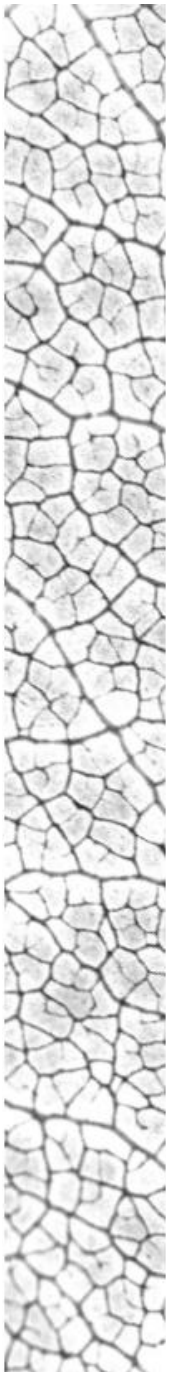

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Enzymatic activity of soil microbiota under different fertilizer systems

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Abstract
Soil enzymes are mostly synthesized by microorganisms and play an essential role in metabolism since they are biological catalysts for the transformation of organic residues. The activity of enzymes is affected by many factors, for instance, the number and activity of soil microbiota, the physicochemical properties of soil, organic matter content, weather conditions during the growing season, incorporation of organic residues into the soil, and the farming system. Among the various groups of soil enzymes, catalase and proteases deserve special attention. Catalase activity associated with the content of organic carbon in the soil is used as an indicator of soil fertility. Soil proteases play a significant role in nitrogen mineralization. The goal of our study was to compare soil enzyme activity in short crop rotation under different fertilizer systems. Methods. Soil catalase activity was determined by gasometrical method. Soil protease activity was determined by the Romeiko method. Results. |Soil protease activity increased from the beginning to the end of vegetation, with the maximum catalase activity being observed approximately in the middle of the growing season of the studied crops. Protease and catalase activities of rhizosphere soils were higher than those of the bulk soil, probably due to higher activity of root-associated microbial communities. The highest indicators of enzymatic activity were recorded under biological fertilizer system. Conclusion. The biological system of crops fertilization of a short crop rotation ensures optimal conditions for the course of enzymatic processes, which, obviously, is a consequence of the activation of soil microbial communities.

Keywords: hydrolases, protease, oxidoreductases, catalase, soil fertility, fertilizer system

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INTRODUCTION

Soil enzymes are mostly synthesized by microorganisms and play an essential role in metabolism since they are biological catalysts for the transformation of organic residues. Among the common sources of soil, enzymes are microorganisms (living and dead), plant roots and plant residues, as well as soil animals. However, the microbiota is the main source of soil enzymes (Kalembasa and Symanowicz, 2012). Soil enzymatic activity initiates the biochemical transformations of organic matter and other nutrients. It is an essential indicator for assessing the cycle of chemical elements, the impact of pollutants, and anthropogenic pressure on the soil, as well as soil health and fertility. In most cases, enzymes enter the soil after the death and lysis of microbial cells and can retain their activity for a long time (Hrynyk et al., 2011; Iutynska and Yamborko, 2005). A wide range of enzymes of different groups mediates soil enzymatic activity. Some enzymes promote the breakdown of organic matter (for example, hydrolases, glucosidases), while others are involved in the mineralization of nutrients (amidase, urease, phosphatase, etc.) (Bowles et al., 2014). Among the various groups of soil enzymes, catalase and proteases deserve special attention. Catalase is a catalyst for the destruction of organic xenobiotics (Borko et al., 2016). Catalase activity is associated with the metabolic activity of aerobic microorganisms and is used as an indicator of

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soil fertility (Trasar-Cepeda et al., 2007). The activity of this enzyme is very stable in the soil and has a significant correlation with the content of organic carbon (Stepniewska et al., 2009). Soil proteases play a significant role in nitrogen mineralization. These enzymes are usually associated with inorganic and organic colloids. Their activity not only reflects the ability of the soil to enzymatically transform the substrate but can also be integral to the ecology of microorganisms (Vranová et al., 2013).

The enzymatic activity of the soil is directly affected by the microbial group composition and development. In this regard, the influence of the environment and farming practices is reflected in the enzymatic activity of the microbial community, and the direction of biochemical processes in the soil can be an indicator of the state of the microbial community (Bardgett, 2005). Enzyme activity is also affected by the soil physicochemical properties, the content of organic matter, weather conditions during the growing season, incorporation of organic residues into the soil, as well as the farming system (Nannipieri et al., 2003; Mekich et al., 2015). For example, in an ecological farming system, in typical chernozem, indicators of enzymatic activity are significantly higher than in the industrial system. In particular, protease activity is higher by 48%, catalase by 19%, and phosphatase by 25%. However, in the biological farming system, catalase activity is 24% lower than in the ecological farming system (Tsyuk et al., 2017).

Along with this, there are data on increasing the enzymatic activity under the effect of mineral (phosphorus-potassium) and organo-mineral fertilizers 1.5–2 times as much (Tsentylo, 2019). It was found that mineral fertilizers increased the activity of invertase, but suppressed the activity of catalase at the same time (Piterson and Greman, 2005).

Studies by other researchers have shown that there is a tendency of increasing soil enzymatic activity from sowing to harvesting. The practice of the biological farming system led to an increase in the diversity of the microbial community in the sugar beet rhizosphere, while the intensive farming system led to a decrease (Patyka et al., 2017; Borko et al., 2016; Chebanova, 2019).

The enzymatic activity of microorganisms and its influence on the formation of soil homeostasis and soil productivity is still an open issue. Especially from the point of view of revealing the features of interaction of the microbial community with new organic fertilizers.

The purpose of the study was to comparatively assess soil enzymatic activity in short crop rotation under different fertilizer systems.

MATERIALS AND METHODS

The research was carried out in the years 2016–2019 at the Bila Tserkva Research and Breeding Station of the Institute of Bioenergy Crops and Sugar Beets NAAS of Ukraine, in the Central Forest-Steppe of Ukraine, in the zone of unstable moisture.

The soil of the experimental plots was typical deep medium loamy coarse chernozem with low humus content (3.5%), the total nitrogen content of 0.31%, hydrolytic acidity of 2.41 mg-equivalent, nearly neutral acidity, the content of easily hydrolyzed nitrogen (N) of 134 mg/1000 g, P_2O_5 content of 276 mg/1000 g, K_2O content of 98 mg/1000 g, and the degree of alkali saturation of 90%. Climatic conditions during the years of the experiment were typical for the zone of unstable moisture of the central Forest-Steppe of Ukraine.

The short crop rotation under study has a four-year rotation cycle and is arranged for the cultivation of the following crops: soybean − winter wheat − sugar beet − maize for grain.

As a control treatment, a contemporary industrial system of crop fertilization was used which stipulates the use of intensive cultivation technologies with the priority of using industrial fertilizers available on the market (mainly mineral fertilizers). In the ecological fertilizer system, a balanced combination of mineral and organic fertilizers at the environmentally regulated application rates was used. In the biological fertilizer system, only contemporary organic fertilizers were applied along with plant residues and humates (**Table 1**).

Soil samples were taken from the plough soil layer of the experimental plots (bulk soil) and from plant rhizosphere soil (Iutynska, 2006).

Soil catalase activity was determined by the gasometrical method based on the measurement of the volume of oxygen released over a time period due to the decomposition of hydrogen peroxide during its interaction with the soil (Tytova and Kozlov, 2012; Gianfreda et al., 2005).

Soil protease activity was determined by the Romeiko method. This method allows determining the degree of proteolytic decomposition of casein by titration with ferric chloride (Tytova and Kozlov, 2012; Gianfreda et al., 2005).

Statistical analysis of research data was performed by the method of analysis of variance using computer software Excel and Statistica - 10 (Prysiazhniuk et al., 2016).

RESULTS

Obtained results indicate that the intensity of soil enzymatic activity is directly affected by the fertilizer system and varies with the cultivated crops.

Table 2 presents the results of the studies on soil enzymatic activity under different fertilizer systems in soybean agrop-hytocoenoses.

Table 1. Fertilizer system for short crop rotation

No	Fertilizer system	Basic fertilization	Pre-seeding fertilization	Fertilization in vegetation
Soybean				
1	Biological	Maize harvest residues (8−12 t/ha) + Biohumus (Vermicompost) “Ekochudo” (200 kg/ha)	Seed treatment with biological fertilizer “Vermisol” (10 l/t)	Quantum– Humate (1 l/ha)
2	Ecological	Harvest residues of maize (8−12 т/га) + $P_{30}K_{30}$	Seed treatment with biological fertilizer “Vermisol” (10 l/t) + N_{30} at cultivation	-
3	Industrial	$P_{60}K_{60}$	N_{60} at cultivation	-
Winter wheat				
1	Biological	Harvest residues of soybean (2−3 t/ha) + Biohumus (Vermicompost) “Ekochudo” (500 kg/ha)	Seed treatment with biological fertilizer “Vermisol” (10 l/t)	Quantum– Humate (0.7 l/ha)
2	Ecological	Soybean harvest residues (2−3 t/ha) + Biohumus (Vermicompost) “Ekochudo” (500 kg/ha) + $N_{22}P_{22}K_{22}$	Seed treatment with biological fertilizer “Vermisol” (10 l/t) + $N_8P_8K_8$ at cultivation	$N_{16,5}$ in spring
3	Industrial	$N_{44}P_{44}K_{44}$	$N_{16}P_{16}K_{16}$ at cultivation	N_{33} in spring
Sugar beet				
1	Biological	Wheat harvest residues (8−10 t/ha) + Biohumus (Vermicompost) “Ekochudo” (1000 kg/ha)	Seed treatment with biological fertilizer “Vermisol” (10 l/t)	Quantum– Humate (0.7 l/ha)
2	Ecological	Wheat harvest residues (8−10 t/ha) + $N_{90}P_{60}K_{90}$	Seed treatment with biological fertilizer “Vermisol” (10 l/t) + N_{55} at cultivation	-
3	Industrial	$P_{90}K_{120}$	$N_{120}P_{30}K_{40}$ at cultivation	$N_{120}P_{20}K_{30}$
Maize for grain				
1	Biological	Sugar beet harvest residues (30−40 t/ha) + Biohumus (Vermicompost) “Ekochudo” (750 kg/ha)	Seed treatment with biological fertilizer “Vermisol” (10 l/t)	Quantum– Humate (1 l/ha)
2	Ecological	Sugar beet harvest residues (30−40 t/ha) + $N_{15}P_{30}K_{30}$	Seed treatment with biological fertilizer “Vermisol” (10 l/t) + N_{30} at cultivation	N_{15}
3	Industrial	$N_{30}P_{60}K_{60}$	N_{60} at cultivation	N_{30}

Table 2. Dynamics of soil enzymatic activity in soybean agro-phytocenoses under different fertilizer systems (2016−2019)

Fertilizer system	Phenological phase					
	Germination		Budding		Browning of beans	
	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)
Bulk soil						
Biological	33.60	8,84	34.78	8.54	36.21	8.43
Ecological	41.52	11.64	42.07	10.20	43.84	9.52
Industrial	39.68	11.53	42.12	10.28	43.78	9.61
Rhizosphere soil						
Biological	33.55	8.93	35.81	9.23	36.62	8.47
Ecological	41.54	11.70	43.21	11.30	44.20	9.89
Industrial	39.62	11.57	43.33	11.40	43.80	9.93
$LSD_{0.05\ soil}$	1.89	0.56	0.41	0.34	0.87	0.49
$LSD_{0.05\ rhizosphere}$	1.95	0.68	0.53	0.47	0.90	0.66

Bulk soil protease activity increased over the soybean growing season with the use of different fertilizer systems. However, when using mineral fertilizers we obtained slightly lower indicators compared to organo-mineral and organic fertilizer systems. Most probably, this may indicate inhibition of bulk soil protease activity due to acidic fertilizer residues (Tsyhichko and Makliuk, 2015).

It is known that the application of moderate rates of mineral fertilizers enhances the protease activity in the soil, while high application rates lead to its suppression (Piterson and Greman, 2005). In view of this, it can be concluded that organo-mineral and organic fertilizer systems provide better conditions for nitrogen nutrition of soybean sowings. Assessment of the soil catalase activity indicates that the decomposition of hydrogen peroxide in the mineral fertilizer system is slower than in organo-mineral or organic fertilizer systems.

According to the other research data, such patterns can be explained by the fact that mineral fertilizers contain a significant amount of nitrate, phosphate, and other physiologically acidic ions. Acidification of the soil caused by these ions also leads to inhibition of catalase activity (Piterson and Greman, 2005).

Particular attention should be given to the peculiarities of assessment of the protease and catalase activity of soybean rhizosphere soil. It is known that the root system of plants not only absorbs water and nutrients but also interacts with the soil by releasing physiologically active substances. However, at the time of full emergence soybean seedlings form rather small root systems, which cannot significantly affect indicators of enzymatic activity.

In the budding phase, when the interaction of soybean with the soil is characterized by intensive growth and formation of generative organs, the protease activity of the rhizosphere soil in the industrial fertilizer system was by 1.03 CFU, in the ecological system by 1.14 and biological system by 1.21 CFU higher compared to similar indicators of the plough soil layer.

Catalase activity in the rhizosphere soil layer was also higher than in the plough layer, with catalase activity in ecological and biological fertilizer systems being slightly higher compared to the industrial system.

Table 3. Dynamics of soil enzymatic activity in soybean agro-phytocenoses under different fertilizer systems (2016−2019)

Fertilizer system	Phenological phase					
	Beginning of booting		Earing		Milky-wax ripeness	
	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)
Bulk soil						
Industrial	25.70	7.87	31.40	7.10	35.00	6.30
Ecological	32.98	11.05	36.90	10.07	38.66	8.43
Biological	33.75	11.94	37.05	10.84	39.13	8.80
Rhizosphere soil						
Industrial	27.88	8.52	35.31	8.78	41.25	6.90
Ecological	33.20	12.10	41.10	11.40	43.23	10.03
Biological	34.20	12.90	42.05	11.74	44.21	10.16
$LSD_{0.05\ soil}$	1.23	0.99	0.77	0.94	0.72	0.60
$LSD_{0.05\ rhizosphere}$	1.40	1.05	0.83	1.10	0.80	0.67

The maximum indicator of catalase activity was observed during the period of intensive vegetation, while in the second half of vegetation it decreased. In our opinion, this can be explained by the activity of the root system of plants not only in terms of absorption of nutrients but also in the release of substances into the soil, including enzymes.

The obtained data on the peculiarities of soil enzymatic activity formation under different fertilizer systems in agro-phytocenoses of winter wheat are shown in **Table 3**.

Soil protease activity in the studied fertilizer systems was increasing during winter wheat vegetation. In the plough soil layer, in the biological fertilizer system, protease activity increased over vegetation. To illustrate, in the phase of the beginning of booting it was 33.75 CFU, in the earing phase 37.05 CFU, and in the phase of milk-wax ripeness 39.13 CFU. Similar patterns of bulk soil enzymatic activity were obtained in the ecological fertilizer system, which confirms the assumption that moderate application rates of mineral fertilizers enhance soil protease activity. However, in the treatment with purely mineral nutrition, bulk soil protease activity was minimal, which was probably caused by changes in acidity due to the mineral components of fertilizers.

Bulk soil catalase activity over winter wheat vegetation decreased; the lowest indicators were recorded in the phase of milk-wax ripeness of wheat grain. In addition, compared to the industrial fertilizer system, the ecological and biological systems, in the booting phase, demonstrated significantly higher rates of catalase activity compared to the mineral fertilizer system.

In the rhizosphere soil layer of winter wheat, the indicators of soil protease and catalase activity were as high as in the plough soil layer. At the same time, the highest indicators were demonstrated by ecological and biological fertilizer systems compared to the industrial one.

If we compare the activity of protease in the plough soil layer and the rhizosphere, it should be noted that the most significant differences were observed in the industrial fertilizer system. Thus, in the phase of the beginning of booting, the protease activity in the rhizosphere was by 2.18, in the earing phase by 3.91 and the phase of milk-wax ripeness by 6.25 CFU higher than in the plough soil layer. In our opinion, this is due not only to the activation of root secretions of plants but also to the fact that a significant part of the mineral component of rhizosphere fertilizers is absorbed by winter wheat plants during vegetation, and therefore local soil deoxidation occurs, which foster enzymatic activation.

The catalase activity of the rhizosphere soil during winter wheat cultivation under the biological fertilizer system was the highest compared to other fertilizer systems. At the beginning of the booting phase it was 12.9 cm^3O_2/min/g, in the phase of earing 11.74 cm^3O_2/min/g, and in the phase of milk-wax ripeness 10.16 cm^3O_2/min/g.

The results of revealing the peculiarities of the soil enzymatic activity formation under different fertilization systems in the agro-phytocenoses of sugar beet are given in **Table 4**.

Analysis of soil protease activity revealed slightly different patterns compared to the crops of a shorter growing season, such as soybean and wheat. Similarly to other crops under study, the activity of proteases increased from the beginning of vegetation, and in the middle of the vegetation of sugar beet (i.e. in summer), it was the highest. In the autumn, at the phase of the technological maturity of sugar beet, peroxidase activity of the soil decreased, which is quite natural. After all, in autumn, all microbiological processes slow down and stop closer to the period of steady fall of temperature.

The lowest values of soil protease activity, in the two-four leaf phase of sugar beet, were observed in the industrial fertilizer system both in the upper soil layers (16.30 CFU) and 0−30 cm layer (18.47 CFU)

Despite the fact that the dynamics of changes in soil protease activity increased during sugar beet vegetation, this treatment was characterized by lower indicators compared to ecological and biological fertilizer systems.

Noteworthy, better indicators of soil protease activity were observed in the 10−30 cm soil layer compared to the 0−10 cm layer. In our opinion, this was facilitated not only by the activation of microbiological activity in the

Table 4. Dynamics of soil enzymatic activity in different fertilizer systems in soybean agro-phytocenoses (2016−2019)

Fertilizer system	Soil layer (cm)	Phenological phase					
		Two−four leaf		Closing rows		Technological maturity	
		Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)
Bulk soil							
Industrial	0−10	16.30	5.32	33.70	7.53	26.20	6.42
	10−30	18.47	5.64	35.90	7.90	28.70	6.63
Ecological	0−10	25.45	7.81	45.00	10.20	30.11	8.35
	10−30	27.10	7.96	47.10	10.63	33.90	8.47
Biological	0−10	32.20	9.12	47.30	11.05	32.20	8.78
	10−30	34.31	9.33	50.20	11.20	36.01	8.98
Rhizosphere soil							
Industrial		18.92	6.79	37.84	8.84	30.25	9.82
Ecological		29.34	8.98	49.98	12.03	34.89	10.00
Biological		35.46	10.85	52.14	14.12	35.63	11.56
$LSD_{0.05\ 0\text{-}10\ cm}$		1.56	0.31	2.14	0.58	1.71	0.44
$LSD_{0.05\ 10\text{-}30\ cm}$		2.35	0.40	2.26	0.68	1.84	0.52
$LSD_{0.05\ rhizosphere}$		2.44	1.02	3.05	0.74	1.92	0.60

Table 5. Dynamics of soil enzymatic activity in different fertilizer systems in soybean agro-phytocenoses (2016−2019)

Fertilizer system	Phenological phase					
	Five-seven leaf		Bolting		Milky-wax ripeness	
	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)	Protease (CFU/40 h/10 g of soil)	Catalase (cm^3O_2/min/g of soil)
Bulk soil						
Industrial	30.15	8.48	33.69	7.92	34.01	7.39
Ecological	37.05	11.47	40.09	10.24	39.65	9.00
Biological	37.22	11.86	40.19	10.66	39.86	9.23
Rhizosphere soil						
Industrial	31.22	8,85	36.16	9.11	37.34	7.71
Ecological	37.17	12.02	42.76	11.45	4212	9.98
Biological	37.41	12.36	43.29	11.67	42.41	10.07
$LSD_{0.05\ soil}$	1.00	0.51	1.09	0.75	1.01	0.46
$LSD_{0.05\ rhizosphere}$	1.12	0.60	1.24	0.89	1.17	0.53

deeper soil layers but also by the fact that primary tillage for sugar beet is performed to a depth of 25−30cm. Therefore microbial community composition significantly varies and harvest residues enter deeper soil layers.

Soil catalase activity at the beginning of the growing season (two-four leaf stage) in the industrial fertilizer system was the lowest.

In the phase of closing the rows, the activity of soil catalase increased in the industrial fertilizer system to 7.53−7.90, and in the biological fertilizer system to 11.05−11.20 cm^3O_2/min/g.

|In the technological maturity phase of sugar beet, soil catalase activity decreased, which may be due to the late harvesting of the roots and the corresponding decrease in the soil microbiological activity in autumn.

Soil protease and catalase activity in the rhizosphere of sugar beet were higher compared to different depths of soil.

The highest indicators of soil protease activity were observed in the biological fertilizer system.

Given in **Table 5** are the peculiarities of enzymatic activity in maize agro-phytocenoses under different fertilizer systems.

Soil protease activity in maize cultivation, as in previous cases, was increasing over vegetation in the studied fertilizer systems. Ecological and biological fertilizer systems demonstrated higher indicators of soil protease activity compared industrial system.

Soil catalase activity decreased over vegetation, with the minimum values falling to the phase of milk-wax ripeness. Compared to the industrial system, the ecological and biological systems ensured better indicators of catalase activity throughout the growing season of maize.

In the rhizosphere layer of grain maize, the indicators of soil protease and catalase activity were higher compared to those in the arable layer.

The catalase activity of rhizosphere soil under the biological fertilizer system was the highest.

Thus, regardless of the type of crop, soil protease activity increased from the beginning to the end of vegetation, with the maximum catalase activity being observed approximately in the middle of vegetation and a decrease to the end of vegetation. The results obtained by us are consistent with the data of other researchers, according to which high levels of humus values increase the activity of hydrolases: proteases, phosphatases, ureases, invertases. At the same time, the activity of oxidoreductase (peroxidase, catalase, polyphenol oxidase) is in inverse dependence on the content of humus in the soil (Tsyuk et al., 2017; Patyka et al., 2017; Borko et al., 2016).

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Taken together, our results indicate that low content of organic matter and humus in the soil is associated

with more intense processes of decomposition of organic residues and synthesis of humic compounds as a result of the activity of redox enzymes: soil protease activity for a growing season increases progressively along with the decrease of catalase activity. According to our observations, the biological system of crops fertilization of a short crop rotation ensures optimal conditions for the course of enzymatic processes, which, obviously, is a consequence of the activation of soil microbial communities.

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