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BIOLOGICAL ACTIVITY OF CHERNOZEMS TYPICAL OF DIFFERENT FARMING PRACTICES

Serhii Rieznik[1], Dmytro Havva[1], Andrii Butenko[2], Konstantin Novosad[1]

[1]*Department of Soil Science, State Biotechnological University, Dokuchaevske Vill, Kharkiv Dist, Kharkiv Reg, Ukraine*
[2]*Sumy National Agrarian University, 160 H. Kondratieva St, UA40021, Sumy, Ukraine*

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Vastutav autor: Corresponding author:	Andrii Butenko

E-mail: andb201727@ukr.net

ORCID:
0000-0002-2703-1021 (SR)
0000-0001-6516-7846 (DH)
0000-0001-9639-9826 (AB)
0000-0003-2043-9160 (KN)

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ABSTRACT. The study aimed to determine the impact of different farming practices (organic and intensive) on the dynamics of potential biological activity of typical chernozem (mollisol). Comparative-profile-genetic, microbiological and mathematical-statistical methods were used for the research. The data obtained during the study of soil biological activity for 2018–2020 were analyzed. The highest population density of Collembola was observed in the variant of fallow soil in the layer of 10–20 cm (111 indiv. $dm^{3\,-1}$) with the lowest amount of Oribatida (32 indiv. $dm^{3\,-1}$). Under conditions of agrogenic use of soils, the predominance of Oribatida over Collembola was recorded. When using green manure in a soil layer of 0–10 cm, the number of Oribatida is 125 indiv. $dm^{3\,-1}$, while Collembola – 50 indiv. $dm^{3\,-1}$. Agrogenic use of chernozems reduces the number of microscopic fungi. The intensive farming system is the reason for the decrease in the number of all ecological and trophic groups of microorganisms in the 0–10 cm layer while increasing their number in the layer of 20–30 cm. Variants of the organic system of agriculture, especially with the use of green manure, contribute to the increase in the number of actinomycetes and amylolytic microbiota, as well as a short-term sharp increase in the number of oligonitrophilic microbiota. Agricultural use of soils reduces the activity of enzymes such as invertase, protease, dehydrogenase and cellulase. However, the activity of urease and catalase – increases in the soils of the organic system of agriculture. Discriminant analysis of biological activity identified three groups of soils, corresponding to different farming systems. This confirms the possibility of using the studied indicators for soil biodiagnostics.

Introduction

At the present stage of soil genesis, the most influential factor there is a human agricultural activity, as evidenced by the huge area ploughed lends and unprecedented rates of soil degradation not only in Ukraine but also around the world (National report..., 2010; FAO, 2020). Agrogenic soil formation is fundamentally different from natural, especially the rate of transformation of organic and mineral parts of the soil. Human agricultural activity often leads to the suppression of soil biosphere functions and harms the ecosystem. The most striking example is arable land, where there is a radical change in the entire biogeocenosis. Intensive tillage, application of mineral fertilizers and pesticides, change of vegetation and alienation of a significant part of biomass and nutrients contribute to the development of degradation processes of soil cover, such as the formation of the arable horizon, erosion, destruction of structural aggregates, compaction, decarbonization, desertification, salinization, reduction of biological diversity *etc*. (FAO and ITPS, 2017; Tikhonenko, 2011; Medvedev, 2008).

In soil biodiagnostics, the most complex sections are biochemical and microbiological characteristics of soils. Microbial communities are a certain ecolotrophic combination of microorganisms of different species that directly affect soil fertility. It is established that a significant part of microorganisms take a active part in the transformation of organic matter and mineral part of the soil. A close positive relationship between crop yields and soil biological activity has been noted by many researchers (Karpenko *et al.*, 2020; Volkohon *et al.*, 2017). The microorganisms studied by us take an active part in the processes of mineralization-synthesis of organic substances in the soil. For example, the number of ammonifying and amylolytic microbiota, which reflect the intensity of mineralization processes, are good indicators of microbiological processes occurring in the soil (Volkohon *et al.*, 2019). Also, various researchers have found that in arable soils there is a decrease in the number of microscopic fungi, but increases the number of phytopathogenic fungi and actinomycetes (Pesakovic *et al.*, 2009; Stanojković *et al.*, 2011).

Nowadays, scientists, not only in Ukraine but all over the world, agree that living organisms are the most informative indicators of changes occurring in the soil (Paz-Ferreiro *et al.*, 2016). Therefore, the study of soil biological activity under different farming practices is especially relevant.

Materials and Methods

Typical deep medium loamy chernozems on the loess (molik, mollisol) located on the territory of Zinkiv district of Poltava region were selected for research (forest-steppe zone, Ukraine). The soils are located on the plateau of the watershed between the rivers Psel and Vorskla.

The selection of individual soil samples took place during 2018–2020 in the fields of farms operating under two radically different systems of agriculture. Organic technology farms abandoned ploughing in 1975, herbicides and other agrochemicals in 1978, and mineral fertilizers a few years later. On another farm, working on traditional technology, a system of different tillage is used: deep loosening, ploughing, disking and cultivation. The technology of growing crops involves the use of seeds, fertilizers and plant protection products only from the best domestic and foreign producers. New agricultural machinery is used in farms and elements of precision agriculture are introduced into production: GPS-monitoring systems, autopiloting, remote sensing methods, yield monitoring, variable sowing rates and differentiated fertilizer application.

The first soil profile is located on a field with an area of 143 hectares, wherein the crop rotation link a vetch yara (*Vicia sativa* L.) is grown for green manure – is a variant of the organic farming system (green manure). The crop rotation is shown in Table 1, and technological operations in Table 2.

The second soil profile is located on a plot that has not been cultivated for over 30 years – it is variant fallow. Legumes, cereals and other wild plants grow in this area. The variant of fallow is controlled.

The third soil profile is located on a field with an area of 94 hectares, where the compost made from cattle manure is applied at a dose of 20 t ha^{-1} – is a variant of the organic farming system (compost).

The fourth soil profile is located on a field with an area of 125 hectares, where use the full range of plant protection products and fertilizers (intensive).

Table 1. Crop rotation and fertilizer system during the research period

Variant	Year		
	2018	2019	2020
Organic farming system (green manure)	Vetch yara on green manure	Winter wheat (green manure, 15 t ha^{-1} of green mass)	Wintering peas – moved corn to silage
Fallow	Weeds		
Organic farming system (compost)	Corn for grain (20 t ha^{-1}of compost from cattle manure)	Oat	Soybeans
Intensive farming system	Corn for grain ($N_{130}P_{30}K_{30}$)	Sunflower ($N_{35}P_{15}K_{30}$)	Corn for grain ($N_{130}P_{30}K_{30}$)

The selection of individual soil samples was carried out in the first decade of May, August and November during 2018–2020. Soil sampling was performed from depths of 0–10, 10–20, 20–30, 30–40 cm. Soil sampling for the study of mesofauna was performed with cylinders with a volume of 1 dm^3. Catch of springtails and oribatides samples were performed in simple Tulgren funnels, followed by their fixation in aqueous-alcohol solution with the addition of 3% glycerol. The number of microarthropods was recalculated by 1 dm^3 in the corresponding soil layer, this method was described by Gilyarov (1975) and Bater (1996).

The number of microorganisms was determined by the method of deep sowing of soil suspension on dense nutrient media, all methods described by Volkohon *et al.* (2010), Shchukovs′kyy *et al.* (2002) and Titova *et al.* (2012). The number of representatives of different ecological and trophic groups of microorganisms was taken into account by sowing dilutions of soil suspension on the following elective nutrient media: meat-peptone agar (MPA), starch-ammonia agar (SAA), peptone-glucose agar – Waxman's agar (PGA), hangry agar (HA), Ashby's agar (ASH), nitrite agar (NA).

The activity of the following soil enzymes was studied: catalase, urease, dehydrogenase, protease and cellulase, all methods described by Khaziev (2005) and Titova *et al.* (2012).

The activity of the enzyme catalase was determined by the gasometric method by Galstyan (Khaziev, 2005). The essence of the method is to determine the amount of oxygen released during the decomposition of hydrogen peroxide. The activity of the enzyme invertase was determined by a modified photocolorimetric (UNICO 1205, USA) method of Khaziev (Khaziev, 2005). The essence of the method is to determine the optical density of the solution after the reduction of copper by glucose and fructose, released during the hydrolysis of sucrose. Urease activity was determined by the colourimetric method of Scherbakova (Khaziev, 2005), by determining the amount of ammonium released using Nessler's reagent (BASF, Germany). Dehydrogenase activity was determined by Galstyan's method, by photocolorimetric determination of the amount of formed triphenylformazan (TFF). Protease activity was determined by the method of Galstyan-Harutyunyan (Khaziev, 2005), a method based on the ability of proteases to decompose the protein substrate into amino acids, followed by photocolorimetric (UNICO 1205, USA) determination of their amount using ninhydrin (Biochem, France). Cellulase activity was determined based on the ability of the enzyme to decompose biopolymers to glucose, the amount of which is determined iodometrically by back titration with sodium hyposulfite (Merck).

Mathematical analysis of the data was performed with Microsoft Excel 2010 and Statgraphics 18.1 trial. Multifactor ANOVA and Discriminant Analysis were used.

Table 2. Technological operations for the period 2018–2020

2018	2019	2020
Variant organic farming system (green manure)		
- harvest of the predecessor in 2017 - earnings of crop residues by a disk cultivator to a depth of 6–8 cm - disking (12–14 cm) - early spring cultivation (4 cm) - pre-sowing cultivation (4 cm) - sowing of vetch yara (continuous sowing) - disking of green manure to a depth (6–8 cm) in two tracks - pre-sowing cultivation (5 cm) - sowing of winter wheat	- early spring harrowing - harvesting by direct combining - harvesting of straw - earnings of crop residues by a disk cultivator (10–12 cm) - cultivation (6–8 cm) - pre-sowing cultivation (5 cm) - sowing of winter peas	- cultivation (6–8 cm) - earnings of crop residues by a disk cultivator to a depth of 12–14 cm - pre-sowing cultivation (6 cm) - sowing corn for silage because the peas are gone - two inter-row cultivation - collection of green mass on a silo - cultivation (12–14 cm)
Variant organic farming system (compost)		
- harvest of the predecessor in 2017 - earnings of crop residues by a disk cultivator (6–8 cm) - disking (12–14 cm) - rolling by heavy ring- spur rollers (spring) - export and application of humus (compost) - cultivation to a depth of 6–8 cm - pre-sowing cultivation (6 cm) - sowing of corn - harrowing of ladders - three inter-row cultivation, and the last with hilling - harvesting - earnings of crop residues by a disk cultivator (6–8 cm) - cultivation to a depth of 12–14 cm	- spring provocative cultivation (3–4 cm) - pre-sowing cultivation (4 cm) - sowing of oats - post-emergence harrowing - harvesting by a separate method - harvesting of straw - earnings of crop residues by a disk cultivator (10–12 cm)	- early spring harrowing - pre-sowing cultivation (4 cm) - soybean sowing - pre-sowing cultivation (4 cm) - new soybean sowing - post-emergence harrowing - three inter-row cultivation - harvesting by direct combining - earnings of crop residues by a disk cultivator (12–14 cm) - deep loosening to a depth of 26 cm
Variant intensive farming system		
- harvest of the predecessor in 2017 - disking (12–15 cm) - deep loosening 35–37 cm (autumn) - application of urea 250 kg ha^{-1} (spring) - cultivation (12–15 cm) - discussing (8–10 cm) - sowing of corn together with the introduction of a diamophos of 120 kg ha^{-1} 10:26:26 - introduction of soil herbicide - care 1–2: application of insurance herbicide + foliar fertilization - care 3: application of insecticide (on the panicle) - harvesting by direct combining - disking (12–15 cm) - ploughing (25–28 cm)	- application of Ammonium Sulfate 100 kg ha^{-1} - cultivation (12–15 cm) - harrowing - sowing of sunflower with the introduction of complex fertilizers 115 kg ha^{-1} 8:24:24 - introduction of soil herbicide - care 1: herbicide around the perimeter of the field and inter-row tillage - care 2: application of graminicide, fungicide, growth regulator and feeding on the leaves (4–5 pairs of true leaves) - care 3: application of insecticide, fungicide and foliar fertilization (asterisk) - harvesting by direct combining - disking (12–15 cm) - deep loosening (35–37cm) - application of urea 250 kg ha^{-1} – cultivation (12–15 cm)	- harrowing - sowing of corn together with the introduction of a diamophos of 125 kg ha^{-1} 9:25:25 - introduction of soil herbicide - care 1: application of insurance herbicide + foliar fertilization (3–5 leaves) - care 2: foliar fertilization (7–8 leaves) - care 3: application of insecticide (on the panicle) - harvesting by direct combining - disking (12–15 cm) - deep loosening (35–37cm) - application of urea 250 kg ha^{-1} - cultivation (12–15 cm)

Results and Discussion

Results (Table 3) indicate a decrease in the number of collembolas and a simultaneous increase in the number of oribatids in the soils of agrocenoses, and their ratio (Acari / Collembola) is 0.5–1.4, which according to other researchers (Ponge *et al.*, 2003; Kalynovskyi, 2014; Coulson *et al.*, 2015) is characteristic of forest cenoses and disturbed soils. Under the fallow area, on the contrary, the number of colembols is 2.2–3.4 times greater. The application of organic fertilizers, especially green manures, in the variants of the organic system of agriculture contributes to the increase in the number of collembolas and oribatids compared to the variant of intensive farming practices.

Studies have shown that the number of microarthropods has a weak correlation with ecolotrophic groups of microorganisms. There was also a moderate correlation between the number of oribatides and cellulase activity r = 0.43 and a significant correlation between the number of colembols and invertase activity r = 0.53.

The largest number of microscopic fungi was recorded in the soil layer of 0–10 cm variant of fallow (PGA = 5.39 CFU*10^3 per 1 g dry soil). Agricultural use of soils reduces the number of micromycetes by 1.5–2.0 times.

Table 3. The average number of microarthropods in typical chernozems under different farming practices (2018–2020)

Variant	Depth, cm	Collembola, indiv. $dm^{3\ -1}$	Oribatida, indiv. $dm^{3\ -1}$
Green manure	0–10	50	125
	10–20	47	70
	20–30	30	51
	30–40	22	51
Compost	0–10	55	75
	10–20	56	53
	20–30	39	28
	30–40	29	27
Fallow	0–10	101	43
	10–20	111	32
	20–30	71	27
	30–40	59	27
Intensive	0–10	50	82
	10–20	56	89
	20–30	37	86
	30–40	49	53
Farm system	S.E.	4.02	4.17
	F-Ratio	9.1	14.32
	P-Value	>0.001	>0.001
	$LSD_{0.5}$	11.18	11.59
Soil layer	S.E.	3.9	4.05
	F-Ratio	28.74	27.23
	P-Value	>0.001	>0.001
	$LSD_{0.5}$	10.85	11.26

Organic farming, especially the application of green manures, increases the number of amylolytic microbiota (Green manure, 0–10 cm – 2.84 CFU*10^6 per 1 g dry soil).

Mathematical and statistical analysis (Table 4) showed no significant difference between the options for the number of actinomycetes, ammonifying and oligotrophic microbiota (P-Value: MPA = 0.9964, ASH = 0.6772, HA = 0.9678, NA = 0.937, SAA actinomycetes = 0.0746). A significant difference was recorded only in the number of micromycetes and amylolytic microbiota ($P < 0.05$).

The number of aerobic microbiota decreases sharply from a depth of 20–30 cm. An intensive farming system causes a decrease in the number of microorganisms in the soil layer 0–10 cm and an increase in their number in the soil layer 20–30 cm compared to other options. This is due to ploughing and mixing different layers of soil with plant debris. Our data are consistent with the results of research by other scientists: Bulyhin *et al.* (2018), Tsova (2016), Araujo *et al.* (2010).

Table 4. The average number of ecological and trophic groups of microorganisms in chernozems typical of different farming practices (2018–2020)

Variant	Depth, cm	PGA	SAA actinomycetes	SAA	MPA	ASH	HA	NA
		CFU*10^3 per 1 g dry soil		CFU*10^6 per 1 g dry soil				
Green manure	0–10	2.75	25.43	2.84	2.62	2.91	4.99	0.69
	10–20	2.14	19.00	1.86	2.69	1.42	4.55	0.44
	20–30	1.08	10.09	0.72	0.63	0.88	1.01	0.26
	30–40	0.86	4.32	0.42	0.53	0.51	0.76	0.20
Compost	0–10	3.06	21.52	2.08	2.45	2.07	4.19	0.58
	10–20	2.15	16.47	1.72	2.48	1.92	4.11	0.50
	20–30	1.09	7.13	0.73	0.92	0.83	0.52	0.25
	30–40	0.63	5.28	0.44	0.62	0.54	0.37	0.20
Fallow	0–10	5.39	16.06	1.78	2.45	1.72	4.26	0.58
	10–20	2.96	11.25	1.07	2.63	1.23	4.69	0.45
	20–30	1.75	5.48	0.63	0.64	0.74	0.61	0.23
	30–40	0.91	3.63	0.30	0.46	0.48	0.47	0.19
Intensive	0–10	3.20	14.02	1.55	2.06	1.70	3.50	0.50
	10–20	2.55	12.61	1.60	2.54	1.55	4.49	0.50
	20–30	1.46	6.05	0.92	1.17	1.11	0.71	0.41
	30–40	1.13	5.73	0.49	0.79	0.75	0.43	0.26
Farm system	S.E.	0.16	1.00	0.09	0.17	0.14	0.54	0.03
	F-Ratio	2.77	2.32	4.36	0.02	0.51	0.09	0.14
	P-Value	0.0411	0.0746	0.0048	0.9964	0.6772	0.9678	0.937
	$LSD_{0.5}$	0.45	2.78	0.23	–	–	–	–
Soil layer	S.E.	0.16	0.97	0.09	0.16	0.14	0.52	0.03
	F-Ratio	49.29	42.42	51.86	31.61	21.17	11.25	29.31
	P-Value	0,0000	>0.001	>0.001	>0.001	>0.001	>0.001	>0.001
	$LSD_{0.5}$	0.44	2.7	0.26	0.46	0.39	1.46	0.09

The obtained data indicate a decrease in the activity of such enzymes as invertase, protease, dehydrogenase and cellulase under agricultural use of chernozems typical compared to the fallow (Table 5). However, in organic farming, the activity of urease and catalase is much higher than in fallow. The activity of enzymes with depth decreases. Only in the case of an intensive system of agriculture because of mixing and rotation of the formation (ploughing), there is a homogenization of the 0–30 cm layer, which leads to a partial alignment of indicators at these depths. A feature of the intensive farming system is not a typical increase in urease activity at a depth of 10–20 cm, which is a consequence of the introduction of urea (14.6 mg NH_3 per 10 g of soil for 24 hours). The application of organic fertilizers in the variants of the organic system of agriculture (especially the use of green manure) increases the activity of all studied enzymes in comparison with the soil of the variant of the intensive system of agriculture, similar results were obtained in studies Kwiatkowski *et al.* (2020), Fließbach *et al.* (2007), Woźniak (2019).

Table 5. Enzymatic activity of chernozems under different farming practices, the average for the years 2018–2020

Variant	Depth, cm	Catalase, cm^3 O_2 per 1 g of soil for 1 min	Invertase, mg of glucose per 1 g of soil for 24 hours	Urease, mg NH_3 per 10 g of soil for 24 hours	Dehydrogenase, mg of TFF per 10 g of soil for 24 hours	Protease, mg of glycine per 1 g of soil for 24 hours	Cellulase, µg of glucose per 10 g of soil for 48 hours
Green manure	0–10	7.54	29.89	25.61	11.68	17.10	5.93
	10–20	7.36	20.94	18.55	9.43	5.42	5.84
	20–30	7.00	12.82	14.03	6.11	3.59	2.60
	30–40	5.79	7.78	12.20	4.28	4.08	2.10
Compost	0–10	6.35	22.47	16.07	11.83	11.65	6.20
	10–20	6.24	18.83	16.15	9.37	5.91	5.22
	20–30	6.12	11.45	13.28	7.32	3.55	2.60
	30–40	5.46	8.44	11.93	5.90	2.44	1.76
Fallow	0–10	5.63	35.83	14.62	12.37	21.96	6.37
	10–20	4.92	19.41	11.96	9.92	6.70	5.30
	20–30	5.02	15.56	12.05	7.82	3.81	3.08
	30–40	4.39	9.40	10.47	4.73	2.04	1.86
Intensive	0–10	4.28	15.92	13.71	8.88	4.72	6.13
	10–20	4.03	16.12	14.60	9.11	3.66	3.76
	20–30	3.97	15.78	12.34	8.68	3.23	3.13
	30–40	3.57	10.64	12.20	6.84	1.70	2.34
Farm system	S.E.	0.11	0.5	0.59	0.18	0.27	0.16
	F-Ratio	88.65	6.35	6.79	3.58	26.97	1.99
	P-Value	0.0000	0.0003	0.0002	0.0138	0.0000	0.1151
	$LSD_{0.5}$	0.29	1.39	1.64	0.51	0.76	0.43
Soil layer	S.E.	0.1	0.49	0.57	0.18	0.27	0.15
	F-Ratio	38.48	225.49	50.77	289.18	232.63	127.13
	P-Value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	$LSD_{0.5}$	0.28	1.35	1.59	0.49	0.74	0.42

Discriminant analysis (Fig. 1) of indicators of biological activity of chernozem soils makes it possible to distinguish different farming systems by a set of predictors, the values of the canonical correlation are 0.24–0.73 under conditions of statistical significance $P < 0.05$. As a result of mathematical and statistical analysis, only those indicators were selected that significantly affect the differentiation of chernozems of different uses ($F < 4.0$), and normalized coefficients of discriminant functions were determined.

Equation of discriminant functions:

1) 1,03302*Catalase + 0,343788*Urease – 0,567763*Dehydrogenase + 0,227503*Protease – 0,346053*PGA – 0,484932*MPA + 0,280742*ASH + 0,504131*HA – 0,398958*Collembola + 0,0209006*Oribatida
2) 0,337853*Catalase –0,681677*Urease + 0,0231899*Dehydrogenase + 0,666935*Protease + 0,0159984*PGA –0,554784*MPA –0,214321*ASH + 0,717943*HA + 0,741564*Collembola – 0,851151*Oribatida
3) 0,266271*Catalase –0,434354*Urease + 1,18612*Dehydrogenase –0,677315*Protease –0,603386*PGA + 0,629841*MPA–0,0744966*ASH –0,491181*HA –0,051398*Collembola –0,52884*Oribatida

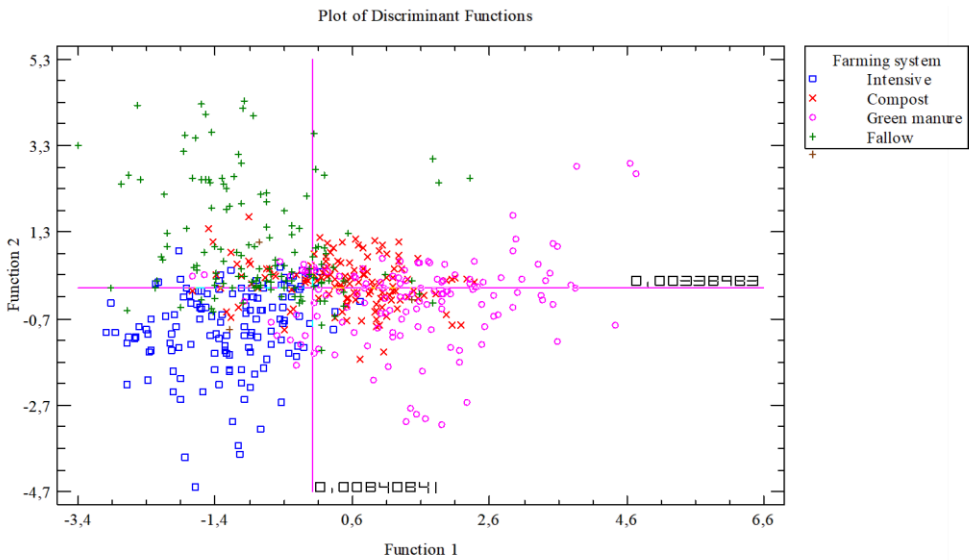

Figure 1. Discrimination of chernozems is typical according to different farming practices on the basis of indicators of biological activity of chernozems (built on Function 1 and Function 2)

According to the equations of functions, the most indicative is the data on the activity of catalase and dehydrogenase, the number of micromycetes, oligocarbophilic and ammonifying microbiota and microsarthropods. Some of these indicators are actively used by other researchers in attempts to mathematically model the relationship of microbiological activity with indicators of fertility and soil formation processes and the impact of weather conditions on them (Steinweg *et al.*, 2012; Demyanyuk *et al.*, 2017; Demydenko, 2021; Hryhoriv *et al.*, 2021; Kvitko *et al.*, 2021).

As a result of the performed discriminant differentiation, 66.5% of the data sample by agricultural systems were reliably classified. For the most part, classification errors occurred between samples of the organic farming system using green manure and compost. According to the above figure, among the clusters of predictors, three groups of indicators are quite clearly distinguished, which are variants of the intensive system of agriculture, fallow and organic system of agriculture

Conclusions

Mathematical modelling development of chernozem soils under different farming practices indicates significant changes in soil formation processes under the influence of human agricultural activity and allows distinguishing "agrochernozem" from natural analogues.

It is recommended to use 10 indicators of potential biological activity for bioindication of chernozem soils, namely: the number of microarthropods, micromycetes, ammonifying and oligotrophic microbiota, catalase activity, dehydrogenase, urease and protease.

Agrogenic soils are characterized by a decrease in the number of colembols with a simultaneous increase in the number of oribatids.

Agrogenic soils are characterized by a decrease in the number of micromycetes and *vice versa* by an increase in the number of actinomycetes and amylolytic microbiota.

Soils in the conditions of the intensive system of agriculture are characterized by a decrease in the activity of soil enzymes in comparison with a fallow. Whereas the organic farming system helps to increase the activity of urease and catalase, even in comparison with fallow land.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

SR – writing a manuscript, analysis and interpretation of data;
SR, DH – acquisition of data, author of the idea, guided the research;
AB – analysis and interpretation of data and is the corresponding author;
KN – critical revision and approve the final manuscript.

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