CATALYTIC POTENTIAL OF SOIL HYDROLASES IN NORTHEAST CHINA UNDER DIFFERENT SOIL MOISTURE CONDITIONS

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ABSTRACT

An incubation test with black soil (Phaeozem), Albic soil (Albic Luvisols), brown soil (Cambisols), and cinnamon soil (Chromic Luvisol) from Northeast China was conducted under the conditions of 10%, 20% and30 % field capacity, and the kinetic parameters of soil urease, phosphatase, and arylsulphatase were determined, aimed to study the changes in the catalytic potential of these enzymes under different soil moisture conditions. All test enzymes exhibited typical Michaelis-Menten kinetic behaviors. The test enzymes exhibited the highest enzyme-substrate affinity ($1/K_m$) at 20% or 30% field capacity. With increasing soil moisture content, the V_{max} of test soil urease decreased, while that of soil phosphatase and arylsulphatase increased, with the maximum V_{max}/K_m of urease at 20% field capacity and that of phosphatases and arylsulphatase at 30% field capacity. To control soil moisture condition could be a feasible way in regulating the biochemical transformation processes of soil nutrients catalyzed by soil hydrolases.

Keywords: Soil enzymatic kinetic parameters, soil hydrolase, soil moisture condition

INTRODUCTION

Soil hydrolases are a group of soil enzymes responsible for the catalytic hydrolysis of soil substances (Tabatabai and Bremner, 1971, 1972; Dick and Tabatabai, 1993; Asmar *et al*., 1994; Amador *et al*., 1997), 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). Soil moisture regime had definite effects on the catalytic potential of soil enzymes (Ross, 1987; García *et al*., 2002; Sardans and Penuelas, 2005). Engasser and Horvath (1976) reported that soil moisture content affects the movement of enzymes and their

substrates in soil, while the diffusion limitation of the substrates may directly affect soil enzyme K_m. Some researches (Burns, 1978; Ladd, 1985; Boyd and Mortland, 1990; Sardans and Penuelas, 2005) also showed that the changes in soil moisture content had significant effects on the kinetic parameters of soil hydrolases. Therefore, to measure the kinetic parameters of soil hydrolases under different soil moisture conditions will help to the understanding of the changes in the substrate affinity and the catalytic activity of soil hydrolases, and further, help to adopt appropriate measures to regulate soil moisture regime to maintain optimal hydrolase activities.

In this paper, black, albic, brown, and cinnamon soil, the main agricultural soils in Northeast China, were sampled, and an incubation test was conducted to study the catalytic potential of urease, phosphatase, and arylsulphatase as affected by different soil moisture conditions, aimed to approach the appropriate soil moisture regime for these enzymes.

MATERIALS AND METHODS

Soil samples collection and preparation

Four sampling sites were installed (Table 1), and 0 – 20 cm soil samples over an approximately 1 ha at each site were collected in early spring before sowing.

In all cases, 50 – 60 subsamples collected were combined into a composite sample, transported to laboratory in isothermal bags, and passed through 2 - mm sieve after removing roots and plant debris. Parts of the subsamples (1000 g, n=3) of each composite sample were pre-incubated at ca. 60% WHC and 25°C for 14 d to stabilize the biological and biochemical characteristics before treatment, and the other parts were air-dried and 2 mm sieved for chemical and physical properties analysis. Some chemical and physical properties of test soils were shown in Table 2.

Incubation test

After pre-incubation, the prepared soil samples were aerobically incubated at room temperature for 14 d. Three treatments with triplicates were installed, i.e., 10%, 20% and 30% field capacity to simulate minimal, normal, and maximum soil humidity, respectively. Distilled water was added daily to compensate the water loss from incubation.

Soil chemical properties analysis

Soil moisture content was determined gravimetrically after oven-dried at 105°C, soil pH was determined by glass electrode (soil:water ratio, 1:2.5), soil total organic carbon and total nitrogen (N) were determined by CNS analyzer Elementar Vario EL III (Matejovic, 1995), soil total phosphorus was determined by UV Spectrophotometer (Carry 50, Varian, American) after digest, soil total sulphur (S) was determined by the turbidimetric method after magnesium nitrate oxidation (Fox, 1987), Alkali-hydrolyzed N was determined by boracic acid absorbing NH_3 released by NaOH., soil available phosphorous (P) extractable with $NaHCO_3$ was determined by Olsen method (Kuo, 1996), and soil available sulfur was determined by the turbidimetric method after acetate and phosphate extraction (Fox, 1987). Particle size distribution was determined by Robinson pipette method and with Calgon as dispersant. These methods are described by Lu (2000).

Table 1: Description of sampling sites

Soil type	Black soil	Albic soil	Brown soil	Cinnamon soil
FAO taxonomy, 1998)	Phaeozem	Albic Luvisols	Cambisols	Chromic luvisol
Location	Hailun Experimental Station of Agricultural Ecology (47 °26' N; 126°58', altitude '240m), Songnen Plain, Heilongjiang Province.	"853" farms (46°26' 42N; 133°02' 04E; altitude'78m), Heilongjiang Province.	Maize experimental field of Shenyang Agricultural University (42° 31' N, 123°46' E, altitude' 70m), Liaoning Province	Chaoyang, (41°41'05N,120 °33'58E, altitude '172m), Liaoning Province
Zone	Temperate zone			
Climatic	Subhumid continental monsoon climate	Humid continental monsoon climate	Semi -humid continental monsoon climate	Semi-arid continental monsoon climate
Cumulative temperature(≥10 °C)	2400 ~ 2500 °C	2400~2500 ºC	3300 ~ 3400ºC	3100~3200°C
Mean annual temperature (°C)	1 – 2	2 - 3	7 - 8	8 - 9
Mean annual precipitation (mm)	500 – 600	500 – 600	650 – 750	430 – 500
Non-frost period (d)	120 – 130	130 – 145	147 – 164	128 – 150

Table 2: Soil properties

	Black Soil	Albic Soil	Brown Soil	Cinnamon Soil
pH (H_2O, 1:2.5)	5.54 a	5.81 b	5.46 a	8.21 c
Organic matter (g kg^{-1})	46.84 d	32.96 c	14.47 b	10.54 a
Total N (g kg^{-1})	2.22 c	1.93 b	0.97 a	0.93 a
Total P (g kg^{-1})	0.79 c	0.56 b	0.25 a	0.30 a
Total S (g kg^{-1})	0.55 c	0.42 b	0.38 ab	0.31a
Organic P (g kg^{-1})	0.42 c	0.36 c	0.10 a	0.22 b
Alkali-hydrolyzed N (mg kg^{-1})	126.57 d	42.62 c	26.48 a	37.45 b
Available P (mg kg^{-1})	102.10 c	29.71 b	11.00 a	10.37 a
Available S (mg kg^{-1})	23.10c	10.74 ab	11.07b	9.50 a
Clay (%)	34.6 c	18.6 a	20.2 b	18.9 a
Silt (%)	51.5 b	67.8 c	50.8 b	35.7 a
Sand (%)	13.9 a	27.9 b	29.0 b	45.4 c

Different letters in columns indicate significant differences between treatments ($p<0.05$)

Soil enzyme activities and kinetic parameters measurement

Enzyme substrates (urea, sodium p-nitrophenyl phosphate, and potassium p-nitrophenyl sulfate) were purchased from Sigma-Aldrich Inc., Seebio Biotech Inc., and J&K China Chemical Ltd., respectively.

Soil urease (EC 3.5.1.5, 37°C) activity was assayed by the method of Tabatabai and Bremner (1994). 6.0g soil samples were reacted with urea at 37°C for 5 h, and the amount of residual urea was determined by using diacetyl monoxime-antipyrine in KCl-acetic phenyl mercury extract. Soil phosphatases (orthophosphoric monoester phosphor-hydrolases, EC 3.1.3.2, pH 6.5, and EC 3.3.3.1, pH 11) activities and arylsulphatase (EC 3.1.6.1, pH 5.8) activity were also assayed by the method of Tabatabai and Bremner (1994). About 1 g soil sample was reacted with sodium *p*-nitrophenyl phosphate or potassium *p*-nitrophenyl sulfate at 37ºC for 1 h, and the released p-nitrophenol was measured

by colorimetry. All the measurements were performed at optimal pH. 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.

The kinetic parameters V_{max} (maximum enzyme velocity) and K_m (substrate affinity constant) were calculated by using Michaelis-Menten equation. Seven concentrations (3, 5, 7, 10, 15, 20, and 30 mmol L^{-1}) of urea solution, six (0.2, 0.5, 1, 5, 15, and 50 mmol L^{-1}) of sodium *p*-nitrophenyl phosphate, and seven (0.5, 1, 5, 10, 15, 25, and 50 mmol L^{-1}) of potassium *p*-nitrophenyl sulfate were used as the substrates of soil urease, phosphatase, and arylsulphatase, respectively. Each determination was also triplicated. The parameters were calculated by nonlinear regression of the statistical software origin 8.0.

Statistical analysis

The experiments followed a completely randomized design. All data were presented as the means of triplicate analyses of triplicate samples. All the values reported were expressed as per g oven-dried soil (105ºC). The effects of soil moisture content were analyzed by variance analysis (one - way ANOVA), Least significant difference at p = 0. 05. (LSD) and Pearson correlation coefficients (r) were calculated by using SPSS 11.0.

RESULTS

Effects of soil moisture regime on soil hydrolases K_m and V_{max} Figure 1 showed that the $1/K_m$ and V_{max} values of test soil enzymes varied with soil moisture content and soil type. The substrate affinity ($1/K_m$) of soil urease increased with soil moisture content, with the peak at 30% field capacity in albic, brown, and cinnamon soils and at 20% field capacity in black soil. Soil phosphatase had the highest $1/K_m$ at 10% filed capacity in albic soil, at 30% field capacity in brown and cinnamon soils, and at 20% and 30% field capacity in blank soil; while soil arylsulphatase had the highest $1/K_m$ value at 10% field capacity in albic soil, at 20% field capacity in blank soil, and at 30% field capacity in brown and cinnamon soils.

The V_{max} of soil urease was the highest at 10% field capacity in black, albic, and brown soils and at 20% field capacity in cinnamon soil. Soil phosphatase had the highest V_{max} at 30% field capacity in blank and albic soils but nearly the same at all test field capacities in brown and cinnamon soils, while that of arylsulphatase was the highest at 30% field capacity in black and albic soils and at 10% field capacity in brown and cinnamon soils.

The $1/K_m$ and V_{max} had larger variations at 20% - 30% field capacity than at 10% - 20% field capacity, suggesting their different responses to different soil moisture regimes.

Effects of soil moisture regime on soil hydrolases catalytic efficiency (V_{max}/K_m)

It's shown in Table 2 that soil urease had higher V_{max}/K_m at 20% field capacity in black soil, at 10% and 30% field capacity in albic and brown soils and at 20% and 30% field capacity in cinnamon soil, soil phosphatase had higher V_{max}/K_m at 20% and 30% field capacity in black and cinnamon soils, at 10% and 30% field capacity in albic soil and at 30% field capacity in brown soil, and soil

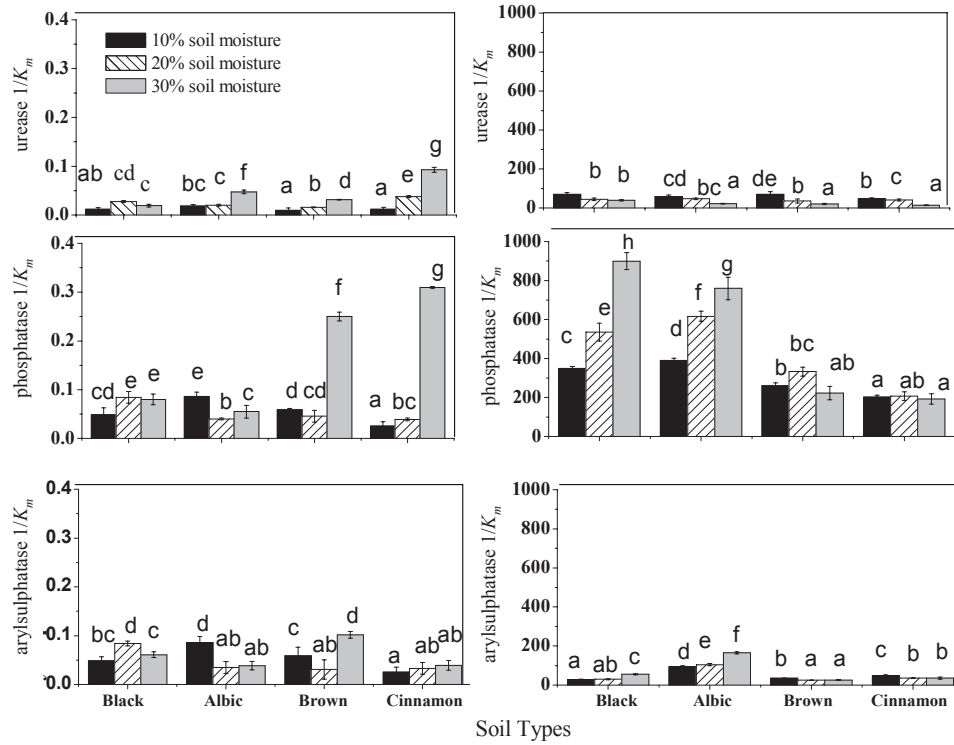

Figure 1: K_m and V_{max} values of soil urease, phosphatase, and arylsulphatase under different soil moisture conditions. (K_m = mmol L^{-1}; V_{max} = µg g^{-1} soil h^{-1}). Different letters indicate significant differences between treatments ($p < 0.05$)

arylsulphatase had higher V_{max}/K_m at 20% and 30% field capacity in black soil and at 10% and 30% field capacity in albic and brown soils, but the same V_{max}/K_m at 10%, 20% and 30% field capacity in cinnamon soil.

DISCUSSION

In general, soil enzyme-substrate affinity ($1/K_m$), similar to free enzyme (Balkan and Ertan, 2007), is increased with increasing soil moisture content because of the enhanced dissolution and translocation of the substrates (Zhou, 1987). However, increasing soil moisture content could decrease substrate concentration, resulting in the decrease of $1/K_m$. The different variation patterns of the $1/K_m$ in test soils depended partly on how the soil moisture regime affected the distribution of the enzymes and their substrates (Boyd and Mortland, 1990).

Some studies suggested that soil enzyme activity was strongly affected by soil moisture regime (Skujins and Mclaren, 1969; Delaune and Patrick, 1970; Kramer and Green, 2000; Wang and Lu, 2006; Yavitt, 2004). There was a

significant correlation between soil phosphatase activity and moisture content (Harrison, 1983; Speir and Coling, 1991; Subhani, *et al*., 2000), and the rank correlation in the study of Bergstrom *et al*. (1998) indicated the significant relationships between soil enzyme activities (urease, phosphatase, and arylsulphatase etc.) and moisture content, which was further confirmed by this study.

The catalytic efficiency of soil enzymes V_{max}/K_m (Gianfreda *et al*., 1995) was highly affected by soil organic matter content and soil texture (Bery *et al*., 1978; Zaman *et al*., 1999; Garcia *et al*., 1993). Higher V_{max}/K_m of test soil enzymes was found in the test soils containing more organic matter and having better texture. In the meantime, less variation of V_{max}/K_m was observed in these soils under effects of different soil moisture condition because of the buffering effects of higher organic matter and clay particle contents.

CONCLUSIONS

The catalytic potential of test hydrolases in the main agricultural soils of Northeast China was affected by the soil moisture regime in some degree, depending on the organic matter content and texture of these soils. The soils with higher organic matter and clay particle contents had less variation of their catalytic potential under different soil moisture conditions. To control soil moisture condition could be a feasible way in regulating the biochemical transformation processes of soil nutrients catalyzed by soil hydrolases.

ACKNOWLEDGEMENTS

This study is supported by the National Basic Research Program (973 Program) (2007CB109307) and the Technology Supporting Program (2006BAD10B01) of China. We thank Professor Zhou LK for his critical review of our manuscript, and the staffs of Department Soil and Plant Nutrition, Institute of Applied Ecology under Chinese Academy of Sciences for their academic and technical assistance.

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