Kinetic and thermodynamic properties of hydrolases in Northeastern China soils affected by temperature

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INTRODUCTION. – Soil enzymes involve in soil nutrient cycling, and can catalyze the conversion of soil nutrients from unavailable to available forms. Soil hydrolases play important roles in the hydrolysis of soil nutrients, among which, urease, phosphatase, and arylsulphatase catalyze the hydrolysis of soil amide N, organic P, and organic S, respectively, being of significance in the N, P, and S uptake by plants (BURNS, 1978; SARAPATKA and KRSKOVA, 1997; TABATABAI and BREMNER, 1970; SPEIR, 1984).

The activities of soil enzymes are directly and indirectly affected by soil temperature (TABATABAI and BREMNER, 1970; MOYO \textit{et al.}, 1989; MCCLAUGHERTY and LINKINS, 1990; DEBOSZ \textit{et al.}, 1999). Their determination at different temperature can not only provide the optimal range of temperature or thermal stability for the activity of certain enzyme (KANAZAWA and MIYASHITA, 1986; KANDELER, 1990; NANNIPIERI \textit{et al.}, 1991; TABATABAI, 1994), but also allow to estimate thermodynamic parameters, e.g., activation energy ($E_a$) and temperature coefficient ($Q_{10}$) (DALAL, 1985). However, limited information was provided regarding the kinetic and thermodynamic behaviors of soil immobilized enzymes at different temperature (DALAL, 1985; SALEEM \textit{et al.}, 2005; TRASAR-CEPEDA \textit{et al.} 2007). Studies on the kinetic parameters of soil hydrolases at different temperature could help us to understand the changes in their affinity to substrate, catalytic activity, and sensitivity to temperature. Temperature influences the movement (the rate of encounter) of

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enzyme and substrate, and hence the $V_{\text{max}}$ and $K_m$. It was reported that soil can also adsorb the substrate (Cervelli et al., 1975; Irving and Gosgrove, 1976), whose adsorption and desorption should be affected by environmental temperature. Some enzyme molecules probably fix on the surface of soil particles by either adsorption or covalent binding. The contents and kinds of organic matter and clay in different soils may affect the substrate- or enzyme diffusion, and hence, the enzyme $V_{\text{max}}$ and $K_m$ (Bery et al., 1978; Garcia et al., 1993; Zaman et al., 1999).

In the present paper, laboratory simulation tests were conducted at three temperatures to study the kinetic and the thermodynamic characteristics of urease, phosphatase, and arylsulphatase in four agricultural soils, i.e., black soil (phaeozem), albic soil (albic luvisols), brown soil (cambisols), and cinnamon soil (chromic luvisol) of Northeast China, aimed to approach the appropriate soil temperature regime for these enzymes.

**Materials and methods.** – **Soil samples collection and preparation.** – Four sampling sites were selected (Table 1), and 60 soil samples (0-20 cm) over an approximately 1 ha at each site were collected in early spring before sowing. Three plots of 50 × 80 m at each site were selected for soil sampling. A random sampling scheme was applied on a grid of 10 × 20 m cells, and each cell corresponds to a sampling target. From each cell, one sample was collected at a minimum distance (approximately 0.5 m) to each other.

The 20 samples from each sampling site were made into a composite sample, transported to laboratory in isothermal bags, and passed through 2.0 mm sieve. A sub-sample was stored at 4°C less than 14 days for enzyme assays, and the remainder was air-dried for chemical and physical properties analysis. Physical and chemical properties of studied soils were listed in Table 1.

**Soil physical and chemical properties determination.** – Soil pH was determined in soil: water suspension (1:2.5 ratio) with a glass electrode. Total C, N, S were determined by element analyzer (Elementar Vario EL) (Matejovic, 1995). Total P was determined by digestion with HClO₄ method (Olsen and Sommers, 1986). Organic P was determined by UV Spectrophotometer (Carry 50, Varian, USA) after extraction by Bowman-Cole method (Bowman and Cole, 1978). Available nitrogen (AN) was determined by micro-diffusion method of boric acid-absorbed NH₃ after alkali-hydrolyzed by NaOH (Bremner and Shaw, 1955; Saghir et al., 1993). Available P extractable with NaHCO₃ was determined by Olsen method (Kuo, 1996). Available S was determined by turbidimetric method after acetate and phosphate extraction (Fox et al. 1987). Particle size distribution was determined by Robinson pipette method and with Calgon as dispersant (Gee and Bauder, 1986). Soil moisture was determined by drying a sample at 105°C for 24 h.

**Incubation and enzyme analysis.** – Parts of the sub-samples (1000 g; n=3) of each composite sample were pre-incubated at room temperature for 14 days to stabilize the biological and biochemical characteristics before treatment. Then, the pre-incubated soils were held to 20% (~60 % WHC) moisture content with distilled water, and incu-
<table>
<thead>
<tr>
<th>Soil type</th>
<th>FAO taxonomy, (1998)</th>
<th>Location</th>
<th>Zone and Climatic</th>
<th>Cumulative temperature (≥10 °C)</th>
<th>Mean annual temperature (°C)</th>
<th>Mean annual precipitation (mm)</th>
<th>Non-frost period (d)</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>Sand (%)</th>
<th>pH (H₂O)</th>
<th>Organic C (g · kg⁻¹)</th>
<th>Total N (g · kg⁻¹)</th>
<th>Total P (g · kg⁻¹)</th>
<th>Total S (g · kg⁻¹)</th>
<th>Organic P (g · kg⁻¹)</th>
<th>Alkali-hydrolyzed N (mg · kg⁻¹)</th>
<th>Available P (mg · kg⁻¹)</th>
<th>Available S (mg · kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black soil</td>
<td>Phaeozem</td>
<td>Hailun Experimental Station of Agricultural Ecology (47°26' N; 126°58', altitude 240m), Songnen Plain</td>
<td>Heilongjiang Province</td>
<td>2400 – 2500 °C</td>
<td>1 - 2</td>
<td>500 – 600</td>
<td>120 – 130</td>
<td>34.6 c</td>
<td>51.5 b</td>
<td>13.9 a</td>
<td>5.54 a</td>
<td>27.17 d</td>
<td>2.22 c</td>
<td>0.79 c</td>
<td>0.55 c</td>
<td>0.42 c</td>
<td>126.57 d</td>
<td>102.10 c</td>
<td>23.10 c</td>
</tr>
<tr>
<td>Albic soil</td>
<td>Albic luvisols</td>
<td>“853” farms (46°26’ 42N; 133°02’ 04E; altitude 78m), Songnen Plain</td>
<td>Heilongjiang Province</td>
<td>2400–2500 °C</td>
<td>2 - 3</td>
<td>500 – 600</td>
<td>130 – 145</td>
<td>18.6 a</td>
<td>67.8 c</td>
<td>27.9 b</td>
<td>5.81 b</td>
<td>19.12 c</td>
<td>1.93 b</td>
<td>0.56 b</td>
<td>0.42 b</td>
<td>0.36 c</td>
<td>42.62 c</td>
<td>29.71 b</td>
<td>10.74 ab</td>
</tr>
<tr>
<td>Brown soil</td>
<td>Cambisols</td>
<td>Maize experimental field of Shenyang Agricultural University (42° 31’ N, 123°46’ E, altitude 70m), Chaoyang, Liaoning Province</td>
<td>Liaoning Province</td>
<td>3300 – 3400°C</td>
<td>7 - 8</td>
<td>650 – 750</td>
<td>147 – 164</td>
<td>20.2 b</td>
<td>50.8 b</td>
<td>29.0 b</td>
<td>5.46 a</td>
<td>8.39 b</td>
<td>0.97 a</td>
<td>0.25 a</td>
<td>0.38 ab</td>
<td>0.10 a</td>
<td>26.48 a</td>
<td>11.00 a</td>
<td>11.07 b</td>
</tr>
<tr>
<td>Cinnamon soil</td>
<td>Chromic luvisol</td>
<td>(41°41’05N,120 33°58E, altitude 172m), Liaoning Province</td>
<td>Liaoning Province</td>
<td>3100–3200°C</td>
<td>8 - 9</td>
<td>430 – 500</td>
<td>128 – 150</td>
<td>18.9 a</td>
<td>35.7 a</td>
<td>45.4 c</td>
<td>8.21 c</td>
<td>6.11 a</td>
<td>0.93 a</td>
<td>0.30 a</td>
<td>0.31 a</td>
<td>0.22 b</td>
<td>37.45 b</td>
<td>10.37 a</td>
<td>9.50 a</td>
</tr>
</tbody>
</table>
bated in an incubation chamber set at 10, 20, and 30 °C for 21 days, respectively. After incubation, the activities of studied enzymes in each temperature treatment were determined. Each treatment was carried out in triplicates.

Urease (EC 3.5.1.5), phosphomonoesterase (EC 3.1.3.2 and EC 3.3.3.1), and arylsulphatase (EC 3.1.6.1) activities were determined as described by Tabatabai (1994). Briefly, urease activity was determined by using 33 mM urea (Sigma, GR) as substrate, incubating at 37 °C for 5 h, and measuring the residual urea colorimetrically by a Continuous Flow Analyzer (Wang et al. 1991). Phosphomonoesterase activity was determined using 50 mM p-nitrophenyl phosphate (Sigma, AR) as substrate, incubating at pH 6.5 modified MUB buffer at 37 °C, and after 1 h, 0.5 M CaCl₂ and 0.5 M NaOH were added to favor soil particle flocculation and the measurement of produced p-nitrophenol. Arylsulphatase activity was determined with 50 mM p-nitrophenyl sulfate (Acros, AR) as substrate, by incubating soil at pH 5.8 (acetate buffer 0.5 M) and 37 °C for 1 h. The same procedures in enzyme activities measurements were followed for the controls, but the substrates were added to the soil samples after incubation and prior to the analysis of residual substrate or reaction product.

**Kinetic parameters.** – Kinetic parameters $V_{\text{max}}$ and $K_m$ of studied hydrolases were calculated from the data obtained at several different substrate concentrations. Seven concentrations (3, 5, 7, 10, 15, 20 and 30 mM) of urea solution, six concentrations (0.2, 0.5, 1, 5, 15 and 50 mM) of sodium p-nitrophenyl phosphate solution, and seven concentrations (0.5, 1, 5, 10, 15, 25 and 50 mM) of potassium p-nitrophenyl sulfate solution were used as the substrates for determining soil urease, phosphatase, and arylsulphatase activities, respectively. The kinetic parameters $V_{\text{max}}$ and $K_m$ were measured by using Lineweaver-Burk equation, the linearization of Michaelis-Menten equation (Tabatabai, 1994):

$$\frac{1}{V} = \frac{K_m}{V_{\text{max}}} \cdot \frac{1}{[S]} + \frac{1}{V_{\text{max}}} ,$$

where $V$ is reaction velocity, $V_{\text{max}}$ is the maximum reaction velocity, $S$ is the concentration of substrate, and $K_m$ is Michaelis constant.

**Thermodynamic parameters.** – The temperature dependence of soil hydrolase activities was described by Arrhenius equation (Trasar-Cepeda et al., 2007):

$$K_{\text{f}} = A e^{(-E_a / RT)} ,$$

where $K_{\text{f}} = v$, initial enzyme reaction velocity, $A$ is the pre-exponential factor, $R$ is the universal gas constant (8.314 J mol⁻¹ K⁻¹), $T$ is the absolute temperature in Kelvin (K), and $E_a$ is the activation of energy (J mol⁻¹ K⁻¹). For each treatment, the $E_a$ and $Q_{10}$ of soil hydrolases were calculated by using the data valid for Arrhenius equation. The temperature coefficient and the enthalpy of activation were calculated by $Q_{10} = e^{10 E_a / RT (T+10)}$ and $\Delta H = E_a - RT$, respectively.

**Statistical analysis.** – All determinations were performed in triplicates, and all values reported were means ± standard deviation, and expressed by per g oven-dried soil (105 °C). Data treatment and statistical analysis were performed by using SPSS 10.0 computer language program. For each variable measured, the data were analyzed by one-way ANOVA. Least significance differences (LSD) at $p = 0.05$ were tested to determine the significant differences between treatment means.
Results. – Effects of temperature on kinetic parameters of soil hydrolases. – All studied soil enzymes exhibited Michaelis-Menten kinetics. The $V_{\text{max}}$ of urease, phosphomonoesterase, and arylsulphatase increased with increasing temperature. Figure 1 is an example for studied enzymes, which showed the effects of temperature on the urease kinetic velocity in black soil, albic soil, brown soil, and cinnamon soil.

Figure 2 showed that the $K_{m}$ and $V_{\text{max}}$ values of studied soil enzymes varied with soil temperature and soil type. The $K_{m}$ of urease and arylsulphatase in the four soils, and of phosphatase in black soil, albic soil and cinnamon soil were increased, i.e., their affinity decreased with increasing temperature. Urease had the nearly same lowest $K_{m}$ at 10 and 20 °C in black soil; phosphatase had the lowest $K_{m}$ (largest affinity) at 20 °C in brown soil, arylsulphatase had the lowest $K_{m}$ at 20 °C in cinnamon soil and the nearly same values at 20 and 30 °C in albic soil.

Enzyme velocity ($V_{\text{max}}$) increased with increasing temperature. The $V_{\text{max}}$ of urease had the peak at 30 °C in all studied soils, whereas that of phosphatase was the lowest at 10 and 20 °C in brown soil, while arylsulphatase had the same nearly highest $V_{\text{max}}$ at 20 and 30 °C in black soil, and at 10 and 30 °C in brown soil (Fig. 2).

Fig. 1. – Lineweaver Burk plots of urease in Chinese soils at increasing temperature (* 10 °C, □ 20 °C, and △ 30°C). $S$: Substrate concentration (mM); $V$: Reaction velocity (μM·g⁻¹·h⁻¹).
The $K_m$ and $V_{max}$ had larger variations at 20 – 30 °C than at 10 – 20 °C, suggesting their different responses to different soil temperature regimes. Less variation of urease was observed in black soil than in other studied soils, and the largest variation of phosphatase was observed in black soil (Fig. 2).

As shown in Table 2, the catalytic efficiency ($V_{max}/K_m$) of urease in black soil, phosphatase in brown soil and cinnamon soil, and arylphatase in cinnamon soil increased with increasing temperature. Urease in albic soil and cinnamon soil had no distinct difference in their $V_{max}/K_m$ among different temperatures, and urease in brown soil had the largest $V_{max}/K_m$ at 10 °C. Phosphatase in black soil and albic soil had the largest $V_{max}/K_m$ at 10 °C. Arylsulphatase of studied soils had the largest catalytic efficiency at 20 °C.
Table 2. – $V_{\text{max}}/K_{\text{m}}$ of soil urease, phosphatase and arylsulphatase.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Temperature</th>
<th>Black Soil</th>
<th>Albic Soil</th>
<th>Brown Soil</th>
<th>Cinnamon Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease</td>
<td>10 ºC</td>
<td>0.144 ±0.018 a A</td>
<td>0.221 ±0.024 b A</td>
<td>0.301 ±0.023 c B</td>
<td>0.219 ±0.018 b A</td>
</tr>
<tr>
<td></td>
<td>20 ºC</td>
<td>0.252 ±0.017 b B</td>
<td>0.198 ±0.021 b A</td>
<td>0.125 ±0.009 a A</td>
<td>0.228 ±0.009 b A</td>
</tr>
<tr>
<td></td>
<td>30 ºC</td>
<td>0.224 ±0.020 b B</td>
<td>0.234 ±0.031 b A</td>
<td>0.163 ±0.008 a A</td>
<td>0.199 ±0.018 a A</td>
</tr>
<tr>
<td>Phosphatase</td>
<td>10 ºC</td>
<td>1.516 ±0.178 c B</td>
<td>2.038 ±0.148 d B</td>
<td>0.475 ±0.063 a A</td>
<td>0.976 ±0.027 b A</td>
</tr>
<tr>
<td></td>
<td>20 ºC</td>
<td>1.157 ±0.110 ab B</td>
<td>1.488 ±0.057 b A</td>
<td>1.015 ±0.154 a B</td>
<td>0.981 ±0.126 a A</td>
</tr>
<tr>
<td></td>
<td>30 ºC</td>
<td>0.887 ±0.147 c A</td>
<td>1.684 ±0.041 c A</td>
<td>1.424 ±0.094 b C</td>
<td>1.344 ±0.14 b B</td>
</tr>
<tr>
<td>Arylsulphatase</td>
<td>10 ºC</td>
<td>0.010 ±0.005 a A</td>
<td>0.024 ±0.019 b A</td>
<td>0.008 ±0.003 a A</td>
<td>0.011 ±0.006 a A</td>
</tr>
<tr>
<td></td>
<td>20 ºC</td>
<td>0.049 ±0.009 a B</td>
<td>0.195 ±0.031 c B</td>
<td>0.035 ±0.006 a B</td>
<td>0.065 ±0.013 b B</td>
</tr>
<tr>
<td></td>
<td>30 ºC</td>
<td>0.015 ±0.027 a A</td>
<td>0.053 ±0.038 b A</td>
<td>0.008 ±0.015 a A</td>
<td>0.055 ±0.044 b B</td>
</tr>
</tbody>
</table>

Different capital letters in columns indicate significant differences between temperature treatments; different lowercase letters in row.
Thermodynamic parameters. – Table 3 showed the values of the activation energy (Ea) (ranged from 30.10 to 173.84 kJ •mol\(^{-1}\) K\(^{-1}\)), enthalpy of activation (∆H) (27.58 – 171.48 kJ •mol\(^{-1}\) K\(^{-1}\)), and temperature coefficients (Q\(_{10}\)) (1.00 to 1.01). The enthalpy of activation (∆H) decreased with increasing temperature, but the decrement had less difference between tested temperatures. The values of Ea, ∆H and Q\(_{10}\) for each studied enzyme differed among tested temperatures, but the thermodynamic parameters (∆H and Q\(_{10}\)) kept constant or slightly decreased.

Discussion. – In general, the K\(_m\) of pure enzyme or soil enzyme decreased with increasing temperature because of the enhanced dissolution and translocation of the substrates, while V\(_{max}\) increased (Dannenberg et al., 1989; Boyd and Mortland, 1990; Zak et al. 1999). However, the increasing K\(_m\) of studied enzymes with temperature was possibly due to the co-effects of soil heterogeneity and temperature. Soil enzyme V\(_{max}\) increased just because the calorific movement of enzyme molecules hurried and the collisions between enzyme and substrate speeded up (Simihaian, 1998). The increasing V\(_{max}\) should result in more substrates transformed during certain periods of time (Agehara and Warncke, 2005). Soil enzymes exhibited different catalytic properties (V\(_{max}/K_m\)), comparing with pure enzymes (Sadhukhan et al., 1993). Catalytic efficiency increased likely because V\(_{max}\) increased markedly and K\(_m\) unobvious increased.

Soil enzyme K\(_m\) and V\(_{max}\) were affected by soil texture and organic matter content (Burns et al. 1972; Bery et al., 1978; Zaman et al., 1999; Garcia et al., 1993; Tietjen and Wetzel, 2003). Soil urease, phosphatase, and arylsulphatase had different K\(_m\) values in different soil types (Tabatabai and Bremner, 1971; Nor, 1982). Higher soil organic C trapped soil urease and prevented the enzyme from affiliating of the substrate, and increased the K\(_m\) values significantly (Kay and Lilly, 1970; Handrikova et al., 1996). Soil organic matter and mineral particles can adsorb enzyme molecules and protect enzymes against microbiological degradation (Plante et al., 2006). Protection function of organic matter on enzymes sometimes enhances their catalytic reaction, and sometimes slows up or inhibits such reaction. Higher organic matter contents result in broader cushion scope and more thermal–stable aggregate (Speir, 1977; Shi et al., 2006). Enzymes exposed to various local concentrations of substrates change their locations and exhibit dif-
Table 3. Thermodynamic parameters $E_a$, $\Delta H$, $Q_{10}$ of soil urease, phosphatase and arylsulphatase in the temperature range 10-30 ºC.

<table>
<thead>
<tr>
<th>Hydrolases</th>
<th>Soil</th>
<th>$E_a$ (kJ·K$^{-1}$·mol$^{-1}$)</th>
<th>$\Delta H$ (kJ·K$^{-1}$·mol$^{-1}$)</th>
<th>$Q_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$10$ ºC</td>
<td>$20$ ºC</td>
<td>$30$ ºC</td>
</tr>
<tr>
<td><strong>Urease</strong></td>
<td>Black soil</td>
<td>76.15 b</td>
<td>73.80 a</td>
<td>73.71 a</td>
</tr>
<tr>
<td></td>
<td>Albic soil</td>
<td>71.51 a</td>
<td>69.16 a</td>
<td>69.07 a</td>
</tr>
<tr>
<td></td>
<td>Brown soil</td>
<td>37.04 a</td>
<td>34.69 a</td>
<td>34.60 a</td>
</tr>
<tr>
<td></td>
<td>Cinnamon soil</td>
<td>41.29 a</td>
<td>38.94 a</td>
<td>38.86 a</td>
</tr>
<tr>
<td><strong>Phosphatase</strong></td>
<td>Black soil</td>
<td>173.84 c</td>
<td>171.48 a</td>
<td>171.40 a</td>
</tr>
<tr>
<td></td>
<td>Albic soil</td>
<td>161.41 c</td>
<td>159.06 a</td>
<td>158.97 a</td>
</tr>
<tr>
<td></td>
<td>Brown soil</td>
<td>111.53 b</td>
<td>109.18 a</td>
<td>109.10 a</td>
</tr>
<tr>
<td></td>
<td>Cinnamon soil</td>
<td>90.22 a</td>
<td>87.86 a</td>
<td>87.78 a</td>
</tr>
<tr>
<td><strong>Arylsulphatase</strong></td>
<td>Black soil</td>
<td>30.10 a</td>
<td>27.74 a</td>
<td>27.66 a</td>
</tr>
<tr>
<td></td>
<td>Albic soil</td>
<td>51.89 c</td>
<td>49.54 a</td>
<td>49.45 a</td>
</tr>
<tr>
<td></td>
<td>Brown soil</td>
<td>45.00 b</td>
<td>42.65 a</td>
<td>42.56 a</td>
</tr>
<tr>
<td></td>
<td>Cinnamon soil</td>
<td>46.98 b</td>
<td>44.63 a</td>
<td>44.55 a</td>
</tr>
</tbody>
</table>

Different capital letters in columns indicate significant differences between treatments; different lowercase letters in row.
ferent activities in different soils even if the enzyme molecules had the same inherent catalytic constants (Engasser and Horvath, 1976).

The larger variation range of $K_m$ and $V_{max}$ during 20 – 30 °C was possibly due to the fact that 30 °C approached to the optimum temperature of enzymes (Moyo et al., 1989; Simhaian, 1998; El-Shora, 2001). The variation of soil urease $K_m$ and $V_{max}$ with temperature was not linear, possibly due to the co-action of temperature and soil physical and chemical properties (Lai and Tabatabai, 1992). There was a significant correlation between soil phosphatase kinetic characters and soil available P at 20 °C and 30 °C, and soil arylsulphatase $K_m$ value had a significant correlation with soil organic matter, total sulfur, and available sulfur at 20 °C.

The $E_a$ values obtained in this study were higher than those reported by Frankenberg and Tabatabai (1982), and the enthalpy of activation ($\Delta H$) obtained by us was lower than that reported by other authors (Lai and Tabatabai, 1992; Tras-Cepada et al. 2007). The high value of enthalpy of activation ($\Delta H$) indicates that a large amount of stretching, squeezing, or even breaking of chemical bonds is necessary for the formation of transition state (Cornish-Bowden, 2004). Enzyme-catalyzed reactions are less sensitive to temperature changes than their un-catalyzed counterparts, whose reaction rates generally increase by a factor of < 2 (Tabatabai, 1994). Temperature coefficients ($Q_{10}$) of enzyme-catalyzed reactions were < 2 (Zeffren and Hall, 1973, Tabatabai and Singh, 1979). Fewer literatures regarding the $Q_{10}$ of soil enzymes were available. Our $Q_{10}$ data ranged from 1.00 to 1.01, and some researchers reported that soil enzyme $Q_{10}$ ranged from 1.10-1.80 (Frankenberg and Tabatabai, 1991). The lower $Q_{10}$ values for studied soil hydrolases indicated a small thermodynamic effect on enzyme velocity.

In conclusion, the effects of soil temperature were more notable on enzyme kinetic behaviors rather than on thermodynamic ones. Most kinetic parameters ($K_m$, $V_{max}$ and $V_{max}/K_m$) increased with increasing temperature, and thermodynamic parameters kept constant. The catalytic characteristics of studied hydrolases in the main agricultural soils of Northeast China were affected by the soil temperature regime in some degree, depending on the organic matter content and texture of these soils. The apparent sensitivity of soil hydrolases to ambient temperature implied that soil warming could have a profound effect on soil nutrients output and soil fertility. Considering that temperature is important for
making appropriate nutrient transformation in cultivation during certain period of time, so controlling soil temperature condition could be a feasible way in regulating the biochemical transformation processes of soil nutrients catalyzed by soil hydrolases.

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REFERENCES


Summary. – With incubation test, the kinetic and thermodynamic parameters of urease, phosphatase, and arylsulphatase in the black (phaeozem), albic (albic luvisols), brown (cambisols) and cinnamon soil (chromic luvisol) of Northeastern China at 10, 20 and 30 ºC were studied. Soil temperature increased kinetic parameters ($K_m$, $V_{max}$ and $V_{max}/K_m$) distinctly, with more distinct variation at 20 ºC-30 ºC than at 10 ºC-20 ºC. The temperature-dependence of the affinity and the rate of substrate hydrolysis varied depending on the enzyme and soil. The values of the activation energy ($E_a$) and temperature coefficient ($Q_{10}$) for each enzyme did not differ much among soils. Less difference was observed in $E_a$ and $Q_{10}$ values than in kinetic parameters within the studied range of temperature suggesting that, the fluctuation of temperature affected more kinetic rather than thermodynamic behaviors of soil hydrolases.