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Regularities in the Development of Soil Biological Activity and Winter Wheat Productivity under Ecologised Fertiliser Systems

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Abstract. Restoration and optimisation of soil processes is an important task of modern agriculture and one of the reserves for increasing agricultural production. Under the current conditions, this becomes possible with the integrated introduction of ecologised fertiliser systems. The purpose of the study is to investigate the regularities of the development of biological features of grey forest soil, the interdependencies between them, and the productivity of winter wheat in ecologised fertiliser systems. The following methods were used in the study: field, laboratory and analytical, biochemical, mathematical and statistical. Patterns of changes *in situ* of cellulolytic, proteolytic, and actual dehydrogenase activities of the soil, the carbon content of labile humus, and the number and weight of winter wheat grains per unit area were similar to each other. There was a decrease in cellulolytic activity, the smallest increase in the remaining biological characteristics of the soil under the use of pea straw, compared to the control. The greatest cellulolytic or proteolytic activity occurred in pea straw + N₃₀P₄₅K₄₅ + biostimulator + humus fertiliser or pea straw + N₃₀P₄₅K₄₅ + biostimulator + microbiological fertiliser, respectively, dehydrogenase – in 2, and the content of labile humus – in the first of these 2 variants. The availability of carbon and nitrogen allowed explaining the identified patterns in a relevant way. The positive Pearson correlation coefficients between plant productivity and soil biological activity, labile humus content, and enzymatic activity, and the insignificant partial correlation coefficients between these variables are partly conditioned by multicollinearity and multivariate interdependencies. In the future, the research would provide a deeper understanding of the patterns of development of biological properties of the soil under ecological fertiliser systems. This would help to improve the elements of greening to adjust the ratio of potential and actual fertility to the optimal level. Scientific results can become a basic basis for the development of effective soil-protecting organic and mineral fertiliser systems for economic and industrial structures of various levels of intensity and financial viability

Keywords: cellulolytic and proteolytic activity, dehydrogenase activity, labile humus, winter wheat productivity, correlation



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INTRODUCTION

Due to the significant decrease in manure production over the last period and the low level of mineral fertiliser use, soil fertility should be maintained mainly by returning organic substances formed during photosynthesis to biological circulation (Hamaliei & Shkarivska, 2009; Tanchyk, 2009). One of the most significant in terms of volumes, relatively cheap, and annually replenished resources of organic matter are by-products of plant origin. Among other things, they have the ability to maintain a sufficient level of fertility, in particular, the content of labile organic matter, the optimal intensity of biochemical processes in the soil (Smetanko *et al.*, 2018; Starchevskiy & Starchevskiy, 2018).

The use of post-harvest residues compensates for the lack of organic matter by 20-25%. However, ploughing straw without fertilisers creates conditions for the consumption of organic and inorganic nitrogen by cellulose-destroying soil microorganisms, thus causing a deterioration in soil fertility. Moreover, the disadvantage of conventional technologies for sealing plant residues is the need to apply a significant amount of nitrogen fertilisers, which, however, have little effect on the assimilation of nutrients contained in straw by plants (Mazur *et al.*, 2003)

Thus, one of the important areas in modern agriculture is the development of agrotechnical approaches aimed at improving the ecological state of the soil, improving its fertility and quality of agricultural products through the use of straw of grain or leguminous crops, preparations of biological compounds, which include beneficial microorganisms, humus substances, plant growth stimulators (Tonkha *et al.*, 2002). Extremely important in this context is the use of chelated microfertilisers, which have the ability to increase the yield and quality of agricultural products (Klymenko, 2017).

Straw, as an annual carbon-containing fertiliser, improves physical and chemical properties, enhances the activity of microflora, and increases the humus content in the soil (Yurkevych & Kovalenko, 2009; Moldovan & Vovkolup, 2012). Under these conditions, the decomposition of fibre is important, the intensity of mineralisation of which covers the area of mobilisation processes in soils, and indicates the provision of available forms of carbon (Trembitska, 2011). According to some researchers, reflecting the most accurate set of conditions affecting the plant, the intensity of cellulose decomposition is one of the indicators of soil fertility (Patyka, 2009). According to the latest cited studies, the use of grain straw + $N_{116}R_{10}K_{90}$ on podzolized chernozem for winter wheat led to a twofold increase in cellulolytic activity compared to the control.

The relationship of microbiological processes with effective soil fertility is characterised by proteolytic activity, as an indicator of the intensity of which the level of soil nitrogen supply largely depends. Proteolytic enzymes catalyse the hydrolysis of soil protein

compounds to amino acids, which, as a result of subsequent biochemical processes, are metabolised with the release of mineral forms of nitrogen. The latter play an important role in the life of soil microorganisms and agricultural crops. In chernozem conditions, typical use of winter wheat straw 3 t/ha + $N_{40}R_{40}K_{40}$ in repeated crops provided activation of proteolytic processes by 11%, relative to the control (Moskalevska & Patyka, 2014; Demianiuk *et al.*, 2016).

In the metabolism of substances and energy in the soil, an important place belongs to biological catalysts of redox reactions involved in the synthesis and decomposition of humus substances. Dehydrogenases are the most widespread among such enzymes in the soil. They catalyse the transfer of hydrogen ions and electrons (dehydrogenation) during the oxidation of organic compounds using metabolic cofactors, in particular NAD (P)⁺ as acceptor molecules. These enzymes are important indicators of the ecological state, including the level of vital activity of microorganisms, and the amount of humus substances that are decomposed by microorganisms. In the conditions of dark grey podzolized soil in the crops of *Bunias orientalis* for the introduction of $P_{60}K_{60}$ dehydrogenase activity was 12% higher than in the control group. However, the use of only nitrogen fertilisers or with $P_{60}K_{60}$ significantly reduced this enzymatic activity. Among other things, this showed an increase in the intensity of biochemical processes of oxidation of organic compounds during the formation of humus substances (Arkhypenko, 2002).

Since the functioning of microbial complexes and their enzymatic activity ensure continuous processes of transformation of organic substances in the soil, the analysis of relevant biochemical processes provides potential opportunities for revealing the directions and mechanisms of the formation of humus substances (Naidonova, 2015). The labile part of the soil organic matter is most sensitive to the effects of agricultural practices and biochemical properties of the soil. Compounds of this fraction have a high level of hydrophilicity, a significant specific amount of nitrogen-containing, carboxyl, and phenolic functional groups, and, accordingly, a low optical density. With the participation of redox and oxidative enzymatic activities, plant residues are mineralised in the soil. One part of them becomes a source of easily accessible nutrients for microorganisms and plants, while the other part forms one of the fractions of labile humus. According to well-established concepts, the content of mobile humus forms largely depends on the intensity of cellulolytic and proteolytic processes aimed at releasing carbon and nitrogen into the soil, and redox enzymatic activities involved in *de novo* formation of humus substances (Nannipieri, 2012; Piotrowska-Długosz, 2019).

At the same time, there are insufficient experimental data and theoretical ideas in the scientific

literature regarding the interdependencies between the intensity of biochemical processes and the content of NaOH-extracted labile organic matter in the soil, and their impact on crop productivity. In addition, the analysis of the peculiarities of using ecologised fertiliser systems for growing agricultural crops is present in the Ukrainian scientific literature in a limited volume. However, significant relevance is inherent in the study and implementation of innovative technologies with a set of ecologised fertiliser systems, aimed, in particular, at achieving optimal intensities of biological processes both in plants and in the soil. In this context, it is important to investigate the influence of these fertiliser systems on the development and interdependence of biological characteristics, in particular, the enzymatic activity of the soil, the formation of labile humus, and the corresponding ratios with the productivity of cultivated crops.

The purpose of the study is to find out and explain the patterns of changes in the biological properties of grey forest soil, the interdependencies between them and the productivity of the winter wheat according to ecological fertiliser systems. To do this, the following tasks were set: 1) investigate the pattern of changes *in situ* of cellulolytic, proteolytic, and potential dehydrogenase activity of the soil, the content of labile humus carbon in it under the conditions of ecologised fertiliser systems; 2) study the regularities of the formation of ear productivity (the number and weight of grains per 1 m²); 3) calculate and explain the values of the obtained correlation coefficients (Pearson, partial) between plant productivity and soil biological activity, labile humus content, and enzymatic activities.

MATERIALS AND METHODS

The study was conducted in the field during 2017-2019 in the experimental field of the Institute of Agriculture of the Carpathian Region in the conditions of an experiment to study the crop rotation productivity in the village of Stavchany, Lviv district, Lviv Oblast. Microplot experiment, plot area – 1 m², the distance between sections – 1 m, repetition – 6 times. The experiment scheme was as follows: 1) control – without fertilisers; 2) pea straw; 3) pea straw + N₃₀P₄₅K₄₅; 4) pea straw + N₃₀P₄₅K₄₅ + BS (biological stimulator); 5) pea straw + N₃₀P₄₅K₄₅ + BS + HF (humus fertiliser); 6) pea straw + N₃₀P₄₅K₄₅ + BS + MF (microbiological fertiliser); 7) pea straw + N₃₀P₄₅K₄₅ + CF (chelated fertiliser). Field studies were conducted with winter wheat (*Triticum aestivum* L.) of the Benefis variety.

Experimental fertiliser systems were assembled on the basis of pea straw + N₃₀P₄₅K₄₅ with the addition of a biostimulator, humus, microbiological, or chelated fertilisers. The characteristics of the drugs are given below.

Terra-sorb biostimulator; preparation composition: 25% – total amount of organic substances, 20% – amino

acids, total amount of nitrogen – 5.5%, B – 1.5%, Fe – 1.0%, Mg – 0.8%, Zn I Mn – 0.1% each, Mo-0.001%.

Humus fertiliser – eco-impulse; it is a concentrated aqueous solution of humic acid salts, composition: mass fraction of organic substances – 43.5%, mass fraction of ash – 56.5%. The preparation increases soil fertility, improves the ecological state – binds products of man-made pollution, prevents the accumulation of nitrates in plant products, and activates maturation.

Microbiological fertiliser – eco-grunt; it contains microorganisms *Basillus subtilis*, *Rhodococcus erythropolis* in the amount of 1,000 mln. units/g⁻¹.

Chelated fertiliser – rozasol 18-18-18+125+ME, its composition: 18% N, P, K; B – 128, Mn – 400, Cu – 94, Fe – 325 and Zn – 287 mg/kg⁻¹. Trace elements, in addition to boron, are in chelated form with EDTA. The preparation ensures uniform development of the crop and the growth of vegetative mass, increases the resistance of plants to temperature stresses and diseases, and is effective on acidic soils.

The preparations were used as follows: the terra-sorb biostimulator (BS) treated winter wheat crops in the phases of spring tillering and entering the tube – 0.5 l/ha⁻¹; humus fertiliser (HF) – eco-impulse was applied to the corresponding plots from autumn when wrapping pea straw – a dose of 3.0 l/ha⁻¹; microbiological fertiliser (MF) was applied at a dose of 3.0 l/ha⁻¹ between the spring tillering phase and tubing; chelated fertiliser (CF) was applied in the tubing phase at a dose of 3.0 kg/ha⁻¹.

The soil of the experimental plots is grey forest, lightly gleyed and loamy. The determination of physicochemical and agrochemical parameters of the soil before laying the experiment is carried out: pH(KCl) – 4.85, hydrolytic acidity Hr – 25.81 mg-eq/kg⁻¹ soil, the sum of absorbed bases S – 55.23 mg-eq/kg⁻¹ soil, easily hydrolysed nitrogen content – 98.0 mg/kg⁻¹, available phosphorus and potassium exchange (0.2 H NCL) – 108.4 and 87.2 mg/kg-1 soil, respectively; the level of total humus according to Tyurin in Nikitin's modification – 2.1%.

Climatic conditions over the years of research (2017-2019) had their own characteristics. This period was characterised by temperature changes and sharp changes in precipitation, especially in the spring and summer periods. The amount of precipitation during the growing season was in the range of 599-602 mm, except for 2017-2018, when it was 114 mm higher than normal. The temperature background for this period was higher than normal by 1.4-1.6°C. In general, the growing conditions of winter wheat were quite satisfactory, the maturation of crops took place in favourable conditions.

In situ cellulolytic, *in situ* proteolytic activities (CA, PA) of the soil were determined according to DSTU 8644:2016 (2017). The substrate of the first among these

fermentative activities was cellulose fibres of linen cloth, the second – gelatine coating of photo paper. Carriers with each of the substrates were placed vertically in the soil at a depth of 30 cm (lower edge) in the field, pressed on the sides, and left for 30 days (for CA) or 10 days (for PA) after the onset of the corresponding phase of ontogenesis of winter wheat – spring tillering, earing. Before and after the application, linen and photo paper were weighed in the soil thickness on analytical scales Radwag AS 220/R2, Poland (± 0.0001 g). Each of the listed enzymatic activities was calculated as a percentage of the mass ratios of carriers together with substrates after and before application: $(\text{final mass} \cdot 100\%) / \text{initial mass}$. In addition, after the onset of the above-mentioned periods of plant development, soil samples were taken (depth 0-30 cm) from experimental variants, and their preparation for laboratory and analytical work was carried out in accordance with DSTU ISO 11464-2001 (2003). The selected soil samples were used to measure potential dehydrogenase activity (DA) according to the Galstyan method according to DSTU ISO 23753-1:2010 (2010), the carbon content of labile humus (C-LH, soluble in 0.2 M NaOH) according to the Tyurin method according to DSTU 4732:2007 (2008). Values of extinction of triphenylformazane formed as a result of dehydrogenase reactions, and Cr^{3+} -containing products of organic carbon oxidation with potassium dichromate $\text{K}_2\text{Cr}_2\text{O}_7$, measured at the wavelength $\lambda = 590$ nm on the KFK-2 electrophotocolorimeter. The listed indicators were determined in 3 repetitions and 2 analytical parallels (in general, $n = 6$), averaged between the specified phases of ontogenesis. Biometric indicators of crop structure elements – the number and mass of grains per unit area of sowing (3 repetitions, 4 plants per repetition, $n = 12$) were determined in the waxy ripeness phase. Analytical scales (± 0.0001 g) (Radwag AS 220/R2, Poland) were used to measure the grain mass. For the calculation of the tested elements of the yield structure, the study

used some principles of generally accepted approaches outlined by V.O. Yeshchenko (2014) and their specifics formulated by R.A. Avramenko and H.V. Kirsanova (2004). The final values of each trait were obtained by averaging their values over two years of research (2017-2019).

Statistical analysis of results, in particular ANOVA (subject to the level of significance $\alpha = 0.05$), performed using Excel 11.0.6560.0. To calculate the estimate of the standard error of the difference between the means – SEDM, values of the Student's t coefficients, and degrees of freedom df , the study used the formulas given in (Woodward, 2014) corresponding to the values of P – function $T.DIST.2T$ of the specified Excel software suite. Data validation for normal distribution was performed in STATISTICA Version 10. For subsequent analyses, the data were logarithmised. Pearson correlation coefficients, partial correlation coefficients, and their significance levels were calculated using STATISTICA Version 10.

RESULTS AND DISCUSSION

The results of the conducted studies (Fig. 1) showed that the application of pea straw alone (variant 2) caused a significant increase ($P < 0.001 - 0.01$) of *in situ* proteolytic activity (PA), potential dehydrogenase activity (DA) of soil, carbon content of labile humus C-LH by 2.20%, 0.006 mg of triphenylformazane g^{-1} soil, 0.27 g/kg^{-1} soil, respectively, but a decrease ($P < 0.01$) of *in situ* cellulolytic activity (CA) by 1.20% compared to the control (variant 1). Application of pea straw + $\text{N}_{30}\text{P}_{45}\text{K}_{45}$, and the specified combination with the addition of elements of greening BS, BS + HF, BS + MF, CF (options 3, 4, 5, 6, 7) has led to simultaneous growth of CA, PA, potential DA, and C-LH content in the soil on 4.71-6.44%, 3.64-7.64%, 0.011-0.019 mg triphenylformazane g^{-1} soil, 1.24-1.93 g/kg^{-1} soil, respectively, compared to variant 1 ($P < 0.001$). In the group of variants 2-7, the lowest level of all these indicators occurred in variant 2, the highest values of CA, DA, C-LH – in variant 5, PA – in variant 6.

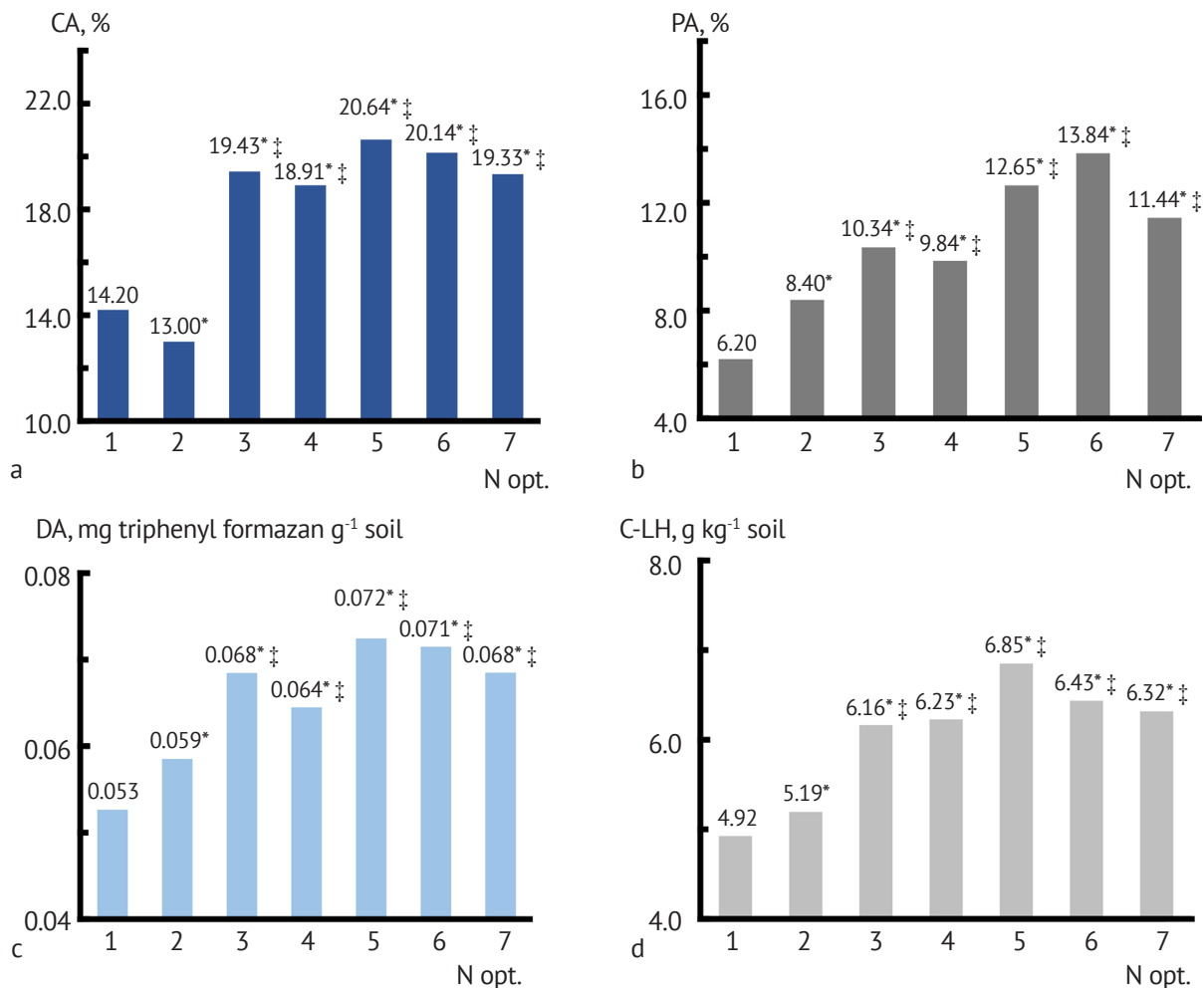


Figure 1. Influence of ecologised fertiliser systems on *in situ* cellulolytic (a), proteolytic (b), potential dehydrogenase (c) activity of soil and carbon content of labile humus (d) (average between spring tillering and earing phases; 2017-2019)
Note: Significance level of differences between the average values according to ANOVA data: a – $P < 0.001$, b – $P < 0.001$, c – $P < 0.001$, d – $P < 0.001$; symbols *, ‡ – the level of significance of the difference relative to options 1, 2, respectively, $P < 0.001-0.01$

Source: compiled by the authors

The authors of the work suggest that in the studied *in situ* CA, PA are involved in depolymerases that catalyse both the hydrolysis of the corresponding high-molecular compounds and the fragments formed from them (Piotrowska-Długosz, 2020; Sobucki *et al.*, 2021). Since enzymes adsorbed on clay minerals and captured by humus substances (Nannipieri *et al.*, 2012; Piotrowska-Długosz, 2019; Piotrowska-Długosz, 2020), it is reasonable to assume that a significant part of CA, PA was caused by “protected”, “attached” enzymes on the applied carriers.

The introduction of legume residues with low C:N into the soil leads to the rapid growth of coprotrophic bacteria, microbial activity and the release of nutrients that meet the needs of the crop (Rao *et al.*, 2019). However, temporary nitrogen immobilisation in the early stages of straw decomposition usually causes nitrogen (N) deficiency in the soil (Li *et al.*, 2019). It is also known that the activity of soil enzymes is induced

by the substrate and suppressed by end products. In particular, β -glucosidase activity is stimulated by organic carbon (OC), NH_4^+ , whereas protease activity is activated by low and suppressed by high concentrations of OC (Vazquez *et al.*, 2019). The addition of inorganic N(IN) inhibits the hydrolysis of OC, protein, and chitin, while the addition of organic N(ON) stimulates these processes (Tian *et al.*, 2022). Obviously, the lack of N in the soil, in particular, is probably NH_4^+ , on suppressed CA in variant 2, compared to variant 1. However, a lack of IN stimulated, and a small lack of ON probably did not significantly affect PA, which was larger under such conditions than in the control.

In general, the intake of organic residues (plants, manure, etc.) causes an increase in the carbon (C) content of microbial biomass (MBC) and soil enzymatic activity as a result of an increase in the availability of a C-substrate that stimulates microbial activity (Zhao *et al.*, 2016). This is consistent with the increase in variant 2

of potential DA – an important indicator of soil microbial activity (Kumar, 2013; Acosta-Martínez & Waldrip, 2014; Piotrowska-Długosz, 2019). A simultaneous decrease in CA, an increase in PA, and a slight increase in C-LH levels may indicate that microbiological processes are focused on compensating for the lack of ON in the first place. This is consistent with the view that increased N availability is required to enhance C uptake by microorganisms (Bowles *et al.*, 2014).

Introduction of straw + N₃₀P₄₅K₄₅ in variant 3 obviously led to a decrease in the C:N ratio in the soil and, consequently, to an increase in the rate of consumption of high-carbon organic compounds by microorganisms compared to the control. The intake of N levelled the restriction of the activity of C-metabolic enzymes, and caused an increase in their activity (Chen *et al.*, 2020), in particular CA. Growth of PA in variant 3, at first sight, contradicts the thesis that mineral forms of N inhibit protease activity (Piotrowska-Długosz, 2020; Tian *et al.*, 2020). Obviously, straw + N₃₀P₄₅K₄₅ reduced N-restriction on microbial biomass formation and P-restriction on metabolic processes in microorganisms (Chen *et al.*, 2017), directly or indirectly activating the growth and reproduction of bacteria and fungi by changing their ratio (Li *et al.*, 2019; Chen *et al.*, 2020). This may, at least in part, be the reason for the activation of enzyme synthesis and secretion (Piotrowska-Długosz, 2019), in particular, the growth of PA. Changes in hydrolase activity were accompanied by a predictable increase, probably in the content of C and N nutrients and, consequently, a documented increase in C-LH, DA (Bowles *et al.*, 2014; Singh *et al.*, 2018).

The obtained results do not coincide with the data of the vegetation experiment by R. Chen *et al.* (2014), according to which the addition of both corn straw and corn straw + mineral N to the substrate led to an increase in β-glucosidase activity (Chen *et al.*, 2014). Leucine aminopeptidase activity increased in the first case and decreased in the second case, while the specific rate of microbial growth was the opposite. The action of the 1st factor was mediated by K-strategists (N-restriction + microbial resource extraction), while the action of the 2nd factor was mediated by r-strategists (stoichiometric decomposition theory). Obviously, more detailed studies are required to determine regularities that are implemented in variants 2, 3.

It is reasonable to assume that the effect of BS or CF on the background of straw + N₃₀P₄₅K₄₅ (variants 4 and 7, respectively) caused the activation of photosynthesis and growth processes in winter wheat plants. At the same time, the stimulation of photosynthesis C₃-plants, for example, with high concentrations of CO₂, leads to an increase in risk deposits and a subsequent increase in the enzymatic activity of C-, N-, and P-cycles in rhizospheric soil. These patterns are accompanied by mineralisation of risk deposits, competition for N between plants and microorganisms (Nannipieri *et al.*, 2012).

Microbial activity in the rhizosphere is stimulated by the entry of labile OC from rhizome deposits and exudates. A sufficient level of OC supply reduces the differences between fermentative activity in the rhizosphere and bulk soil (Tian *et al.*, 2020). BS or CF on the background of straw + N₃₀P₄₅K₄₅ caused the growth of CA, PA, and DA, primarily in the rhizosphere, and in general, in the soil in variants 4 and 7, compared to the control. A slight decrease in the listed indicators, especially PA in variant 4, compared to variant 7, at least in part, may have been caused by stronger competition between microorganisms and plants for N and C in the first case. Changes in the C-LH content under such conditions were closest to CA, DA.

Adding HF to straw + N₃₀P₄₅K₄₅ + BS (variant 5), obviously, led to the enrichment of the soil with easily accessible forms of OC, ON (humic acid salts), which, in turn, led to an increase in microbial biomass and enzymatic activity as a result, in particular, an increase in the availability of C-substrate (Zhao *et al.*, 2016), and was also one of the reasons for the increase in the content of C-LH in the soil, relative to the control. After applying MF on the background of straw + N₃₀P₄₅K₄₅ + BS (variant 6) the soil was enriched with microorganisms *Bacillus subtilis*, *Rhodococcus erythropolis*, which are known destructors of straw (stubble). Obviously, this led to the formation of additional “exogenous” microbial activity, which contributed to the total fermentative activity of the soil and probably indirectly activated autochthonous microorganisms, generally causing an increase in the C-LH value.

According to the current soil continuum model, the decomposition and stabilisation of soil organic matter (SOM) depend on the ability of reducing organisms to access SOM and on the protection of SOM by soil minerals. The stability of SOM is determined not so much by its chemical composition, but by the interaction of the latter with environmental factors, in particular, with solubility, the size of molecules, and their functional properties. Microorganisms, their metabolites, and decomposition products are considered as the main input of SOM (Schmidt *et al.*, 2011; Lehmann & Kleber, 2015; Rao *et al.*, 2019). Plants and their residues, microbial exoenzymes, are also important for SOM formation (Paul, 2016; Rao *et al.*, 2019). This pattern of SOM persistence suggests that it exists in forms ranging from less degraded to more decomposed and eventually undergoes oxidation by soil microorganisms (Schmidt *et al.*, 2011; Lehmann & Kleber, 2015). Consequently, the idea of operationally obtained humus fractions loses its original meaning, but remains important for understanding their meaning and functions (Lehmann & Kleber, 2015). D.C. Olk *et al.* (2019) argue the relevance of NaOH-extracted humic and fulvic acids (HA, FA-humic substances, HS) for use in environmental and agricultural research. The authors of this study concluded that HS contains biological molecules and humification

products with a molecular weight mostly lower than previously thought. In this case, the decomposition of SOM can occur from the most (oxidised) to the least (reduced) thermodynamically stable form. Consequently, permanganate-oxidised OC (POXC) is a weakly processed (moderately stable), easily oxidised pool of soil OC (SOC). In addition, the fraction of OC extracted with cold water (CWEOC) contained a higher proportion of aliphatic and carboxyl groups, while the fraction extracted with pyrophosphate or NaOH contained more aromatic groups. This means that CWEOC is also poorly treated by microorganisms, while NaOH-HS, on the contrary, is a more "mature" SOC (Culman *et al.*, 2021; Fox *et al.*, 2017). It is likely that the patterns inherent in CWEOC, at least in part, are also characteristic of hot water-extracted HWEOC. Often, studies use one of the most labile fractions – dissolved OC (DOC), which is collected using lysimeters (Błońska *et al.*, 2021), or extracted with K_2SO_4 (Culman *et al.*, 2021).

POXC has been shown to be sensitive to agricultural management methods, in particular, to tillage, organic fertilisers, and positively correlated with crop productivity (Culman *et al.*, 2021). Straw application caused an increase in HWEOC, but did not affect DOC, while nitrogen addition in fertilisers acted the other way around (Li *et al.*, 2019). It is known that the activity of soil enzymes positively correlates with the content of SOM, water-soluble humus WEOC (Lazcano *et al.*, 2013). A strong positive correlation between β -glucosidase activity and POXC, HWEOC, has been documented, with an accumulation of 1st due to OC from plant residues and 2nd due to rhizosphere (Hok *et al.*, 2018). A strong

positive correlation was demonstrated between the activity of β -glucosidase, β -D-cellobiase, xylanase, N-acetyl- β -D-glucosaminidase (based on POXC), and DOC (lysimeric) released from plant litter (Błońska *et al.*, 2021). This is mediated by the stimulation of microbial activity under the influence of DOC and subsequent stabilisation of the latter in the "heavy" fraction by binding to minerals. HA and FA are important for providing plants with Fe, Ca, K and are sensitive to land management methods, in particular, ploughing plant residues, organic fertilisers (manure), etc. (Olk *et al.*, 2019). In addition, SOM affects yield by improving, maintaining soil quality and health, and providing plants with nutrients (Lal, 2020). At the same time, it was not possible to find in the scientific literature the substantiation and explanation of the interdependencies between C-, N-hydrolysing fermentative activities of the soil, signs of redox microbial activity, and NaOH-extracted labile humus, and productivity.

In connection with the above, indicators of productivity of the ear of winter wheat were determined (Fig. 2) for the purpose of subsequent analysis of the interdependencies between them and the studied signs of biological activity of the soil under the conditions of ecologised fertiliser systems. In variant 2 there was an increase in the number and mass of grains by 1.12 thsd. units/m², 0.025 kg/m², respectively, compared to variant 1 (control). In variants 3-7, these indicators experienced an increase of 4.28-7.31 thsd. units/m², 0.155-0.271 kg/m², respectively, with respect to the control. In general, the pattern of changes in the characteristics of winter wheat productivity was similar to the patterns of variation in the experimental variants of CA, PA, DA, and C-LH values.

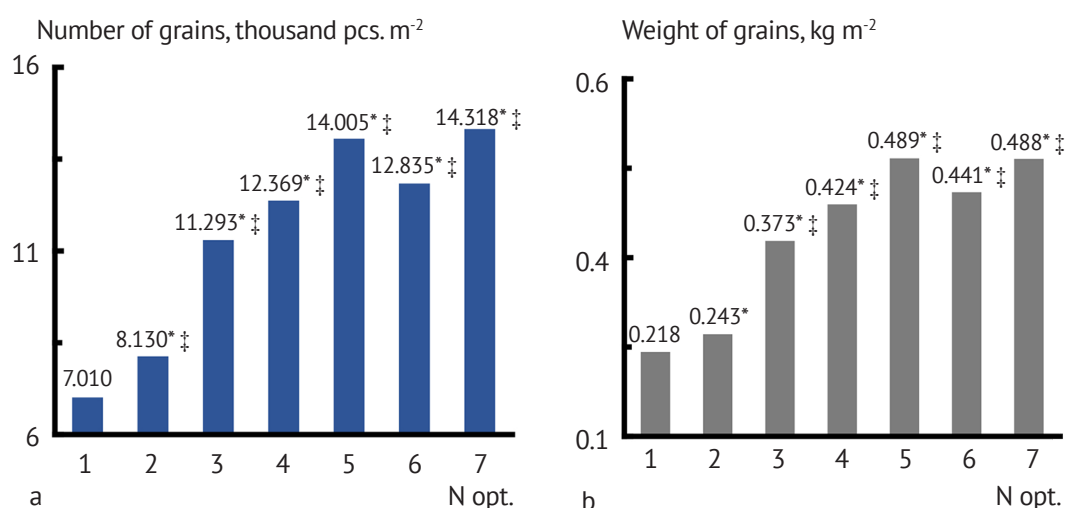


Figure 2. Influence of ecologised fertiliser systems on the number (a) and weight (b) of grains per crop area of winter wheat (waxy ripeness phase; 2017-2019)

Note: Significance level of differences between the average values according to ANOVA data: a, b – $P < 0.001$; symbols *, ‡ – the level of significance of differences relative to variants 1, 2, respectively, $P < 0.001$

Source: compiled by the authors

High and positive Pearson correlation coefficients were obtained between logarithmic values of the number and weight of winter wheat grains per 1 m² (NGA, WGA, respectively) and studied signs of soil biological activity (CA, PA, DA, C-LH): 0.901-0.975 ($P < 0.001-0.01$) (Table 1). The Pearson correlation coefficients between C-LH and the enzymatic activity of CA, PA, DA, and between C-LH and NGA, WGA, were significant and amounted to 0.926-0.967 ($P < 0.001-0.01$), 0.972-0.975 ($P < 0.001$), respectively. Such results indicate likely significant positive interdependencies between NGA or WGA and CA, PA, DA, C-LH, and between C-LH and CA, PA, DA. At the same time, the partial correlation coefficients between the listed variables in the specified order were significantly smaller and insignificant: -0.389-0.668 ($P > 0.05$), 0.023-0.683 ($P > 0.05$). This, at least to some extent, may be conditioned by the high level of multicollinearity of the original correlation matrix. In particular, the above Pearson correlation coefficients between the characteristics of ear productivity and soil biological activity were accompanied by a significant level of interdependence ($r > 0.7$) between C-LH and CA, PA, DA, and between the listed enzymatic activities. The latter among these regularities determined a significant level of multicollinearity between the studied biological characteristics of the soil. Another reason for the relationships found between the Pearson partial and correlation coefficients was probably the involvement of unaccounted factors in the interdependencies under consideration.

In particular, the above-mentioned POXC, CWEOC, HWEOC, DOC can affect the values of CA, PA, DA, and serve as output pools of OC for the formation of a more "mature" C-LH. It is possible that DOC (80°C), dissolved organic nitrogen DON (25, 80°C), and hot water-extracted organic nitrogen HWEOC (Li, 2019; Zhao *et al.*, 2016) of IN form in fertilisers (Chen *et al.*, 2020) and in the soil, easily hydrogenated nitrogen, MBC, N microbial biomass MBN, etc. Notably, the health, quality, productivity, and fertility of the soil are characterised by complex indices, which include enzymatic activities within one or more processes, the content of soluble forms of OC, soil respiration, pH, etc. It is known that there are almost always no direct proportional interdependencies between the potential enzymatic activity of the soil and *in situ* productivity of agricultural plants (Piotrowska-Długosz, 2019; Nannipieri *et al.*, 2012). The determination of *in situ* CA, *in situ* PA did not alleviate this situation in the context of interpretations of partial correlation coefficients, among other things, probably for the following reasons: 1) involvement of many hydrolases specific to the respective biological polymers and oligomers formed from them; 2) complex processes of sorption, desorption of enzymes, intermediate and final products on the respective carriers, etc.; 3) involvement of at least 3 phases – substrate carrier, soil solution, soil colloids; 4) other important enzymatic activities, bio-, physicochemical properties of soil, etc., are not taken into account (Table 1).

Table 1. Pearson correlation coefficients and partial correlation coefficients between the values of the studied soil characteristics and the productivity of the ear of winter wheat

Pearson correlation coefficients						
	ln(NGA)	ln(WGA)	ln(CA)	ln(PA)	ln(DA)	ln(C-LH)
ln(WGA)	0.998 ^a					
ln(CA)	0.922 ^b	0.942 ^b				
ln(PA)	0.911 ^b	0.901 ^b	0.838 ^c			
ln(DA)	0.939 ^b	0.931 ^b	0.902 ^b	0.975 ^a		
ln(C-LH)	0.972 ^a	0.975 ^a	0.951 ^b	0.926 ^b	0.967 ^a	
Partial correlation coefficients						
ln(PA)			-0.033			
ln(DA)			0.143	0.816		
ln(C-LH)			0.683	0.023	0.456	
ln(NGA)			0.055	0.249	-0.221	0.615
ln(WGA)			0.281	0.353	-0.389	0.668

Note: NGA, WGA – the number and weight of grains, respectively, based on the area of sowing; indices a, b, c – significance level of the correlation coefficient $P < 0.001$, $P < 0.01$, $P < 0.05$, respectively; the logarithm sign indicates the logarithmic average values of the calculated values

Source: compiled by the authors

X. Wan *et al.* (2019) cite the results of studies according to which the removal of sediments contributed

to an increase in the number of fungi and changed the composition of the bacterial community in favour of

species predisposed to use more recalcitrant OC. The authors of this study found that the treatment of trees, their roots, and the removal of precipitation, which lead to a decrease in OC intake in the soil, also cause a decrease in enzymatic activity. At the same time, a significant negative correlation between DOC or NH was found integrally by season, and cellulohydrolase, β -glucosidase, β -1,4-N-acetylglucosaminidase, and acid phosphatase. At the same time, phenol oxidase or peroxidase significantly positively correlated with NH_4^+ or DON, respectively. These patterns suggest that a certain level of competition between microorganisms and plants for C and N in the experimental variants probably led to a decrease in the values of OC entry into the soil, cursory activation and/or an increase in the number of bacteria and fungi effective in the decomposition of resistant OC. Such a sequence of events may well lead to a decrease in the partial correlation coefficients between C-LH and the studied soil enzymatic activity or plant productivity.

Higher, however insignificant, partial correlation coefficients between C-LH and CA, as well as NGA, WGA and C-LH – 0.683 ($P > 0.05$), 0.615–0.668 ($P > 0.05$), at least to some extent, could be caused by a small sample size: $n = \text{number of variants} = 7$. Corresponding coefficients of determination: 0.4665, 0.3782–0.4462. This interpretation suggests that for larger n , the formation of C-LH or NGA, WGA values by at least 46.65% or 37.82–44.62% depends on CA or C-LH, respectively. In turn, this is consistent with the data cited above and interpretations that labile fractions of OC (POXC, DOC) have the ability to stimulate C-hydrolysing fermentative activity of the soil (Hok et al., 2018; Błońska et al., 2021). At the same time, it is possible that the transition of DOC to the heavy fraction (Błońska et al., 2021), and the likely “maturation” of POXC to NaOH-HS, were accompanied by the accumulation of the C-LH under study, including CA. It is reasonable to assume that C-LH is one of the SOC and/or SOM fractions involved in the known effects of the latter on crop yields (Lal, 2020).

In general, the results obtained indicated the presence of complex ambiguous causes that direct changes in the studied features of soil biological activity and, probably, multifactorial interdependencies between C-LH and *in situ* CA, PA, potential DA under the conditions of ecologised fertiliser systems. Obviously, ambiguity and multifactorial nature are also characteristic of the relations between the productivity indicators of winter wheat per sown area and CA, PA, DA, C-LH. In turn, this supports the view that C-, N-hydrolysing fermentative

activity of soil should be studied in relation not only to C-LH, but also to other forms of OC and/or ON, in particular, WEOC, NaOH-extracted HA, FA, total SOC, easily hydrolysed nitrogen, probably also IN (NH_4^+ , NO_3^-). It is expedient to involve other potential enzymatic activities in such studies, for example, β -glucosidase, xylanase, invertase, N-acetyl- β -D-glucosaminidase, leucine aminopeptidase.

CONCLUSIONS

The ecologised fertiliser systems under study caused, in general, unidirectional growth *in situ* of cellulolytic, proteolytic, potential dehydrogenase activities of grey forest soil under winter wheat and the content of carbon NaOH-soluble labile humus (average between the phases of spring tillering and earing). The exception is the decrease *in situ* of cellulolytic activity under the conditions of pea straw application, compared to the control. The highest level of the above hydrolytic enzymatic activities of the soil in the specified order was achieved under the conditions of pea straw + $\text{N}_{30}\text{P}_{45}\text{K}_{45}$ + biostimulator + humus fertiliser or pea straw + $\text{N}_{30}\text{P}_{45}\text{K}_{45}$ + biostimulator + microbiological fertiliser, dehydrogenase – in two, while the highest content of labile humus – in the first of these two experimental variants. The smallest values of the studied biological characteristics of the soil among the applied ecological fertiliser systems occurred with the application of pea straw. The largest number and weight of grains per unit area of wheat sowing was observed in the case of using pea straw + $\text{N}_{30}\text{P}_{45}\text{K}_{45}$ + biostimulator + humus fertiliser or pea straw + $\text{N}_{30}\text{P}_{45}\text{K}_{45}$ + chelated fertiliser, the smallest – after applying pea straw.

High, positive and significant Pearson correlation coefficients between the number and weight of winter wheat grains per 1 m² and the studied signs of biological activity of the soil, including the carbon content of labile humus and the studied enzymatic activities (0.901–0.975, $P < 0.001$ –0.01), significantly smaller and insignificant coefficients of partial correlation between these variables (–0.389–0.683, $P > 0.05$) are caused, in particular, by the multicollinearity and multifactorial nature of such interdependencies.

There is a significant scientific feasibility of substantiating changes in the studied biological characteristics of the soil by the availability of carbon and nitrogen substrates obtained from straw, mineral fertilisers, their impact on biomass, features and intensity of functioning of soil microflora, enzymatic activity, which determines the prospects for further research

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Закономірності формування біологічної активності ґрунту, продуктивності пшениці озимої за екологізованих систем удобрення

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Анотація. Відновлення та оптимізація ґрунтових процесів є важливим завданням сучасного землеробства і одним з резервів збільшення виробництва сільськогосподарської продукції. В існуючих умовах це стає можливим у разі комплексного запровадження екологізованих систем удобрення. Мета досліджень – вивчення закономірностей розвитку біологічних ознак сірого лісового ґрунту, взаємозалежностей між ними та продуктивністю пшениці озимої за екологізованих систем удобрення. У дослідженнях використано такі методи: польові, лабораторно-аналітичні, біохімічні, математико-статистичні. Паттерни змін *in situ* целюлозолітичної, протеолітичної, актуальної дегідрогеназної активностей ґрунту, вмісту у ньому вуглецю лабільного гумусу, кількості і маси зерен пшениці озимої з розрахунку на одиницю площі посіву були подібними між собою. Відмічено зниження целюлозолітичної активності, найменший приріст решти серед вивчених біологічних ознак ґрунту за умов застосування соломи гороху, порівняно з контролем. Найбільша целюлозолітична або протеолітична активність, мали місце за соломи гороху + $N_{30}P_{45}K_{45}$ + біостимулятор + гумусне добриво або соломи гороху + $N_{30}P_{45}K_{45}$ + біостимулятор + мікробіологічне добриво, відповідно, дегідрогеназна – у 2-х, а вміст лабільного гумусу – у першому з цих 2-х варіантів. Доступність вуглецю і азоту дозволили релевантно пояснити виявлені закономірності. Додатні коефіцієнти кореляції Пірсона між продуктивністю рослин і біологічною активністю ґрунту, вмістом лабільного гумусу і ферментативними активностями, а також незначущі коефіцієнти часткової кореляції між цими змінними частково зумовлені мультиколінеарністю й багатофакторністю взаємозалежностей. У перспективі дослідження дозволять глибше зрозуміти закономірності формування біологічних властивостей ґрунту за екологізованих систем удобрення. Це сприятиме удосконаленню елементів екологізації з метою коригування до оптимуму співвідношень потенційної і актуальної родючості. Наукові результати можуть стати базовою основою розробки ефективних ґрунтоохоронних органо-мінеральних систем удобрення для господарсько-виробничих структур різного рівня інтенсивності та фінансової спроможності

Ключові слова: целюлозолітична і протеолітична активності, дегідрогеназна активність, лабільний гумус, продуктивність пшениці озимої, кореляція
