

SOIL NUTRIENT CONTENTS AND ENZYMATIC CHARACTERISTICS AS AFFECTED BY 7-YEAR NO TILLAGE UNDER MAIZE CROPPING IN A MEADOW BROWN SOIL

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ABSTRACT

No tillage is being popularized for the rainfed maize production in Northeast China. In order to evaluate its effects on the nutrient contents and enzymatic characteristics in upland soils of Northeast China, surface (0-20 cm) meadow brown soil samples were collected from the plots under no tillage and conventional tillage in a 7-year field experiment under maize cropping in Shenyang, with the soil pH, contents of total C, N, P and S and available N, activities of α - and β -galactosidase, α - and β -glucosidase, urease, protease, phosphomonoesterase, phosphodiesterase, and arylsulphatase, and kinetic parameters of β -glucosidase, protease, phosphomonoesterase, phosphodiesterase, and arylsulphatase determined. Comparing with conventional tillage, no tillage increased the contents of soil total C, N, and S and available N, the activities of test enzymes, and the V_{max}/K_m of soil urease, protease, and phosphomonoesterase, but decreased the activity of soil α -galactosidase and the V_{max}/K_m of soil β -glucosidase significantly. All the results suggest that long term no tillage for the maize production on meadow brown soil of Northeast China could enhance soil nutrients storage and the turnover of soil N and P, but had definite negative effects on the transformation of soil C.

Keywords: Conservation tillage, conventional tillage, soil enzyme activity, enzyme kinetic properties.

INTRODUCTION

Northeast China abounds in rainfed maize (*Zea mays* L.) and soybean (*Glycine max* L.), being one of the most susceptible regions to climate change. Its maize production occupies 28% of China's. The intensive maize cropping based on conventional tillage has been traditional in Northeast China for several decades, which led to the continuous soil degradation with an increasing

cost/benefit ratio (Mannering and Fenster, 1983; Stonehouse, 1997; Roldán, *et al.*, 2005; Wang, *et al.*, 2006). No tillage, an alternative to conventional tillage, has been adopted by many countries to avoid soil erosion, and offered numerous benefits that conventional tillage could not match (Uri, 2000; Borie *et al.*, 2002; Wang *et al.*, 2006).

Soil nutrient contents relate to the soil productivity, while soil enzymatic characteristics can provide information about the status of key biochemical reactions that participate in the rate-limiting steps of the transformation of soil nutrients. Soil enzymes had rapid responses to the changes of soil management modes (Angers *et al.*, 1993; Mullen *et al.*, 1998; Marx *et al.*, 2001; Jimenez *et al.*, 2002; Alvear *et al.* 2005), being of significance in characterizing the effects of farming system on soil properties (Farrell, *et al.*, 1994).

The aim of present study is to understand the changes in the nutrient contents and enzymatic characteristics of meadow brown soil, a typical agricultural soil for rainfed maize production in Northeast China, under 7-year no tillage and maize cropping, and to evaluate the effects of no tillage on the improvement of soil quality under rainfed farming.

MATERIALS AND METHODS

Study site and field experiment

A 7-year field experiment was conducted on a meadow brown soil (Luvisol, FAO, 1982) at National Field Research Station of Shenyang Agroecosystems, Chinese Academy of Sciences (41°31'N, 123°24'E). This Station is located at Lower Liaohe River Plain, with a warm and semi-humid continental monsoon climate. The mean annual air temperature is about 7-8°C, cumulative temperature ($\geq 10^\circ\text{C}$) is 3300-3400°C, mean annual precipitation is 650-700 mm, and non-frost period is about 147-164 d.

Two treatments were installed, *i.e.*, conventional tillage (CT) and no tillage (NT). The experimental design was a randomized complete block in a split plot arrangement. Each treatment was in 19.0 m \times 9.0 m plots with 4 replicates, and

applied with 150 kg N hm^{-2} and 40 kg P hm^{-2} . The test crop was maize (*Zea mays L.*).

Soil collection

Surface (0-20 cm) soil samples were collected after maize harvested in October 2005. The fresh samples from at least 15 locations in each plot of each treatment were taken, gently mixed, and sieved to remove root material. Parts of the samples were used for the determination of moisture content, available N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$), and enzyme activities; parts of them were air-dried at room temperature and ground for the determination of soil total C, N, and S.

Assay of soil chemical properties

Soil moisture content was determined gravimetrically after drying at 105°C; Soil pH was determined in soil: water suspension (1: 2.5 ratios) with a glass electrode (Lu, 2000). Soils were analyzed for total carbon (TC), total nitrogen (TN) and total sulfur (TS) with one CNS analyzer - Elementar Vario EL III (Matejovic, 1995). Total phosphorus (TP) was determined by digestion with $\text{H}_2\text{SO}_4\text{-HClO}_4$ method (Kuo, 1996). Available nitrogen (inorganic N) (AN) was analyzed using the Continued-Flow Analysis (CFA) after extraction by KCl (2 mol L^{-1}) (Keeney, 1982).

Assay of soil enzyme activities

Enzyme substrates were purchased from Sigma-Aldrich. Inc., SeeBio Biotech, Inc., and J&K China Chemical Ltd., respectively. Enzyme activities were measured according increase of resultant or decrease of substrate by colorimetric determination methods. Soil enzyme activities were assayed within 2 weeks after sampling. During this time, samples were stored at 4°C. Soil urease (E.C.3.5.1.5), phosphomonoesterase (E.C.3.1.3.2, pH 6.5; PMase),

phosphodiesterase (E.C.3.1.4.1, pH 8.0; PDase), arylsulfatase (E.C.3.1.6.1, pH 5.8; ArSase), α -D-glucosidase (E.C.3.2.1.20, pH 6.0; α -GLUase), β -D-glucosidase (E.C.3.2.1.21, pH 6.0; β -GLUase), α -D-galactosidase (E.C.3.2.1.22, pH 6.0; α -GALase) and β -D-galactosidase (E.C.3.2.1.23, pH 6.0; β -GALase) activity was assayed by the method of Tabatabai (1994). Soil samples (6.0 g) were reacted with 0.2% urea as substrate 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 with a continuous flow auto analyzer (BRAN+LUEBBE). For PMase, PDase, ArSase, α -GLUase, β -GLUase, α -GALase, and β -GALase, soil sample (1.0 g) was reacted with substrate (50 mM sodium *p*-nitrophenyl phosphate, sodium Bis-*p*-nitrophenyl phosphate, potassium *p*-nitrophenyl sulfate, *p*-nitrophenyl α -D-glucoside, *p*-nitrophenyl β -D-glucoside, *p*-nitrophenyl α -D-galactoside, *p*-nitrophenyl β -D-galactoside as substrates respectively at optimal pH. After incubation for 1 h (37°C), CaCl₂-NaOH or CaCl₂-Tris (hydroxymethyl aminomethane) was added to stop enzymatic reactions, precipitate humic molecules responsible for brown coloration and extract *p*-nitrophenol. The colored product was measured colorimetrically at 410 nm. Soil protease (E.C.3.4.21-24, PRase) activity was assayed by the method of Ladd and Butler (1972). Briefly, fresh soil sample (1.0 g) was reacted with 2% Na-caseinate as substrate and Tris buffer for 2 h (50°C) at optimal pH and the residual casein was precipitated with 10 % trichloroacetic acid and filtrate was reacted with Na₂CO₃ and Folin-Ciocalteu reagent. The tyrosine concentration was measured colorimetrically at 700 nm after 1h incubation at room temperature. All the determinations were performed at optimal pH. For all

enzyme assays, controls were included for each soil sample analyzed. The same procedure as for the enzyme assay was followed for the controls

Determination of kinetic parameters

Seven concentrations (0.005, 0.010, 0.015, 0.020, 0.035, 0.020 and 0.040 mol·L⁻¹) of urea solution, six concentrations (0.0005, 0.001, 0.0025, 0.005, 0.015 and 0.050 mol·L⁻¹) of sodium *p*-nitrophenyl phosphate solution, six concentrations (0.0005, 0.00075, 0.01, 0.015, 0.03 and 0.050 mol·L⁻¹) of sodium bis (*p*-nitrophenyl) phosphate solution, seven concentrations (0.0005, 0.001, 0.005, 0.01, 0.015, 0.025 and 0.05 mol·L⁻¹) of potassium *p* - nitrophenyl sulfate solution and six concentrations (0.003, 0.007, 0.010, 0.020, 0.030, 0.050 mol·L⁻¹) of β -D-Glucoside solution were used as the substrates of urease, phosphomonoesterase, phosphodiesterase, arylsulfatase and β -glucosidase, respectively. The kinetic parameters V_{max} and K_m were calculated by nonlinear regression of the statistical software origin 8.0.

Statistical analysis

All determinations were performed in triplicate, and all the values reported are means and expressed by per g oven-dried soil (105°C). Data treatment and statistical analysis were performed by using SPSS 10.0 Program. For each variable measured, the data were analyzed by one-way ANOVA and differences of means were carried out by using the *t*-test at $P=0.05$.

RESULTS

Soil nutrient contents

No significant differences were observed in soil pH, total P and total S between

treatments NT and CT, but the contents of soil total C, total N and available N in NT increased significantly compared with those in treatment CT (Table 1).

Soil enzyme activities

Comparing with CT, NT increased the activities of soil urease, protease,

phosphomonoesterase, phosphodiesterase and arylsulfatase, but decreased the activity of soil α -D-galactosidase significantly. No significant differences were observed in the soil β -D-galactosidase, α -D-glucosidase, and β -D-glucosidase activities between NT and CT (Figure 1).

Table 1. Effect of tillage on soil chemical properties (NT-no tillage; CT-conventional tillage)

	pH	Organic carbon (g kg ⁻¹)	Available nitrogen (mg kg ⁻¹)	Total nitrogen (g kg ⁻¹)	Total phosphorus (g kg ⁻¹)	Total sulfur (g kg ⁻¹)
CT	4.8±0.02 a	12.36±0.25 b	41.16±1.40 b	11.8±0.67 b	6.99±0.18 a	3.47±0.17 a
NT	4.95±0.06 a	11.68±0.16 a	34.86±1.03 a	10.1±0.37 a	6.35±0.19 a	3.1±0.11 a

Values in columns sharing different letters differ significantly ($P \leq 0.05$) as determined by the t-test.

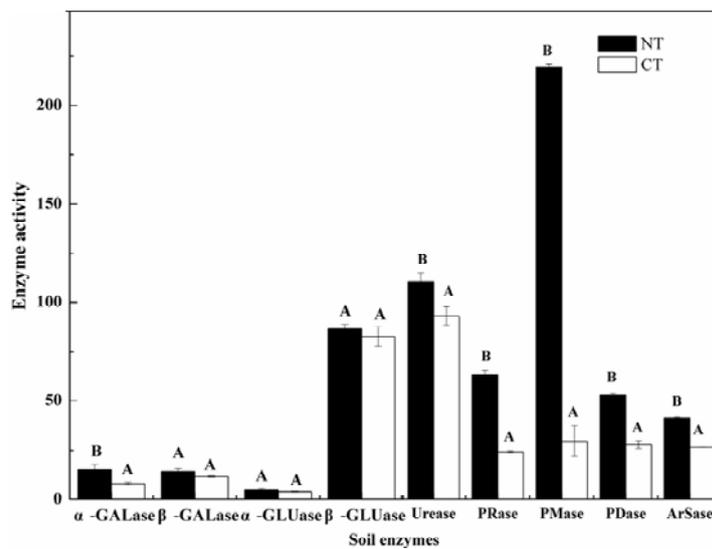


Figure 1. Effect of tillage on activities of soil enzymes involved in carbon, nitrogen, phosphorus and sulfur cycling (NT-no tillage; CT-conventional tillage; α -GLUase: α -D-glucosidase; β -GLUase: β -D-glucosidase; α -GALase: α -D-galactosidase and β -GALase: β -D-galactosidase; Urease; protease: PRase; PMase: phosphomonoesterase; PDase: phosphodiesterase; ArSase: arylsulfatase; Enzyme activity: $\mu\text{g g}^{-1} \text{h}^{-1}$). Values in columns sharing different letters differ significantly ($P \leq 0.05$) as determined by the t-test.

Soil kinetic parameters

As shown in figure 2, the V_{max} of soil protease, phosphomonoesterase, phosphodiesterase and arylsulfatase was higher in NT, while that of β -D-glucosidase and urease was higher in CT. The K_m of soil protease, phosphomonoesterase, and phosphodiesterase was higher in NT than in CT, while that of soil urease was in

reverse. No significant difference was observed in the K_m of soil β -D-glucosidase, and arylsulfatase between NT and CT (Figure 2).

The NT increased the V_{max}/K_m of soil urease, protease, and phosphomonoesterase but decreased that of β -D-glucosidase, and had lesser effects on the V_{max}/K_m of phosphodiesterase and arylsulphatase (Table 2).

Table 2. V_{max}/K_m value of soil enzymes under no tillage (NT) and conventional tillage (CT) (β -GLUase: β -D-glucosidase; Urease; protease: PRase; PMase: phosphomonoesterase; PDase: phosphodiesterase; ArSase: arylsulphatase. K_m : mM; V_{max} : $\mu\text{g g}^{-1} \text{h}^{-1}$).

	β -GLUase	Urease	Prase	PMase	PDase	ArSase
CT	34.04 b	2.79 a	64.60 a	74.42 a	10.54 a	0.82 a
NT	26.58 a	4.30 b	80.67 b	105.42 b	10.96 a	1.08 a

Values in columns sharing different letters differ significantly ($p \leq 0.05$) as determined by t-test.

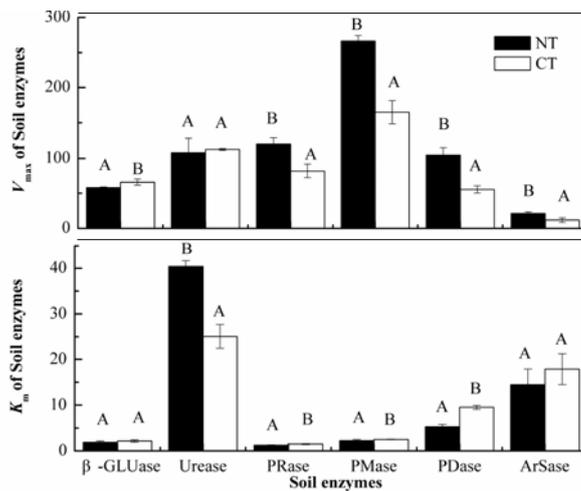


Figure 2. Effect of tillage on kinetic parameters (V_{max} and K_m) of soil enzymes involved in soil carbon, nitrogen, phosphorus and sulfur cycling (NT-no tillage; CT-conventional tillage; β -GLUase: β -D-glucosidase; Urease; protease: PRase; PMase: phosphomonoesterase; PDase: phosphodiesterase; ArSase: arylsulphatase. K_m : mM; V_{max} : $\mu\text{g g}^{-1} \text{h}^{-1}$). Values in columns sharing different letters differ significantly ($P \leq 0.05$) as determined by the t-test.

DISCUSSION

Conventional tillage often destroys soil structure, allowing a faster mineralization of soil organic matter (Alvear *et al.*, 2005), while no tillage can improve soil aggregation (Dao, 1998; Green, *et al.*, 2007). In our study, no tillage increased soil organic matter content, being beneficial to the maintenance of soil structure and the reserve of soil moisture. NT treatment increased five enzymatic activities contributing to the distribution of nutrients and organic C turnover without plowing (Dick *et al.*, 1996). Factors contributing to the higher activities under no-tillage may include the absence of the disturbing, which mitigate the problem of runoff of soil nutrient (Bandick and Dick, 1999). Mullen *et al.* (1998) pointed that increased total soil organic matter produces an increase in enzymatic activities, especially acid phosphomonoesterase. These results are confirmed by many researchers (Angers *et al.*, 1993; Bandick and Dick, 1999). Higher α -D-galactosidase activity in present study could be the effect of crop residues left on soil, which provide C and substance for these enzymatic activities (Wick *et al.*, 1998), increasing soil humus amount and protecting enzymatic fraction (Martens *et al.*, 1992). Mijangos (2006) reported that higher values were found in NT plots except for phosphatase activity. Study of Roldán *et al.* (2005) showed that NT positively influenced urease and phosphatase, while not influenced dehydrogenase, β -D-glucosidase and protease.

Our results showed that NT could enhance soil nutrients storage and the turnover of soil N and P, while not C. Similarly with most enzymes activity, Vmax values of phosphomonoesterase,

phosphodiesterase and arylsulfatase, and V_{max}/K_m of urease, phosphomonoesterase and phosphodiesterase were higher in NT. Soil urease had lower activity and Vmax, but higher catalytic potential V_{max}/K_m in NT. Lower kinetic velocity (V_{max}) and catalytic potential (V_{max}/K_m) of β -D-glucosidase confirmed its' lower activity in NT, although some research (Mullen *et al.*, 1998; Bandick and Dick, 1999; Alvear *et al.*, 2005) showed that β -D-glucosidase was significantly higher in NT than CT. α -galactosidase, β -galactosidase, α -D-glucosidase and β -D-glucosidase play important role in the C cycle in soil, being positively related to total soil carbon in general (Eivazi and Tabatabai, 1990), while our study showed α -galactosidase, β -galactosidase, α -D-glucosidase had no distinctly relationship with SOC. Seven years' no-tillage for the maize production on meadow brown soil of Northeast China had definite negative effects on the transformation of soil C.

In many soils, increases in various soil enzyme activities have been associated with decreases in tillage intensities, and were highly correlated with C contents (Green *et al.*, 2007). While urease, β -D-galactosidase, α -D-glucosidase and β -D-glucosidase activity had no distinctly changes in NT system as compared to CT at the end of 7 years. Therefore, the correlation found in other soils did not hold true for meadow brown soil.

CONCLUSIONS

Our data of chemical, biochemical properties in the surface layer of one soil located at the temperature humid zone (North-China) were coincident and clearly showed a further improvement of soil quality upon no tillage systems over a 7-year period as compared to conventional tillage. Here, it was concluded that soil

enzyme characters (activities and kinetic parameters V_{max} , K_m and V_{max}/K_m) have great value as early and sensitive indicators of changes in soil properties induced by different farm management systems. To be practical use, indicators of soil quality must be responsive to soil management practices in a relatively short time. The results also indicate that soil enzyme kinetic properties could be measures as indicators of changes induced by tillage systems.

ACKNOWLEDGEMENTS

The authors are grateful for the help received by the anonymous reviewers. This study was supported by the Chinese Government Science and Technology Supporting Program (2006BAD10B01) and NSFC Program (40771004).

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