Soil hydrolase activities and kinetic properties as affected by wheat cropping systems of Northeastern China

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ABSTRACT

Agricultural practices that reduce soil degradation and improve agriculture sustainability are important particularly for dry hilly land of Chaoyang County in the Liaoning Province, North-east China, where cinnamon soils are widely distributed and mainly for wheat production. The impacts of 10-year cropping systems (wheat-cabbage sequential cropping, wheat-corn intercrop, wheat-sunflower rotation, wheat-soybean rotation) on soil enzyme properties of surface-soil (0–20 cm) were studied. Total carbon, nitrogen, phosphorus and sulfur, and nine soil hydrolases related to nutrient availabilities (β-galactosidase, α-galactosidase, β-glucosidase, α-glucosidase, urease, protease, phosphomonoesterase, phosphodiesterase, arylsulphatase) and five enzymes kinetic characters were examined. Wheat-corn intercrop systems had higher total C, total N, total P and total S concentrations than wheat-soybean and wheat-sunflower rotation systems. Most test enzyme activities (α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, urease, protease, phosphomonoesterase and arylsulphatase) showed the highest activities under wheat-corn intercropping system. Urease, protease and phosphodiesterase activities of wheat-cabbage sequential cropping system were significantly higher than two rotation systems. The maximum reaction rates of enzymes (V_{max}) were higher than apparent enzyme activity, which suggests larger potential activity of enzymes, while not all kinetic parameters were adaptive as soil quality indicators in dry hilly cinnamon soil.

Keywords: cinnamon soil; wheat cropping systems; soil enzyme activities; kinetic properties of soil enzymes

One current trend in the global agriculture was to search for highly productive, sustainable and environmentally friendly cropping systems (Crew and Peoples 2004). Crop rotating and intercropping systems, which greatly provide pest and weed control benefits, conserve organic matter, minimize the degradation of soil resources, improve water infiltration and stimulate microbial activity, also received considerable attention in China (Ae et al. 1990, Karpenstein-Machan and Stuelpnagel 2000, Li et al. 2001).

Soil enzymes are more important in the agriculture for their role in the cycling of the nutrients and were considered to be early indicators of specific biochemical reactions in soil because of their relationship to soil biology and rapid response to changes in soil managements (Bandick and Dick 1999, Kandeler et al. 1999). Visser and Parkinson (1992) considered that the most suitable biological and biochemical properties for estimating soil quality were those related to the cycling of biogenic elements and to the transformation of organic matter in the soil. Kinetic parameters of soil enzymes (V_{max} and K_m) imply splitting velocity of enzyme-substrate complexes into enzyme and reaction products and reflect the conjunction af-

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finity between enzyme and substrate. To measure the kinetic parameters of soil hydrolases under different cropping systems will help in improving the understanding of the changes in the substrate affinity and the catalytic activity (Zhang et al. 2009).

Much information is available on the relation between soil managements and soil enzyme activities (Angers et al. 1993, Bandick and Dick 1999), but very few studies focus on activities and kinetic properties of soil enzymes under different cropping systems in dry hilly areas of China, where plow lands are widely distributed (Dodor and Tabatabai 2003, Balota et al. 2004, Green et al. 2006). Wheat production in Chaoyang city is 1/3 of that in Liaoning province, which has been one of the higher crop production provinces in China. The objectives of this study were to study (1) responses of soil enzyme activities involved in nutrient cycling (β-galactosidase, α-galactosidase, β-glucosidase, α-glucosidase, urease, protease, phosphomonoesterase, phosphodiesterase, arylsulphatase) to different wheat cropping system; (2) soil enzyme kinetic properties involved in C, N, S and P cycling due to long-term wheat cropping systems.

MATERIALS AND METHODS

Site description and cropping managements. The study site was located at 41°N latitude and 120°E longitude in dry hilly land in the west of Liaoning Province, Northeastern China, southeast to Horqin sand land, with semiarid continental climate. The soil type is cinnamon soil (Hapli-Usti-Luvisol). The annual average precipitation of the research site is 173.8–440 mm with 70.0% occurring during summer, about 14.2% is received during fall, the other 14.5% in spring. The plots were established in a completely randomized block design with three field replicates of four different cropping systems (10 years), including wheat-cabbage sequential cropping, wheat-corn intercrop, wheat-soybean rotation, and wheat-sunflower rotation. Fertilizer was applied to each plot according to soil tests obtained each year and projected crop yield for each individual crop in a rotation.

Soil samples collection and preparation. Soil samples of all the treatments were taken during October 2005 after the growing season. Four sampling sites were selected, and 60 soil samples (0–20 cm) over an approximately 1 ha at each site were collected. 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 activities assays, and the remainder was air-dried for chemical properties analysis.

Soil chemical properties assay. Soil pH was determined in soil: water suspension (1: 2.5 ratios) with a glass electrode. Total C, N and S were determined by element analyzer (Elementar Vario EL) (Matejovic 1995). Total P was determined by digestion with $HClO_4$ method (Kuo 1996). Available nitrogen (AN) was determined by micro-diffusion method of boric acid-absorbed NH_3 after alkali-hydrolyzed by NaOH (Miller and Keeney 1982). Soil moisture was determined by drying a sample at 105°C for 24 h.

Soil enzyme activities assay. Enzyme substrates were purchased from the Sigma-Aldrich Inc., Seebio Biotech, Inc. and J & K China Chemical Ltd., respectively. Enzyme activities were measured according to an 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), arylsulphatase (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) activities were 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, Norderstedt, Germany). For phosphomonoesterase, phosphodiesterase, arylsulphatase, α-D-glucosidase, β-D-glucosidase, α-D-galactosidase, and β-D-galactosidase, soil sample (1.0 g) was reacted with substrate (50 mmol 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*-ni-

trophenyl β-D-galactoside, respectively) at optimal pH. After incubation for 1 h (37°C), $CaCl_2$-NaOH or $CaCl_2$-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. The same procedures in the 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. 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 the filtrate was reacted with Na_2CO_3 and Folin-Ciocalteu reagent. The tyrosine concentration was measured colorimetrically at 700 nm after 1 h incubation at room temperature.

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 by adding substrate solution after incubation.

Kinetic parameters measurements. Six concentrations (0.005, 0.010, 0.015, 0.020, 0.035, and 0.040 mol/l) of urea solution, six concentrations (0.0005, 0.001, 0.0025, 0.005, 0.015 and 0.050 mol/l) of sodium *p*-nitrophenyl phosphate solution, six concentrations (0.0005, 0.00075, 0.01, 0.015, 0.03 and 0.050 mol/l) 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) of potassium *p*- nitrophenyl sulfate solution and six concentrations (0.003, 0.007, 0.010, 0.020, 0.030,0.050 mol/l) of β-D-glucoside solution were used as the substrates of urease, phosphomonoesterase, phosphodiesterase, arylsulphatase and β-glucosidase, respectively. The kinetic parameters V_{max} and K_m were measured by using Lineweaver-Burk linearization of Michaelis-Menten equation:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

Statistical analysis. All determinations were performed in triplicate, and all the values reported were means and expressed by per g oven-dried soil (105°C). Data treatment and statistical analysis were performed by using the SPSS 10.0 computer language program and original 8.0. For each variable measured, data were analyzed by one-way ANOVA and the means were separated using the least significant difference (*LSD*) method at $P = 0.05$.

RESULTS

Soil pH and nutrient contents. Values of pH under the intercropped plants and wheat-sunflower were higher than wheat-cabbage sequential cropping and wheat-soybean rotation, and the increase from 7.86 to 8.02 was found to be statistically significant (Table 1). Total organic C (TOC) concentrations of intercropped corn increased significantly compared with two rotation cropping, followed by wheat-cabbage sequential cropping, and there was no significant difference between them. The total N and total S concentrations of wheat grow with corn increased significantly compared with other systems. Total P concentrations of wheat-cabbage sequential cropping was the highest, followed by intercropping, wheat-soybean rotations and wheat-sunflower rotation, and the differences among them were distinct.

Soil enzyme activities. The enzyme activities involving soil C cycling (α-galactosidase, β-galactosidase, α-glucosidase, and β-glucosidase),

Table 1. Soil chemical properties under different cropping systems

	pH (1:2.5)	Available N (mg/kg)	TOC	Total N	Total P	Total S	C/N ratio
			(g/kg)				
Wheat-cabbage sequential cropping	$7.86^{a} \pm 0.02$	$80.08^{d} \pm 2.50$	$13.14^{b} \pm 0.60$	$1.12^{a} \pm 0.07$	$1.24^{d} \pm 0.03$	$0.24^{ab} \pm 0.03$	$11.73^{a} \pm 0.62$
Wheat-corn intercropping	$8.02^{b} \pm 0.03$	$56.02^{c} \pm 6.60$	$15.35^{c} \pm 0.35$	$1.42^{b} \pm 0.05$	$1.07^{c} \pm 0.03$	$0.27^{b} \pm 0.01$	$10.81^{a} \pm 0.31$
Wheat-soybean rotation	$7.88^{a} \pm 0.03$	$24.21^{a} \pm 3.21$	$11.73^{a} \pm 0.31$	$1.05^{a} \pm 0.02$	$0.55^{b} \pm 0.02$	$0.22^{a} \pm 0.01$	$11.17^{b} \pm 0.15$
Wheat-sunflower rotation	$8.00^{b} \pm 0.04$	$43.32^{b} \pm 5.47$	$11.61^{a} \pm 0.43$	$1.02^{a} \pm 0.02$	$0.47^{a} \pm 0.02$	$0.20^{a} \pm 0.02$	$11.38^{b} \pm 0.49$

Values in columns sharing the different letter differ significantly ($P < 0.05$) as determined by the *LSD* test

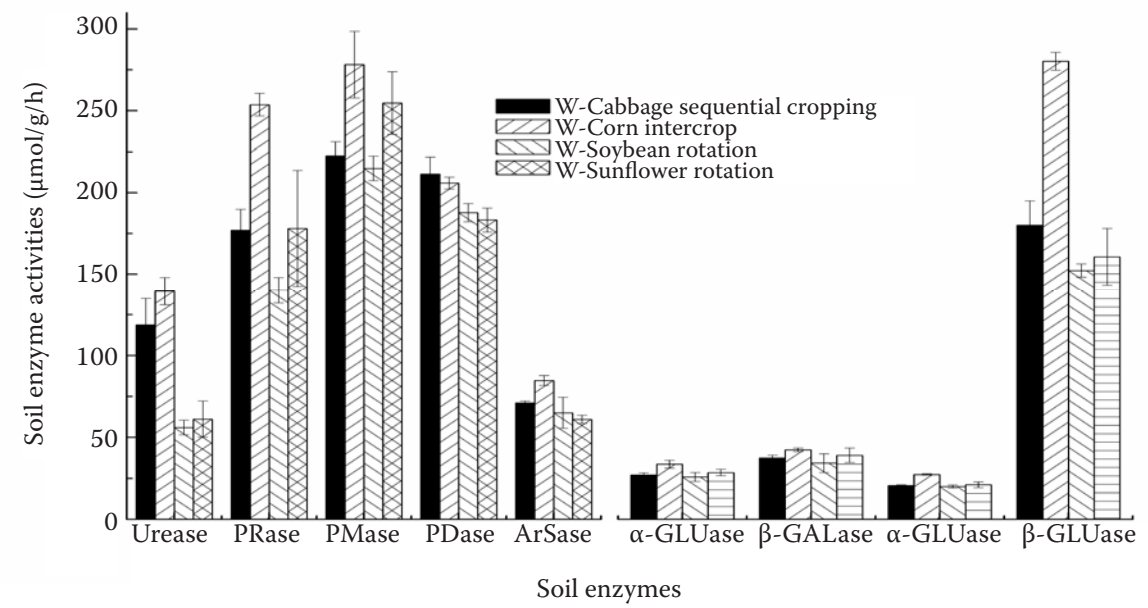

Figure 1. Effect of different cropping systems on soil enzyme activities. Wheat-cabbage sequential cropping; wheat-corn intercrop; wheat-soybean rotation; wheat-sunflower rotation. α-GALase – α-galactosidase; β-GALase – β-galactosidase; α-GLUase – α-glucosidase; β-GLUase – β-glucosidase; PRase – protease; PMase – phosphomonoesterase; PDase – phosphodiesterase; ArSase – arylsulphatase

phosphomonoesterase, arylsulphatase and the soil N cycling enzyme activities (urease and protease) showed the highest activities in wheat-corn intercropping system. Urease, protease and phosphodiesterase activities of wheat-cabbage sequential cropping were higher significantly than two rotation patterns, while other enzymes activities had no distinct difference between sequential cropping and two rotation patterns (Figure 1).

Kinetic parameters of soil enzymes. Figure 2 showed that V_{max} were higher than apparent activity in Figure 1, which suggests larger potential

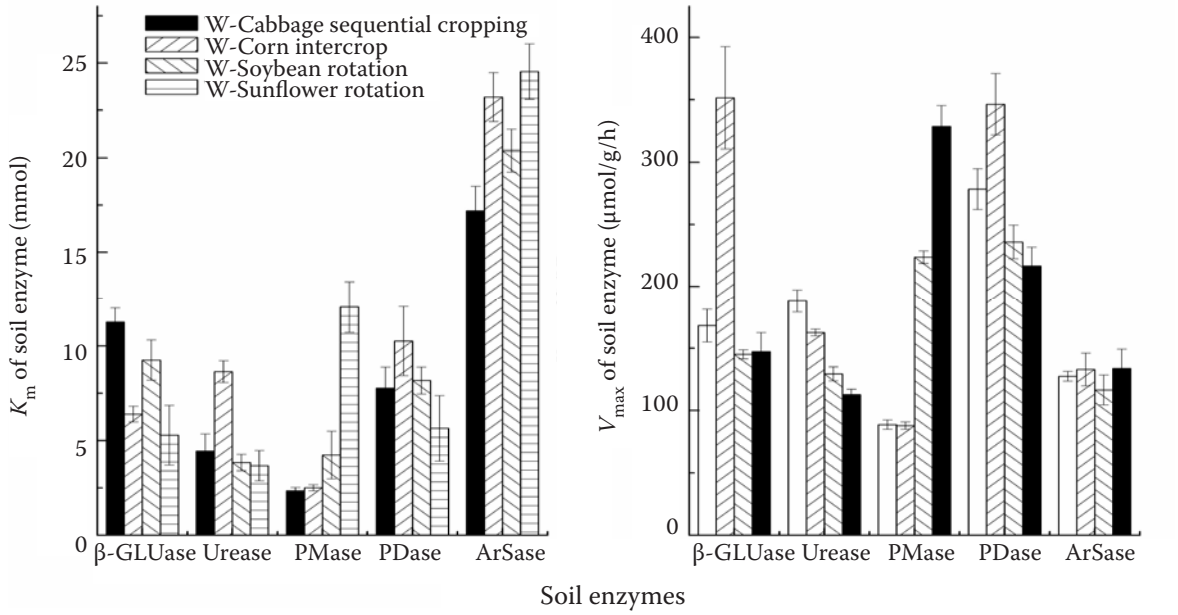

Figure 2. Effect of different cropping systems on V_{max} and K_m of soil enzymes. Wheat-cabbage sequential cropping; wheat-corn intercrop; wheat-soybean rotation; wheat-sunflower rotation. β-GLUase – β-glucosidase; PMase – phosphomonoesterase; PDase – phosphodiesterase; ArSase – arylsulphatase

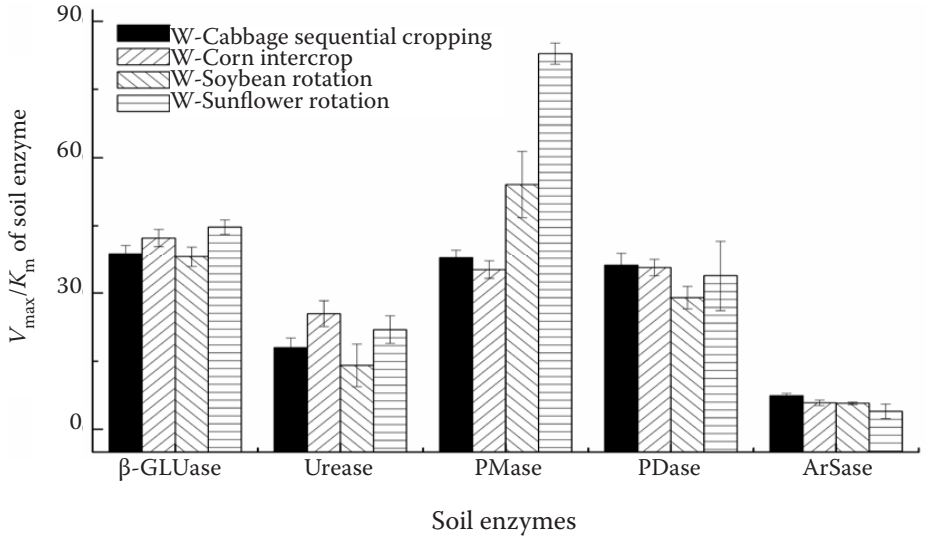

Figure 3. Effect of different cropping systems on V_{max}/K_m of soil enzymes. Wheat-cabbage sequential cropping; wheat-corn intercrop; wheat-soybean rotation; wheat-sunflower rotation. β-GLUase – β-glucosidase; PMase – phosphomonoesterase; PDase – phosphodiesterase; ArSase – arylsulphatase

activity of soil. Wheat-corn intercropping had the highest V_{max} of β-glucosidase and phosphodiesterase; wheat-cabbage sequential cropping had higher V_{max} of urease than that under wheat-corn intercropping and wheat-soybean rotation; wheat-sunflower rotation had the highest V_{max} of phosphomonoesterase. Sequential cropping and intercropping had no potential to activate ability of soil arylsulphatase.

Figure 2 showed that K_m had no distinct relationship with V_{max}, which suggests that affinity had no positive relationship with enzyme amounts. Wheat-corn intercropping had the lowest K_m values of soil β-glucosidase and phosphomonoesterase.

Higher V_{max}/K_m of soil arylsulphatase was found in the wheat-cabbage soil which contained more organic matter, and the highest catalytic efficiency of phosphomonoesterase in wheat-sunflower rotation (Figure 3).

DISCUSSION

The benefits of crop rotations in maintaining soil organic matter levels was well established (Engels et al. 1995, Acosta-Martínez et al. 2007). The present study has suggested that intercropping and sequential cropping improved soil quality, followed by wheat-cabbage sequential cropping, and wheat-sunflower rotation cropping system led to a decrease in soil organic matter, as seen from Table 1. Soil C/N ratio did not change significantly with different cropping patterns, and there was no drastic change in the pH of cinnamon soil in present study, same as illuminated in the study of Chander et al. (1997). Another study showed that there were no differences in soil organic carbon content in an early assessment (after 5 years) comparing continuous crop rotation system that included fallow periods (rye (Secale cereal)-cotton-wheat-fallow) in semiarid soils from West Texas (Acosta-Martínez et al. 2004).

Crop management practices significantly affect enzyme activities, with microbial parameters being influenced by soil organic matter contents (Jordan et al. 1995). The type of organic matter was shown to influence the activities of invertase, cellulose, and amylase more than the quantity of organic matter (Balota et al. 2004). Although some studies reported that the organic C content was significantly related to the levels of soil enzymes activities (Eivazi and Tabatabai 1990), linear regression analyses in this study showed that the activities of phosphodiesterase were significantly correlated with total P ($P < 0.05$), activities of arylsulphatase and β-glucosidase with the organic C content ($P < 0.05$), activities of arylsulphatase, α-glucosidase, and β-glucosidase with the total N content ($P < 0.05$), urease and arylsulphatase activities with total S content ($P < 0.05$) in soil.

Some studies showed that crop rotation strongly influenced soil enzyme activities (Dodor and Tabatabai 2003, Inal et al. 2007). It is believed that differences in chemical properties of soils such as pH, organic C and N among four test cropping systems are primarily responsible for differences in soils enzyme activities. Intercropping and sequential cropping provided the opportunity to return residues of different C/N

ratios to the soil, which could influence microbial activity and dynamics and positively affect soil productivity through the increased microbial activity associated with the diverse root rhizosphere. Klose and Tabatabai (2000) found that soils under crop rotations with high input and diversity of organic materials generally contained higher concentrations of microbial biomass and enzyme activities. Enzyme activities under wheat-sunflower cropping were lower than those under wheat-soybean, perhaps because leguminous plants excreted enzymes into the rhizosphere (Burns 1978) and sunflower depleted more nutrients than soybean (Chander et al. 1997). Miller and Dick (1995) reported that enzymes activities were found to be significantly higher for a legume-vegetable rotation than for the traditional vegetable rotation. The response of different enzymes to cropping systems is mostly related to the development of the microbial community structure and influenced by soil properties and environmental factors (Dodor and Tabatabai 2003). We should go deep into study on the amount of biomass carbon returned to the soil, turnover and distribution of active and passive pools of soil organic carbon.

Crop rotations have diverse crop sequences and change the soil habitat environment due to their differences in extract nutrients, depth of roots, amounts of residue which remain in soil and differences in their components. Some research pointed that the catalytic efficiency of soil enzymes V_{max}/K_m was highly affected by soil organic matter content (Zaman et al. 1999). Yet, in the present study, not all test soil enzymes in the test soils containing more organic matter had higher V_{max} and V_{max}/K_m, perhaps due to the soil enzymes were also affected by crop kinds, microorganisms and soil texture.

It is concluded that there exists a strong relationship between soil enzyme activities and management practices in long-term experiment fields such as Chaoyang. Also, activities of selected soil enzymes that are sensitive to rotational practices may have the potential to be used as indicators of soil quality and sustainability. However, not all kinetic parameters that were adaptive in dry hilly cinnamon soil can be used as indicators monitoring soil health and quality.

REFERENCES

Acosta-Martínez V., Mikha M.M., Vigil M.F. (2007): Microbial communities and enzyme activities in soils under alternative crop rotations compared to wheat-fallow for the Central Great Plains. Applied Soil Ecology, *37*: 41–52.

Acosta-Martínez V., Zoveck T.M., Allen V. (2004): Soil microbial, chemical and physical properties in continuous cotton and integrated crop-livestock systems. Soil Science Society of America Journal, *68*: 1875–1884.

Ae N., Arihara J., Okada Yoshihara T.K., Johansen C. (1990): Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science, *248*: 477–480.

Angers D.A., N'dayegamiye A., Nielsen D.C. (1993): Tillage-induced differences in organic matter of particle size fractions and microbial biomass. Soil Science Society of America Journal, *57*: 512–516.

Balota E.L., Kanashiro M., Colozzi A., Andrade D.S., Dick R.P. (2004): Soil enzyme activities under long-term tillage and crop rotation systems in subtropical agro-ecosystems. Brazilian Journal of Microbiology, *35*: 300–306.

Bandick A.K., Dick R.P. (1999): Field management effects on soil enzyme activities. Soil Biology and Biochemistry, *31*: 1471–1479.

Burns R.G. (1978): History of abiontic soil enzyme research. In: Burns R.G. (ed.): Soil Enzymes. Academic Press, London, 1–33.

Chander K., Goyal S., Mundra M.C., Kapoor K.K. (1997): Organic matter, microbial biomass and enzyme activity of soils under different crop rotations in the tropics. Biology and Fertility of Soils, *24*: 306–310.

Crew T.E., Peoples M.B. (2004): Legume vervus fertilizer source of nitrogen: ecological tradeoffs and human needs. Agriculture, Ecosystem and Environment, *102*: 279–297.

Dodor D.E., Tabatabai M.A. (2003): Amidohydrolases in soils as affected by cropping systems. Applied Soil Ecology, *24*: 73–90.

Eivazi F., Tabatabai M.A. (1990): Factors affecting glucosidase and galactosidases in soils. Soil Biology and Biochemistry, *22*: 891–897.

Engels E.A., Becker M., Ottow J.G.G., Ladha J.K. (1995): Influence of phosphorus or phosphorus-potassium fertilization on biomass and dinitrogen fixation of the stem-nodulating green-manure legume *Sesbania rostrata* in different marginally productive wetland rice soils. Biology and Fertility of Soils, *20*: 107–112.

Green V.S., Stottb D.E., Diack M. (2006): Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. Soil Biology and Biochemistry, *38*: 693–701.

Inal A., Gunes A., Zhang F., Cakmak I. (2007): Peanut/maize intercropping induced changes in rhizosphere and nutrient concentrations in shoots. Plant Physiology and Biochemistry, *45*: 350–356.

Jordan D., Kremer R.J., Bergfield W.A., Kim K.Y., Cacnio V.N. (1995): Evaluation of microbial methods as potential indicators of soil quality in historical agriculture fields. Biology and Fertility of Soils, *19*: 297–302.

Kandeler E., Palli S., Stemmer M., Gerzabek M.H. (1999): Tillage changes microbial biomass and enzymes activities in particle-size fractions of a Haplic Chernozem. Soil Biology and Biochemistry, *31*: 1253–1264.

Karpenstein-Machan M., Stuelpnagel R. (2000): Biomass yield and nitrogen fixation of legumes mono-cropped and intercropped

with rye and rotation effects on a subsequent maize crop. Plant and Soil, *218*: 215–232.

Klose S., Tabatabai M.A. (2000): Urease activity of microbial biomass in soils as affected by cropping systems. Biology and Fertility of Soils, *31*: 191–199.

Kuo S. (1996): Phosphorus. In: Sparks D.L. (ed.): Methods of Soil Analysis, Part 3, Chemical Methods, SSSA Book series No 5. Soil Science of America, Madison, 869-919.

Ladd J.N., Butler J.H.A. (1972): Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biology and Biochemistry, *4*: 19–30.

Li L., Sun J.H., Zhang F.S., Li X.L., Yang S.C., Rengel Z. (2001): Wheat/maize or wheat/soybean strip intercropping. I. Yield advantage and inter specific interactions on nutrients. Field Crops Research, *71*: 123–137.

Matejovic I. (1995): Total nitrogen in plant material determined by means of dry combustion: a possible alternative to determination by Kjeldahl digestion. Communications in Soil Science and Plant Analysis, *26*: 2217–2229.

Miller M., Dick R.P. (1995): Thermal stability and activities of soil enzymes as influenced by crop rotations. Soil Biology and Biochemistry, *27*: 1161–1166.

Miller R.H., Keeney D.R. (1982): Nitrogen-availability indices. In: Page A.L., Miller R.H., Keeney D.R. (eds): Methods of Soil Analysis. Part II: Chemical and Microbiological Properties. 2nd Edition. Soil Science Society of America, Madison, 711–733.

Tabatabai M.A. (1994): Soil enzymes. In: Weaver R.W., Angle J.S., Bottomley P.S. (eds): Methods of Soil Analysis. SSSA, Madison, 775–833.

Visser S., Parkinson D. (1992): Soil biological criteria as indicators of soil quality: soil microorganisms. American Journal of Alternative Agriculture, *7*: 33–37.

Zhang Y.L., Sun C.X., Chen L.J., Duan Z.H. (2009): Catalytic potential of soil hydrolases in northeast China under different soil moisture conditions. Journal of Soil Science and Plant Nutrition, *9*: 116–124.

Zaman M., Di H.J., Cameron K.C. (1999): A field study of gross of N mineralization and nitrification and their relationships to microbial biomass and enzyme activities in soils treated with dairy effluent an ammonium fertilizer. Soil Use and Management, *5*: 188–194.

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