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MICROBIOLOGICAL AND ENZYMATIC ACTIVITY OF TYPICAL CHERNOZEM UNDER DIFFERENT TILLAGE AND FERTILIZATION SYSTEMS

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Abstract

Studies (2021-2023) conducted on a typical low humus chernozem (Bila Tserkva, Ukraine) showed that in the arable layer, microbiota on starch-ammonium agar (SAA) is highest in non-fertilized plots under differentiated tillage and in fertilized plots under disc tillage. On meat peptone agar (MPA), the highest microbiota activity is observed under differentiated tillage across all plots. Microbiota on pectin-glucose agar with soil extract (PGASE), as well as nitrifying bacteria and actinomycetes, had the highest number under differentiated tillage, while the mineralization and pedotrophic coefficients had the lowest values. The lowest number of denitrifying bacteria and the highest number of nitrogen bacteria were recorded in the differentiated tillage. The activity of invertase, phosphatase dehydrogenases and polyphenol oxidases is higher, while catalase activity is lower under differentiated tillage compared to mouldboard and mouldboardless tillage. The coefficient of humus accumulation was higher under differentiated tillage and significantly lower under chisel and disking tillage. Increasing organic and mineral fertilizer rates with mouldboardless and disc tillage enhances the biological diversity of the arable layer.

Key words*: fertilization, crop rotation, microbiota, enzymes, activity, productivity.*

INTRODUCTION

Soil monitoring in Ukraine is primarily focused on controlling agro-physical and agrochemical fertility indicators, and in some cases, assessing potential weed infestation. However, the concept of soil fertility requires consideration of its biotic parameters, not only in terms of quantity but also in relation to the structure of the biota (megafauna, macrofauna, mesofauna, microfauna, and microflora) (Prymak et al., 2023). This will ensure soil "health" through microbial diversity (Frunze N., 2015). In one gram of soil, the number of bacteria ranges from 300,000 to 95 million, and in some cases, up to 4 billion (Storchous, 2013) according to other researchers, this number ranges from 3.7 to 5.7 billion (Ostapchuk et al., 2016). Understanding the processes of microbial communities in agricultural soils is essential for developing optimal and energy-efficient agroecosystems (Shi et al., 2019).

The most important indicators of soil fertility are the abundance and structure of the microbiota, with enzymes playing a crucial role. Soil enzymes are the most stable component of its biological activity, as they can be adsorbed by soil particles after cell death. The primary source of enzymes in the soil is plant residues (Frunze, 2013). Soil microorganisms are the primary source of enzymes and play a crucial role in maintaining key nutrient cycles in the soil (C, N, P, S) by transforming them from organic matter (Aon et al., 2001).

In a broad sense, the enzymes present in the soil can be classified as intracellular and extracellular (Dick, 1994). The first category mainly includes enzymes associated with viable proliferating cells, while extracellular enzymes are those released into the surrounding environment through secretion or lysis, as well as active enzymes linked to dead cells and other non-living soil fractions (Sinsabaugh & Moorhead, 1994). The activity of soil enzymes is a key candidate for "sensors," as they integrate information, on the one hand, from the microbial state, and on the other hand, from the physico-chemical conditions of the soil (Aon et al., 2001).

Experiments suggest that soil biota depends not only on the ecological properties and tillage of the soil but also on the historical context, meaning the colonisation and extinction of certain species (Bender et al., 2016). The importance of historical contingency for community assembly has also been demonstrated in the secondary succession of vascular plants (Ejrnæs et al., 2006). Shafi et al. (2007) demonstrated that crop rotation systems increase the amount of nitrogen (N) available in the soil, thereby reducing the need for fertilisation, while promoting plant growth and health. Crop rotation, combined with long-term fertilisation, led to an increase in microbial diversity in the maize rhizosphere. A higher abundance of Acidobacteria, Actinobacteria, and Proteobacteria was found in fields where crop rotation was practised, compared to monoculture systems (Soman et al 2017).

The predominance of pathogenic or symbiotic microorganisms also depends on the physical properties of the soil: density, porosity, aeration, water-air and thermal regimes, as well as the systems of its cultivation (Prymak et al., 2022; Usharani et al., 2019).

Deep ploughing creates a shock state for the soil microbiota, with aerobic microflora being transferred to the anaerobic conditions of the lower part of the plough layer, while anaerobic microflora is, conversely, exposed to aerobic conditions. As a result, there is a significant decrease in the abundance of soil microbiota. This can be altered by using a non-inversion tillage system over 4-5 years. In most cases, with such soil cultivation, cellulose-degrading microbiota are more abundant in the upper part of the plough layer, while their numbers in the lower part remain the same or even lower (Nadtochii et al., 2010).

The activity of three enzymes (dehydrogenase, phosphatase, arylsulfatase) was higher in fields without tillage than in fields with conventional tillage (Bergstrom et al., 1998). No-tillage (NT) systems lead to an increase in the concentration of nutrients, organic matter, and pesticides on the soil surface (Dick, 1992). The activity of soil urease, acid phosphatase, and protease at a depth of 0–10 cm, as well as the activity of acid phosphatase, dehydrogenase, arylsulfatase, invertase, amylase, and urease at a depth of 0-7.5 cm, was significantly higher in the soil without tillage (NT) than with ploughing (Bolton et al., 1985).

The composition of the rhizosphere bacterial community can vary significantly under different soil cultivation methods. Rhizosphere bacterial communities under no-tillage conditions are found to be more stable than under plough tillage, which may be related to the relatively low disturbance of the soil and the unique environment of the rhizosphere under no-tillage conditions (Wang et al., 2020).

Mouldboard & mouldboardless (differentiated) tillage activates biological processes in typical chernozem, including the release of carbon dioxide and its assimilation by soil microbiota, cellulolytic, nitrification, and enzymatic activity. Under differentiated tillage, nitrifying bacteria prevail, while under disking (continuous shallow) tillage, ammonifying bacteria dominate (Voitovyk & Zhovtun, 2024).

The results of a long-term soil tillage experiment conducted on sandy soil in Lithuania showed that the use of sustainable soil cultivation can protect soils from biological degradation and maintain soil quality compared to conventional tillage. The intensity of soil tillage positively affected microbial substrate utilisation and urease activity, but negatively impacted dehydrogenase activity, the number of bacteria and fungi, and the Shannon microbial community diversity index. Higher total porosity stimulated higher enzyme activity; however, microbial activity negatively correlated with bulk density (Janušauskaite et al., 2013).

Legrand et al. (2018) assessed the impact of soil tillage on bacterial and fungal diversity and found an effect on community composition, along with a somewhat positive impact of reduced tillage on diversity indices, but not on saturation. Snell Taylor et al. (2018) noted that long-term management with no-tillage significantly increased the size of soil microbial communities while negatively affecting bacterial diversity. Sengupta A. & Dick W. A. (2015) concluded that higher soil tillage intensity leads to a lower number of dominant bacterial species.

Soil tillage prepares the soil through mechanical disruption and has a number of negative impacts on the soil environment of agricultural lands, causing degeneration and

serious disruption of the soil ecosystem (Knowler & Bradshaw, 2007). In particular, prolonged soil tillage leads to negative consequences for soil health, such as nutrient loss and soil compaction (Foley et al., 2011). As an alternative, zero tillage, as a conservative practice for minimal soil disturbance, is widely used to increase and stabilise yields (Knapp & van der Heijden, 2018). Long-term implementation of zero tillage improves soil quality and the soil environment (Scopel et al., 2013). Zero tillage can increase the diversity of soil microbial communities (Wang et al., 2017; Mathew et al., 2012; Dong et al., 2017). Overall, enzyme activity in the soil increases with the minimisation of mechanical tillage. An active microbial community in the rhizosphere of winter rye plants with an intensive enzymatic complex is observed under strip-till cultivation (Vilnyi & Maklyuk, 2014).

Soil fertilisation is necessary to meet the growing demand for livestock feed and human food. However, fertilisation has both short-term and long-term effects on soil microbiota. This, in turn, can influence plant viability and growth (Kurzemann et al., 2020; Grabovskyi et al., 2024).

Mineral and organic fertilisers change the composition of the microeukaryote community, with only organic fertilisers having a dose-dependent effect on the microeukaryote community in the soil (Ding et al., 2019). However, compared to mineral fertilisers, organic fertilisers can retain a greater diversity of microbes in the soil environment (Ye et al., 2019).

The use of chemical fertilisers changes the abundance of microbial populations and stimulates their growth by adding nutrients. However, overall data showed that chemical fertilisers do not have a significant impact on the richness and diversity of bacteria and fungi. Instead, a large number of individual bacterial or fungal species were sensitive to fertiliser application, which was primarily explained by changes in the chemical properties of the soil caused by mineral or organic fertilisation. Among the negative effects of chemical fertilisation, a decrease in enzymatic activity has been highlighted in several studies, especially in soils that received the highest amounts of fertilisers, along with losses of organic matter (Dincă et al., 2022).

Based on the analysis of microbial communities from datasets of 64 long-term trials worldwide, it was concluded that the application of mineral fertilisers leads to a 15.1% increase in microbial biomass compared to non-fertilised control plots; additionally, the use of nitrogen (urea and ammonium fertilisers) can have a temporary or stable effect on increasing soil pH (Geisseler & Scow, 2014; Wang et al., 2020).

The application of nitrogen increases the potential nitrification speed but reduces nitrogen use efficiency and changes the beta-diversity of ammonia-oxidising bacteria (AOB), decreasing the relative abundance of *Nitrosospira* (nitrite-oxidising bacteria) and increasing the relative abundance of *Nitrosomonas* (which oxidise ammonia to nitrite) (Zou et al., 2022). On the other hand, the application of mineral and organic fertilisers significantly increased the species richness and alpha-diversity of AOB (Tao et al., 2017).

In general, organic and mineral fertilisers typically have a positive effect on numerous soil bacteria, the most common of which is *Azotobacter*. For example, the use of mineral fertilisers on semi-arid alfisols led to an increase in *Azotobacter* abundance (Cinnadurai et al., 2013). Similar results were obtained in maize crops (Adediran et al., 2003). Alternatively, intensive fertilisation with mineral nitrogen can negatively impact other specific bacterial groups (e.g., diazotrophs, beta-proteobacteria), which are important rhizospheric microbes with symbiotic N-fixing interactions with leguminous plants.

Mineral fertilisers have also been noted to have a negative impact on soil microbiota. Long-term use of mineral fertilisers leads to the loss of soil organic matter (SOM), particularly in arid and semi-arid regions or where monoculture practices (e.g., maize) are employed (Luo et al., 2015; Zhang et al., 2010). Prolonged use of mineral fertilisers can be detrimental, especially with high nitrogen fertiliser dozes, as it leads to increased gaseous nitrogen losses and deterioration of the soil's physical, chemical, and biological properties (Schjønning et al., 2018). It has also been found that mineral fertilisers cause a reduction in soil porosity and nutrient availability (Song

et al., 2015). Additionally, mineral fertilisers have a significant effect on the abundance of microorganisms and the qualitative selection of entire soil microbial communities (Doran & Zeiss, 2000). Dangi et al. (2020) suggested that the use of organic fertilisers or amendments may potentially mitigate the harmful effects of inorganic fertilisers on the environment in agroecosystems, but they may also impact soil microorganisms, whose specific effects are not yet clearly defined. They found that soil amendments, such as biochar or the addition of other organic fertilisers over approximately two years, influenced microbial community biomass, composition, and yield.

Based on a review of the literature, the impact of specific soil cultivation and fertilisation technologies on agricultural crops has been established (Fernandez et al., 2019; Legrand et al., 2018; Huang et al., 2013; Mikanová et al., 2009; Mathew et al., 2012; Prymak et al., 2024; Grabovskyi et al., 2023). However, information on the microbiological condition of soils under different cultivation technologies and fertilisation systems is insufficient and fragmented.

The aim of our research was to determine the impact of basic soil tillage systems and fertilisation on the change in the microbial community and enzymatic activity of the plough layer of typical chernozem.

MATERIALS AND METHODS

The research was conducted in the experimental field of Bila Tserkva National Agrarian University in 2021-2023 on typical deep low-humus black soil in a stationary five-field crop rotation.

The scheme of the experiment included four systems (variants) of basic tillage (factor A) (Table 1) and four fertilizers (factor B) systems were studied: 0 - without fertilizers, 1 - 6 t/ha of manure + $N_{54}P_{48}K_{48}$, 6 t/ha of manure + $N_{92}P_{66}K_{90}$, 3 – 6 t/ha of manure + $N_{120}P_{92}K_{110}$.

Table 1. Systems of basic tillage in crop rotation

№ field	Crop	Tillage (factor A)			
		I	II	III	IV
		mouldboard (control)	mouldboardless	mouldboard & mouldboardless (differentiated)	disking (continuous shallow)
		Depth, cm			
1	Soybean	10-12	20-22	10-12	10-12
2	Winter wheat	6-8	6-8	6-8	6-8
	white mustard on green manure	10-12	10-12	10-12	10-12
3	Corn	25-27	25-27	25-27	10-12
4	Spring barley	10-12	10-12	10-12	10-12
	white mustard on green manure	10-12	10-12	10-12	10-12
5	Sunflower	25-27	25-27	25-27	10-12

Crop rotation fields were fully deployed in space and time. In the experiment, threefold repetition was placed completely on the area, plots of the first order (tillage system) – sequentially in one tier, the second (fertilizer rates) – sequentially in four tiers. The sown area of the elementary plot was 171 m^2 (9 x 19 m) and the accounting area was 112 m^2 (7 x 16 m). The area of each field is 7835.6 m^2 (76×103.1) and the total area under the experiment is 3.7 hectares. Cattle manure, ammonium nitrate, simple granulated superphosphate, and potassium salt were used as fertilizers. Microbiological and enzymatic activity of typical chernozem was determined by the methods Hrytsayenko et al., 2003, Gorodniy et al., 2005.

RESULTS AND DISCUSSIONS

The soil microbiota in areas with crop rotation consists of 76-93% bacteria, 9-25% actinomycetes, and 0.2-0.5% fungi (Table 2). The placement of organic and mineral fertilisers, as well as plant residues in the upper part of the plough layer (0-10 cm), under mouldboardless and disking tillage systems, facilitated the intensive development of

microbiota. In the lower part of the plough layer (20-30 cm), this process was hindered due to the lack of energy material for the microbiota.

Table 2. Microbiota abundance in the plough layer (0-30 cm) of typical chernozem under different tillage and fertilisation systems, individuals/g of dry soil

Indicators	Soil cultivation systems (factor A)							
	mouldboard (control)		mouldboardless		mouldboard & mouldboardless (differentiated)		disking (continuous shallow)	
	Fertiliser systems (factor B)							
	without fertilizers	6 t/ha of manure + $N_{120}P_{92}K_{110}$	without fertilizers	6 t/ha of manure + $N_{120}P_{92}K_{110}$	without fertilizers	6 t/ha of manure + $N_{120}P_{92}K_{110}$	without fertilizers	6 t/ha of manure + $N_{120}P_{92}K_{110}$
Microbiota on SAA, mln	27.67	42.71	27.46	43.81	28.37	42.38	28.24	46.31
Microbiota on MPA, mln	11.81	14.12	11.21	13.45	12.94	15.06	10.94	13.29
Actinomycetes, mln	3.70	6.23	3.53	5.80	4.00	7.13	3.24	5.40
Fungi, ths.	12.42	17.20	14.71	21.33	13.22	18.77	15.15	22.04
Ammoniators, ths.	28.60	83.30	25.70	72.30	28.00	80.10	24.90	71.00
Cellulose-degrading bacteria, ths.	112.40	154.5	105.70	140.80	110.30	150.80	101.1	126.70
Denitrifiers, ths.	42.30	71.20	45.10	78.30	39.30	66.10	46.7	80.50
Nitrifiers, ths.	4.20	7.30	4.03	6.80	4.48	7.80	3.88	6.58
Microbiota on pectin-glucose agar with soil extract (PGASE), mln	19.68	33.79	15.26	23.42	21.63	35.47	15.87	26.36
Phosphorus-binding bacteria, ths.	30.30	41.30	29.00	39.6	29.80	40.5	28.40	38.70
Mineralisation ratio (SAA:MPA)	2.34	3.00	2.45	3.26	2.19	2.81	2.58	3.48
Pedotrophicity index (PGASE:MPA)	1.67	2.39	1.36	1.74	1.67	2.36	1.45	1.98
Azotobacter, colonies on soil plates	141	190	134	177	143	194	132	175

The number of microbiota in the 0-10 cm soil layer on meat peptone agar (MPA) and starch-ammonium agar (SAA) (which consume organic and mineral nitrogen, respectively), including actinomycetes, fungi, cellulolytic and nitrifying bacteria, was higher by 16% and 28%, 24%, 29%, 27%, and 25% under mouldboardless tillage, and by 53% and 86%, 34%, 43%, 41%, and 32% under disking tillage, compared to the control.

In the 20-30 cm soil layer, these values were lower by 37 and 10, 38, 33, 35, and 44% under chiselling and 39 and 6, 62, 41, 42, and 61% under disking compared to the control. Conducting deep ploughing during the crop rotation period in one field noticeably, and in two fields – significantly, reduces the heterogeneity of the arable layer based on these indicators.

In the arable layer of soil, the microbiota counts on SAA increased by 2.6% only on fertilized plots under chiseling compared to the control. The highest values were observed on unfertilized plots under differentiated soil tillage and on fertilized plots under disking. On MPA, the microbiota was highest under mouldboard-mouldboardless tillage and lowest under disking.

The coefficient of mineralization of soil organic matter on fertilized and unfertilized plots is higher by 5% and 9% under mouldboardless tillage and 10% and 16% under disking compared to the control. Under differentiated tillage, it is 6% lower than the control. Incorporating plant residues into the lower part of the arable layer of typical chernozem increases this coefficient in the 0-10 cm soil layer compared to disking. A narrower carbon-to-nitrogen ratio in humus increases the proportion of microbiota on SAA relative to its

total count in the soil environment, ultimately raising the mineralization coefficient.

In the lower part (20-30 cm) of the arable layer of typical chernozem, the ratio of microbiota on SAA and MPA equalizes on the plots treated with mouldboard ploughing. Under mouldboardless and disking tillage, this ratio increases due to the significant reduction or absence of plant residues entering the lower part of the arable layer.

The number of actinomycetes in the arable layer on non-fertilized and fertilized plots is 5% and 7% lower under mouldboardless tillage, 12% and 13% lower under disking tillage, and 8% and 14% higher under differentiated tillage, compared to the control. The fungal microbiota count is higher in the mouldboardless tillage variants by 18% and 24%, in the differentiated tillage variants by 13% and 19%, and in the disking tillage variants by 22% and 28%.

The highest number of ammonifying bacteria was recorded in the arable layer under mouldboard & disking tillage; under mouldboardless, differentiated, and disking tillage, their count was 10% and 13%, 2% and 4%, and 13% and 15% lower, respectively, on non-fertilized and fertilized plots. A similar pattern was observed for cellulose-degrading bacteria, where the decrease was 6% and 9%, 2%, 10%, and 18%, respectively. Being typical representatives of the mineralization block of the soil microbiota, their numbers significantly respond to the mass and quality of plant residues. Their share in the upper, middle, and lower parts of the arable layer was 34%, 35%, and 31% under mouldboard & disking tillage; 45%, 34%, and 21% under mouldboardless tillage; 40%, 34%, and 26% under mouldboardless-mouldboard tillage; and 51%, 31%, and 18% under disking tillage.

Denitrifying bacteria in the arable layer were 8% and 12% higher under mouldboardless and disking tillage, respectively, and 7% lower under differentiated tillage compared to the control. For nitrifying bacteria, the opposite trend was observed: their count was 4-7% and 8-10% lower on chisel and mouldboardless-mouldboard tillage plots, respectively, and 7% higher under disking tillage compared to the control. A similar pattern, even more pronounced, was observed regarding the microbiota count on pectin-glucose agar with soil extract (PGAE). In this regard, the first tillage variant showed an advantage over the second and fourth variants by 22-31% and 19-22%, respectively.

The pedotrophic index, which characterizes the intensity of organic matter utilization in the soil, was almost at the same level under mouldboard & mouldboardless tillage and was 19-27% and 13-17% lower under chisel and disking tillage, respectively, compared to the control.

The population of *Azotobacter* in the plough layer, whose activity largely depends on the soil aeration, exceeded the control by only 1-2% under differentiated soil tillage, while under chisel and disc tillage, it was reduced by 5-7% and 6-8%, respectively.

Invertase activity depends on the fertilisation system, depth, and method of tillage of typical chernozem. The localisation of plant residues in the upper part of the plough layer under mouldboard and disc tillage increased this indicator by 8% and 12%, respectively, compared to ploughing at a depth of 25-27 cm. In the lower layers (10-20 and 20-30 cm), the opposite trend was observed: invertase activity was 11% and 21% higher in the ploughed plots. Overall, in the crop rotation, invertase activity in the plough layer was 5-7% lower under mouldboard and shallow tillage and 5-7% higher under mouldboard & mouldboardless tillage, compared to the control (Table 3).

Urease activity in the upper part of the plough layer was also higher under mouldboard and disc tillage by 11% and 16%, respectively, compared to the control. Overall, across the crop rotation, urease activity in the plough layer was nearly the same under mouldboard & mouldboardless and differentiated tillage, but significantly lower in the fertilised plots with mouldboard and disc tillage compared to the control.

Protease activity characterises the intensity of hydrolysis of organic compounds of a protein nature into peptides, which are subsequently broken down into amino acids. These amino acids undergo ammonification and nitrification, leading to the formation of plant-accessible forms of soil nitrogen. A certain proportion of amino acids condenses with oxidised forms of aromatic compounds, contributing to the formation of soil humus. Protease activity in

different layers of the arable soil (0-10, 10-20, and 20-30 cm) varied across soil tillage options similarly to invertase and urease activity. Overall, across the crop rotation, the protease activity in the 0-30 cm soil layer under mouldboardless tillage slightly decreased, remained unchanged under differentiated tillage, and significantly decreased under disking, compared to the control.

Table 3. Enzymatic activity of tilthy soil under different tillage and fertilisation systems

Soil cultivation systems (factor A)	Fertiliser systems (factor B)	Invertase, mg of glucose per 1 g of soil per 24 hours	Urease, mg of $N\text{-}NO_3$ per 100 g of soil in 3 hours	Proteases, mg of amine nitrogen per 100 g of soil per 20 hours	Phosphatase, mg P_2O_5 per 100 g of soil per 48 hours	Dehydrogenases, Lenard optical density units	Catalase, ml of dry soil O_2 per 1 minute	Polyphenol oxidases (mg of purpurgalin per 100 g of soil in 30 minutes)	Peroxidases (mg of purpurgalin per 100 g of soil in 30 minutes)	Humus accumulation coefficient
mouldboard (control)	0	8.01	2.37	111	1.6	0.148	2.31	58	108	54
	1	9.23	3.31	141	2.7	0.291	2.58	81	117	69
	2	10.04	3.54	150	3.5	0.341	2.71	96	123	78
	3	10.83	3.78	158	3.8	0.360	2.78	107	132	81
mouldboardless (chisel)	0	7.49	2.24	108	1.7	0.142	2.45	51	102	50
	1	8.67	3.13	136	2.9	0.279	2.75	73	112	65
	2	9.47	3.34	144	3.7	0.325	2.89	88	121	73
	3	10.27	3.58	152	4.0	0.341	2.99	101	133	76
mouldboard &mouldboardless (differentiated)	0	8.42	2.39	110	1.6	0.163	2.23	66	113	58
	1	9.74	3.35	140	2.8	0.325	2.47	92	121	76
	2	10.62	3.57	151	3.7	0.385	2.58	105	125	84
	3	11.52	3.84	160	4.0	0.409	2.63	112	132	85
disking (continuous shallow)	0	7.50	2.23	104	1.5	0.136	2.55	50	104	48
	1	8.61	3.10	132	2.5	0.260	2.88	70	111	63
	2	9.33	3.30	142	3.3	0.310	3.04	85	120	71
	3	10.03	3.51	150	3.6	0.326	3.13	101	136	74
SD_{05}		0,52	0.15	7	0.4	0.017	0.17	6	8	4

Phosphatases, by catalysing the hydrolysis of organophosphorus compounds, orthoesters of alcohols, and phenols (detaching phosphate groups from organophosphates), facilitate the biochemical mobilisation of organic phosphorus, making it available to plants. The activity of phosphatases in the arable soil did not undergo significant changes across the tillage variants, although a trend of increased activity was observed on fertilised plots under chisel and differentiated tillage, while it decreased under disking.

Dehydrogenases catalyse the dehydrogenation of hydrogen from organic substances in the soil (carbohydrates, aromatic acids, humic acids, amino acids, alcohols, etc.), acting as an intermediate carrier of hydrogen. The detached hydrogen from carbohydrates and organic acids is transferred to organic compounds like quinones or to the oxygen in the air. The oxidation of these organic substances occurs both during mineralisation and humification. Dehydrogenase activity reflects the vitality of the microbiota and the content of humic substances that can be decomposed by it. The dehydrogenase activity under mouldboardless, differentiated, and disking tillage systems is higher in the upper part of the plough layer, while it is lower in the lower part by 13.6% and 23.0%, compared to the control. On fertilised plots, the dehydrogenase activity under disking tillage decreases significantly, whereas it increases significantly under differentiated tillage. Mouldboardless tillage, however, lags behind the control in this indicator.

Catalase activity, which reflects the intensity of soil respiration, is significantly higher in the plough layer under disking tillage compared to mouldboard-disking tillage. The difference increases with higher fertiliser doses: on

unfertilised plots, it is 10%, and on fertilised plots with the highest dose, it is 13%. Under differentiated tillage, this indicator decreases insignificantly, while under chiselling tillage, it increases by 6-8%.

Polyphenol oxidases catalyse the oxidation of phenols to quinones. Through heteropolymer condensation of the latter with polypeptides and amino acids, primary humus compounds are formed under suitable conditions. Polyphenol oxidases and peroxidases are typically measured simultaneously. The latter catalyse the oxidation of polyphenols in the presence of hydrogen peroxide or organic peroxides, which are produced by microbial activity or the action of certain oxides. The intensity of humus mineralisation largely depends on reactions involving peroxidases, while humification is driven by polyphenol oxidases, as the latter significantly affect the conversion of aromatic organic compounds into humus components. Therefore, the intensity of humus formation is determined by the ratio of polyphenol oxidase activity to peroxidase activity, which is known as the humus accumulation coefficient. The activity of polyphenol oxidases was significantly lower in the second and fourth soil tillage treatments compared to the first. The differentiated tillage treatment exceeded the control by 5-14% for this indicator, with the difference being insignificant only at the highest fertilizer dose.

No significant changes in peroxidase activity were observed in the arable layer across the soil tillage treatments, but with the increase in fertilizer levels, it, like the activity of other enzymes, increased. However, on non-fertilized plots with mouldboard and disc tillage, peroxidase activity decreased by 2-6%, while it increased under differentiated tillage compared to the control.

The humus accumulation coefficient decreased by 6-7% and 7-11% for the second and fourth soil tillage treatments, respectively, and increased by 5-10% for the third treatment, compared to the control.

CONCLUSIONS

The use of deep ploughing throughout the crop rotation significantly reduces the heterogeneity of the arable layer in terms of microbiota population. Increasing organic and mineral fertilizer rates with mouldboardless and disc tillage enhances the biological diversity of the arable layer.

In the arable layer, microbiota on starch-ammonium agar (SAA) is highest in non-fertilized plots under differentiated tillage and in fertilized plots under disc tillage. On meat peptone agar (MPA), the highest microbiota activity is observed under differentiated tillage across all plots.

The highest population of microbiota on pectin-glucose agar with soil extract (PGASE), nitrifying bacteria, and actinomycetes was observed under differentiated tillage, while the mineralization and pedotrophic coefficients had the lowest values.

The highest fungal microbiota population was found under mouldboardless and disc tillage, while ammonifying and cellulolytic bacteria were most abundant under mouldboard & disc tillage. Under differentiated tillage, the lowest population of denitrifiers and the highest of *Azotobacter* was recorded.

The activity of invertase, phosphatases (on fertilised plots), dehydrogenases, and polyphenol oxidases is higher, while catalase activity is lower under differentiated tillage compared to mouldboard and mouldboardless tillage. The protease and urease activities in the arable layer are nearly at the same level for these tillage variants.

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