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Original Article

Activity of Enzymes Involved in Nitrogen and Phosphorus Circulation in Cropland Soils

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Abstract

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According to the data of the Mongolian Ministry of Agriculture the area of rapeseeds increases every year, and for today it makes up about 15% from entire agriculture area. In our country the crop rotation occurs as wheat-rape-wheat-rape, which leads to loss of soil fertility and yield reduction. Study on fertility changes of agricultural soil, especially influence of cultivation on soil fertility is lucking. That is why in this study we tried to evaluate the intensity of biochemical processes in soil by comparing activity of enzymes involved in nitrogen and phosphorus cycle (protease, urease, acid and alkaline phosphatases) of the wheat, rape soils with enzymes of soils were seeding crops did not produced. The results show that in cropland soils, acidity of all soils was increased, amount of available phosphorus decreased, activity of acid and alkaline phosphatases noticeably changed compared to the control soil. From these results we can see that crop cultivation influence the biological processes in soil. So we have to take it into consideration for further farming and management systems, and plant cultivation activities.

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Introduction

Soil is a complex system wherein chemical, physical and biochemical factors are held in dynamic equilibrium. The alteration of this equilibrium by natural or anthropogenic activity, may cause instability and stress (Doran & Parkin, 1994). Usually microbial biomass and activity can be considered good indicators of soil biological status (Masciandaro *et al*., 2012), however, soil fertility largely depends on the activity of biological processes in the soil in which soil enzymes actively participate. Studies on enzyme activities provide information on the biochemical processes occurring in the soil.

There is growing evidence that soil biological parameters may be potential, sensitive indicators of soil ecological stress or restoration (Kizilkaya & Bayrakli, 2005), and management-induced changes in soil quality (Kennedy & Papendick, 1995). Hydrolytic enzymes as phosphatases, protease and urease are involved in the decomposition of organic nitrogen and phosphorus compounds in soil. They play a key role in formation of soil biological activity. Phosphatases are a group of enzymes that catalyze hydrolysis of phosphoric acid esters and anhydrides (Nannipieri *et al*., 2011). Apart from being a good indicator of soil fertility, phosphatase enzymes play key roles in the soil system (Eivazi & Tabatabai, 1977). Proteases (EC 3.4.) in the soil originate from a number of different sources, including microorganisms, plants, animal excrements (urine and faces), decomposition of dry and wet deposition (including leaching from vegetation). The significance of these sources varies according to the type of ecosystem and applied management

(Vranova, 2013). Urease (EC EC 3.5.1.5, urea amidohydrolase) was the first enzyme ever to be extracted and studied. This enzyme decomposes urea into CO_2 and NH_3. In soil, urease may originate from plant residues, plant waste or soil microbes (Askin & Kizilkaya, 2005).

Material and Methods

Soil sampling. Soil samples were taken in 2014-2016 from the Jargalant farm wheat (N48^{0}47'56.85" E106^{0}13'06.57", 1059 m a.s.l.), rape fields (N48^{0}47'85.41" E106^{0}17'51.6", 1066 m a.s.l.), and fields next to them (N48^{0}47'56.85" E106^{0}13'06.57", 1059 m a.s.l.; N48^{0}47'85.41" E106^{0}17'51.6", 1066 m a.s.l.), where these crops do not grow. The steppe soil (N48^{0}43'13.72" E106^{0}18'84.51", 1121 m a.s.l.) was taken as a control. The soil subsamples were taken from the four places at depth 0-25 cm from the soil surface, mixed, sieved (2 mm), stored and kept in freezer until laboratory analysis.

Methods. For estimation of acidity of the soil samples were shaken with 0.9% NaCl for 30 minutes after what was filtered and titrated by 0.001 N NaOH; available phosphorus was estimated by colorimetric assay with hydroquinone, which based on the bicarbonate method developed by Olsen *et al*. (1954). After adding 2 ml of Na_2CO_3 and Na_2SO_3 into soil filtrate, mixture of 1% hydroquinone was added and the absorbance was measured at 750 nm (Jones, 2001). Calculations were made according to the phosphate calibration curve (0.05 mg/ml). Amount of available phosphorus was expressed in μg/g of soil sample. Protease activity was estimated by Kunitz's method (Kochetov, 1980; Purev, 2012), urease activity – by Romeiko and Malinskaya colorimetric assay (Khaziev, 2005), activity of acid (EC 3.1.3.2) and alkaline phosphatases (EC 3.1.3.1) were estimated by Tabatabai-Bremner assay with para-nitrophenylphosphate (PNPP) as a substrate (Khaziev, 2005). Enzymatic activities were expressed in units (U): 1U of phosphatases activity were defined as the amount of enzyme protein in 1 g of soil, which forms 1 mg of para-nitrophenol in 1 hour; 1U of protease activity was taken as 1μg of tyrosine formed in 1g of soil in 1 hour; 1U of urease activity was taken as 1 μg nitrogen formed from NH_3 by urease of 1g soil in1 hour.

Data analysis. All experiments were carried out with 3-5 repetitions and mean values were taken. Pearson correlation coefficient (r) was calculated between chemical properties and soil enzymes activities. One-way ANOVA was used for analysis of soil chemical character and enzyme activities. Least significant difference (P = 0.05) was taken to determine whether means differ significantly.

Results and Discussion

Our results show that the acidity of the soils was increased in all soil samples, for the last year

Table 1. Soil acidity and available phosphorus.

№	Soil	Soil depth, cm	Titrative acidity, mg/g			Available phosphorus, mg/g		
			2014	2015	2016	2014	2015	2016
1	Wheat field (WS)	0-7	0.072	0.111	0.648	0.047	0.170	0.023
		7-15	0.078	0.111	0.491	0.051	0.123	0.019
		15-25	0.078	0.109	0.424	0.044	0.088	0.009
2	Next to wheat field (NWS)	0-7	0.067	0.118	0.618	0.047	0.222	0.029
		7-15	0.069	0.114	0.703	0.051	0.142	0.022
		15-25	0.064	0.103	0.654	0.037	0.064	0.015
3	Rape field (RS)	0-7	0.075	0.144	0.241	0.019	0.055	0.009
		7-15	0.075	0.129	0.285	0.020	0.031	0.009
		15-25	0.075	0.108	0.237	0.017	0.027	0.009
4	Next to rape field (NRS)	0-7	0.067	0.105	0.352	0.047	0.082	0.016
		7-15	0.067	0.096	0.284	0.051	0.030	0.011
		15-25	0.064	0.087	0.249	0.037	0.026	0.009
5	Control (CS)	0-7	0.090	0.133	0.976	0.031	0.038	0.014
		7-15	0.098	0.153	0.723	0.035	0.022	0.011
		15-25	0.076	0.122	0.440	0.033	0.017	0.007

available phosphorus was decreased from 1.6 (next to rape field soil) to 3.3 times (wheat field soil) in the cropland soils (Table 1).

Soil titrative activity showed the following order in the various fields: CS > NWS > WS > RS ≥ NRS and available phosphorus NWS > WS > NRS > CS > RS. Activity of acid phosphatase was higher than the activity of alkaline phosphatase, because all soils were acidic. Protease activity increased, but the activity of urease decreased (Table 2). In cropland soils these changes were observed significantly compared to the control soil. Hu *et al*. (2014) estimated the influence of fertilizers on wheat field soil pH, organic C, total N, values of available phosphorus and activity of dehydrogenase, urease, alkaline phosphatase and invertase. They found that fertilization increases the mean values of available P, total N, soil organic C, soil properties related to the soil depth. According to our data the activity of soil enzymes have dependence from soil depth and showed highest activity in surface soil layer (0-7 cm).

Speir and Cowling (1991) estimated that at the low-fertility site, where organic phosphorus provides most plant phosphorus, herbage and root phosphatase activities were significantly correlated with available organic phosphorus. The results of our studies show that the activity of the estimated enzymes is more correlated with the soil titrative acidity than with the available phosphorus content (Table 3). Gaind and Nain (2007) conducted a field experiment to evaluate the relative contribution of organic and inorganic fertilizers in improving pH, C, N, humus, microbial biomass, dehydrogenase, phosphatase, cellulase, β-glucosidase and xylanase activities of soil under wheat crop. Their results show that alkaline phosphatase activity varied with the different treatment applied to soil. Its activity directly related with the quantity of microbial biomass contained in the added material and has an inverse relation with available phosphorus.

Our results show that by three years, the activity of acid and alkaline phosphatases decreased in all soil samples. The activity of both acid and alkaline phosphatase showed the most activity in CS, which can indicate this soil

Table 2. Activity of estimated enzymes.

№	Soil	Soil depth, cm	ACP, U			ALP, U			Protease, U			Urease, U		
			2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016
1	WS	0-7	1.58	1.65	0.052	0.72	0.84	0.080	2.94	4.87	6.83	29.90	12.69	6.21
		7-15	2.03	0.91	0.077	1.30	0.44	0.050	3.32	4.09	5.68	19.80	14.92	2.86
		15-25	1.63	0.91	0.022	0.69	0.28	0.005	2.99	3.27	4.78	16.70	6.60	2.81
2	NWS	0-7	0.46	1.87	0.008	0.24	1.72	0.040	4.00	8.75	7.68	11.30	7.99	10.48
		7-15	0.40	1.70	0.050	0.25	0.95	0.044	3.75	6.79	5.04	10.50	9.96	12.67
		15-25	0.23	1.53	0.040	0.19	0.81	0.003	3.42	5.16	4.01	9.30	9.54	6.41
3	RS	0-7	0.42	4.09	0.003	0.30	0.69	0.003	3.25	3.16	4.13	10.70	7.51	6.45
		7-15	0.28	1.95	0.075	0.22	1.30	0.060	4.08	2.74	3.83	8.80	10.98	1.72
		15-25	0.20	0.60	0.057	0.19	0.88	0.060	3.42	2.68	3.62	8.00	10.83	1.63
4	NRS	0-7	0.46	2.27	3.880	0.24	1.71	1.08	4.00	6.26	11.26	11.30	13.20	12.23
		7-15	0.40	1.75	0.267	0.25	0.71	0.290	3.75	3.53	6.59	10.50	12.03	9.74
		15-25	0.23	0.62	0.029	0.19	0.38	0.020	3.42	2.68	4.00	9.30	9.94	2.06
5	CS	0-7	5.81	4.11	3.7	4.54	2.48	2.350	3.84	9.93	17.16	38.90	20.48	17.65
		7-15	6.48	1.60	0.790	3.06	0.82	0.610	1.84	6.32	12.09	24.00	17.99	12.88
		15-25	3.46	0.61	0.260	1.43	0.45	0.270	1.33	3.83	5.93	16.70	16.63	5.74

Table 3. Pearson correlation coefficients (*r*) between chemical properties and soil enzyme activities.

Correlation	2014				2015				2016			
	ACP	ALP	Protease	Urease	ACP	ALP	Protease	Urease	ACP	ALP	Protease	Urease
Titrative acidity	0.8959	0.8452	-0.4597	0.6704	0.5404	0.2695	0.2967	0.3639	0.3393	0.5395	0.6780	0.6982
Available phosphorus	-0.0615	-0.0993	0.1073	0.1157	0.0015	0.1661	0.4278	-0.3356	0.0170	-0.0331	0.1683	0.3626

Table 4. One-way ANOVA analysis of soil chemical character and enzyme activities.

	DF	Titrative acidiy	Available phosphorus	ACP	ALP	Protease	Urease
CS vs WS	40	0.8925	0.1268	0.0293*	0.0046**	0.1934	0.4113
CS vs NWS	40	0.9948	0.0620	0.0098**	0.0040**	0.6527	0.8721
CS vs RS	40	0.3882	0.9999	0.0166*	0.0023**	0.0475*	0.6888
CS vs NRS	40	0.3925	0.9390	0.0438*	0.0066**	0.4641	0.9756

more rich in organic C and microbial biomass. The following results confirm these data and for urease we can see similar trend. For example, Hu *et al*. (2014) found that the activities of dehydrogenase, urease, alkaline phosphatase and invertase were generally higher in the fertilized than in the unfertilized treatments, and the application of organic fertilizer produced higher activity than addition of inorganic fertilizer treatments.

There was a close positive correlation between dehydrogenase and available phosphorus and soil organic C correlated with all enzymes measured. Smith and Powlson (2003), Balezentiene and Klimas (2009) showed that the presence of readily available organic N stimulated urease activity. Urease had a strong positive relationship with pH value (Hu *et al*., 2014). According to our data, the activity of urease has a positive correlation with soil titrative acidity and decrease of this correlation may indicate the decrease of organic N in soil. We revealed that in three year average soil enzymes the following order: protease – CS > NWS > WRS > WS > RS; urease – CS > NRS > NWS > RS > WS; ACP – CS > NRS > WS > RS > NWS and ALP – CS > NRS > WS ≥ NWS > RS. This data confirms results of previous researchers and we can suggest that activity of biological processes in CS occurs more intensively, crop cultivation influences the biological processes in soils. Changes in soil ALP and ACP occurred more noticeable in comparison with control soil and activity of protease significantly changed in the rape field soil (Table 4). Our study revealed that crop growth significantly influences on soil enzymatic and biological activities.

Conclusion

Our results indicate that land use strongly affects soil enzyme activities, which are important in soil functioning, such as nutrient mineralization and cycling, decomposition and formation of soil organic matter. Soil enzymes provide a sensitive biological indicator for soil quality. So we have to take it under consideration when accessing soil erosion and changes in soil fertility and study soil-plant relationship.

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