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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.

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