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Original article

Hydrolase kinetics to detect temperature-related changes in the rates of soil organic matter decomposition

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ABSTRACT

To evaluate the importance of the role of temperature on the decomposition of soil organic matter (SOM), we investigated the SOM decomposition rates and the kinetics of two hydrolases involved in carbon (C) and nitrogen (N) cycling, namely β-1,4-glucosidase (βG) and β-1,4-*N*-acetylglucosaminidase (NAG), and their sensitivity to temperature in representative temperate forests. Soils were collected from three spatially-separate replicate plots at study sites distributed at heights of 1233, 1060, and 825 m a.s.l. along an elevation gradient on the southern slopes of Laotuding Mountain, Northeast China. Soils were incubated at temperatures between 4 and 40 °C at 6 °C intervals for 7 days in our laboratory. Decomposition rates of SOM responded positively to temperature, and, at the same temperature, were highest in the soil from 1233 m a.s.l. than in the soils from the other elevations. SOM decomposition rates were positively correlated with the maximum activity (V_{max}) of βG and the contents of total and particulate organic C, but were negatively correlated with the soil silt and clay contents. The V_{max} and the Michaelis constant (K_m) of the two hydrolases were positively correlated and were also correlated with increases in temperature, suggesting that the K_m values could offset increases in V_{max} with increases in temperature. These correlations also highlight the enzymatic tradeoff between the maximum catalytic rate and the substrate binding affinity for the two hydrolases. The catalytic efficiencies of the two hydrolases were highest at 1060 m a.s.l., followed by 1233 m a.s.l., and were lowest at 825 m a.s.l. The catalytic efficiencies were positively correlated with the soil water contents and macroaggregate contents (>250 μm), but negatively with the soil C/N ratios. The temperature sensitivities (Q_{10}) of the SOM decomposition rates were similar at the different elevations ($P > 0.05$), but generally increased as the temperature increased ($P < 0.05$). The $Q_{10(Vmax)}/Q_{10(Km)}$ values of βG and NAG increased significantly as the temperature increased from 22 to 40 °C ($P < 0.001$), and were generally similar between elevations ($P > 0.05$). Our results suggest that, in spite of the negative effects of increased temperatures on enzyme substrate affinity, increases in hydrolytic activity will lead to accelerated SOM decomposition in temperate forests.
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1. Introduction

Extracelluar enzymes, or exoenzymes, are involved in microbial nutrient transformations and they can influence rate limiting steps of soil organic matter (SOM) decomposition considerably [1]. Among these exoenzymes, β-1,4-glucosidase (βG: EC 3.2.1.21) and β-1,4-*N*-acetylglucosaminidase (NAG:EC 3.2.1.52) are particularly important [2,3]: βG catalyzes the final step in the breakdown of

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cellulose compounds, mediates the subsequent release of simple glucose to microorganisms, and plays a central role in the carbon (C) cycle [2,3]. In contrast, NAG hydrolyzes the *N*-acetylglucosamine of fungal chitin and bacterial murein (peptidoglycan), two very abundant organic substrates in soils that are provided by microbial biomass and is therefore linked to the microbial turnover of C and nitrogen (N) [3].

Soil enzyme kinetic parameters, including the maximum activity (V_{max}) and Michaelis constant (K_m), reflect the splitting velocity of enzyme-substrate complexes into enzyme and reaction products and the aggregated affinity between the enzyme and substrate, respectively [4]. Estimations of V_{max} can provide information about rates of decomposition under saturated substrate concentrations, and the K_m values can help to understand enzymatic responses to varying substrate concentrations [5]. Furthermore, the V_{max}/K_m ratio has been suggested as a better proxy of the catalytic efficiency than the individual parameters [3].

Along with the SOM decomposition rate, the kinetics of the V_{max} and K_m enzymes are temperature sensitive [5–7]. Relative temperature responses are commonly compared with a Q_{10} index, defined as the change in a reaction rate for a temperature increase of 10 °C [6]. The Q_{10} of the SOM decomposition rate has attracted significant interest because of its importance in global carbon cycling and the potential for feedbacks to global warming [8]. The relative changes in both V_{max} and K_m parameters in response to temperature increases may control the sensitivity of the SOM decomposition rate to temperature [5,9]. Given current concerns about the effects of climate warming on the processes affecting SOM decomposition and the essential role of soil enzymes in these processes, the Q10 of soil enzyme kinetic parameters should be considered as a useful tool to characterize the microbially mediated SOM decomposition under climate warming scenarios.

Studies which have explored the relationship between SOM decomposition rates and temperature provided inconsistent results [7,10]. In temperate forest soils, the Q_{10} of SOM decomposition rates decreased with increases in the incubation and soil temperatures [7]. On the contrary, a study of arctic soils reported that the Q_{10} of the SOM decomposition rate was lower, or equal to 1, at low temperatures (3–9 °C) and was about 3 at high temperatures (9–15 °C) [10]. The authors suggested that the substrate availability for microbes, including the quantity and quality of SOM, influenced the temperature dependence of SOM decomposition [10]. Physical protection is another important factor that influences temperature dependence of SOM decomposition [8,11]. The substrate for microbes may become physically protected in the interior of soil aggregates where microorganisms and their enzymes may only have limited access and where oxygen concentrations could also be low [8,11]. The substrate quality and quantity control the abundance and composition of the microbial community and alter their enzyme systems [12]. Simultaneously, the shifts that occur in the composition of microbial communities, such as increases in the abundances of Gram-positive bacteria with temperature, also influence SOM decomposition rates [13].

The sensitivity of enzyme kinetic parameters to temperature is dependent on the *in situ* temperature [5,14]. A cross-latitudinal study showed that the Q_{10} of K_m for βG declined from cold to warm environments [5]. The ability of enzyme proteins to change their structural conformation with temperature, thereby altering the active sites, could impact the Q_{10} of K_m in soils [5,14]. In cold habitats, the high catalytic efficiency has been reported to offset the low enzyme activity common at low temperatures [15]. However, a laboratory incubation study indicated that the temperature of the static K_m should be low to intermediate (0–20 °C) to ensure a high catalytic efficiency is maintained [16]. In spite of the useful information derived from these studies, there is a lack of information about the enzyme kinetic parameters that influence SOM decomposition in temperate forests.

For this study, we selected sites at three different elevations in temperate forests on Laotuding Mountain (Northeast China). Temperate forests cover about 767 million hectares worldwide and store about 14% of global forest C [17]. The forests of this study were natural, intact, vertically zoned forest ecosystems. We determined the decomposition rates of SOM, kinetic parameters of βG and NAG, and their sensitivity to temperature at the three different elevations. We hypothesized that SOM decomposition rates, catalytic efficiencies, and their temperature sensitivities would be greater at higher elevations (colder environment) than at lower (warmer environment), and that the SOM decomposition rates and the catalytic efficiencies would be correlated.

2. Material and methods

2.1. Study site

The study sites were on Laotuding Mountain (41°11′–41°21′N, 124°41′–125°5′E) in the Changbai Mountains, Liaoning Province, Northeast China. The area has a continental temperate monsoon climate. The temperature ranges from a maximum of 37.2 °C to a minimum of −37.5 °C on this mountain, and a mean annual temperature of about 6.0 °C has been recorded at 675 m above sea level (a.s.l.) over the past 20 years. The mean annual temperature decreases about 1 °C for a 100 m increase in elevation. From the lowest part of the mountain (675 m a.s.l.) to the summit (1365 m a.s.l.), the mean annual precipitation increases from 900 to 1200 mm [18]. There are distinct vertical changes in the vegetation distribution on the southern slopes of Laotuding Mountain. From the lowest part of the mountain (675 m a.s.l.) to the summit (1365 m a.s.l.), the vegetation includes *Larix kaempferi* forest at 675–825 m a.s.l., *Pinus koraiensis* forest at 825–950 m a.s.l., deciduous broad-leaved forest (*Quercus mongolica* and *Acer truncatum*) at 950–1050 m a.s.l., spruce-fir mixed maple forest (*Picea jezoensis* mixed *Acer truncatum*) at 1050–1150 m a.s.l., spruce-fir forest (*Picea jezoensis*) at 1150–1230 m a.s.l., *Betula ermanii* forest at 1230–1306 m a.s.l., and mid-mountain meadow at 1306–1365 m a.s.l. The vertical vegetation belt comprises horizontal bands of forests and soils typical of temperate to frigid zones. We selected three different sites along an elevation gradient at 1233, 1060, and 825 m a.s.l. At each site, we chose three spatially-separate plots as replicates. The site at 1233 m a.s.l. is dominated by *Betula ermanii* secondary forest. Shrubs include *Acer komarovii Pojark*, and herbs include *Athyrium multidentatum*, *Ligularia fischeri,* and *Cacalia hastata*. The dominant tree species at 1060 m a.s.l. are *Picea jezoensis*, *Abies nephrolepis*, *Acer truncatum*, and *Quercus mongolica*. Herbs present are *Diarrhena mandshurica* and *Thalictrum tuberiferum*. The site at 825 m a.s.l. is dominated by *Pinus koraiensis* trees that are 30–40 years old. Herbs comprise *Syringa velutina*, *Euonymus alatus*, *Acanthopanax senticosus*, *Philadelphus schrenkii* and *Ampelopsis brevipedunculata*. Soils developed from granite residual parent materials, and are dark brown soils (Luvisols) at 1233 and 1060 m a.s.l., and brown soils (Cambisols) at 825 m a.s.l [18].

2.2. Soil sampling

Soil samples were collected along the southern slope of the mountain in August 2014 from 3 independent plots at each of the three elevations. The plots measured 10 m × 10 m, and the distance between each plot was at least 10 m. A total of nine plots were sampled. To reduce the spatial heterogeneity in the soil parameters at each elevation level, we collected soils from five points in each plot. We first removed the surface organic litter from the forest

Table 1
Properties of soil at the study sites.

Sites (m)	pH	SWC (%)	SOC (g kg^{-1})	TN (g kg^{-1})	C/N	POC (g kg^{-1})	>250 μm (%)	53–250 μm (%)	<53 μm (%)
1233	5.3 ± 0.2b	60 ± 3a	77 ± 11a	6.4 ± 0.7a	12 ± 1b	44 ± 9a	58	19	23
1060	5.3 ± 0.2b	57 ± 2a	67 ± 4a	5.6 ± 0.3a	12 ± 1b	36 ± 5a	61	9	30
825	6.1 ± 0.1a	35 ± 1b	56 ± 7b	3.8 ± 0.3b	15 ± 1a	30 ± 5a	57	13	30

Different letters within same column indicate significant differences between the sampling sites when $P < 0.05$. SWC: soil water content; SOC: soil organic carbon; TN: total nitrogen; C/N: ratio of SOC to TN; POC: particulate organic carbon. The same abbreviations are used in the following figures and table.

floor, and then collected the soil samples from the top 10 cm of the mineral soil from the A horizon in each plot using a soil corer with a diameter of 2 cm. The samples collected from the five points in each site were then pooled to form a composite sample and were packed in polyethylene bags, immediately stored in a portable refrigerator, and transported to the laboratory. The fresh soil samples were passed through a 2 mm sieve to remove fine roots from the soil, and then were stored at 4 °C until analysis for their physical and chemical properties, SOM decomposition rates, and hydrolysis kinetics.

2.3. Soil analyses

Soil pH was measured at a soil-to-water ratio of 1:2.5 using a digital pH meter. The soil water content was measured on soil that was oven-dried at 105 °C to constant weight. The soil aggregates of different size categories (<53 μm, 53–250 μm, 250 μm–2 mm) were separated by wet sieving and then were freeze-dried, as outlined by Six et al. [19]. Samples of the particle fractions 53 μm–2 mm were dispersed at a soil-to-sodium hexametaphosphate solution (5 g L^{-1}) ratio of 1:3 by shaking for 15 h. After shaking they were passed through a 53 μm sieve, and the material that was retained on the sieve was oven-dried at 50 °C [20]. The particulate organic carbon contents (53 μm–2 mm) were determined by an elemental analyzer (Elementar, Vario Max CN, Germany). Soil organic carbon and total nitrogen were also determined with an elemental analyzer (Elementar, Vario Max CN, Germany) [21]. The properties of the soils at the study sites are shown in Table 1.

Decomposition rates of SOM were measured using a self-developed laboratory incubation system that comprised a CO_2 analyzer (Li-COR, Li-7000) [22]. Briefly, samples of field-moist fresh soil, equivalent to 40 g of dry soil, were put into 150 mL incubation bottles. All samples were pre-incubated at 20 °C for 4 d to stimulate microbial activities, and then were incubated at 7 different temperatures (4, 10, 16, 22, 28, 34, and 40 °C) for 7 d. Soil moisture contents were kept constant during the course of the entire incubation period by weighing each sample and spraying with distilled water to compensate for any water loss. The SOM decomposition rates were measured once for 75 s [22]. Each sample was analyzed in triplicate, and a total of 189 samples were analyzed. The SOM decomposition rate was expressed in units of μg C g^{-1} soil d^{-1}.

The measured SOM decomposition rates and the incubation temperatures were fitted to Equation (1) to obtain the best fitting values for α and β [22]:

$$R = \alpha \times \exp^{(\beta \times T)} \quad (1)$$

where R was the measured SOM decomposition rate (μg C g^{-1} soil d^{-1}), and T was the incubation temperature. Then, we used the values of β derived from Equation (1) to calculate the Q_{10} values with Equation (2):

$$Q_{10(Rmin)} = \exp^{10 \times \beta} \quad (2)$$

The two hydrolytic enzymes, βG and NAG, were measured using the fluorimetric method of Saiya-Cork et al. [23]. The 4-methylumbelliferone (MUB) labeled substrates, 4-MUB-β-D-glucoside and 4-MUB-*N*-acetyl-β-D-glucosaminide, were selected to represent βG and NAG activities, respectively. Briefly, 2 g fresh soil was homogenized in 250 ml of a 50 mmol L^{-1} acetate buffer prepared at a similar pH for each soil (Table 1). The microplates were incubated in the dark at 7 different temperatures (4, 10, 16, 22, 28, 34, and 40 °C) for 4 h. The activities were measured using a microplate fluorometer (Synergy^{H4}, BioTek, USA) at excitation and emission rates of 365 and 460 nm, respectively. The βG and NAG activities were expressed in units of nmol h^{-1} g^{-1} soil. The kinetic parameters were measured at MUB-substrate concentrations of 5, 10, 20, 30, 40, 60, 100, and 200 μmol L^{-1}. The kinetic parameters (V_{max} and K_m) were estimated by fitting the Michaelis-Menten equation to the activities using hyperbola in SigmaPlot (SigmaPlot for Windows, Version 10.0) [24].

$$V = (V_{max} \times [S])/(K_m + [S]) \quad (3)$$

where V was the reaction velocity. The maximum velocity of the decomposition of the enzyme-substrate complexes into enzyme and reaction products (V_{max}, nmol h^{-1} g^{-1} soil) represents the theoretical maximal velocity of enzyme catalysis when the enzyme has been saturated by substrate. The Michaelis constant (K_m, μmol L^{-1}), i.e., the substrate concentration at a velocity equal to half of the maximum velocity, reflects the affinity of the enzyme to the substrate. The concentration of the MUB-labeled substrate was represented by [S].

The hydrolases catalytic efficiency (K_{cat}) was calculated with Equation (4) [4]:

$$K_{cat} = V_{max}/K_m \quad (4)$$

where K_{cat} reflects the total enzyme catalytic process that combines the enzyme-substrate complex dissociation (V_{max}) and the rate at which the enzyme-substrate complex forms (K_m).

The temperature sensitivities (Q_{10}) of βG and NAG kinetic parameters were calculated at 7 different temperatures (4, 10, 16, 22, 28, 34, and 40 °C) using Equation (5) [9].

$$Q_{10} = \exp^{(S \times 10)} \quad (5)$$

where S was the slope of the linear regression between the natural logarithm-transformed enzyme kinetic parameters (i.e. V_{max} and K_m) at the incubation temperature and Q_{10} was the temperature sensitivity of each hydrolase kinetic parameter.

2.4. Statistical analysis

All values were reported as means ± standard errors (n = 3). One-way analysis of variance (ANOVA) was used to test for significant differences between the properties of the soils from the three elevations. The effects of elevation and incubation temperature on the SOM decomposition rate, kinetic parameters and their Q_{10}, and

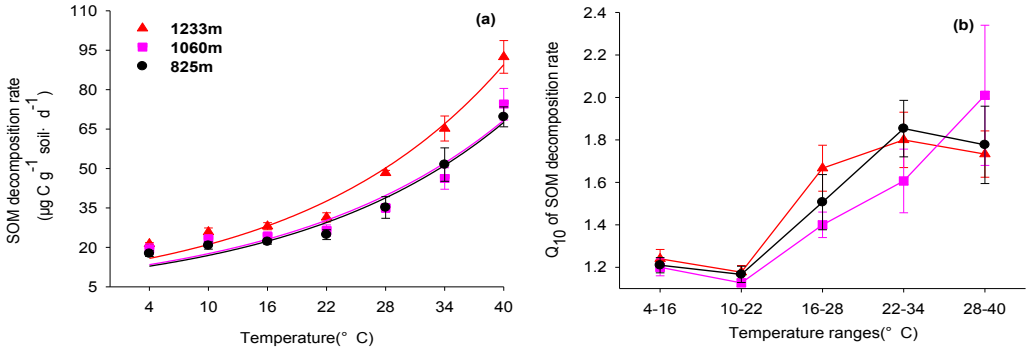

Fig. 1. Decomposition rates (a) and temperature sensitivity (b) of soil organic matter (SOM) decomposition rates at three elevations depending on incubation temperatures. Values are mean ± SE (n = 3). (a): Elevation $P < 0.01$, Temperature $P < 0.001$. (b): Temperature $P < 0.001$.

catalytic efficiency were examined by covariance analysis (ANCOVA) with elevation as the main effect and incubation temperature as the covariate. Pearson analysis was used to determine the correlations between SOM decomposition rates, hydrolytic kinetic parameters, and soil environmental factors. Linear regression analysis was used to determine the relationships between V_{max} and K_m for βG and NAG. SPSS 17.0 for Windows was used for all statistical analyses. A significance level of $P < 0.05$ was applied.

3. Results

3.1. Temperature sensitivity of SOM decomposition rates

The SOM decomposition rates varied widely from 17.7 to 92.5 μg C g^{-1} soil d^{-1}. The mean value (92.5 μg C g^{-1} soil d^{-1}) was highest for samples from 1233 m a.s.l. incubated at 40 °C (Fig. 1). The mean rate (17.7 μg C g^{-1} soil d^{-1}) was lowest in samples from 825 m a.s.l. incubated at 4 °C (Fig. 1). The SOM decomposition rates increased with temperature and were, at any given temperature, higher at 1233 m a.s.l. than at 825 and 1060 m a.s.l. ($P < 0.01$, Fig. 1).

The Q_{10} of the SOM decomposition rates ranged from 1.1 (at 825 m a.s.l. for temperatures between 10 and 22 °C) to 2.0 (at 825 m a.s.l. for temperatures between 28 and 40 °C). The Q_{10} values of the SOM decomposition rates were higher at temperatures from 22 to 40 °C than at lower temperatures (between 4 and 22 °C) ($P < 0.001$, Fig. 1). However, the between-elevation variations in the Q_{10} of SOM decomposition rates were not significant ($P > 0.05$, Fig. 1).

3.2. Enzyme kinetics and their temperature sensitivity

Variations in the βG and NAG activities in response to increases of between 5 and 200 μmol L^{-1} in substrate concentrations followed the Michaelis-Menten equation (Fig. 2). The V_{max} and K_m of NAG were higher at 825 m a.s.l. than at 1233 and 1060 m a.s.l. ($P < 0.001$, Fig. 3). However, the catalytic efficiencies of βG and NAG were highest at 1060 m a.s.l., followed by 1233 m a.s.l., and were lowest at 825 m a.s.l. ($P < 0.001$, Fig. 3). The enzyme kinetic parameters (V_{max} and K_m) for βG and NAG were positively correlated, and the slope for βG was steeper than for NAG ($P < 0.01$, Fig. 4). The range of catalytic efficiencies of βG (from 3.3 to 10.0 h^{-1}) was larger than the range for NAG (ranged from 3.0 to 7.8 h^{-1}) (Fig. 3). The V_{max} and K_m of βG and NAG increased with temperature ($P < 0.001$, Fig. 3). The catalytic efficiencies of the two hydrolases decreased from 4 to 22 °C, but increased between 28 and 40 °C (Fig. 3).

The Q_{10} values of the βG kinetic parameters were higher than those of NAG (Fig. 5). The $Q_{10(Vmax)}/Q_{10(Km)}$ ratios of βG increased with temperature which was mainly caused by changes in $Q_{10(Vmax)}$, while increases in the $Q_{10(Vmax)}/Q_{10(Km)}$ ratios of NAG with temperature were generally caused by $Q_{10(Km)}$ (Fig. 5). However, the $Q_{10(Vmax)}/Q_{10(Km)}$ ratios of βG did not vary between elevations ($P > 0.05$, Fig. 5). By comparison, the $Q_{10(Vmax)}/Q_{10(Km)}$ ratios of NAG were higher at 825 m a.s.l. than at 1060 and 1233 m a.s.l. ($P < 0.001$, Fig. 5).

3.3. Relationships between SOM decomposition rates, enzyme kinetic parameters, and soil environmental factors

The SOM decomposition rates were positively correlated with the V_{max} of the βG, TN, SOC, and POC contents, but negatively correlated with the silt and clay contents (<53 μm) ($P < 0.05$, Table 2). Enzyme kinetic parameters (V_{max} and K_m) of NAG were positively correlated with the soil pH and C/N ratio, but were negatively correlated with TN (Table 2). The catalytic efficiencies of βG and NAG were positively correlated with the soil macroaggregate and soil water contents, but negatively correlated with the C/N ratio (Table 2).

4. Discussion

4.1. Temperature sensitivity of SOM decomposition rates

In agreement with our hypothesis, SOM decomposition rates were greater at higher elevations than at lower elevations. In fact, the maximum SOM decomposition rate was observed at 1233 m a.s.l. However, contrary to our expectations, the SOM decomposition rates were not affected by the catalytic efficiencies of the two hydrolases. Correlation analyses showed that the SOM decomposition rates were positively correlated with substrate availability (soil TN, SOC, and POC contents), but negatively with the soil silt and clay contents. Large pools of partially decomposable soil C accumulate under cold, wet conditions [25], and the soil labile C content tends to be higher under broad-leaved litter than under coniferous litter [26]. In this study, at 1233 m a.s.l., high SOC, TN, and POC contents were attributed to the wet and cold weather conditions, and the broad-leaved plant species. In addition, decomposition rates of SOM are generally lower in soils with high clay concentrations [27]. The lower protection from mineral particles at 1233 m a.s.l. than at the other two lower elevations increased the potential for SOM decomposition. In this study, the V_{max} of βG was positively correlated with the SOM decomposition rate, which suggests that the SOM decomposition rate was affected by the potential enzyme activity of βG, and shows that βG enzyme activities can be useful to explain SOM decomposition rates.

The variations in the Q_{10} values for the SOM decomposition rates were significant between temperatures, but not between elevations. The variations were similar to those in the $Q_{10(Vmax)}/$

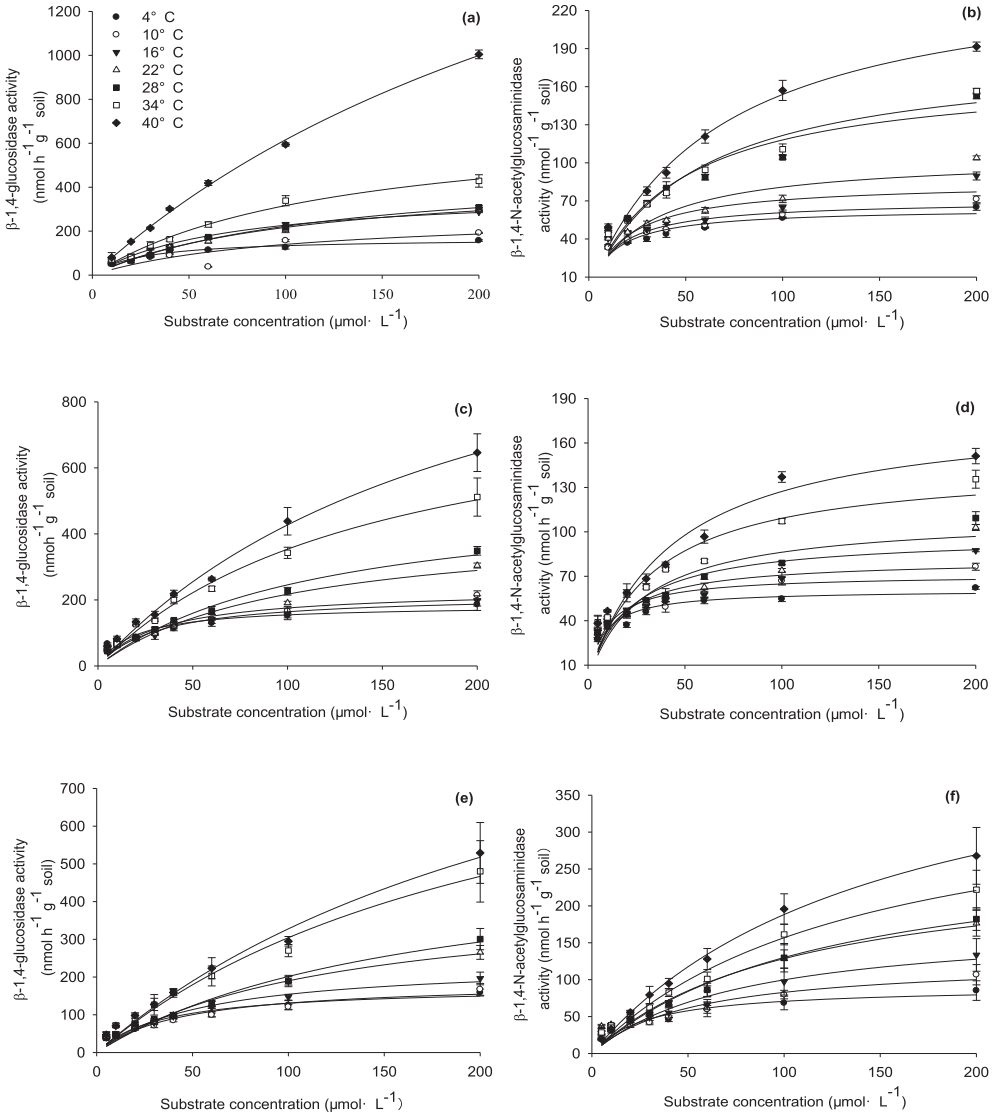

Fig. 2. Michaelis-Menten plots of β-glucosidase and β-1,4-*N*-acetylglucosaminidase at 1233 m a.s.l. (a, b), 1060 m a.s.l. (c, d), and 825 m a.s.l. (e, f) depending on incubation temperatures, values are mean ± SE (n = 3).

$Q_{10(Km)}$ ratios of βG, which suggests that the $Q_{10(Vmax)}/Q_{10(Km)}$ ratios of βG might explain the temperature sensitivity of SOM decomposition rates. This also suggests that enzymatic kinetics of K_m should be considered when assessing the sensitivity of the SOM decomposition rates to temperature. The Q_{10} values of the SOM decomposition rates were higher between 28 and 40 °C than between 4 and 22 °C. The uncommonly high temperatures of >20 °C probably activated the growth of more thermophilic members of the microbial community [28]. These temperatures are uncommon in temperate climates where the annual soil temperature is 6 °C, so this finding shows that the composition of microbial communities may shift in response to different temperature ranges [6].

4.2. Factors influencing enzyme kinetics at different elevations

The catalytic efficiencies of βG and NAG were highest at 1060 m a.s.l., followed by 1233 m a.s.l., and were lowest at 825 m a.s.l. The differences in the soil environmental characteristics (C/N ratio, pH, soil water content, and >250 μm aggregate) induced by changes in weather, elevation, and plant species affected the soil enzyme catalytic reaction characteristics (Tables 1 and 2). The soil macroaggregate contents were positively correlated with the catalytic efficiency. Enzymes can be immobilized on the outer surfaces of organo-mineral particles or entrapped in micro-aggregates [29]. The enzymes that are not entrapped have more contact with the solution and are less likely to be influenced by limitations in the substrate diffusion than those within the pores of micro-aggregates [29]. Therefore, the catalytic efficiencies of enzymes in different places may differ, even if the soil enzymes have the same inherent catalytic constants [29]. The higher macroaggregate content at 1060 m a.s.l. suggests that there were more enzymes on the outer surfaces of particles, which resulted in an increase in the catalytic efficiency.

The kinetic parameters (V_{max} and K_m) of βG were similar between the three elevations. However, the kinetic catalytic efficiencies of βG and NAG were lower at 825 m a.s.l. than at 1233 and 1060 m a.s.l. The C/N ratios were positively correlated with V_{max} and K_m, indicating that the enzymatic activity and substrate affinity were regulated by the soil C/N ratios [30]. The C/N ratios were higher under coniferous forest at 825 m a.s.l than under broad-

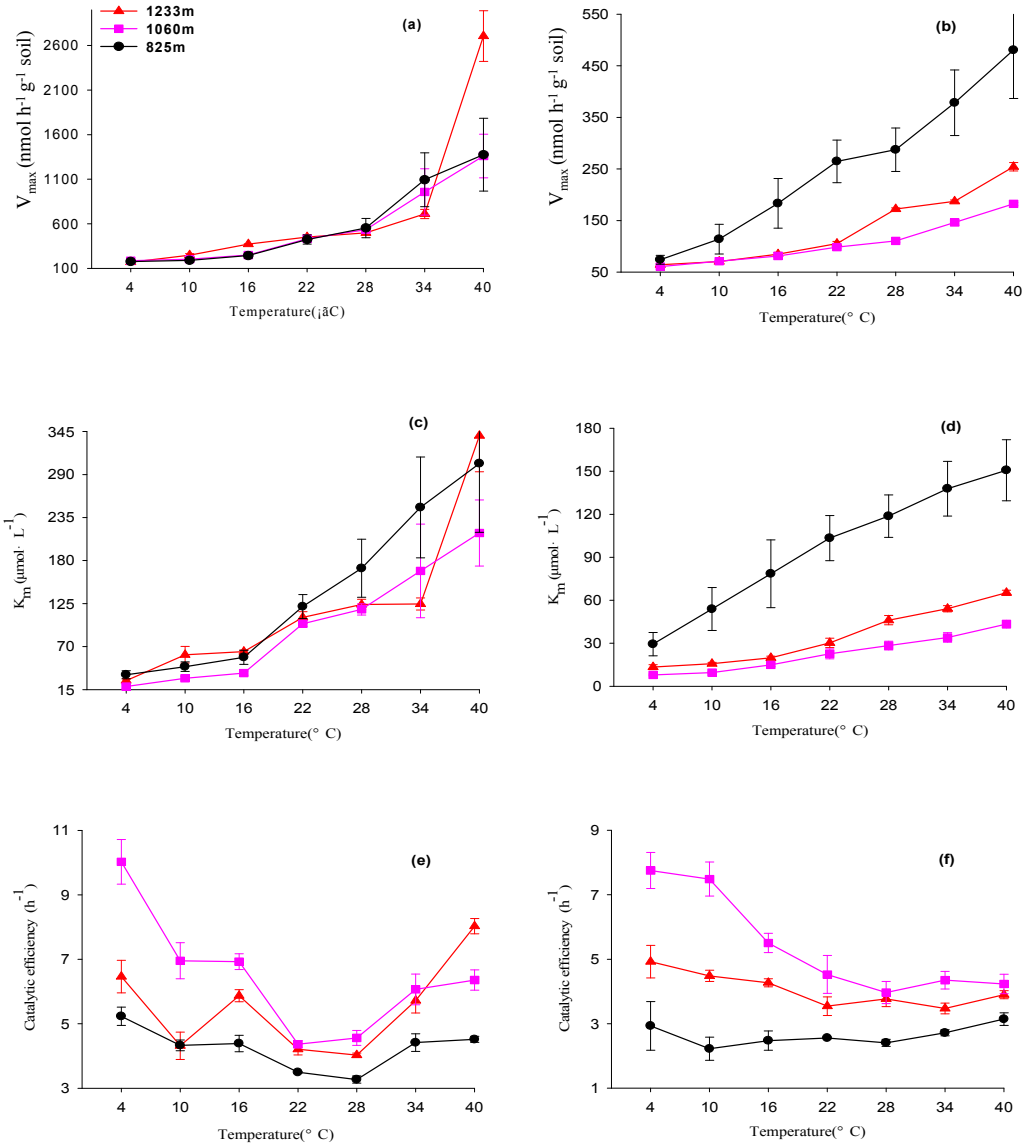

Fig. 3. The maximum activity (V_{max}), the Michaelis constant (K_m) and catalytic efficiency (V_{max}/K_m) for β-glucosidase (a, c, e) and *N*-acetyl-glucosaminidase (b, d, f) at three elevations depending on incubation temperatures. values are mean ± SE (n = 3). (a): Temperature P < 0.001, (b): Elevation P < 0.001, Temperature P < 0.001, (c): Temperature P < 0.001, (d): Elevation P < 0.001, Temperature P < 0.001, (e): Elevation P < 0.001, (f): Elevation P < 0.001, Temperature P < 0.001.

leaved forest at 1233 m a.s.l. and coniferous mixed broad-leaved forest at 1060 m a.s.l. Thus, the lower substrate affinity induced by higher C/N ratios meant that the catalytic efficiency was lower at 825 m a.s.l. than at the other elevations.

The catalytic efficiency was also positively correlated with soil water contents. Diffusions of the substrates and enzyme molecules benefitted from the high soil water [31]. The low soil water content meant that the catalytic efficiency was low at 825 m a.s.l. In addition, the kinetic characteristics of the two hydrolases were influenced by the soil pH. Potential βG enzyme activity may be stimulated in relatively acidic soils [32]. Our results confirmed that the catalytic efficiency of βG might be preferentially stimulated in acidic soil. However, in acidic soil, the relatively high soil pH contributed to the low catalytic efficiency at 825 m a.s.l.

Both the V_{max} and K_m of βG and NAG were positively correlated, and responded positively to temperature (Figs. 3 and 4), which suggests that a decrease in the substrate affinity would offset any increase in the potential enzyme activity with temperature. Consistent with the findings of a study of three temperate forest soils in central Massachusetts, USA, the catalytic efficiencies of βG were higher than those of NAG [33]. The fact that the catalytic efficiencies of βG were higher than those of NAG may be related to the fact that cellulose is much more abundant in soil than chitin, which does not normally accumulate in nature [34]. The catalytic efficiencies of the enzymes decreased from low to intermediate temperatures (4–22 °C), reflecting the potential thermal adaptation of catalytic efficiencies to seasonal temperature variations, and indicating that the efficient enzymatic functioning would reduce in response to warming during spring and autumn periods. However, the catalytic efficiencies increased from 28 to 40 °C, which suggests that the efficient enzymatic functioning would increase in response to warming during summer periods in a temperate climate.

5. Conclusions

Decomposition rates of SOM responded positively to

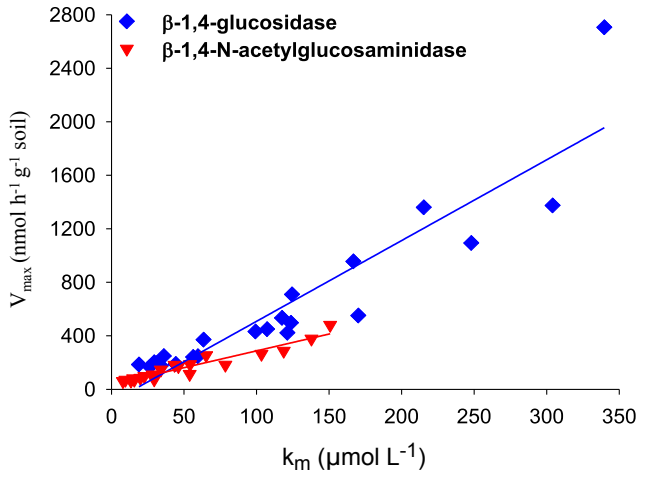

Fig. 4. Relationships between the maximum activity (V_{max}) and the Michaelis constant (K_m) for β-glucosidase and β-1,4-*N*-acetylglucosaminidase. β-glucosidase: $R^2 = 0.85$, $P < 0.01$; β-1,4-*N*-acetylglucosaminidase $R^2 = 0.91$, $P < 0.01$.

temperature, and, at the same temperature, were higher at 1233 m a.s.l than at the other two lower elevations, indicating that there was higher potential for SOM decomposition in cold environments than in warm environments in this temperate forest. Correlation analysis showed that the SOM decomposition rates were positively correlated with the V_{max} of βG, which shows that the V_{max} of βG was a better indicator of the SOM decomposition rates than that of NAG. The SOM decomposition rates were also positively correlated with the substrate contents (soil organic carbon and particulate organic carbon contents), but negatively with the soil silt and clay contents, indicating the SOM decomposition rates were influenced by soil properties (chemical properties and physical properties).

The V_{max} and K_m of βG and NAG were positively correlated and were also correlated with increases in temperature, suggesting that the K_m values could offset increases in V_{max} with increases in temperature. The catalytic efficiencies of the two hydrolases ranged as 1060 m a.s.l.> 1233 m a.s.l.> 825 m a.s.l, and were positively correlated with the soil water contents and macroaggregate contents (>250 μm), but negatively with the soil C/N ratios, indicating the soil properties are more important than soil temperature

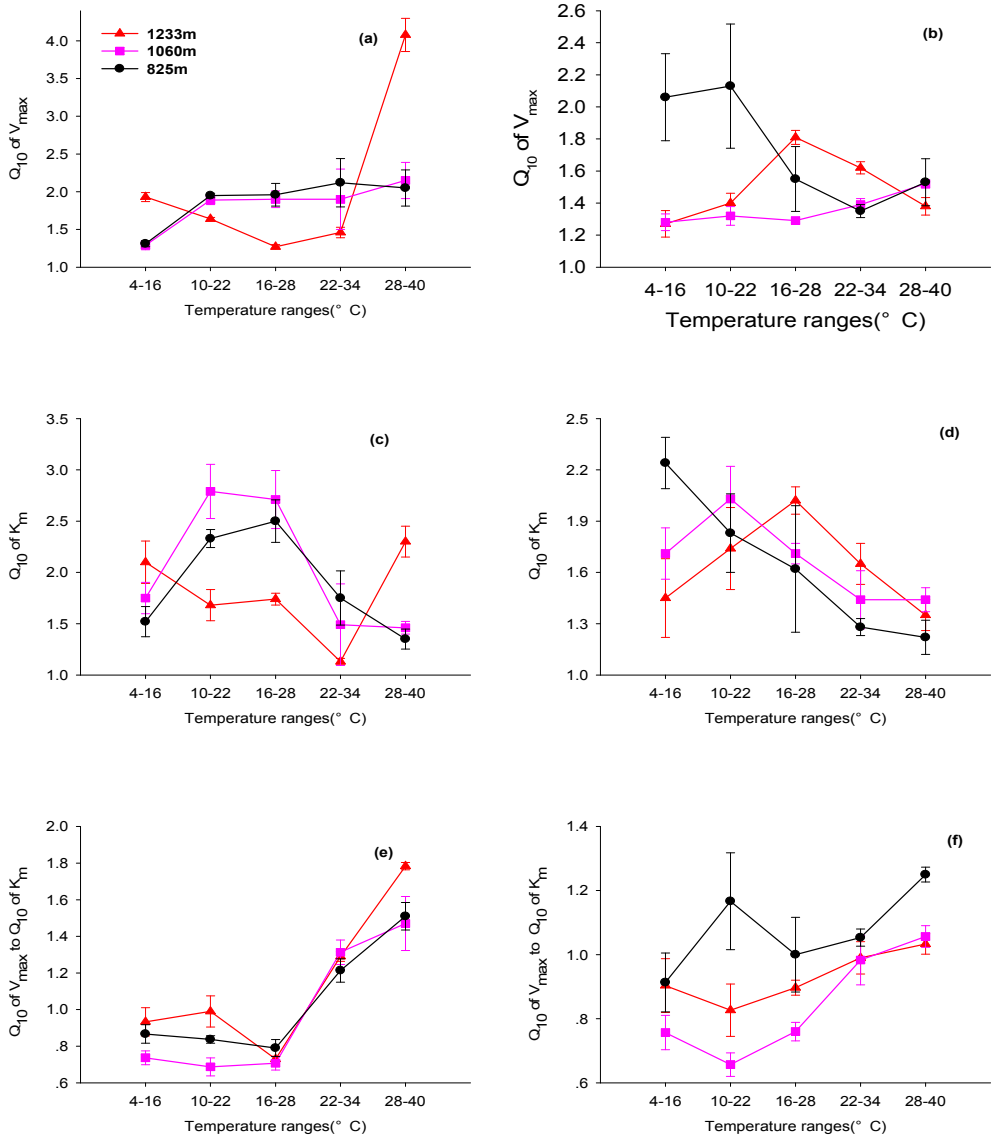

Fig. 5. Temperature sensitivity (Q_{10}) of the maximum activity (V_{max}), the Michaelis constant (K_m) and their ratios for β-glucosidase (a, c, e) and *N*-acetyl-glucosaminidase (b, d, f) at three elevations depending on incubation temperatures. (a): Temperature $P < 0.001$; (b): Elevation $P < 0.001$; (d) Temperature $P < 0.01$; (e) Temperature $P < 0.001$, (f) Elevation $P < 0.001$, Temperature $P < 0.001$.

Table 2
Pearson correlation coefficients (R^2) between SOM decomposition rates, soil environmental factors, and hydrolytic kinetic parameters.

Variables	C_{min}	pH	SWC	TN	SOC	C/N	POC	<53 μm	>250 μm
C_{min}	1	NS	NS	0.71*	0.77*	NS	0.72*	−0.69*	NS
βG-V_{max}	0.67*	NS	NS	NS	NS	NS	NS	NS	NS
βG-K_m	NS	NS	NS	NS	NS	0.86**	NS	NS	NS
NAG-V_{max}	NS	0.72*	−0.85**	−0.69*	NS	0.95**	NS	NS	NS
NAG-K_m	NS	0.73*	−0.87**	−0.71*	NS	0.94**	NS	NS	−0.71*
βG-K_{cat}	NS	−0.70*	0.87**	0.79*	NS	−0.75*	NS	NS	0.73*
NAG-K_{cat}	NS	NS	0.70*	NS	NS	−0.64	NS	NS	0.80**

C_{min}: soil organic matter decomposition rate, βG: β-glucosidase, NAG: β-1,4-*N*-acetyglucosaminidase. V_{max}: maximum activity, K_m: Michaelis constant, K_{cat}: catalytic efficiency. The same abbreviations are used in the following figures and table. Significance levels are marked as follow: NS ($P > 0.05$), * ($P < 0.05$); and ** ($P < 0.01$).

differences in controlling the catalytic efficiency in temperate forests.

The Q_{10} values of the SOM decomposition rate increased with temperature, but did not differ between the three elevations, suggesting that there was little variation in the sensitivity of the SOM decomposition rate to temperature along the temperate elevation gradient; the sensitivity, however, will increase under global warming conditions. The $Q_{10(Vmax)}/Q_{10(Km)}$ ratios of βG and NAG increased at 22–40 °C, indicating that the maximal reaction rate was more sensitive to warming than the substrate affinity was to warming in the temperate forest. Our results suggest that, in spite of the negative influence of increased temperatures on enzyme substrate affinity, increases in hydrolytic activity will lead to accelerated SOM decomposition in temperate forests.

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