether microbial CUE is temperature sensitive, this predicted offset may range from 30% to nearly all of the C loss for the highest K_m temperature sensitivities we modeled. Although we only ran the model with β-glucosidase parameters, the K_m temperature sensitivity of the other enzymes was generally positive and often at the high end of the range observed for β-glucosidase (Table 5). Given that V_{max} temperature sensitivity was relatively similar

Table 6 Modeled microbial biomass (mg cm^{-3}) and soil organic carbon pool sizes (mg cm^{-3}) with 5 °C warming over mean annual temperature for the five field sites

	Constant CUE			CUE temp sensitive		
	C_{MIC}	C_{SO} (constant K_m)	C_{SO} (sensitive K_m)	C_{MIC}	C_{SO} (constant K_m)	C_{SO} (sensitive K_m)
AK						
0°C	2.00	270	270	3.41	174	174
5°C	2.00	162	192	2.44	139	164
ME						
5°C	2.00	152	177	2.44	130	152
10°C	2.00	95	129	1.77	103	141
WV						
9°C	2.00	109	140	1.89	113	146
14°C	2.00	73	108	1.37	96	142
CA						
17°C	2.00	77	96	1.12	123	154
22°C	2.00	57	76	0.77	127	170
CR						
26°C	2.00	29	35	0.54	82	98
31°C	2.00	21	26	0.31	102	127

The model was run with constant and temperature-sensitive carbon use efficiency for microbial assimilation, and with constant and temperature-sensitive half-saturation constants (K_m) for soil carbon decomposition.
CUE, carbon use efficiency; C_{MIC}, microbial biomass carbon; C_{SO}, pool size of soil organic carbon; AK, Alaska; ME, Maine; WV, West Virginia; CA, California; CR, Costa Rica.

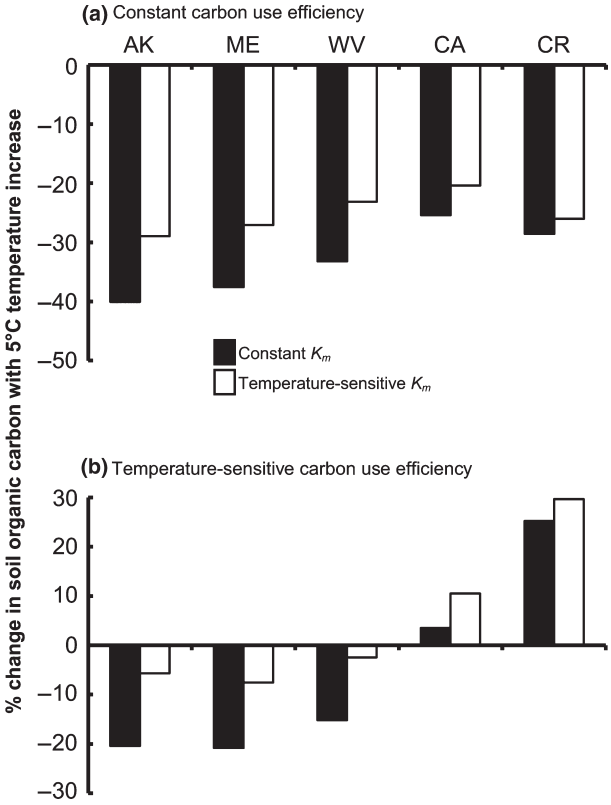

Fig. 4 Results of a mathematical model depicting the percent change in soil organic carbon in response to 5 °C warming as a function of sampling location with constant (a) and temperature-sensitive (b) carbon use efficiency. The effects of temperature-sensitive half-saturation constants (K_m) are also shown for each site. AK, Alaska; ME, Maine; WV, West Virginia; CA, California; CR, Costa Rica.

across enzymes (Table 4), the offsetting effect of K_m temperature sensitivity would apply to most of the enzymes we studied (β-xylosidase is an exception in some sites).

Some of our model conclusions may depend on the parameters we chose, but the potential for K_m and V_{max} temperature sensitivities to have offsetting effects on soil C loss appears robust. Based on a similar model, Davidson *et al*. (2006) also predicted that the two quantities could nearly cancel each other if their magnitudes were equal and substrate concentrations were relatively low. By coupling soil C stocks and microbial biomass, our model goes further and illustrates the relative importance of measured V_{max} and K_m temperature sensitivities for hypothetical C pools. Interestingly, our model predicts that the fraction of soil C lost under warming, and the canceling effect of K_m temperature sensitivity, are both independent of the absolute magnitude of K_m. As K_m changes, the equilibrium soil C pool size changes along with it (Eqn 8), leaving the relative importance of K_m unchanged. This relationship is important because it means that our qualitative predictions of temperature responses are valid even if we do not know the absolute K_m values in field soils.

In contrast, the warming response and K_m offset are sensitive to the absolute magnitude of V_{max}. Lower V_{max} values tend to increase soil C concentrations (Eqn 8), and higher C substrate availability makes the temperature sensitivity of K_m less important (Davidson *et al*., 2006). Higher substrate availability due to low temperatures and decomposition rates also explains why our model predicts relatively greater C losses from the Alaska site with warming; the apparent temperature sensitivity of soil respiration (Eqn 5) increases at higher substrate availability (Davidson *et al*., 2006), which also may have significant effects in locations with high SOM concentrations, like Bear Brook, Maine, in this study (Table 1).

Without more data for model validation, it is not clear which if any of the model scenarios in Table 6 best approximates reality. Although we measured the apparent temperature sensitivities of the enzyme parameters, we did not quantitatively fit the model to microbial biomass or soil C stocks at the research sites. Thus, our analysis should be viewed as a relative comparison of V_{max} and K_m temperature sensitivity effects rather than an attempt to make quantitative predictions about soil C responses to global warming. However, we speculate that the actual response of our study ecosystems would lie somewhere within the range of predictions in Table 6. Microbial biomass is unlikely to be constant across the research sites, especially since soil C changes so dramatically in the 'Constant CUE' scenarios. Microbial biomass is typically 1–4% of soil organic C (Fierer *et al*., 2009), so the predictions for Costa Rica (~10% of soil C) are clearly unrealistic. The ratios of microbial biomass to soil C are more reasonable in the temperature-sensitive CUE scenarios, but the changes in microbial biomass and soil C stocks are probably too extreme for Costa Rica. It might not be valid to use the same CUE-temperature function for all of the sites, since microbial biomass in the warmer sites may be adapted to higher temperatures and therefore have higher CUE than predicted. Nonetheless, our model captures fundamental microbial and enzymatic processes, is simple enough to be solved analytically, and could easily be scaled up and parameterized to make quantitative predictions about soil C stocks.

In conclusion, we found support for our hypothesis that at least some microbial EHEs from higher

latitudes are more sensitive to temperature increases than those from lower latitudes, indicating that there is local adaptation of microbial EHE function (especially K_m) to temperature. This is important because the adaptation of K_m to a particular temperature regime may sustain microbial decomposer activity under warming, especially with varying substrate concentrations. Moreover, our model suggests that variation in enzyme temperature sensitivity could affect rates of C cycling in soils under different temperature regimes. Clearly, we need more studies of isolated microbial EHEs from soils at different latitudes to make more definitive statements regarding specific protein–temperature responses, and to improve our ability to model the effects of temperature on decomposition. Nevertheless, the data provided here on bulk soil enzymatic responses to temperature are a step in that direction. Finally, given the uncertainty of the effects of temperature on CUE (e.g., Steinweg *et al.*, 2008; Dijkstra *et al.*, 2011), we conclude with a call for more detailed studies of the CUE–temperature relationship in soil microbes from a range of habitats. This will drastically improve our ability to model the effects of warming on decomposition.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Maximal biochemical activity (V_{max}) and Michaelis–Menten constants (K_m) for five microbial digestive enzymes measured at seven temperatures in soils from Alaska, California, and Costa Rica.
Table S2. Maximal biochemical activity (V_{max}) and Michaelis–Menten constants (K_m) for five microbial digestive enzymes measured at seven temperatures in soils from Maine and West Virginia.

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