Chapter 18

# DIRECTIONALITY OF MICROBIOLOGICAL PROCESSES IN THE RHIZOSPHERE OF WINTER WHEAT UNDER THE INFLUENCE OF BIOLOGICAL PRODUCTS AZOTOHELP® AND GROUNDFIX®

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## INTRODUCTION

The agricultural sector is facing climate change, which intensifies extreme climatic events, leading to deterioration of soil fertility, increased harmfulness of pathogens and pests, and subsequently to a decrease in grain productivity.[1,2,3,4,5]

Losses of major crops from adverse environmental stressors, such as drought, salinity, extreme temperatures, nutrient imbalances, heavy metals and others, have reached 50-82% and pose a serious threat to agriculture and

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food security.[6,7,8] The use of plant growth-promoting bacteria (PGPB) can provide a new approach to increasing crop yields under such conditions.[5,9]

Soil and plant-associated microorganisms play a key role in ecosystem functioning by carrying out numerous biogeochemical cycles and decomposition of organic matter.[10] For this reason, biostimulants and biofertilisers are considered to be the most important components of sustainable agriculture that have a lasting impact on soil fertility.[11]

Soft winter wheat (*Triticum aestivum* L.) is one of the main food grains, which is a leading food crop in about 50 countries, including Ukraine. In this regard, increasing gross grain production of this crop is a key strategic objective, especially under martial law, which will ensure Ukraine's food and economic security.

## 1. Identifying the background to the problem and formulating the problem

Climate change, excessive use of chemical fertilizers and pesticides significantly increase the harmfulness of abiotic stresses, which affects the biological activity of the soil and the productivity of grain crops, including winter wheat. For example, the frequency, intensity and unpredictability of

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floods are increasing, affecting 10-15 million hectares of wheat annually, resulting in yield losses of 20-50%.[12,13,14]

Recently, there has been a growing interest worldwide in the use of microbial biological products, namely biological polyfunctional plant nutrition products (biofertilisers), which can actively participate in regulating the resistance of crops in a warming climate. The global market for biological multifunctional plant nutrition products (biofertilisers) containing microorganisms and their physiologically active substances is estimated at USD 3.72 billion in 2025 and is expected to reach USD 5.97 billion by the end of 2024, growing at a CAGR of around 9.94% over 2025-2030.[15] The bacteria that make up biological products stimulate plant growth and increase plant productivity through the assimilation of nutrients, production of biologically active substances, induction of a cascade of defence reactions and development of plant systemic immunity, which helps to minimize the harmful effects of biotic and abiotic stresses.[16,17]

Many studies in the USA, Europe and Asia have been devoted to the impact of biological products on cereals. Considerable material has been accumulated on the multifaceted effects and mechanisms of PGPB's positive impact on plants: production of biologically active substances, stimulation of plant systemic resistance, etc. The joint use of several strains with different properties and mechanisms of interaction with the plant is being investigated as a way to improve the efficiency of inoculation. The relevance of using biological products in winter wheat crops is also due to the expanding use of organic and integrated farming technologies in Ukraine and the development of measures to adapt plants to climate change.

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## 2. Analyze existing methods of solving the problem and formulate the task

Increasingly, in cereal crop technologies today, biological products with different mechanisms of action, such as arbuscular mycorrhizal fungi, plant growth-promoting bacteria, *Trichoderma* fungi, endophytes and seaweed extracts (SWE), are used to increase their resistance to stressful conditions.[18,19,20]

For example, Radzikowska-Kujawska, D., et al. (2023) found that BactoComplex and *Bacillus velezensis-based* preparations had a positive effect on the physiological state and yield of winter wheat under different moisture conditions, especially on improving the resistance of wheat plants to drought, and the least damage to the photosynthetic apparatus in plants.[21]

The yield of winter wheat and the biological activity of the soil are directly related, so the ways of activating biological processes in the soil are of great importance. Anli, M. et al. (2022).[22] For example, Dobrzyński, J. et al. (2025) studied the effect of the PGPB consortium, namely *Pseudomonas* sp. G31 and *Azotobacter* sp. PBC2 (P1A), on the soil bacterial community of wheat in the field. Compared to the control, a significant increase in the content of nitrates and available phosphorus in the rhizosphere during the growing season and plant productivity was found. The metataxonomic study showed that the used consortium had no significant effect on the diversity of local soil bacteria, however, 3 weeks after application, P1A increased the relative abundance of

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*Nitrospira*, which could have influenced the increase in nitrates in the rhizosphere, and reduced the number of *Bdellovibrio*.[23]

The production of osmoprotectants by Bacillus spp. bacteria, namely proline, polyamines, glutamate and total free amino acids, also contributes to salinity and drought resistance in plants. Exopolysaccharides (EPS) bind toxic $Na^+$ and limit the supply of $Na^+$ to the roots. Soil aggregation due to EPS formation and changes in root exudate secretion helps to moisten the rhizosphere and improve water and nutrient uptake. EPS also increases root adhesion to the soil.[24]

Biofertilisers are especially important for wheat cultivation in conditions of soil phosphorus deficiency. For example, Yahya, M., et al. (2022) found that biofortification of Enterobacter spp. strains DSM 109592 and DSM 109593 using filter sludge (FM) increased soil-available phosphorus (8.5-11%) and phosphatase activity (4-5%), and subsequently wheat grain yield (4-9%) and seed phosphorus levels (9%).[25]

The use of a biopreparation based on *Azotobacter vinelandii* in the agrocenosis of winter wheat contributed to the mineralization of plant residues, accelerated decomposition processes and weakened their mechanical characteristics.[26]

El-Nahrawy, S, et al. (2020) studied isolates of the genus Azotobacter, which demonstrated an increase in salinity tolerance of winter wheat plants, seed germination, increase in wet and dry weight, root and shoot length.[27]

Ji et al. (2020, 2022) found that the action of *Bacillus subtilis* HG-15 and *Enterobacter cloacae* HG-1 increased salt tolerance, growth and development

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of inoculated wheat plants.[28,29] Joint inoculation with diazotrophic bacteria (*Paenibacillus beijingensis* BJ-18) and phosphate-solubilising bacteria (*Paenibacillus* sp. B1) significantly increased plant biomass, nitrogen content in plants (~ 30%) and soil (12%), and nitrogenase activity (69%).[30,31]

In this regard, it is promising to use multifunctional fertilizers based on microorganisms that can activate soil biota and all biological processes in the soil, affecting plant resistance and productivity, as an element of biologisation of farming systems.

***The aim and objectives of the study*** were to determine the role of additive and synergistic effects of different concentrations of Azotohelp® and Groundfix® in winter wheat cultivation in the Western Forest-Steppe of Ukraine.

## 3. Research conditions and methods

The research was conducted at the experimental field of the Khmelnytsky State Agricultural Research Station of the Institute of Feed and Agriculture of Podillya (Khmelnytsky State Agricultural Research Station of the Institute of Feed and Agriculture of Podillya, Samchyky village, Starokostiantynivskyi district, Khmelnytsky region, northwestern part of the Right-Bank Forest-Steppe of Ukraine) during 2020-2022.

In the experiment, winter wheat of the Bogdana variety was sown. The main soil tillage was ploughing. The soil of the experimental plot is a slightly podzolised medium loamy black soil, medium-powerful, low-humus on loess loam of brownish-palmy colour.

Agrochemical characteristics of the soil: humus (according to Tyurin) – 2.8-2.9%, pH – 5.8-6.2; hydrolytic acidity 1.9-2.3 mg/equiv. per 100 g; gross reserves of nitrogen 0.153-0., phosphorus 0.136-0.; alkaline hydrolysed nitrogen 17-19.3 mg, mobile forms of phosphorus and potassium (according to Chirikov) 20.8-22.6 and 8-12 mg per 100 g, respectively. In terms of mechanical composition, it is a medium loamy soil with a lumpy-dusty structure.

[28] Ji C., Liu Z., Hao L., Song X., Wang C., Liu Y., Li H., Li C., Gao Q., & Liu X. Effects of *Enterobacter cloacae* HG-1 on the Nitrogen-Fixing Community Structure of Wheat Rhizosphere Soil and on Salt Tolerance. *Frontiers in plant science*, 2020. Vol. 11, P. 1094. https://doi.org/10.3389/fpls.2020.01094

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Spring 2021 was slightly cooler than normal, especially in March (average 2.3 °C vs. 0.4 °C), and precipitation (188.6 mm) in May was almost three times higher than normal. The summer of 2021 was very wet and warm, with record rainfall in July (349.2 mm), which inhibited the growth of certain aerobic microorganisms. The spring of 2022 was warm and moderately humid, and the summer was more stable compared to 2021, however, the average rainfall since spring has been insufficient.

To grow winter wheat in the experiments, we used conventional agricultural practices for the conditions of the Western Forest-Steppe.[32,33,34]

The experiments with Groundfix® and Azotohelp® (biotechnology company BTU, Ukraine) were set up in the last decade of September and the first half of October in accordance with generally accepted agronomic practices. The biological product Groundfix® contains the following microorganisms: *Bacillus velezensis (Bacillus subtilis), Bacillus subtilis, Priestia megaterium (Bacillus megaterium var.phosphaticum), Agrobacterium pusense (Azotobacter chroococcum), Agrobacterium salinitolerans (Enterobacter), Paenibacillus polymyxa* (titer 0.5-1.5 × $10^9$CFU/cm³). Azotohelp® is based on nitrogen-fixing bacteria *Agrobacterium pusense (Azotobacter chroococcum*) and biologically active products of their vital activity (titer 1.0 × $10^9$CFU/cm³).

Scheme of the experiment: 1 – Control; 2 – Groundfix® 3 l/ha for pre-sowing cultivation; 3 – Azotohelp® 3 l/ha for pre-sowing cultivation; 4 – Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha for pre-sowing cultivation. The experiment was replicated four times. Harvesting and accounting of winter wheat was carried out in the last decade of July.

The ratio of ecological and functional groups of microorganisms indicates the direction of microbiological processes in the soil. The number of microorganisms of the main ecological and functional groups was determined in accordance with DSTU 7847:2015[35], by microbiological sowing of soil suspension of appropriate dilution on selective nutrient media.[36]

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[33] Методологія і практика використання мікробних препаратів у технологіях вирощування сільськогосподарських культур / В. В. Волкогон та ін.; за ред. В. В. Волкогона. Київ: Аграрна наука, 2011. 156 с.

[34] Виробництво насіння пшениці озимої та ярої (Методичні рекомендації) / За ред. кандидатів с.-г. наук А.А. Сіроштана, В.П. Кавунця. Миронівка, 2021. 49 с.

[35] ДСТУ 7847:2015. Якість ґрунту. Визначення чисельності мікроорганізмів у ґрунті методом посіву на тверде (агаризоване) живильне середовище. [Чинний від 2016-07-01]. Київ: УкрНДНЦ, 2016. 12 с. Експериментальна ґрунтова мікробіологія / За ред. В.В. Волкогона. Київ: Аграрна наука, 2010. 464 с.

[36] Експериментальна ґрунтова мікробіологія / За ред. В.В. Волкогона. Київ: Аграрна наука, 2010. 464 с.

Selective media were used to determine the presence of certain groups of microorganisms and their associations: Zvyagintsev (total number of microorganisms that use mainly organic nitrogen compounds), Ashby (Azotobacter and rhizobia), starvation agar (oligotrophs), wort agar (yeasts), Vinogradsky (cellulose degraders), starch-ammonia agar (SAA) (nitrifying and actinomycetes) and soil agar (SA) (pedotrophs). The soil moisture content was determined in parallel. The number of microorganisms in 1 g of moist soil was calculated by the following formulas:

$$N_c = \frac{a \times 10^n}{m \times (1-\omega)} \text{x}10$$

where $N_c$ is the number of CFU in 1 g of raw soil, a is the average number of CFU, $10^n$ is the dilution factor and the serial number of dilution (n), m is the mass of soil in the first dilution, ω is the mass fraction of moisture in the sample in%;

$$\omega = \frac{m1\text{x}100}{m2},$$

where, m1 is the mass of moisture, m2 is the mass of the soil sample. The research was conducted at the Institute of Applied Biotechnology of the BTU biotechnology company.

Statistical processing of the data was performed using Microsoft Office Excel® 2010 for Microsoft Windows®, mean values were compared using analysis of variance (ANOVA) with $p \leq 0.05$.

## 4. Research results and discussion

According to the results of the study of the direction of microbiological processes in the soil, biological multifunctional preparations for plant nutrition (biofertilisers) Roundfix® and Azotohelp®, applied for pre-sowing cultivation, had a positive effect on the microbiological activity of the soil in the agrocenosis of winter wheat, which subsequently affected the productivity of the crop.

The flowering and early grain filling phases of winter wheat (BBCH 61-83) are critical for plants in response to drought or waterlogging. Due to the cool spring and significant precipitation in May 2021 (188.6 mm), which was almost 3 times higher than normal, soil conditions were unfavourable for the development of aerobic microorganisms. Waterlogging leads to changes in the soil microbial community that affect both its overall structure and the activity of plant growth stimulation. The study of the main ecological and functional groups showed that under the influence of bacteria and their metabolites, which are part of the Groundfix® and Azotohelp®, namely *Bacillus velezensis (Bacillus subtilis), Bacillus subtilis, Priestia megaterium (Bacillus megaterium var.phosphaticum), Agrobacterium pusense (Azotobacter chroococcum), Agrobacterium salinitolerans (Enterobacter),* *Paenibacillus polymyxa* and *Agrobacterium pusense (Azotobacter chroococcum),* the activity of soil microbiota can stabilise under adverse conditions. It is known that mineralisation-immobilisation processes are more intense under the influence of active strains of cellulose-degrading microorganisms. Thus, in the variants with biological products, the number of cellulose-degrading microorganisms increased significantly (1.0-1.2 x$10^4$ CFU 1 g of dry soil) compared to the control – 7.2 x$10^3$CFU 1 g of dry soil.[37]

The total number of heterotrophic microorganisms that use mainly organic nitrogen compounds and perform key functions in the nitrogen cycle and transformation of organic matter in the soil in the rhizosphere of winter wheat (in the growth phase BBCH 61-83) was in the range of $2.9\times10^5$-$1.7\times10^6$CFU/g dry soil. The highest number of microorganisms was found in the variant with Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha for pre-sowing cultivation (variant 4). The same variant also had the highest content of bacteria of the genus *Azotobacter* 3.9 x $10^5$CFU/g dry soil compared to other variants (1.0-1.9 x $10^5$CFU/g dry soil). In the variants with the use of biological products, a higher number of actinomycetes was found (2.2-2.9 x $10^5$vs. 1.9 x $10^5$CFU/g dry soil), which have high physiological activity, produce antibiotics, and play a significant role in soil rehabilitation.

An increase in the coefficient of mineralisation-immobilisation of nitrogen (hereinafter referred to as Km-i) was found in the variants with biological products compared to the control (Fig. 1).

The ratio of the number of microorganisms immobilising mineral nitrogen and ammonifiers (mineralisation-immobilisation ratio) characterises the intensity of plant residues mineralisation processes.[38]

This indicator was the highest in variant 4 – 0.88, with the combined use of Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha compared to the control (0.49), in variants with separate use of preparations – 0.58 and 0.54, respectively. The approach of this indicator to 1 indicates the absence of intensity of mineralisation processes in the soil in variant 4, and an increase in the available forms of nitrogen for plants.

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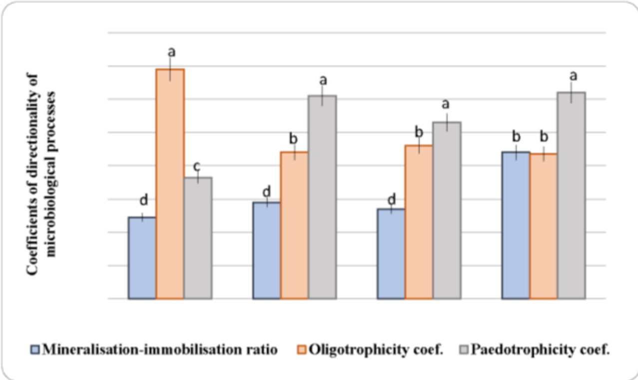


**Fig. 1. Coefficients of direction of microbiological processes in the soil during the cultivation of winter wheat, in the phase BBCH 61-83, 2021: 1 – Control; for pre-sowing cultivation: 2 – Groundfix® 3 l/ha; 3 – Azotohelp® 3 l/ha; 4 – Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha (Letters above the histograms correspond to the ranking of Tukey's test at $p < 0.05$)**

It was found that in the variant with the combined use of Groundfix® and Azotohelp®, the number of spore-forming bacteria was two orders of magnitude higher than in the other variants and amounted to 1.0 x $10^6$CFU/g of dry soil, while in other variants this figure ranged from 9.7-$10^4$to 2.0 x $10^5$CFU/g of dry soil (Fig. 2).

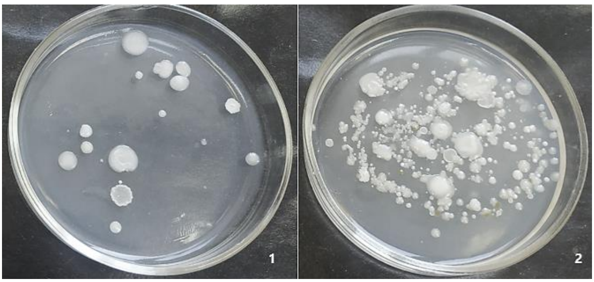

**Fig. 2. Spore-forming bacteria: 1 – control; 2 – Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha for pre-sowing cultivation**

The high intensity of spore formation in response to waterlogging may indicate the resistance of bacterial strains and their ability to maintain viability and restore their activity under optimal conditions. Thus, biofertilisers increase the regulation of the tolerance mechanism of grain crops, provide the necessary mineral nutrients and increase their adaptability and productivity in drought conditions by stimulating the antioxidant defence system and photosynthetic activity.[39,40]

PGPBs contribute to the increase of plant tolerance to abiotic stresses by inducing antioxidant, hormonal, photosynthetic and other metabolic pathways in plants.[41] In particular, *Bacillus* spp. initiate the physiological response of plants to cold stress, including the synthesis of cold shock proteins, antioxidant enzymes, initiation of signal transduction pathways, osmotic regulation, and membrane transport. Biofilm formation for resistance to abiotic stresses (waterlogging, cold stress, etc.) is one of the important features of bacteria that allows bacterial communities to survive and produce their metabolites under stressful conditions. Zubair et al. (2019) found that *Bacillus* spp. CJCL2 and *B. velezensis FZB42* produce ACC deaminase and can form biofilms under cold stress. Inoculation of wheat with these cold-resistant Bacillus strains contributed to the induction of a stress response in plants exposed to cold stress by regulating abscisic acid, lipid peroxidation, and proline accumulation pathways.[42]

The intensity of microbiological processes, the supply of nutrients to plants in the variant with the combined use of Groundfix® and Azotohelp®, is also evidenced by the coefficients of oligotrophicity and pedotrophicity – 0.87 and 1.24, respectively (in the control variant, respectively, 1.38 and 0.73). The coefficient of pedotrophicity in the variant with a separate application of Groundfix® was also high – 1.22, indicating a sufficient content of mobile organic compounds. The oligotrophicity coefficient in the variants with biological products was less than 1, which indicates a good supply of soil microbiota with easily digestible organic substances and the formation of optimal conditions for the functioning of the soil microbial complex.

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[39] Senko H., Kajić S., Huđ A., … Brkljačić L., Sviličić P. Will the beneficial properties of plant-growth promoting bacteria be affected by waterlogging predicted in the wake of climate change: A model study. *Applied Soil Ecology.,* 2024. Vol. 198. P. 105379. 10.1016/j.apsoil.2024.105379.

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[41] Tiwari S., Prasad,V. Chauhan P.S. and Lata C. Bacillus amyloliquefaciens Confers Tolerance to Various Abiotic Stresses and Modulates Plant Response to Phytohormones through Osmoprotection and Gene Expression Regulation in Rice. *Front. Plant Sci.,* 2017. Vol. 8. P. 1510. https://doi.org/10.3389/fpls.2017.01510

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The summer of 2021 was very wet and warm, with record rainfall in July (349.2 mm), which affected the inhibition of the growth of certain aerobic microorganisms. However, even under such unfavourable conditions, the combined use of Groundfix® and Azotohelp® in the developmental phase of wheat BBCH 87-89 helped to balance microbiological soil processes. Thus, the Km-i was the highest and amounted to 0.67 (in the control variant 0.35) (Fig. 3)

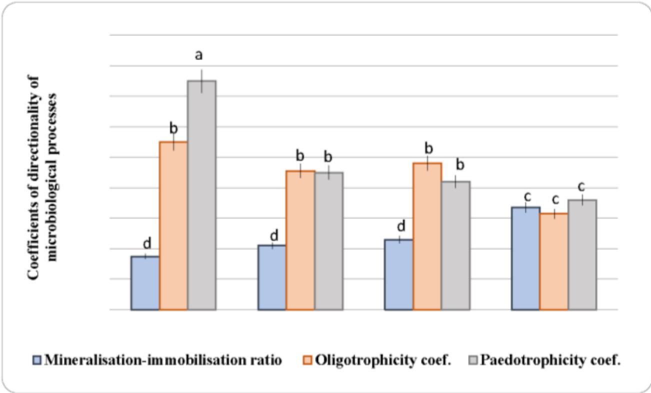


**Fig. 3. Coefficients of direction of microbiological processes in the soil during the cultivation of winter wheat in the BBCH 87-89 phase, 2021: 1 – Control; for pre-sowing cultivation: 2 – Groundfix ® 3 l/ha; 3 – Azotohelp® 3 l/ha; 4 – Groundfix ® 1.5 l/ha + Azotohelp® 1.5 l/ha**

Letters above the histograms correspond to the ranking of Tukey's test at $p < 0.05$

In the same variant, the coefficients of oligotrophic and pedotrophic were 0.63 and 0.72, respectively. The reduction of nutrients in the soil in the last phase of wheat development correlated with high crop yields. Also, in this variant and the application of Azotohelp® separately, the highest numbers of *Azotobacter* spp. were observed (3.8-4.2 x$10^5$vs. 2.4-2.7 CFU/g of dry soil). The variant with the use of Azotohelp® (3 l/ha) for pre-sowing cultivation for certain indicators of soil ecological and functional groups showed slightly lower activity compared to Groundfix® (3 l/ha), which may be due to the influence of unfavourable climatic conditions in 2021 on its effectiveness.

For example, similar results were observed by Silletti, S. and others (2021), in whose studies *Azotobacter chroococcum* was less effective for the growth of winter wheat in excessively moist conditions with suboptimal fertilisation and increased plant resistance only under extreme drought stress.[43]

However, in the variant with Azotohelp® (3 l/ha), the yield of winter wheat was higher compared to Groundfix® (3 l/ha). This may be due to the formation of metabolites that indirectly increase plant resistance to stresses of various nature by active strains of *Agrobacterium pusense (Azotobacter chroococcum.* According to scientific sources, such metabolites may include indole-3-acetic acid, which stimulates root growth, improves water absorption, siderophores, vitamins that act as antioxidants, protecting cells from oxidative stress, exopolysaccharides, and antibiotics).

The combined application of Groundfix ® 1.5 l/ha+ Azotohelp® 1.5 l/ha for pre-sowing cultivation revealed additive and synergistic effects. Even with extreme precipitation during the growing season of 2021, the metabolites of bacteria – components of biological products – contributed to an increase in soil biogenicity and plant resistance.

The spring of 2022 was warm and moderately humid, and the summer was more stable compared to the previous year, which created optimal conditions and contributed to the biological activity of the soil microbiota and the formation of the crop. It is the more stable weather conditions that can explain the insignificant differences between the variants in the number of the main ecological and functional groups in the developmental phase of winter wheat BBCH 61-83. There was a significant difference in the number of oligotrophs, the highest was found in the control – 49.3 million CFU/g of dry soil (indicating a decrease in the content of nutrients in the soil). In the variants with separate application of Groundfix® and Azotohelp®, these figures were 28.5-29.2 million CFU/g dry soil, and with the combined use of biofertilisers – 20.1 million CFU/g dry soil. The coefficient of oligotrophicity for the soil under the combined use of preparations and in the variant with Groundfix® (3 l/ha) was 0.68 and 0.63, respectively (in the control – 0.97), which indicates a good supply of soil microbiota with easily digestible organic substances and the formation of optimal conditions in the soil microbiota (Fig. 4).

[43] Silletti, S., Di Stasio, E., Van Oosten, M. J., Ventorino, V., Pepe, O., Napolitano, M., Marra, R., Woo, S. L., Cirillo, V., & Maggio, A. (2021). Biostimulant Activity of *Azotobacter chroococcum* and *Trichoderma harzianum* in Durum Wheat under Water and Nitrogen Deficiency. *Agronomy, 11*(2), 380. https://doi.org/10.3390/agronomy11020380

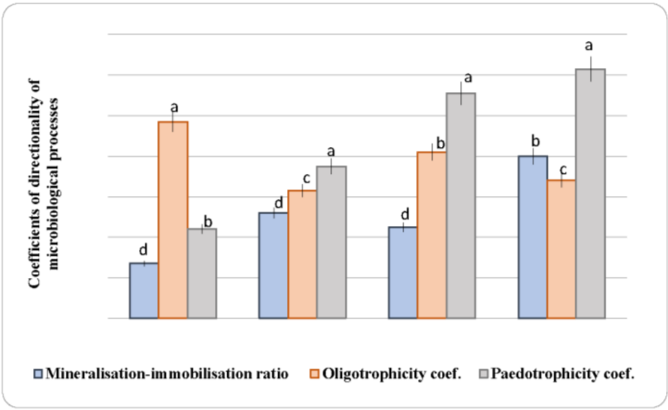


**Fig. 4. The coefficients of microbiological processes in the studied soil samples (in the phase BBCH 61-83, 2022): 1 – Control; for pre-sowing cultivation: 2 – Groundfix® 3 l/ha; 3 – Azotohelp® 3 l/ha; 4 – Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha**

Letters above the histograms correspond to the ranking of Tukey's test at $p < 0.05$

With the combined use of Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha, an increase in the available forms of nitrogen for plants, stability of mineralisation processes, the index of mineralization and immobilisation of nitrogen was the highest – 0.80, compared to the control (0.27), in variants with separate use of preparations – 0.52 and 0.45, respectively

The coefficients of pedotrophicity under the combined use of preparations and Azotohelp® 3 l/ha were 1.23 and 1.11, respectively, indicating a sufficient content of mobile organic compounds, active mineralisation processes and high microbial activity. The control variant was characterised by the lowest value of this coefficient of 0.44, which indicates a decrease.

In the development phase BBCH 61-83, winter wheat goes from flowering to early ripening, which is a key stage in the formation of the crop. At this time, optimal nutrition and moisture supply is very important, which was greatly facilitated by the use of Groundfix® + Azotohelp.®

Gudz S. O., Skivka L. M. (2020) found that the indicators of the nitrogen mineralisation-immobilisation coefficient were the lowest under the biological fertilisation system, which indicates a balance between mineralisation and immobilisation processes. At the same time, an increase in the value of the pedotrophicity coefficient, which indicates an increase in the intensity of decomposition of soil organic matter, in particular humus compounds, was observed under the industrial fertilisation system. Indicators of the oligotrophicity coefficient under ecological and biological fertilisation systems indicate the provision of soil microbiota with easily digestible organic matter and the formation of optimal conditions for the functioning of the soil microbial complex.[44]

In 2022, in the phase of full ripeness and grain ripening (BBCH 87-89), before harvesting, in the control variant, a slowdown in transformation processes, a decrease in the intensity of microbiological processes, the number of the main ecological and functional groups of microorganisms was observed, which was due to the depletion of readily available nutrients due to the death of the root system and a decrease in the activity of root exudation, competition between microorganisms.

However, the introduction of biological products Roundfix® and Azotohelp® affected plant metabolism, production of exudates, which led to prolonged activity of the rhizosphere microbiota, namely the storage of a certain number of nitrogen fixers, pedotrophs, microorganisms that use organic nitrogen compounds. Thus, in the control variant, compared to the combined use of Groundfix® and Azotohelp®, the lowest total number of bacteria was observed, respectively, 13.1 vs. 141.7 million CFU/g of dry soil, the number of bacteria of the genus *Azotobacter* – 5.4 vs. 16.6 million CFU/g of dry soil, the coefficient of mineralisation and nitrogen immobilisation – 0.31 vs. 0.52 (Fig. 5).

Letters above the histograms correspond to the ranking of Tukey's test at $p < 0.05$.

A synergistic effect on soil microbiota was found under the influence of the combined application of Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha, which was manifested in an increase in the number of many ecological and functional groups, so the total number of bacteria increased from 27.4 to 141.7 million CFU/g of dry soil. This indicates a balanced effect of biological products on soil microbiota, which contributes to the improvement of its functional diversity and stability.

Indicators of the oligotrophicity coefficient in the first three variants ($K_{O.}$= 1.78-2.32) indicate the depletion of soils with easily assimilated nutrients, while with the combined use of biological products this indicator was 0.75, which indicates a sufficient supply of nutrients. The increase in the level of assimilation of nutrients from soil reserves by the microbiota was more intensive in the control variant compared to the variants with the use of biological products ($K_P$= 1.19 vs. 0.81-1.02).

[44] Гудзь С. О., Сківка Л. М. Особливості формування еубактеріального комплексу ризосфери пшениці озимої (Triticum durum) за різних систем удобрення. *Вісник Київського національного університету імені Тараса Шевченка. Біологія*, 2020. Т. 81. № 2. С. 31–36. http://nbuv.gov.ua/UJRN/VKNU_biol_2020_2_8.

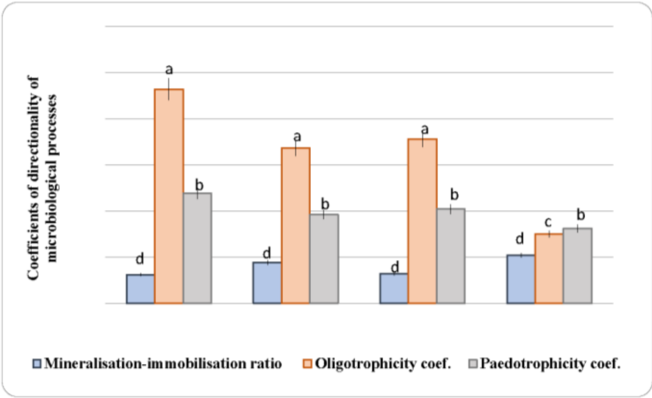


**Fig. 5. Coefficients of microbiological processes in the studied soil samples (in the BBCH 87-89 phase, 2022): 1 – Control; for pre-sowing cultivation: 2 – Groundfix® 3 l/ha; 3 – Azotohelp® 3 l/ha; 4 – Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha**

The processes of plant growth and development, especially under waterlogged or arid conditions, depend on morphological, physiological and biochemical adaptation, as well as on the gene regulation of plants that modulates such traits. The use of Groundfix® and Azotohelp® for pre-sowing cultivation results in the formation of more favourable conditions for the active functioning of the winter wheat rhizosphere microbiota, activation of microbiological processes, and active transport of assimilants.

The use of biological products helps to stabilise plant growth and development and affects the final yields through microbial-plant interaction. The analysis of winter wheat plant yields in 2021-2022 showed that the use of biological polyfunctional plant nutrition products (biofertilisers) Roundfix® and Azotohelp® for pre-sowing cultivation increases productivity by an average of 10.2-16.6% compared to the control. The highest yields were observed in the variant with the combined use of Roundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha – 6.61 t/ha (with separate application, respectively, 6.25 and 6.41 t/ha) (Table 1).

Table 10

**Winter wheat yield, t/ha**

| Variant | Biological products | Yield, t/ha | | | Increase to control (average for 2 years) | |
|---|---|---|---|---|---|---|
| | | 2021 | 2022 | Average | t/ha | % |
| 1 | Control | 5,34 | 5,99 | 5,67 | 0 | 0 |
| 2 | Groundfix® 3 l/ha | 5,84 | 6,65 | 6,25 | 0,58 | 10,23 |
| 3 | Azotohelp® 3 l/ha | 6,05 | 6,76 | 6,41 | 0,74 | 13,05 |
| 4 | Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha | 6,27 | 6,95 | 6,61 | 0,94 | 16,58 |
| | $p < 0.05$ | *0,28* | *0,31* | | | |

It is the complex interaction of the bacteria constituents of Groundfix® and Azotohelp® and their metabolites that can explain the improvement in the growth and development of winter wheat. Similar data were obtained by other researchers. For example, Yahya M. and others (2022) found that the use of biofertilisers based on phosphate-mobilising bacteria not only contributed to the solubilisation of phosphate in the soil, but also improved the growth parameters of wheat plants, increased the content of available phosphorus in the soil and phosphate activity.[45]

Combining several PGPMs with different mechanisms of action into consortia can increase their effectiveness and adaptability in the field compared to single-strain inoculants, compensating for traits that are not present in others and leading to an enhanced overall effect. Thus, the use of a consortium containing Erwinia sp. EU-B2SNL1 (N-fixer), *Chryseobacterium arthrosphaerae* EU-LWNA-37 (P-solubiliser) and *Pseudomonas gessardii* EU-MRK-19 (K-solubiliser) improved growth and physiological parameters, including root/shoot length and biomass, chlorophyll, carotenoids, phenols, flavonoids and soluble sugar content in barley crops.[46]

The EU's Biodiversity Strategy and 2030 Action Plan include a reduction in the use of chemical fertilisers by at least 20% and the use of chemical plant

[45] Yahya M., Rasul M., Sarwar Y., Suleman M., Tariq M., Hussain S. Z., Sajid Z. I., Imran A., Amin I., Reitz T., Tarkka M. T., & Yasmin S. Designing Synergistic Biostimulants Formulation Containing Autochthonous Phosphate-Solubilising Bacteria for Sustainable Wheat Production. *Frontiers in microbiology,* 2022. Vol. 13, P. 889073. https://doi.org/10.3389/fmicb.2022.889073

[46] Kaur T., Devi R., Kumar S., Sheikh I., Kour D., & Yadav A. N. Microbial consortium with nitrogen fixing and mineral solubilising attributes for growth of barley (*Hordeum vulgare* L.). *Heliyon,* 2022. Vol. *8*(4), P. e09326. https://doi.org/10.1016/j.heliyon.2022.e09326

protection products by 50%, and the allocation of at least 25% of EU agricultural land for organic farming by 2030. For example, El-Sorady, G. A. et al. (2022) found that the use of mineral forms of N (75%) and Azotobacter spp. demonstrated high and statistically similar efficiency, leading to an increase in winter wheat growth parameters by 30-50%.[47]

Li, J., et al. (2023) found that the use of biostimulants improved nitrogen uptake and transport, meeting the needs of the crop in the mid-late stages of growth, and improved yields with a corresponding reduction in the application of mineral nitrogen compounds.[48]

Rossini, A., Ruggeri, R. & Rossini, F. (2025) found that wheat plants treated with biostimulants outperformed control plants both in terms of root development by more than two times and in terms of chlorophyll content (+75% to +82%). In terms of grain yield, the use of biostimulants was most effective at medium doses of mineral nitrogen. In particular, a mixture of seaweed and microbial extracts at medium doses of mineral nitrogen (100 kg $^{ha-1)}$allowed wheat to achieve grain yields similar to the control group at standard nitrogen doses (150 kg $^{ha-1)}$, thus saving 33% of nitrogen. In terms of grain quality, foliar treatment with biostimulants increased the protein content of the grain by 4%.[49,50]

The main mechanisms of bacteria that promote plant growth under salinity or drought stress are the production of phytohormones, ACC deaminase, exopolysaccharides, promotion of nutrient availability from the soil, modification of biomass and root system morphology, induction of antioxidant enzymes synthesis, stimulation of photosynthetic apparatus, accumulation of osmolytes in crops, ionic homeostasis in plants and induction of genes encoding resistance to extreme abiotic factors.[51]

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[47] El-Sorady G. A., El-Banna A. A. A., Abdelghany A. M., Salama E. A. A., et.al. Response of Bread Wheat Cultivars Inoculated with Azotobacter Species under Different Nitrogen Application Rates. *Sustainability,* 2022. Vol. 14. No. 14, P. 8394. https://doi.org/10.3390/su14148394

[48] Li J., Ma H., Ma H., Lei F., He D., Huang X., Yang H., & Fan G. Comprehensive Effects of N Reduction Combined with Biostimulants on N Use Efficiency and Yield of the Winter Wheat-Summer Maize Rotation System. *Agronomy,* 2023. Vol. 13. No. 9, P. 2319. https:/ /doi.org/10.3390/agronomy13092319

[49] Rossini A., Ruggeri R. & Rossini F. Combining nitrogen fertilisation and biostimulant application in durum wheat: Effects on morphophysiological traits, grain production, and quality. *Italian Journal of Agronomy,* 2025. Vol. *20.* P. https://doi.org/100027. 10.1016/j.ijagro.2025.100027.

[50] Etesami H., & Maheshwari D. K. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. *Ecotoxicology and environmental safety,* 2018. Vol. 156, P. 225-246. https://doi.org/10.1016/j.ecoenv.2018.03.013

[51] Samantaray A., Chattaraj S., Mitra D., Ganguly A., Kumar R., Gaur A., Mohapatra P. K. D., Santos-Villalobos S. L., Rani A., & Thatoi H. Advances in microbial based bio-inoculum for amelioration of soil health and sustainable crop production. *Current research in microbial sciences,* 2024. Vol. 7, P. 100251. https://doi.org/10.1016/j.crmicr.2024.100251

The implementation of sustainable agricultural practices is becoming increasingly important to address global food security and environmental issues, and microbial bioinoculants are becoming a promising approach to preserving soil health and promoting sustainable crop production.

## CONCLUSIONS

Biological multifunctional preparations for plant nutrition (biofertilisers) Groundfix® and Azotohelp®, applied for pre-sowing cultivation, have influenced the formation of more favourable conditions for the active functioning of the microbiota of the winter wheat rhizosphere, increasing the available forms of nutrients for plants. This is due to the direction of mineralisation-immobilisation processes, development of ecological and functional groups of microorganisms, including oligotrophs and pedotrophs in the wheat rhizosphere.

Mineralisation-immobilisation processes are more intense under the influence of active strains of cellulose-degrading microorganisms. Thus, in the variants with biological products, the number of cellulose-degrading microorganisms increased by 1.4-1.7 times compared to the control. The use of Roundfix® and Azotohelp® biofertilisers compared to the control contributed to an increase in the total number of microorganisms, the number of bacteria of the genus *Azotobacter, and* actinomycetes, which are bioindicators of soil health. The best variant was the one with the combined application of Groundfix ® 1.5 l/ha + Azotohelp® 1.5 l/ha for pre-sowing cultivation compared to the separate application of biological products at a rate of 3 l/ha.

The additive and synergistic effect of the combined use of cultivation of Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha on the microbiological activity of soils, as well as on the formation of winter wheat plant productivity, was established.

Extreme weather conditions such as drought or waterlogging are critical for plants during the flowering and early grain filling phases (BBCH 61-83) of winter wheat. The studied vegetation periods were characterized by significant precipitation (almost 3 times higher than normal). Groundfix® and Azotohelp® indirectly contributed to the increase of plant resistance to adverse climatic conditions.

The use of Groundfix® and Azotohelp® biofertilisers for pre-sowing cultivation increased the productivity of winter wheat plants by 6.25-6.61 t/ha on average in 2021-2022 compared to the control.

The prolonged effect of the introduction of biological products Groundfix® and Azotohelp® in the phase of full ripeness and ripening of winter wheat grain (BBCH 87-89) was revealed. Despite the decrease in the formation of root exudates during this period, the activity of the rhizosphere microbiota was observed, namely the storage of a certain number of nitrogen fixers, pedotrophs, microorganisms that use organic nitrogen compounds.

Thus, the use of biofertilisers under the influence of Groundfix® and Azotohelp® allows to maintain microbiological balance even in the later phases of crop development, which leads to a slowdown in biological soil depletion after harvesting, the formation of organo-mineral balance due to the stable operation of spore-forming and pedotrophic forms, and creates prerequisites for better soil structure and effective destruction of post-harvest residues.


## SUMMARY

Climate change, excessive use of chemical fertilizers and pesticides significantly increase the harmfulness of abiotic stresses, which affects the biological activity of the soil and the productivity of grain crops, including winter wheat. Recently, there has been a growing interest worldwide in the use of microbial biological products, namely biological polyfunctional plant nutrition products (biofertilisers), which can actively participate in regulating the resistance of crops in a warming climate. The global market for biological multifunctional plant nutrition products (biofertilisers) containing microorganisms and their physiologically active substances is estimated at USD 3.72 billion in 2025 and is expected to reach USD 5.97 billion by the end of 2024, growing at a CAGR of around 9.94% over 2025-2030._The relevance of using biological products in winter wheat crops is also due to the expanding use of organic and integrated farming technologies in Ukraine and the development of measures to adapt plants to climate change.

The aim and objectives of the study were to determine the role of additive and synergistic effects of different concentrations of Azotohelp® and Groundfix® in winter wheat cultivation in the Western Forest-Steppe of Ukraine.__Biological multifunctional preparations for plant nutrition (biofertilisers) Groundfix® and Azotohelp®, applied for pre-sowing cultivation, have influenced the formation of more favourable conditions for the active functioning of the microbiota of the winter wheat rhizosphere, increasing the available forms of nutrients for plants._The use of Groundfix ® and Azotohelp® biofertilisers compared to the control contributed to an increase in the total number of microorganisms, the number of bacteria of the genus *Azotobacter, and* actinomycetes, which are bioindicators of soil health. The additive and synergistic effect of the combined use of cultivation of Groundfix® 1.5 l/ha + Azotohelp® 1.5 l/ha on the microbiological activity of soils, as well as on the formation of winter wheat plant productivity, was established. The use of Groundfix ® and Azotohelp® biofertilisers for pre-sowing cultivation increased the productivity of winter wheat plants by 6.25-6.61 t/ha on average in 2021-2022 compared to the control.

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