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BIOLOGICAL PROCESSES IN GRAY FORESTAL SOIL UNDER WINTER WHEAT BY ECOLOGIZED FERTILIZING SYSTEMS

The results of effectiveness studies of the ecologized fertilizers systems use (EFS), arranged on the basis of the optimal rate of mineral fertilizers ($N_{60}P_{90}K_{90}$), treatment of plants with the biostimulator (BS) terra-sorb with addition of humus or microbiological fertilizer (HF – eco-impulse or MF – eco-soil, respectively) on biological processes in the soil under winter wheat are presented. The studies were carried out in 2018–2020 in the stationary experiment on productivity of short crop rotations on the experimental field of the Institute of Agriculture of Carpathian region NAAS of Ukraine. The soil of the experimental field is gray forestal surface-gleyed, in which biochemical (enzymatic) activity was diagnosed, in particular, cellulolytic, proteolytic, dehydrogenase and content of labile (mobile) organic matter was determined taking into account yield of winter wheat. Using fertilizers systems $N_{60}P_{90}K_{90}$ or $N_{60}P_{90}K_{90}$ + BS, soil's cellulolytic and proteolytic activity increased markedly relative to the control. The highest increase level in destructive ability of corresponding soil hydrolases among the EFSs used was provided by the factor of organic components added to the soil (HF or MF on the background of $N_{60}P_{90}K_{90}$ + BS). Under such conditions, cellulolytic and proteolytic activities in the soil were 34.80–37.70 and 11.90–11.40%, respectively. Under conditions of $N_{60}P_{90}K_{90}$, $N_{60}P_{90}K_{90}$ + BS, a clear decrease of the dehydrogenase activity in the soil by 13.12–19.67% was noted, while after the addition of HF or MF there was a trend towards increase of this activity (+4.92–11.48%) relative to control. The applied fertilizers systems led to increase of the accumulation levels of labile humus carbon in the soil by 13.35–26.46% compared with the control variant. Introduction of HF or MF together with BS on the mineral background, provided the highest content of carbon of the mobile organic matter (588.4 and 561.7 mg/100 g of soil, respectively). Such an integrated application of elements of the ecologization (BS – influence on the plant, HF or MF – effect on the soil) was the most effective in dirrection of improving winter wheat productivity. The yield of the studied crop on the background of $N_{60}P_{90}K_{90}$ was 5.16 t/ha, whereas after addition of ecologization elements (BS, BS + HF, BS + MF), the values of this indicator experienced an increase by 3.00–9.65% in relation to the variant $N_{60}P_{90}K_{90}$. The results obtained make it possible to form separate elements of the scientific foundations, that are necessary for development of ways and methods of improving the biological state of soils, obtaining high and sustainable crop yields.

Key words: ecologized fertilizers systems, winter wheat, biostimulant, humus and microbiological fertilizer, enzymatic activity, labile humus.

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Біологічні процеси у сірому лісовому ґрунті під пшеницею озимою за екологізованих систем удобрення

Наведено результати досліджень з вивчення ефективності застосування екологізованих систем удобрення (ЕСУ), скомпонованих на базі оптимальної норми мінеральних добрив ($N_{60}P_{90}K_{90}$), обробки рослин біостимулятором (БС) терра-сорб та з додаванням гумусного або мікробіологічного добрива (відповідно ГД – еко-імпульс або МД – еко-ґрунт) на біологічні процеси в ґрунті під пшеницею озимою. Дослідження проведено в 2018–2020 рр. у стаціонарному досліді з вивчення продуктивності короткоротаційних сівозмін на експериментальному полі Інституту сільського господарства Карпатського регіону НААН. Ґрунт дослідного поля – сірий лісовий поверхнево оглеєний, в якому здійснено діагностування біохімічної (ферментативної) активності, зокрема целюлозолітичної, протеолітичної, дегідрогеназної, а також визначено вміст лабільної (рухомої) органічної речовини й облік урожайності пшениці озимої. За використання систем удобрення $N_{60}P_{90}K_{90}$ або $N_{60}P_{90}K_{90}$ + БС помітно зростала целюлозолітична та протеолітична активність ґрунту щодо контролю. Найбільший рівень підвищення руйнуючої здатності відповідних ґрунтових гідролаз серед застосованих ЕСУ забезпечував фактор органічних складових, доданих у ґрунт (ГД або МД на фоні $N_{60}P_{90}K_{90}$ + БС). За таких умов целюлозолітична та протеолітична активності ґрунту становили відповідно 34,80–37,70 та 11,90–11,40 %. За умов $N_{60}P_{90}K_{90}$, $N_{60}P_{90}K_{90}$ + БС було відзначено чітке зниження дегідрогеназної активності ґрунту на 13,12–19,67 %, тоді як після додавання ГД або МД – тенденцію до зростання зазначеної активності (+4,92–11,48 %) щодо контролю. Застосовані системи удобрення зумовлювали збільшення накопичення вуглецю лабільного гумусу в ґрунті на 13,35–26,46 % порівняно з контрольним варіантом. Внесення ГД або МД сумісно з БС на мінеральному фоні забезпечувало найвищий вміст вуглецю рухомої органічної речовини (віповідно 588,4 та 561,7 мг/100 г ґрунту). Таке інтегроване застосування елементів екологізації (БС – вплив на рослину, ГД або МД – вплив на ґрунт) було найефективнішим у напрямі поліпшення продуктивності пшениці озимої. Врожайність дослідженої культури на фоні $N_{60}P_{90}K_{90}$ становила 5,16 т/га, тоді як після додавання елементів екологізації (БС, БС + ГД, БС + МД) величина цього показника зазнавала зростання на 3,00–9,65 % щодо варіанта $N_{60}P_{90}K_{90}$. Отримані результати дозволяють сформувати окремі елементи наукових основ, що потрібні для розвитку шляхів

і способів поліпшення біологічного стану ґрунтів, отримання високих та сталих врожаїв сільськогосподарських культур.

Ключові слова: екологізовані системи удобрення, пшениця озима, біостимулятор, гумусне та мікробіологічне добриво, ферментативна активність, лабільний гумус.

Introduction. Modern technological process in agricultural production leads to problems associated with degradation of soil fertility. One of the important aspects in this context is to achieve ecologized sustainability of agroecosystems, their ability to maintain bioproductivity during exploitation by high quality of production [9, 16, 20]. The above-mentioned agricultural territories must ensure the functioning of mechanisms aimed at restoring soil fertility. The effectiveness of many technologies depends on how well they fit into the system of natural processes and patterns, rather than acting against them. Understanding the specifics of soil functioning in agro- and biocenoses can help in the formation of tools for systematic optimization of soil fertility parameters [4, 13, 19].

Modern environmentally friendly fertilizer systems include: microbiological, humic fertilizers, biostimulators of plant growth, appropriate biological products, etc. [2, 31]. Thus, a rational system of fertilizers is a basic link in the formation of optimal agri-environmental functions of soils, their sustainable fertility and obtaining high level of yield and product quality. The most important and effective measure to harmonize the ecologized and productive functions of soils may be alternative biologized or greened fertilizer systems. However, the most sensitive diagnostic criterion for soil fertility is the functioning of the microbiological coenosis and level of enzymatic activity.

According to the authors [5, 14], the use of fertilizer systems containing humic fertilizers, as well as biological preparations-destructors of stubble, provided improved biological activity of soil and in particular the activity of redox enzymes [1, 26], which in turn led to increased content of labile humus.

According to many authors [25, 27, 29], the activation of microbial-plant interaction with the help of microbiological preparations and biostimulators of growth is a significant factor in increasing the productivity of agroecosystems, the potential of which is used very poorly in modern conditions. The use of microbiological preparations for seed inoculation or crop treatment increases the soil's biochemical activity and quantitative composition of the microflora [3, 28].

Biostimulants intensify the activity of plant organisms, accelerating their biochemical processes, including nutrition. Thanks to growth regulators, the growth of the root system improves, which in turn promotes the development of rhizobial microflora, microbiota [11, 19]. The direction of these processes determines the level of organic matter destruction, the cycle of nutrients, improving fertility. In the conditions of modern agriculture of great scientific and practical importance are the studies of biological features of soil, in particular its biochemical activity, which is the most informative indicator of the ecologized condition of the soil [15, 30].

Of all the organic carbon compounds, cellulose is the most common. In this regard, the intensity of fiber decomposition in the soil is an important indicator of its biological activity [24, 32]. The intensity of fiber mineralization covers the direction of mobilization processes and indicates the provision of different types of soils with available forms of carbon. No less important is the study of conversion processes of organic matter, given the anthropogenic impact on soil and its properties. It should be borne in mind that destruction of organic matter and assimilation by microbial groups and plants available products of their decomposition increases the productivity of agroecosystems, in particular in terms of restoring, preserving and improving soil fertility [21].

Equally important is the level and features of organic compounds' proteolysis in the soil, decomposition processes of which are provided by appropriate enzymes that hydrolyze proteins, peptides and other organic compounds [10, 23].

In the study of soil fertility, special attention is paid to the study of reactions that are catalyzed by redox enzyme systems of microorganisms. The activity of these enzymes, in particular dehydrogenases, polyphenol oxidases, peroxidases, is a leader in the regulation of organic compounds' metabolism in soil phases and plays a crucial role in humification. A number of researchers [1, 12] indicate an increase in the intensity of biochemical processes of oxidation of organic compounds under conditions of sufficient amount of nitrogen fertilizers application on the background of phosphorus-potassium fertilizers. However, the regularity of these processes is not fully understood and requires more detailed study, in particular, the use of EFSs filled with relevant biologized elements.

It should be noted that scientific literature has accumulated data on the gradation of the fertilizer systems' impact on the content of labile humus in different soils under one or another treatment of plants. Indeed, researchers [18, 22] found that the mineral fertilizer system provides a lower content of labile humus than organo-mineral on chernozem podzolic heavy-loamy in the conditions of the Right Bank Forest-Steppe of Ukraine. At the same time, on the sod-podzolic soil of the Northern Forest-Steppe of Ukraine the use of green manure on organo-mineral background and inoculation of wheat grain with polymyxobacterin content of labile humus was 0.26%, while in the organic system – 0.24 %. Thus, by the influence on the content of labile humus, the considered fertilizer systems can be located in the direction: mineral < organic < organo-mineral. However, there are insufficient data on changes in the humus state of the soil and its enzymatic activity under ecologized fertilizer systems. The study of these soil properties is of great relevance in the conditions of ecologized fertilizer systems and is the subject of our research.

Materials and methods. The research was conducted in 2018–2020 in the conditions of a stationary experiment on the study of the scientific basis of productivity management of short crop rotations in the Carpathian region. The object of research was the crops of winter wheat *Triticum aestivum* L. variety Benefis, sown after peas for grain with a seeding rate of 5.5 million germinated seeds/ha. Seed treatment was performed with a disinfectant vitavax 200 FF at a rate of 3.0 l/t. At signs of diseases, the fungicide amistar-extra was applied at the rate of 0.75 l/ha. Weeds treatment – the herbicide grodil-maxi at the rate of 100 ml/ha. The scheme of the experiment included 5 options:

1. Control (without fertilizers);
1. $N_{60}P_{90}K_{90}$;
2. $N_{60}P_{90}K_{90}$ + BS;
3. $N_{60}P_{90}K_{90}$ + BS + HF;
4. $N_{60}P_{90}K_{90}$ + BS + MF.

BS – biostimulator terra-sorb which includes: organic matter – 25 %, amino acids – 20 %, nitrogen (total amount) – 5.5 %, B – 1.5, Fe – 1.0, Mg – 0.8, Zn and Mn – 0.1 and Mo – 0.001 %. HF – humus fertilizer eco-impulse – concentrated aqueous solution of humic acids' salts: mass fraction of organic matter – 43.5 %, mass fraction of ash – 56.5 %. MF – microbiological fertilizer eco-soil which includes microorganisms *Bacillus subtilis*, *Rhodacoccus erytropolis*.

The soil of experimental plots – gray forestal surface-gleyed, light-loamy with the corresponding agrochemical characteristic: pH_{salt} – 4.85, nitrogen content according to Cornfield – 9.8, available phosphorus and potassium according to Kirsanov (0.2 n HCl) – 10.8 and 8.7 mg/100 g of soil, level of total humus – 2.1 %.

Cellulolytic and proteolytic activity was determined *in situ* by the intensity of decomposition, respectively, of linen and gelatin on photographic paper [17]. These substrates of enzymatic activities were placed vertically in the soil at a depth of 30 cm (lower edge), pressed on the sides, and left for 10–30 days after the onset of the earing phase. In the same phase of ontogenesis, soil samples were taken (depth 0–30 cm; DSTU ISO 11464-2001 [7]) to measure dehydrogenase activity by the Galstian method according to DSTU ISO 23753-1:2010 [8] and carbon content of labile humus by the method Tiurin according to DSTU 4732:2007 [6]. The value of economic harvest was determined in the phase of wax ripeness after harvesting and threshing grain of winter wheat.

Results and discussion. The processes of fiber destruction in terrestrial ecosystems are important. This cellulose-destroying activity is involved in the transformation of soil organic matter and significantly affects the level of its fertility. At the same time, the intensity of cellulose decomposition closely depends on abiotic, agrotechnical factors, in particular types and norms of fertilizers. The latter among the listed patterns are reflected in the results of this scientific work.

The pattern of *in situ* changes in the cellulolytic activity of gray forestal soil under winter wheat in the earing phase (relative units of activity – the degree of decomposition of fiber) is shown in Figure a. In the soil of the control variant (Var. 1) processes of cellulose decomposition were the slowest, which is apparently due only to the actual activity of currently available cellulose-destroying microorganisms and the lack of anthropogenic energy from outside during the testing period. Under conditions of mineral fertilizer background in norm $N_{60}P_{90}K_{90}$ in var. 2–5, the intensity of cellulose decomposition was higher than in the control. Application of only mineral fertilizers (var. 2) increased cellulolytic activity by 1.3 times relative to control. Obviously, this is due to the intensification of microbiological processes, which in turn led to an increase in the constant rate of stubble destruction, and ultimately – increase in microbial mass. The addition of BS (var. 3) in combination with mineral fertilizers did not change the level of cellulose-destroying ability of the soil, in relation to option 2 (Figure, a).

In var. 4, 5 – with carrying, respectively, BS + HF or BS + MF on the background of $N_{60}P_{90}K_{90}$, there was an increase in the hydrolysis of cellulose by 13.4–16.3 % in relation to var.1. Also in var. 4, 5 cellulosolytic activity was higher by 6.3–9.2 % compared with var. 2, and by 5.7–8.6 %, relative to var. 3 (Figure, a).

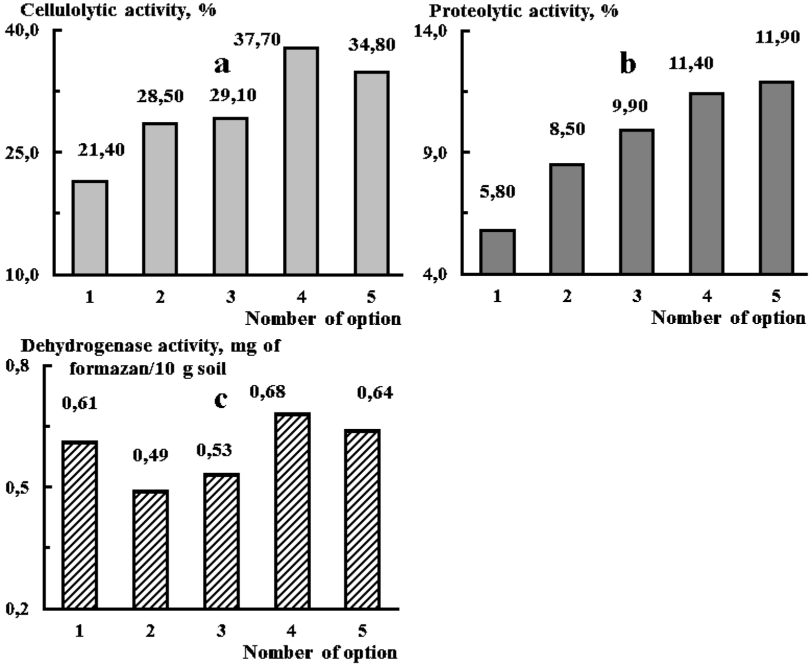

Fig. Changes in the enzymatic activity of gray forestal soil under winter wheat depending on the EFS (2018–2020). Contents of options by numbering – see section "Materials and methods".

It is important that the decomposition of biological substrates of protein and polypeptide nature in the soil is carried out with the participation of proteases. Regularities of *in situ* formation of proteolytic activity of gray forestal soil under winter wheat in the earing phase (relative units of activity – degree of gelatin decomposition) are shown in Figure, b. On var. 1 proteolytic activity of the soil was the lowest among the studied fertilizer technologies (var. 1–5) and amounted 5.80 %. Obviously, this is due to the lack of fertilizers, primarily organic components in this control version (Figure, b). In fact, on var. 2 this activity increased by only 2.70 %, while in the var. 3 (+ BS) – already by 4.10 % relative to control. However, the use of BS + HF or BS + MF in fertilizer systems on var. 4, 5, respectively, led to a more significant increase in the proteolytic activity of the soil – 5.60-6.10 % relative to control. Also the magnitude of proteolytic activity on var. 4, 5 were higher by 2.90–3.40 % compared to var. 2, by 1.50–2.00% relative to var. 3.

Enzymes of the oxidoreductase class, in particular dehydrogenases, play an important role in the formation of soil fertility. The probable transfer of hydride anion (proton + 2 electrons) from oxidizable organic matter in the soil to molecular oxygen or renewable organic matter during dehydrogenase reactions has created opportunities for diagnostic use of this activity to determine the intensity and direction of oxidative restorative processes, their participation in the formation and modification of humus components. In this context there are 2 stages of humification: 1) decomposition of the original organic residues to simpler – amino acids, peptides of phenolic compounds – breakdown products of lignins, tannins, etc. with the participation, in particular, hydrolytic, oxidative enzymes; 2) synthesis of organic compounds with the formation of high molecular weight humic substances of specific nature (condensation) with the participation, in particular, dehydrogenases, polyphenol oxidases.

The data shown in the figure showed that in var. 2, 3 there was a decrease of 13.11–19.67 %, while in var. 4, 5, tendencies to increase of dehydrogenase activity by 4.98–11.48 % relative to var. 1.

Thus, both the mineral fertilizer system (var. 2) and fertilizer systems with the use of ecologization elements (BS, HF, MF – var. 3–5) had the ability to increase the intensity of biochemical processes of humus formation of stage 1 – cellulosolytic, proteolytic activity. However, only under conditions of BS + HF or BS + MF on the background of $N_{60}P_{90}K_{90}$ (var. 4, 5, respectively) there was a slight increase, while by the use of only $N_{60}P_{90}K_{90}$, or $N_{60}P_{90}K_{90}$ + BS (var. 2, 3 respectively) – noticeable decrease in the intensity of the humification processes of stage 2 – dehydrogenase activity relative to var. 1. The reasons for the found ambiguous ratios of cellulosolytic or proteolytic activity on the one hand, and dehydrogenase – on the other, remains unknown and their disclosure requires more detailed research. However, it is reasonable to assume that the simultaneous activation of stages 1 and 2 on var. 4, 5, is at least partly due to the influence of ecologization elements on both the plant (BS) and the soil (HF or MF), the interaction of these influences with each other and with $N_{60}P_{90}K_{90}$.

In general, the functioning of soil enzymes ensures the destruction of organic residues, their transformation into humus and subsequent mineralization of the latter. These processes are a significant driving force in the evolution of soils, maintaining their homeostasis, including biochemical, within the stationary conditions of the ecosystem. It is obvious that the level of accumulation and/or intensity of transformation of humic substances is an integral indicator of biological changes in soil, both in natural biogeocenoses and in crops under anthropogenic factors. The importance of the accumulation of labile limiting substance is indisputable, as the compounds that are part of it are directly involved in plant nutrition, serve as energy material for microorganisms, perform protective functions against conservative organic matter, and so on. It is clear that for ecologic and economically successful cultivation of winter wheat it is advisable to take into account not only the enzymatic activity in the soil, but also the immediate results of the intensity of humus-forming processes, including changes in carbon content of labile organic matter.

Among the studied technologies, the lowest carbon content of labile humus took place on var. 1 – 465.3 mg/100 g of soil (Table 1). On var. 2, 3 values of this indicator experienced approximately the same increase – by 13.3–14.6 % compared with the control. EFS on var. 4, 5 caused a larger increase in the carbon content of labile humus – 20.7–26.5 %, relative to var. 1.

It is reasonable to assume that the increase in carbon content of labile humus in the soil on var. 2, 3, compared with var. 1, at least in part, was due to higher activity of cellulases and proteases (compare Figure a, b and Table 1). The close values of the first of these indicators in both cases indicate, most likely, that the action of BS on the background of $N_{60}P_{90}K_{90}$ did not cause, or caused only a slight increase in the studied activities compared to $N_{60}P_{90}K_{90}$ (var. 3 vs. var. 2). There is also no reason to assume a significant participation of dehydrogenase activity in the formation of a pool of labile humus in the soil on var. 2, 3.

1. Influence of EFS on carbon content (C) of labile humus in gray forestal soil under winter wheat (2018–2020)

variant №	C of labile humus, mg/100 g of soil
1	465,3
2	533,2
3	527,4
4	588,4
5	561,7

Note. Content of variants by numbering – see section "Materials and methods".

It is noteworthy that the size of carbon pool of labile humus, as well as the values of studied enzymatic activities, were the largest on var. 4, 5. Taking into account the above results and interpretations, the following assumption is relevant regarding the transient effects of ecologization elements on the plant (BS), soil (MF or MF), the interaction of these effects with each other, and with $N_{60}P_{90}K_{90}$. First of all, these mechanisms led to the development of microbiota with higher activity of hydrolytic enzymes and dehydrogenases, compared with the substrate in the absence of fertilizers, only a full dose of mineral fertilizers, or combination of the latter with BS (var. 4, 5 vs. var. 1, 2, 3). The increase in these enzymatic activities ultimately led to an increase in labile humus pools in the soil, and thus, among other things, to improving the nutrient supply of plants and the preconditions for higher levels of final productivity of winter wheat.

In this regard, in the final stages of the research, the values of the economic yield of winter wheat were determined under conditions of studied fertilizer systems. The lowest level of this indicator was on var. 1 – 2.71 t/ha (Table 2). In var. 3, 4 the yield of winter wheat increased by 91.14–96.68 %, while in var. 4, 5 – by 101.48–109.59 % compared to the control.

2. Yields of winter wheat depending on the EFS (2018–2020)

variant №	Harvest, t/ha
1	2,71
2	5,18
3	5,33
4	5,68
5	5,46
$LSD_{0,05}$	1,67

Thus, the last part of the obtained results to some extent confirms the above hypothesis that the simultaneous effect of BS + HF or BS + MF on the mineral background (Var. 4, 5) can cause greater yield gains relative to control than in the case of $N_{60}P_{90}K_{90}$ or $N_{60}P_{90}K_{90}$ + BS (var. 2, 3 respectively). It is advisable to anticipate the presence of differences in, so to speak, the mechanisms of action of the studied fertilizer systems in the direction of formation of the studied biological characteristics of the soil and effective fertility on var. 4, 5 on the one hand, and var. 2 and / or var. 3 on the other. In this context, it is important to note small differences between the values of the tested indicators on var. 4 and var. 5. In fact, the EFS on var. 4 led to higher levels of cellulolytic, dehydrogenase activity, carbon content of labile humus and yield, compared with var. 5. However, proteolytic activity was higher in var. 5, compared with var. 4. The meaning of such patterns remains unclear, however, hypothetically, they may mean differences in a number of aspects of the EFS on the var. 4 and var. 5.

The low intensity of microbiological processes and, consequently, low levels of enzymatic activity required for the formation of labile and conservative organic matter in gray forestal soils, as well as the ability of used EFS to improve potential and actual fertility under such conditions, create opportunities for important research. In particular, it will be expedient to deepen and expand the study of patterns of productivity of these plants depending on the intensity of enzymatic processes in the soil, especially polyphenol oxidase, peroxidase activities, group or fractional composition of humus using correlation and variance analysis.

Conclusions. The gradual increase of proteolytic, close to the gradual increase of cellulolytic activity, the content of labile humus in the soil, the economic yield of winter wheat, and the lack of these patterns for dehydrogenase activity in the same substrate in var. 1 (control), var. 2 ($N_{60}P_{90}K_{90}$), var. 3–5 (elements of BS, BS + HF, BS + MF ecologization, respectively, against the background of $N_{60}P_{90}K_{90}$) was revealed. The highest levels of cellulolytic (37.70 %), dehydrogenase activities (0.68 mg of formazan/10 g of soil), carbon of labile humus (588.4 mg/100 g of soil), yield (5.68 t/ha) were achieved in var . 4, the greatest proteolytic activity – on var. 5 (11.90 %). The second place in terms of the level of these indicators in this order was occupied by var. 5 (34.80 %, 0.64 mg of formazan/10 g of soil, 561.7 mg/100 g of soil, 5.46 t/ha), var. 4 (11.40 %), respectively. Hypotheses are proposed according to which EFS with biological components that simultaneously affect the plant and soil (containing BS + HF or BS + MF on the background $N_{60}P_{90}K_{90}$ – var. 4 or 5) are characterized by different mechanisms, ways of action on the formation of studied biological soil characteristics and effective fertility, compared with fertilizer systems on var. 2 and/or var. 3 ($N_{60}P_{90}K_{90}$, $N_{60}P_{90}K_{90}$ + BS respectively). For the implementation of the first of these mechanisms it is important to simultaneously increase the studied hydrolytic and dehydrogenase activities of the soil, while for the second – a moderate increase in hydrolytic, decrease in dehydrogenase activities of the soil relative to control. In the first case, there were larger pool sizes of labile organic matter of the soil, and higher yield levels than in the second. Emphasis is also placed on the probable differences in aspects of the modes of action of the EFS in var. 4, 5 on the formation of the studied indicators.

The obtained results can be used to create a general plan of final productivity formation patterns for winter wheat plants depending on the biological properties of gray forestal soil under EFS conditions.

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