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EFFECT OF PROBIOTIC TREATMENT ON THE MICROBIOLOGICAL ACTIVITY OF UKRAINIAN TYPICAL BLACK SOIL

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ABSTRACT

The search for novel substances that promote the establishment of a microbial community and facilitate optimal humification processes while increasing soil organic matter content offers an opportunity for land restoration. The objective of study was to determine the abundance of ecological and trophic groups of soil microorganisms and the intensity of microbiological processes when subjected to probiotic treatment at various concentrations and doses in typical black soil. The eco-trophic groups of soil microorganisms were identified by inoculating dilutions of soil suspensions onto selective nutrient media. The direction and intensity of soil microbiological processes were assessed using the mineralization-immobilization, oligotrophy, and pedotrophy indexes. The results demonstrate that probiotics has a positive impact on the microbiological activity of the soil, leading to an increase in the number of ecological and trophic groups of soil microorganisms during both spring and autumn seasons. Notably, a significant effect on the soil microorganism conditions was observed after 30 days of probiotic treatment. Probiotics exhibit a favorable influence on microbiological processes within the soil, fostering conducive conditions for the development of soil microorganisms and the formation of humus. The most effective concentration of probiotics for promoting the functionality of microbial communities in black soils is determined to be 10%, accompanied by a dose of 100 l ha^{-1}. Consequently, the application of probiotic treatment at a concentration of 10% and a dose of 100 l/ha^{-1} holds the potential to enhance the biological state of the soil, restore soil microbial diversity, and serve as an environmentally safe fertilizer.

Keywords: soil, probiotics, soil micro-organisms, microbial activity, soil microbiological processes

INTRODUCTION

Soil biodiversity, unlike other aspects of soil science, remains poorly understood, particularly regarding the anthropogenic impacts on the diversity of microbes and soil fauna that reside unseen in the soil. The provision of essential ecosystem services, such as carbon sequestration, nutrient cycling, water retention, and the production of nutritious food, heavily relies on the significant contribution of soil biodiversity (**Marinari *et al*., 2006; Baumann *et al*., 2012; Kucharski *et al*., 2015**). However, the transition from grasslands to cultivated crops on black soils has resulted in the loss of a substantial portion of the original biodiversity, the extent of which is not well-documented due to the passage of time since these changes occurred. Restoring these soils to their pristine or near-pristine state is challenging, so a future challenge is to recover at least some of the lost biodiversity.
The impact of reducing soil biodiversity on soil functioning was investigated in an experiment by **Wagg *et al*. (2014)**, where certain groups of soil organisms were eliminated along a gradient, resulting in decreased abundance and richness of fungal and bacterial communities. The decline in plant species diversity is closely linked to the reduction in soil biodiversity and the simplification of soil communities. This finding supports previous research indicating that the composition of plant communities is influenced by the diversity and species composition of various soil organisms. Changes in soil biodiversity and soil community composition also affect nutrient cycling processes (**FAO, 2022**). The implementation of military operations within Ukraine's territory results in the destruction of soil biodiversity, potentially triggering adverse processes of soil degradation, reducing arable land, and potentially causing a food crisis. Soil contamination caused by military activities can arise from the utilization of nitro-aromatic explosive compounds. **(FAO and UNEP, 2021)**. These compounds have a high persistence and once entered the soil, they tend to remain, harming the local biota and reducing the soil health and fertility. The detrimental impacts of incendiary weapons that contain white phosphorus arise from the accompanying contaminants and residues produced during combustion. These weapons have the potential to contaminate soil with trace elements, hydrocarbons, organic solvents, surfactants, synthetic phenols, cyanide, dioxins, and radionuclides. Such contamination reduces soil fertility, crop yields, and poses risks to human health and the environment (FAO, 2022). Consequently, it is crucial to prioritize the restoration of damaged lands, their reintegration into agricultural use, and the promotion of sustainable functioning in agro-ecosystems. Under the current conditions, it is actually to search the ways which can support the restoration of soil biodiversity, improve the biological indicators of the soil and optimize of soil humification process. This will make it possible to develop and substantiate of resource-saving, environmentally safe systems of using new types of fertilizers and plant protection systems in specific soil and climatic conditions.
Our research works **(Pysarenko *et al*., 2019; Pysarenko *et al*., 2021; Pysarenko *et al*., 2022)** showed positive effect of probiotic application on phytosanitary state of agrocenoses, microbial remediation of petroleum polluted soil. However, the application of probiotics to enhance the activity of soil microorganisms is currently a poorly researched and significant topic in today's context. Therefore, there is an expediency in determining the optimal doses of probiotic application for the restoration of degraded soils and the functioning of ecologically sustainable agroecosystems.
The objective of this study was to determine the abundance of key ecological and trophic groups of soil microorganisms and the intensity of microbiological processes when subjected to probiotic treatment at various concentrations and doses in typical black soil.

MATERIAL AND METHODS

The field experiment took place at the research field of Poltava State Agrarian University from 2016 to 2021. Poltava State Agrarian University is located in the east part of Poltava region, central part of Ukraine. The experiment was based on three replications and conducted in the spring (April-May) and in the autumn (October).
The experiment focused on investigating the effects of applying a probiotic derived from *Bacillus subtilis* on the ecological and trophic groups of soil microorganisms. Specifically, the study examined the impacts on ammonifying bacteria, streptomycetes, amylolytic microorganisms, pedotrophic microorganisms, oligonitrophilic microorganisms, and microscopic fungi. Additionally, the experiment assessed the influence of the probiotic application on various microbiological processes occurring in the soil. Experiment included following factors: Factor A – concentration of probiotic: 10%; 1%; 0.1%; Factor B – dose of probiotic application: 50 l ha^{-1}; 100 l ha^{-1}; 150 l ha^{-1}. Control variant was soil without probiotic treatment.

The main soil characteristics site (0–30 cm depth) were the following: soil type – typical black soils, soil organic matter 3.5%; NH^3-N – 134 mg kg^{-1} dry soil; P_2O_5 – 276 mg kg^{-1} dry soil; K_2O – 98 mg kg^{-1} dry soil; acidity (pH) – 6.8.
The abundance of major microorganism groups in the soil was quantified by counting the number of cells per gram of completely dry soil. For the purpose of microbiological analysis, 10 grams of soil were collected from each experimental variant. The samples were moved into sterile mortars and microorganisms were dispersed. Dilutions 1:10 of the initial soil suspension were used for sowing on selective environment.
The ecological and trophic groups of soil microorganisms were identified by inoculating dilutions of soil suspensions onto selective nutrient media. **(Volkogon *et al.*, 2010)**: ammonifying bacteria – on the on meat peptone agar (MPA); streptomycetes and bacteria using mineral nitrogen (amylolytic) – on the starch-ammonia agar (SAA); pedotrophic – on the soil agar; number of microscopic fungi – on the agarized Chapek's medium with lactic acid; oligonitrophilic microorganisms – on the hungry agar. Following the inoculation of nutrient media, they were incubated at a temperature of 28°C for a period ranging from 5 to 14 days, depending on the growth rate of specific microorganism groups. The abundance of microorganisms was quantified as colony-forming units (CFU) per gram of completely dry soil. The moisture content of the soil sample was determined using the thermostatic weight method. To calculate the number of colony-forming units, the soil moisture and the dilution of the soil suspension were taken into account. The microbiological assessment of ecological and trophic groups of soil microorganisms was conducted on the 15th and 30th day after the commencement of the experiment. The results were processed statistically. The reported results were presented as the average of all replicate experiment sand their standard errors (x±SE). Mathematical analysis of the data was performed with Microsoft Excel 2010.
The direction and intensity of microbiological processes in the soil were assessed using the mineralization-immobilization, oligotrophy, and pedotrophy indexes (**Volkogon *et al.*, 2010**). The mineralization-immobilization index (IMI) represents the ratio of amylolytic microorganisms utilizing ammonia (mineral) nitrogen to ammonifying microorganisms assimilating organic nitrogen found in the soil's protein substances. An IMI value greater than 1 indicates an acceleration in humus decomposition or unfavorable conditions for microorganism development. The oligotrophy index (IO) denotes the ratio of oligotrophic microorganisms responsible for the complete mineralization of soil organic compounds to ammonifying microorganisms that absorb organic nitrogen. An IO value greater than 1 suggests unfavorable degradation processes occurring in the soil. The pedotrophy index (IP) reflects the ratio of pedotrophic microorganisms involved in transforming the water-soluble fraction of soil nutrients to ammonifying microorganisms that assimilate organic nitrogen IP> 1 indicates the restoration of SOM.

RESULTS AND DISCUSSION

1. *The impact of probiotic application on the abundance of ecological and trophic groups of soil microorganisms.*

The results of the study showed that the application of probiotics contributed to the establishment of a certain level of biological activity in the surface layer of the soil. This created specific conditions for the transformation of organic matter and increased the productivity of the agrobiocenosis. The analysis of the ecological and trophic groups of microorganisms showed that the soil had a higher microbial richness in spring compared to autumn, which can be attributed to the active recovery of the microbiota during this season.
Consequently, during the spring season, the average values of total bacterial counts for all examined variants were 1.24 to 1.64 times higher on the 30th and 15th day of microbiological assessment, respectively, in comparison to the autumn period. Results are shown in the figure 1. The trends observed for the abundance of pedotrophic, oligotrophic, ammonifying, and amylolytic bacteria were consistent. During the spring season, the average values of these bacterial groups were 1.37 to 1.56 times higher on the 30th day and 1.43 to 1.08 times higher on the 15th day of microbiological assessment compared to the autumn period.

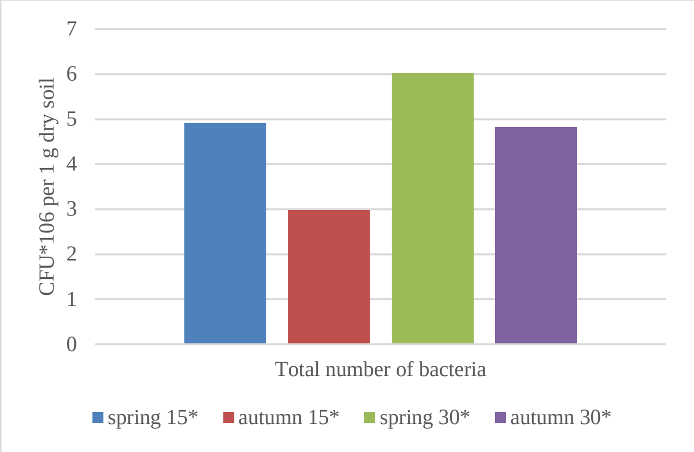

Figure 1 The total number of bacteria according to the seasons of the year.
*Note: * - day of microbiological indication after probiotic treatment.*

The number of actinomycetes varied significantly between different seasons. On average, the abundance of actinomycetes was 3.33 to 4.82 times higher in the spring compared to the autumn. On the other hand, the number of microscopic fungi was higher in the spring but did not show a significant difference, ranging from 1.4 to 1.44 times higher compared to the autumn. Results are shown in the figure 2.

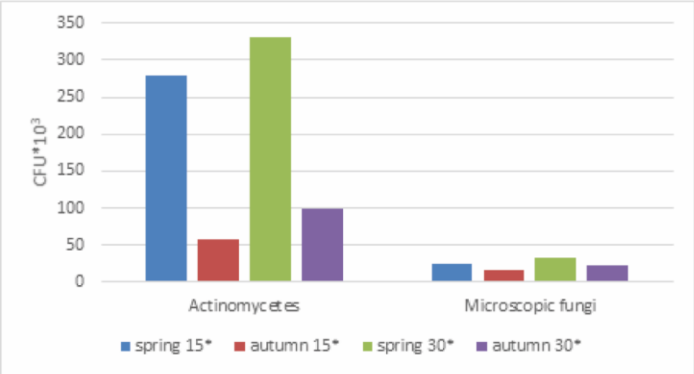

Figure 2 The total number of actinomycetes and microscopic fungi according to the seasons of the year.
*Note: * - day of microbiological indication after probiotic treatment.*

The research results indicate that the effect of probiotics on soil microorganisms is influenced by the dosage, concentration of probiotic application and the duration of the aftereffect. The highest microbial abundance was observed on the 30th day after probiotic application, with a noticeable activation of microbiological processes occurring on the 15th day. It was found that the most effective experimental variant for increasing soil microbial activity in both the spring and autumn seasons was to apply the probiotic at a concentration of 10% and a dosage of 100 liters per hectare. Specifically, the total bacterial count in the soil increased by 6-33% when the probiotic was applied at a 10% concentration compared to the control. The highest value of total bacteria in the soil was observed when using a dose of 100 liters per hectare and a concentration of 10%, resulting in a 33% increase in spring and a 25% increase in autumn compared to the control. The same trend was observed for other groups of soil micro-organisms.
The application of probiotics at a concentration of 10% resulted in a significant increase in the number of pedotrophic bacteria. On the 15th day after application, there was a 47-78% increase compared to the control, while on the 30th day, the increase ranged from 50-173%. Furthermore, when using a probiotic concentration of 10% and a dose of 100 liters per hectare, the highest increase in pedotrophic microorganisms was observed. In the spring, there was a 78% increase compared to the control, and in the autumn, there was a remarkable 173% increase. In contrast, applying a probiotic concentration of 1% and a dose of 100 liters per hectare in the spring resulted in a lower increase in pedotrophic microorganisms, reaching 15.5 CFU10*6 per 1 gram of dry soil on the 30th day. This value was 1.3 times lower compared to the application of a 10% probiotic concentration at the same dose. Similarly, in the autumn, the application of a 1% probiotic concentration and a dose of 100 liters per hectare resulted in an increase to 10.1 CFU10*6 per 1 gram of dry soil on the 30th day, which was 1.53 times lower compared to the application of a 10% probiotic concentration at the same dose. Applying a probiotic concentration of 0.1% showed a moderate increase in pedotrophic microorganisms, not exceeding 20% compared to the control. Therefore, the highest activity of pedotrophic microorganisms was observed on the 30th day when using a probiotic concentration of 10% and a dose of 100 liters per hectare. Results are shown in the figure 3.

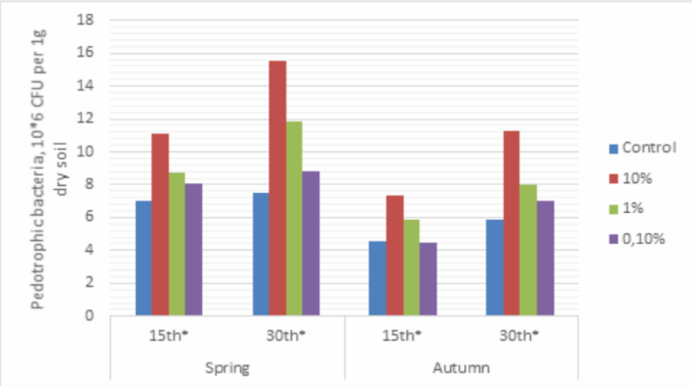

Figure 3 The number of pedotrophic bacteria depending on concentration of probiotic.
*Note: * - day of microbiological indication after probiotic treatment.*

The number of oligotrophic microorganisms showed a decrease of 1-9% on the 15th day when applying probiotic concentrations of 10% and 1%, compared to the control. However, on the 30th day of probiotic application, the number of oligotrophic microorganisms either increased slightly or approached the control level. Thus, no significant effect of probiotic application on the activity of oligotrophic soil microorganisms has been established. Results are shown in the figure 4.

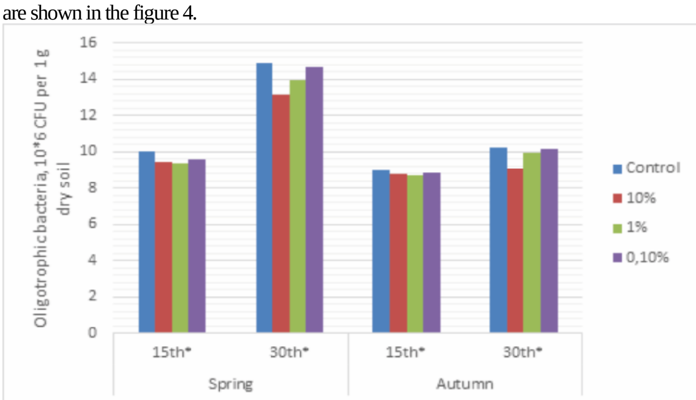

Figure 4 The number of oligotrophic bacteria depending on concentration of probiotic.
*Note: * - day of microbiological indication after probiotic treatment.*

Ammonifying and amylolytic bacteria play a vital role in the nutrient cycle, particularly nitrogen (N). The number of ammonifying bacteria increases by 3-17% in the spring and by 7-38% in the autumn when applying a probiotic concentration of 10%, compared to the control. Results are shown in the figure 5. Significant increases in the number of ammonifying bacteria were observed only at a dose of 100 l ha^{-1} during application of probiotic concentrations of 1% and 0.1% (by 3-9% in the spring and by 8-15% in the autumn compared to the control).

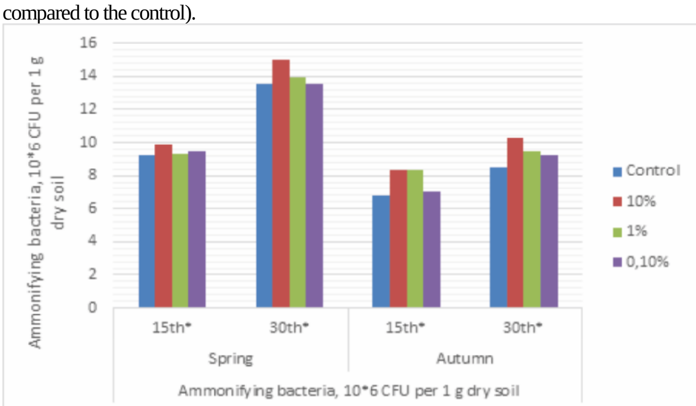

Figure 5 The number of ammonifying bacteria depending on concentration of probiotic.
*Note: * - day of microbiological indication after probiotic treatment.*

The number of amylolytic microorganisms decreases by 3-4% in the spring with application of probiotic concentration 10% on the 15th day compared to the control and approximates to the control variant on the 30th day of application. In the autumn application of probiotic concentration 10% leads to slight decrease in number of amylolytic microorganisms. Therefore, application of probiotic (10%) supports to increase the number of soil microorganisms which assimilate organic N, and reduce (not significant) the number of soil microorganisms which assimilate ammonia N (mineral), compared to the control. Results are shown in the figure 6.

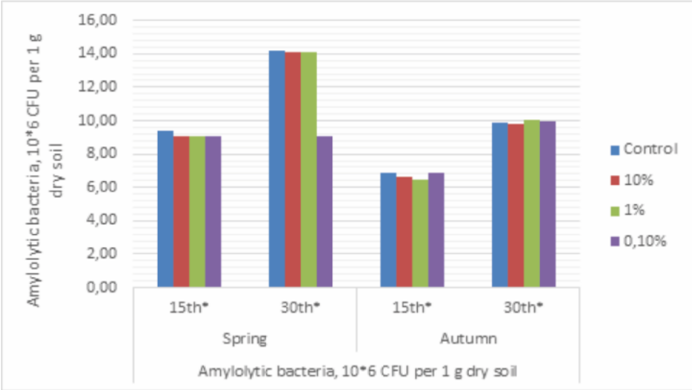

Figure 6 The number of amylolytic bacteria depending on concentration of probiotic.
*Note: * - day of microbiological indication after probiotic treatment.*

The number of actinomycetes increases by 1.2-1.5 times compared to the control when applying a probiotic concentration of 10% at a dose of 50 and 100 l ha^{-1} in both the spring and autumn. The highest number of microorganisms in this group was observed during the application of a probiotic concentration of 10% at a dose of 100 l ha^{-1} (0.452 $CFU10^6$ per 1 g dry soil on the 15th day; 0.590 $CFU10^6$ per 1 g dry soil on the 30th day in the spring, and 0.069 $CFU10^6$ per 1 g dry soil on the 15th day; 0.112 $CFU10^6$ per 1 g dry soil in the autumn). Results are shown in the figure 7.

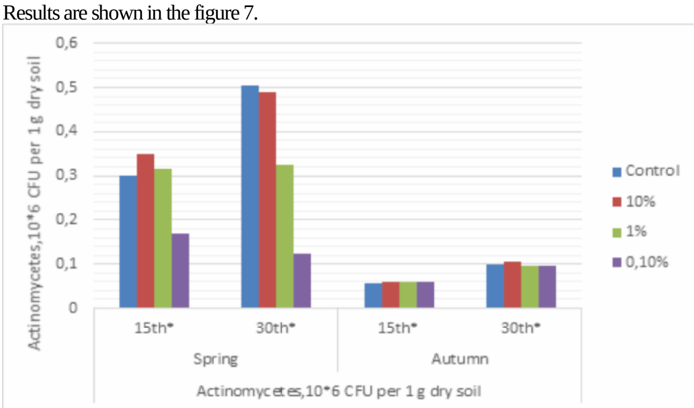

Figure 7 The number of actinomycetes depending on concentration of probiotic.
*Note: * - day of microbiological indication after probiotic treatment.*

The research results of soil microbiological indication indicate an increase in the total number of microscopic fungi with the application of probiotics. The number of microscopic fungi was significantly higher by 10-55% on the 15th day and by 5-31% on the 30th day during the application of a probiotic concentration of 10%. It was also observed that the highest increase in the number of microscopic fungi occurred on the 15th day Results are shown in the figure 8.

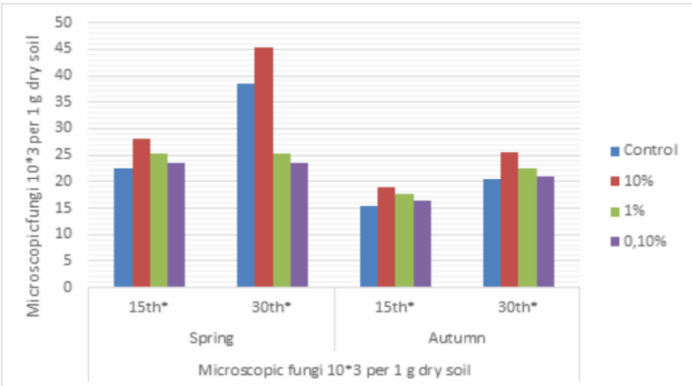

Figure 8 The number of microscopic fungi depending on concentration of probiotic.
*Note: * - day of microbiological indication after probiotic treatment.*

Therefore, the application of probiotics has an influence on the microbiological activity of the soil. The number of ecological and trophic groups of soil microorganisms increases during probiotic application, with the exception of oligotrophic and amylolytic bacteria. In the spring, there is a higher activity of soil microorganisms during probiotic application compared to the autumn. The influence of probiotics on soil microorganisms depends on the concentration and dose of the probiotic. The research results have shown that the most effective concentration of probiotic is 10% with a dose of 100 l ha^{-1}.

2. The impact of probiotic application on the direction of microbiological processes in the soil.

The analysis of soil samples without the application of probiotics (control variant) revealed an IMI value greater than 1 in both spring and autumn, indicating a prevalence of organic matter decomposition processes over its synthesis. However, when probiotics were applied, the IMI index was influenced, resulting in some variants showing IMI values below 1. This signifies a reduction in the rate of humus decomposition and the creation of favorable conditions for the growth and development of soil microorganisms. Among the tested probiotic concentrations, the most effective one was found to be 10% with a dose of 100 l ha^{-1}, as indicated in Tab 3 and 4. In the spring, the application of this concentration resulted in a decrease of IMI values by 12-14%, while in the autumn, the reduction reached 28-31% compared to the control variant. On the other hand, the application of probiotic concentrations of 1% and 0.1% had a less pronounced positive impact on soil microorganisms, and this effect was observed only at a dose of 100 l ha^{-1}. Specifically, in the spring, the IMI values decreased by 6-13% and 3-7% respectively, while in the autumn, the decrease ranged from 16-25% and 5-15% respectively, compared to the control variant.

Table 3 Soil microbiological processes in the spring.

Microbiological indexes	Concentrations of probiotic									
	Control	10%			1%			0.1%		
	Doses of probiotics, l ha^{-1}									
	Control	50	100	150	50	100	150	50	100	150
15th day*										
IMI	1.02	0.96	0.87	0.91	1.01	0.89	1.01	1.00	0.92	0.97
IP	0.76	1.08	1.21	1.06	0.90	1.03	0.86	0.95	0.83	0.79
IO	1.09	1.00	0.92	0.95	1.06	0.97	0.99	1.07	0.97	1.02
30th day*										
IMI	1.05	0.98	0.92	0.93	1.01	0.99	1.02	1.06	0.97	1.05
IP	0,56	1.11	1.30	0.70	0.76	1.11	0.68	0.67	0.70	0.57
IO	1.10	0.97	0.79	0.87	1.01	0.96	1.04	1.09	1.05	1.10

Note: * - day of microbiological indication after probiotic application.

The pedotrophy index (IP) serves as an indicator of the decomposition intensity of soil organic matter and the humification process. In the control variant, the IP values ranged from 0.56 to 0.76 (IP<1), suggesting a low level of humus recovery during both the spring and autumn periods. However, the application of probiotics was found to influence the pedotrophy index, resulting in higher IP values compared to the control in both seasons. Among the studied probiotic concentrations, the most effective one was determined to be 10% at a dose of 100 l ha^{-1}. In the spring, the IP values increased by 59-132%, while in the autumn, the increase ranged from 48-96% compared to the control variant. This indicates that the application of probiotics at a concentration of 10% led to an enhanced decomposition intensity of soil organic matter, aligning with the nutrient requirements of plants and activating the process of soil humification.

Table 4 Soil microbiological processes in the autumn.

Microbiological indexes	Concentrations of probiotic									
	Control	10%			1%			0.1%		
	Doses of probiotics, l ha^{-1}									
	Control	50	100	150	50	100	150	50	100	150
15th day*										
IMI	1.01	0.80	0.69	0.91	0.82	0.76	0.76	1.01	0.96	0.97
IP	0.68	0.70	1.01	0.92	0.65	0.92	0.51	0.71	0.80	0.40
IO	1.32	1.15	0.88	1.16	1.10	0.93	1.11	1.32	1.22	1.27
30th day*										
IMI	1.16	1.08	0.83	1.00	1.09	0.98	1.14	1.15	0.99	1.10
IP	0.69	0.90	1.35	0.99	0.72	0.99	0.82	0.77	0.73	0.77
IO	1.20	1.13	0.73	0.83	1.04	0.99	1.15	1.15	1.00	1.15

*Note: * - day of microbiological indication after probiotic application.*

It was determined that in the control variant, the index of oligotrophy (IO) was greater than 1, indicating the presence of degrading processes in the soil during both the spring and autumn periods. The research results showed a decrease in the oligotrophy index in both study periods when probiotics were applied at various concentrations and doses. The most effective application was observed with a concentration of 10% and a dose of 100 l ha^{-1}, resulting in a decrease of IO by 15-28% in the spring and 33-39% in the autumn compared to the control. This decrease indicates an increase in nutrient availability for soil microorganisms, suggesting improved conditions for their growth and activity.

Furthermore, the application of a 1% probiotic concentration did not show a significant impact on the oligotrophy index, as it remained similar to the control variant across all doses. This suggests that the concentration of 0.1% was not effective in promoting changes in nutrient availability.

Overall, the application of a 10% probiotic concentration enhances the supply of nutrients available to soil microorganisms. The most favorable results were observed on the 15th and 30th days, indicating the activation of probiotics and their positive influence on the nutrient content for different ecological and trophic groups of soil microorganisms. Moreover, the analysis of mineralization-immobilization, oligotrophic, and pedotrophic indexes demonstrates that probiotic application contributes to a decrease in humus decomposition rate and degrading processes, while promoting the restoration of humus and an increase in nutrient availability for soil microorganisms.

Soil fauna plays a crucial role in agricultural productivity, influencing nutrient cycling, soil organic matter dynamics, and plant growth. The presence of a diverse and stable microbial network, with abundant and diverse keystone taxa, primarily bacterial communities, is vital for efficient nutrient cycling and optimal plant health and fitness, which are key factors contributing to crop productivity (**Patyka *et al*., 2014; Betancur-Corredor *et al*., 2023**). Several studies have investigated the application of natural materials to activate soil microbial species and develop soil remediation practices for areas contaminated with residual chemicals (**Ni *et al*., 2023; Fu *et al*., 2022**). **Ni *et al*. (2023**) explored the effect of applying corn straw biochar (CB) combined with low-molecular-weight organic acids (LMWOAs) on the mechanism of removing napropamide residues in the rhizosphere. Their research revealed that the combined treatment increased the relative abundances of microbial species involved in napropamide biodegradation, leading to enhanced xenobiotic degradation in the soil. Additionally, the desorbed napropamide from the soil was effectively biodegraded. The combined application of biochar significantly increased the relative abundances of keystone species participating in nutrient cycling and herbicide removal. **Fu *et al*. (2022**) investigated the impact of biochar on the active bacterial communities involved in the mineralization of rhizodeposits and soil organic carbon (SOC). Their research demonstrated that biochar application shifted the rhizosphere communities towards lower richness and evenness, while stimulating the growth of *Actinobacteria* (including genera affiliated with Micrococcaceae) and other oligotrophic organisms. This response was likely due to the neutralization of soil acidity (increasing the pH from 4.53 to 6.17) and the increase in recalcitrant organic carbon content (from 10.69 to 25.77 g·kg−1). Overall, these studies highlight the importance of soil fauna and microbial communities in agricultural systems and provide insights into the potential of natural materials, such as biochar and organic acids, for improving soil health, nutrient cycling, and the removal of chemical residues.

Green manure and biofumigant crops are practices that can help maintain or increase agricultural soil yield by preventing degradation and protecting important ecosystem services. **Walker *et al*. (2022, 2023**) describe the benefits of these practices, including improvements in organic carbon levels, soil aggregation, erosion prevention, and overall soil fertility. Both ryegrass green manuring and biofumigation were found to impact soil microbial communities, favoring copiotrophic bacteria and fungi involved in organic matter degradation. These treatments also significantly increased the relative abundance of arbuscular mycorrhizal fungi (AMF) by 800% to 1400% compared to fallow land in two out of three years. The plots treated with ryegrass and biofumigants consistently showed higher soil aggregate stability, and there was a 20.4% increase in soil carbon observed in one section of the trial site.

Crop rotation is an environmentally friendly and highly sustainable cultivation method. **Liu *et al*. (2023**) conducted a meta-analysis of 76 studies to evaluate the effects of crop rotation on soil microbial indicators. The research results demonstrated that proper use of crop rotation, in conjunction with other agricultural practices, led to increased soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), fungal biomass, and bacterial Shannon's diversity index. Compared to continuous monoculture, crop rotation significantly increased MBC by 13.43% and MBN by 15.84%. Introducing other crops into continuous legume monoculture for rotation resulted in a significant increase in fungal biomass (45.50%), particularly in regions with mean annual precipitation values of 600 to 1000. Additionally, rotation increased Shannon's diversity index for soil bacteria by 7.68% compared to monoculture practices. The application of organic farming methods also has an impact on soil microbial activity and diversity. **Khatri *et al*. (2023)** conducted studies showing that bacterial community diversity indices were significantly higher in soil from organic fields. The study identified key taxa such as *Flavobacterium, Bacillus, Pseudomonas, and Planctomycetes*, which have the potential to contribute to disease suppressiveness in organic fields. This finding lays the foundation for the development of synthetic microbial communities that can induce suppressiveness in otherwise conducive soil.

Our research confirms that the application of natural methods such as probiotics contributes to the activation of soil microbial communities and microbiological processes. Through increased microbial activity and diversity, the use of natural methods and technologies in agriculture offers a sustainable alternative for preventing soil-borne plant diseases.

CONCLUSION

Application of probiotic has positive effect on microbiological activity of the soil. The total number of soil micro-organisms increases by 33% when probiotics are applied in the spring and by 25% in the autumn (using a dose of 100 liters per hectare and a concentration of 10%). A significant effect on soil microbial conditions was observed on the 30^{th} day of applying probiotics.

Application of probiotics has a positive influence on the direction of microbiological processes in the soil, creating favourable conditions for the development of soil microorganisms and humus formation.

The effect of probiotic on the soil microorganisms depends on the concentration and dose of probiotic application, as well as the time of the aftereffect. The most effective concentration of probiotic for the functioning of microbial cenosis of black soils is 10% and a dose 100 l ha^{-1}. The results of the research showed that probiotic application at a concentration of 10% and a dose of 100 l ha-1 can be used as an environmentally friendly fertiliser in organic farming, which will contribute to the improvement of soil biological indicators.

Conflict of interest: The author declares that there is no conflict of interest regarding the publication of this paper.

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