Panfilova et al.
CABI Agriculture and Bioscience (2026) 7:1, 0061
https://doi.org/10.1079/ab.2026.0061

CABI Agriculture and Bioscience

RESEARCH

OPEN ACCESS

Combined legume-based sowing systems and microbial biopreparations for restoring degraded chernozem fertility in the Southern Steppe of Ukraine

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Abstract

Introduction: Soil degradation in the Southern Steppe of Ukraine requires effective biological approaches to restoring chernozem fertility. *Methods*: This study evaluated the combined use of legume crops, mixed sowings and microbial biopreparations during a 3-year field experiment conducted in 2022–2024. Agrochemical, microbiological and statistical methods were used to assess changes in soil properties and crop productivity. *Results*: The integrated B5 treatment, combining mixed sowings with Azotokhelp, Helprost Universal, Phytocid and Organic-Balance, produced the strongest response. Humus content increased by 31%, readily available nitrogen by 40%, and the total number of soil microorganisms by 3.2 times. Nitrogen-fixing bacteria increased by 6.4 times, while dehydrogenase activity increased by 4.1 times. These improvements were accompanied by higher crop productivity: soybean yield increased by 75%, chickpea by 64% and lucerne green mass yield by 59%. Protein content and other yield-quality indicators also improved. *Conclusion*: The findings indicate that integrated legume-based biological management can enhance soil fertility, microbial activity and crop productivity. This approach may support regenerative agriculture and the restoration of degraded agroecosystems, although its long-term stability and economic feasibility require further evaluation.

Keywords: degraded chernozems, soil fertility restoration, legume crops, microbial biopreparations, mixed sowings, soil microbiota, regenerative agriculture

Introduction

The current state of Ukraine's agricultural sector is shaped by a combination of challenges caused by both degradation processes in the soil cover and the consequences of hostilities on large areas of agricultural land. These problems are particularly acute in the Southern Steppe zone, where intensive farming has led to the depletion of organic matter, a decline in biological activity and a loss of fertility in chernozem soils.

As noted by Gamajunova *et al*. (2021), in the last few years (2020–2025) the condition of soils in the Southern Steppe of Ukraine has shown a decrease in fertility to 2.8–3.2% (with the optimum being 4.5–5.0% for chernozems) due to non-compliance with crop rotations, a significant reduction in the area under legumes and perennial grasses, and the absence of manure. The authors state that about 65% of agricultural land in the southern region of Ukraine shows signs of moderate and severe degradation, manifested in a decrease in humus content to 2.8–3.2% and disruption of the microbiological balance. The authors also point out that biopreparations are an effective tool for restoring soil fertility, as they improve its structure, enrich it with beneficial microorganisms and promote efficient uptake of nutrients by plants. Biopreparations help to decompose organic residues, fix atmospheric nitrogen and create favourable conditions for the development of plant root systems (Chen *et al*., 2024; Khan *et al*., 2025). It has been established that the use of biopreparations for pre-sowing seed treatment contributed to an increase in the grain yield of winter wheat, which indicates favourable conditions for plant growth and development.

Post-war recovery of agriculture requires the development of effective and environmentally safe technologies for remediating disturbed agroecosystems (Bilan *et al*., 2017; Vincevica-Gaile *et al*., 2022). Traditional approaches based on the use of mineral

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Received: 03 June 2026. Accepted: 04 August 2026. Published: 20 August 2026

fertilisers are not always accessible and economically feasible under conditions of limited resources and may also create additional pressure on the environment. In this context, biological methods of restoring soil fertility are becoming particularly relevant, making it possible to re-establish the natural mechanisms of soil formation and enhance the self-regenerating capacity of agroecosystems, as confirmed in the work of Yuvaraj *et al.* (2020).

The relevance of this approach extends beyond Ukraine, as soil degradation, organic matter depletion, declining microbial activity and increasing drought stress affect agricultural regions worldwide. Therefore, the combined use of legume crops and microbial biopreparations may also be applicable to other degraded soils, particularly in regions where reducing dependence on mineral fertilisers and restoring biological soil functions are priorities.

Recent studies (2020–2025) demonstrate the considerable potential of legume crops and microbial biopreparations in restoring soil fertility. Rajput *et al.* (2024) and Mosier *et al.* (2021) point to the ability of legumes to biologically fix atmospheric nitrogen, improve soil structure and enhance microbial activity. The research of Martinelli *et al.* (2022) emphasises the importance of shifting the focus from short-term yield increases to long-term restoration of soil functions. Oviatt (2023), within the LEGU-MED project, proved the effectiveness of using legume crops in crop rotations to restore degraded soils in the Mediterranean.

However, the synergistic effects of combining different legume crops in mixed sowings remain insufficiently studied. Further research is particularly needed on their integration with biopreparations that have different mechanisms of action under the conditions of the Southern Steppe of Ukraine. In particular, the optimal ratios of crops in mixed sowings, schemes of biopreparation application and the impact on soil microbiological indicators under stress caused by hostilities and climate change require clarification, as noted in the work of Chatzistathis *et al.* (2021). Ayilara *et al.* (2023) studied in detail the potential of soybean endophytic bacteria to increase the productivity and stress tolerance of this crop, which is especially relevant in the context of climate change. However, these studies were carried out mainly on monocultures and did not cover the integrated impact on soil ecosystems in the conditions of combined sowings.

Previous studies have mainly focused on the individual effects of legume crops or microbial preparations on soil fertility improvement, plant productivity and nutrient availability. However, limited attention has been paid to the combined influence of mixed legume sowings and complex microbial biopreparations on the restoration of degraded chernozems under the specific environmental conditions of the Southern Steppe of Ukraine. Unlike previous studies that primarily investigated monocultures or single microbial inoculants, the present study evaluates the synergistic effect of different legume combinations together with biopreparations containing microorganisms with complementary mechanisms of action. In addition, this research integrates agrochemical, microbiological and productivity indicators to provide a comprehensive assessment of soil fertility restoration over a 3-year field experiment. Therefore, the novelty of this study lies in establishing the effectiveness of an integrated biological approach for restoring degraded chernozem soils under post-war agricultural recovery conditions.

Taking the above into account, the aim of this study was to evaluate the effectiveness of the integrated application of legume crops and microbial biopreparations with different mechanisms of action for restoring the fertility of degraded chernozems in the Southern Steppe of Ukraine. The study addressed the following research questions: (1) How do monoculture and mixed legume sowings combined with different biopreparation application schemes affect the agrochemical indicators of degraded chernozem soils? (2) How do these treatments influence the abundance, diversity and metabolic activity of soil microorganisms? (3) To what extent are changes in soil agrochemical and microbiological properties associated with the productivity and quality indicators of legume crops? Accordingly, the objectives were to assess changes in humus content, available nitrogen, phosphorus and potassium, and soil pH; to determine changes in microbial abundance, diversity, dehydrogenase activity and soil respiration; and to evaluate legume productivity and its relationship with the measured soil fertility indicators.

The significance of this study extends beyond the restoration of individual soil fertility indicators. By integrating mixed legume sowings with microbial biopreparations, the research contributes to the development of biologically based and resource-efficient approaches to regenerative agriculture. This is particularly important for regions where soil degradation, climate-related stress and restricted access to mineral fertilisers limit conventional recovery strategies. The study also provides field-based evidence on the relationship between soil microbiological activation, nutrient availability and crop productivity, thereby supporting a more integrated understanding of soil restoration processes. In the broader context, the findings may inform the development of sustainable soil management technologies for degraded chernozems and comparable agroecosystems affected by intensive cultivation or post-conflict disturbance.

Methods

The study was carried out during 2022–2024 on the experimental fields of the Educational, Scientific and Practical Centre of Mykolayiv National Agrarian University, located in the village of Blahodarivka, Mykolayiv region, in the Southern Steppe zone of Ukraine. The soil of the site was southern chernozem, residually slightly solonetzic, heavy loam on loess. The reaction of the soil solution was neutral (pH 6.5–6.7). Humus content in the 0–30 cm layer was 3.1–3.3%.

Monitoring of meteorological conditions was carried out using a Pessl Instruments (iMETOS) automatic weather station equipped with high-precision sensors. The mean air temperature for the growing season (April–September) was 18.9 ± 2.1°C (2022), 19.3 ± 2.3°C (2023) and 18.7 ± 2°C (2024), with a long-term norm of 18.5 ± 1.8°C. The total precipitation amounted to 285 ± 45 mm (2022), 245 ± 38 mm (2023) and 265 ± 42 mm (2024), with a norm of 290 ± 50 mm. Soil moisture in the 0–20 cm layer was measured every 10 days using a TDR-350 portable moisture meter (FieldScout, USA) and was 18.5 ± 2.1% (2022), 16.8 ± 1.9% (2023) and 17.9 ± 2% (2024), with a long-term norm of 19.2 ± 2.2%.

As research objects, seeds of legume crops that met the varietal quality requirements of State Standard of Ukraine (2002a) were used: field pea (*Pisum sativum* L.) cv. 'Kruiz', cultivated soybean (*Glycine max* [L.] Merr.) cv. 'ES Pallador', chickpea (*Cicer arietinum* L.) cv. 'Zodiak', food lentil (*Lens culinaris* Medik.) cv. 'Khryzolit', lucerne (*Medicago sativa* L.) cv. 'Zoriana', as well as lacy phacelia (*Phacelia tanacetifolia* Benth.) as a green manure crop.

Commercial biological preparations manufactured by BTU-Center (Ukraine) were used in the experiment. Azotokhelp®, SC was a liquid suspension concentrate containing viable cells of the natural nitrogen-fixing bacterium *Azotobacter chroococcum* and its biologically active metabolites, including amino acids, vitamins, phytohormones and fungicidal compounds. The viable-cell concentration was not less than 1.0 × 10^9 CFU/cm^3. The manufacturer identified the microorganism at the species level but did not publicly disclose its strain accession or deposit number.

Phytocid®, SC was a liquid biological fungicide containing viable cells and spores of Bacillus subtilis strain 221, deposited under registration number 393, at a concentration of not less than 1.0 × 10^9 CFU/cm^3. Seed treatment at 2 L/t corresponded to a minimum microbial dose of 2.0 × 10^{12} CFU/t of seed. Foliar application at 2 L/ha in 300 L/ha of water corresponded to 6.67 mL/L of working solution and at least 2.0 × 10^{12} CFU/ha per application. Two foliar applications provided a cumulative minimum dose of 4.0 × 10^{12} CFU/ha.

Helprost® Universal, SC was an integrated liquid fertiliser containing, per litre, up to 33.0 g nitrogen, 18.0 g phosphorus, 32.0 g potassium, 8.5 g sulfur, 1.5 g zinc, 1.5 g magnesium, 1.5 g boron, 8.5 g manganese, 2.5 g copper, 0.15 g molybdenum and 0.01 g cobalt, together with 27.0 g organic acids and 5.5 g amino acids. At the application rate of 3 L/ha, the preparation supplied 99 g N/ha, 54 g P/ha, 96 g K/ha, 25.5 g S/ha, 4.5 g Zn/ha, 4.5 g Mg/ha, 4.5 g B/ha, 25.5 g Mn/ha, 7.5 g Cu/ha, 0.45 g Mo/ha, 0.03 g Co/ha, 81 g organic acids/ha and 16.5 g amino acids/ha. The preparation was diluted in 300 L/ha of water, corresponding to 10 mL/L of working solution. The commercial formulation also contained a microbial complex; however, the manufacturer did not disclose its taxonomic composition, individual strain designations or viable-cell concentrations.

Organic-Balance®, SC was a liquid suspension concentrate containing a microbial consortium composed of Bacillus velezensis 221 (393), formerly identified as Bacillus subtilis 221 (393), at 40 ± 10%; Agrobacterium pusense 3064 (IMV B-7090), formerly identified as Azotobacter chroococcum 3064 (IMV B-7090), at 30 ± 10%; Paenibacillus polymyxa 1718 (IMV B-7027) at 10 ± 5%; Enterococcus hirae LK-50 (392), formerly identified as Enterococcus faecium LK-50 (392), at 10 ± 5%; and Lactobacillus delbrueckii subsp. bulgaricus 9702 (IMV B-7085) at 10 ± 5%. The total concentration of viable microorganisms was 1.0 × 10^8–1.0 × 10^9 CFU/cm^3. In addition to viable microbial cells, the formulation contained microbial metabolites, including phytohormones, amino acids, vitamins, enzymes and compounds with fungicidal and bactericidal activity.

The experiment was conducted as a multifactorial field trial with four replications. A randomised block design was used. Each plot covered 25 m^2 (5 m × 5 m). Blocks were formed according to the site microrelief and soil fertility gradient, and the experimental variants were randomly allocated within each block. To minimise cross-effects of green manure crops and microbial inoculants, buffer strips at least 0.5–1 m wide were left between plots. To eliminate systematic error and control edge effects, a fully randomised design with stratification along the main soil fertility gradient was applied. The placement of variants within each of the four blocks was carried out using random permutations generated in the R software environment (version 4.2.2). Each experimental plot was assigned a unique numerical code reflecting the block number (1–4), treatment variant (K, B1-B5) and coordinates within the block (for example, 2-B3-15). To minimise edge effects, protective strips 1.5 m wide, exceeding standard recommendations, were left around each accounting plot (15 m^2).

The randomised block field design was selected to account for the natural spatial heterogeneity of soil fertility and microrelief within the experimental site and to reduce their influence on treatment effects. The inclusion of an untreated control and five treatment variants enabled the separate and combined effects of mixed sowings and microbial biopreparations to be compared. Four replications provided independent observations for statistical analysis, while the 3-year study period allowed treatment responses to be evaluated under varying meteorological conditions. Repeated measurements of agrochemical and microbiological indicators were analysed using mixed-effects models to account for temporal dependence and variability among experimental blocks.

Six variants (n = 6) were studied: control (legume monoculture without biopreparations); variant 1 (B1) – legume monoculture with seed inoculation with ‘Azotokhelp’; variant 2 (B2) – combined sowings (soybean + phacelia, chickpea + lucerne) without treatment; variant 3 (B3) – combined sowings with seed inoculation with ‘Azotokhelp’; variant 4 (B4) – combined sowings with complex treatment using ‘Helpros Universal’ and ‘Phytocid’; variant 5 (B5) – combined sowings with complex treatment using ‘Azotokhelp’, ‘Helpros Universal’, ‘Phytocid’ and ‘Organic-Balance’ (Table 1).

This detailed scheme made it possible to assess both the individual effect of the inoculant and the synergistic effect of combining mixed sowings with integrated application of biopreparations with different mechanisms of action on the restoration of soil fertility. The field stage began in March 2022 with site preparation, which included harrowing and cultivation to a depth of 10–12 cm after autumn ploughing. Sowing was carried out manually at optimal agrotechnical dates in April of each study year, in accordance with zonal seeding rates for each crop. All plots received identical care, including mechanical weed control. Foliar treatments were applied

Table 1. Scheme of the experiment (n = 6 variants, 2022–2024; spraying – L/ha, seed treatment – L/t).

Variant	Type of sowing	Biological preparations and method of application	Rate of preparation	Working solution/ method
K (control)	Monocultures (pea, soybean, chickpea, lentil, lucerne)	No treatment	–	–
B1	Monocultures	Seed inoculation with Azotokhelp	0.8 L/t seed	10 L/t water; sowing ≤3 h after inoculation
B2	Combined sowings: • soybean + phacelia • chickpea + lucerne	No biopreparations	–	–
B3	Combined sowings (as in B2)	Seed inoculation with Azotokhelp	0.8 L/t seed	10 L/t water; sowing ≤3 h after inoculation
B4	Combined sowings (as in B2)	• Soil application of Helpros Universal, SC • Seed treatment with Phytocid	Helpros: 3 L/ha Phytocid: 2 L/t seed	300 L/ha water; spraying of the soil surface with incorporation to 5–7 cm
B5	Combined sowings (as in B2)	• Seed inoculation with Azotokhelp • Soil application of Helpros Universal • Foliar spraying during vegetation with Phytocid (2 times) • Treatment of residues with Organic-Balance	Azotokhelp: 0.8 L/t seed Helpros: 3 L/ha Phytocid: 2 L/ha Organic-Balance: 0.5 L/ha (≈ 1–2 L/ha on residues)	Azotokhelp: 5–10 L/t water Helpros/Phytocid/ Organic-Balance: 300 L/ha water; spraying + incorporation/loosening

using a Solo 475 backpack sprayer (Solo, Germany) in the morning in calm weather. Soil sampling was conducted in accordance with State Standard of Ukraine (2004). On each accounting plot (area 15 m^2), at least 15 elementary samples were taken at a depth of 0–20 cm with an auger 2–3 cm in diameter using the 'envelope' method and then combined into a composite sample. All samples were labelled, recorded in a field journal and transported to the laboratory.

In the laboratory, agrochemical and microbiological analyses of the collected samples were performed. Humus content was determined by the Tyurin method according to State Standard of Ukraine (2015) using a spectrophotometer; pH was measured potentiometrically in a salt extract (1:2.5) in accordance with State Standard of Ukraine (2022); cation-exchange capacity and total exchangeable bases were determined by standard agrochemical methods using ammonium acetate buffer at pH 7 according to State Standard of Ukraine (2022); available forms of phosphorus and potassium were determined by the Chirikov method (State Standard of Ukraine, 2002b); and total nitrogen was determined by the Kjeldahl method (State Standard of Ukraine, 2001).

Total numbers of microorganisms were determined by plating on meat-peptone agar with incubation at 30°C for 72 h; numbers of nitrogen-fixing bacteria were determined on Ashby medium at 28°C for 5–7 days. The microbial diversity coefficient was assessed using Shannon (H′) and Simpson (1-D) indices based on the quantitative composition of colony-forming units isolated from different trophic groups of microorganisms (nitrogen-fixing, phosphorus-mobilising, cellulose-decomposing). Colony counts were performed after incubation on nutrient media, with subsequent enumeration of visible colonies on Petri dishes after 5–7 days. For each variant, at least three replicates were taken, and only colonies within the statistically reliable range (30–300 CFU per plate) were considered. Calculations of diversity indices were carried out using the PAST v.4.09 software package, which provides statistical processing of microbiological data and an integrated assessment of the structural complexity of soil communities.

Dehydrogenase activity was determined by the Casida–Klein–Santoro method (1964) in a modification for soils, based on the reduction of triphenyltetrazolium chloride to triphenylformazan. For analysis, 5 g of air-dry soil (sieved through a 1-mm mesh) was taken, to which 5 ml of a 1% solution of triphenyltetrazolium chloride and 5 ml of phosphate buffer (pH 7.6) were added. To prevent acidification of the sample, 0.1 g of $CaCO_3$ was additionally introduced. The samples were incubated at 30°C for 24 h in the dark. After incubation, triphenylformazan was extracted with 25 ml of 95% ethanol (alternatively acetone) and filtered through filter paper. The optical density of the extract was measured on a spectrophotometer at λ = 485–490 nm relative to control samples without soil. Results were expressed in µg triphenylformazan/(g 24 h).

Soil respiratory activity was determined using incubation chambers with alkaline CO_2 absorbers in a modification using 0.1 M NaOH as an absorbent. Hermetic chambers (diameter 10 cm, height 15 cm, surface area S = 0.00785 m^2) were installed on the soil surface of the plots; an open container with 20 ml of 0.1 M NaOH was placed inside each chamber. Incubation was carried out for 24 h at 25 ± 1°C under field conditions. To control the effect of water vapour, parallel chambers with distilled water or $Ca(OH)_2$-based absorbent (soda lime) were used. After exposure, excess NaOH was titrated with 0.05 M HCl in the presence of phenolphthalein, and blank values from control chambers without soil were determined in parallel. The amount of CO_2 evolved was calculated according to formula (1):

$$R = \frac{(\text{Vcontr.} - \text{Vsample}) \times C \times 12}{t \times S}, \quad (1)$$

where R – respiration rate, mg C-CO_2 m^{-2} h^{-1}; V(contr.–sample) – difference in the volumes of HCl used for titration of the blank and sample (L); C – concentration of HCl (mol L^{-1}); 12 – atomic mass of carbon (g mol^{-1}); t – exposure time (h); S – soil surface area under the chamber (m^2). Results were expressed in terms of C-CO_2 (mg C-$CO_2 \cdot m^{-2}$ h^{-1}), in line with international practice.

Assessment of agrocenosis productivity as an indicator of soil fertility restoration was carried out by determining the yield and qualitative characteristics of legume crops. Yield was recorded manually from the accounting area of 15 m^2, with subsequent conversion to per-hectare values. For soybean, chickpea and lentil, total yield (t ha^{-1}), mass of 1000 seeds (g) (Adventurer Pro AV264C analytical balance, Ohaus, USA), number of pods per plant and seed protein content by the Kjeldahl method (State Standard of Ukraine, 2003) (Kjeldahl apparatus, Velp Scientifica, Italy) were determined. For lucerne, green mass yield (t ha^{-1}) for each cut (MS4202L technological scales, Mettler Toledo, Switzerland) and crude protein content (SPECORD 210 PLUS spectrophotometer, Analytik Jena, Germany) were taken into account. All measurements were performed in four replications for each variant.

The obtained numerical data were subjected to statistical analysis using the Statistica 12.0 software package (StatSoft Inc., USA). For yield and morphostructural parameters, multifactor analysis of variance (ANOVA/GLM) was applied. For microbiological and agrochemical characteristics with repeated measurements over time, mixed models with the restricted maximum likelihood method were used. Year and treatment were included in the mixed model as fixed effects because the study covered three specific growing seasons characterised by different climatic conditions. The Year × Treatment interaction was also included as a fixed term to determine whether treatment effects differed among years. Block and experimental plot were treated as random effects, with repeated observations nested within plots. The significance of the interaction was assessed at $p < 0.05$; where significant, treatment effects were interpreted separately for each year. Correlation analysis was carried out using Pearson's coefficient (r) for normally distributed data and Spearman's coefficient (ρ) in cases of deviation from normality (assessed by the Shapiro–Wilk test). Before analysis, linearity of relationships and homogeneity of variances were checked. To exclude multicollinearity in multifactor models, values of the Variance Inflation Factor were calculated. Statistical significance of correlation coefficients was determined at $p < 0.05$. Homogeneity of variances was tested using Levene's and Brown-Forsythe tests. For normally distributed data, multiple comparisons were performed using Tukey's HSD test; for non-normally distributed data, the non-parametric Mann–Whitney U-test was applied. Results were presented as arithmetic mean (M) ± standard error of the mean (m).

Results

DYNAMICS OF AGROCHEMICAL SOIL INDICATORS DEPENDING ON THE APPLIED APPROACHES

Over the 3-year research period, a pronounced positive trend was observed in the key agrochemical indicators of soil fertility, which statistically correlated with the applied agrotechnological measures. The humus content, as an integral indicator of fertility, showed stable growth in all experimental variants; however, the most pronounced dynamics were recorded in the variants with the combined application of biopreparations and mixed sowings. At the beginning of the study, the baseline humus content was 2.8–3%, which is typical for degraded ordinary chernozems of the region, whereas by autumn 2024, in the control variant (K), this indicator showed a slight increase to 3.1 ± 0.12%. This increase was due to the natural processes of mineralisation of plant residues and partial humification of organic matter.

The most noticeable dynamics were observed in the variants with the complex application of biopreparations. In variant B4, where mixed sowings were used in combination with the preparations 'Helpros Universal' and 'Phytocid', the humus content reached 3.4 ± 0.15%, which exceeded the control values by 9.7% ($p < 0.05$). The most effective was variant B5 with the full scheme of biopreparation application, where the humus content was 3.8 ± 0.18%, exceeding the control by 22.6% and variant B4 by 11.8% ($p < 0.05$). This increase in humus was associated with several interacting processes. *Azotobacter chroococcum* in 'Azotokhelp' promoted nitrogen fixation, while 'Organic-Balance' enhanced the microbial decomposition of plant residues. 'Helpros Universal' and 'Phytocid' also improved the availability of mineral nutrients.

The concentration of readily available nitrogen forms ($N\text{-}NO_3$ + $N\text{-}NH_4$) varied considerably among the experimental treatments and across plant growth stages. In the variants with seed inoculation by the preparation 'Azotokhelp' (B1, B3, B5), a consistently increased content of mineral nitrogen was observed during the vegetation period, which on average was 25–40% higher compared with the control. For example, in the critical flowering stage of soybean, when the nitrogen demand rises sharply, in variant B3 this indicator reached 12.8 ± 0.9 mg/kg of soil, whereas in the control variant it did not exceed 8.2 ± 0.7 mg/kg of soil ($p < 0.01$) (Table 2). This indicated the effective activity of nitrogen-fixing bacteria and the ability to supply plants with available forms of nitrogen in critical development periods.

The acidity of the soil environment remained relatively stable throughout the study period, fluctuating within the slightly acidic range (pH 6.5–6.8). However, in variants B4 and B5 a clear trend towards a slight increase in pH by 0.2–0.3 units by the end of the experimental period was observed. This effect may have been caused by the intensification of microbial transformation of organic matter and the formation of alkaline metabolic products of microorganisms, as well as by reduced leaching of basic cations as a result of improved soil structure. Additional indicators of the effectiveness of the applied agrotechnologies were changes in the content of available forms of phosphorus and potassium; in the variants with the application of the preparation 'Helpros Universal', an increase in available phosphorus by 13–25% and exchangeable potassium by 13–22% compared with the control was recorded, which is explained by microbial mobilisation of hardly accessible mineral compounds of these elements.

Statistical processing of the obtained results demonstrated significant differences between the experimental variants ($p < 0.05$), with the highest values of the coefficient of determination (R^2 = 0.87–0.92) observed in relation to the complex use of biopreparations and the dynamics of humus content, which indicates the direct influence of the applied agrotechnological measures on the restoration of soil fertility.

MICROBIOLOGICAL AND RESPIRATORY ACTIVITY OF THE SOIL ON THE STUDIED PLOTS

The results of microbiological studies revealed differences between the experimental variants, indicating a profound influence of the applied agrotechnological practices on the soil microbiota. Analysis of the main microbiological indicators showed a clear dependence on the type of treatment and the combination of biopreparations.

The total number of microorganisms was a key indicator of the effectiveness of the applied measures. In variant B5 with the complex application of biopreparations, an exponential growth of the microbial pool was observed, which by the end of the 2024 vegetation period reached a maximum value of 8.7 ± 0.4 × 10^7 CFU/cm^3. This value was 3.2 times higher than the control (2.7 ± 0.3 × 10^7 CFU/cm^3) ($p < 0.001$), indicating the creation of optimal conditions for the development of microorganisms. The variants using only the inoculant 'Azotokhelp' (B1, B3) showed intermediate results in the range of 4.1–4.9 × 10^7 CFU/cm^3, reflecting the importance of microbiological activation of the soil (Table 3).

Table 2. Final agrochemical indicators of soil fertility after application of different restoration approaches (2024).

Indicator	Control (K)	Variant B1	Variant B2	Variant B3	Variant B4	Variant B5
Humus, %	3.4 ± 0.13	3.5 ± 0.14	3.4 ± 0.13	3.7 ± 0.16	3.8 ± 0.15	4 ± 0.18*
Readily available nitrogen ($N\text{-}NO_3$ + $N\text{-}NH_4$), mg/kg	8.2 ± 0.7	10.5 ± 0.8*	9.1 ± 0.7	11.8 ± 0.9*	10.2 ± 0.8	12.8 ± 0.9*
Soil pH	6.5 ± 0.2	6.6 ± 0.2	6.6 ± 0.2	6.7 ± 0.2	6.7 ± 0.2*	6.8 ± 0.2*
Available phosphorus, mg/kg	45.2 ± 2.1	48.7 ± 2.3	47.3 ± 2.2	51.2 ± 2.4*	53.8 ± 2.5*	56.4 ± 2.6*
Exchangeable potassium, mg/kg	135 ± 6	145 ± 7	142 ± 6	152 ± 7*	158 ± 7*	165 ± 8*

Note: K – legume monocultures without biopreparations; B1 – monocultures with Azotokhelp seed inoculation; B2 – mixed soybean–phacelia and chickpea–lucerne sowings without biopreparations; B3 – mixed sowings with Azotokhelp seed inoculation; B4 – mixed sowings with Helprost Universal soil application and Phytocid seed treatment; B5 – mixed sowings with Azotokhelp seed inoculation, Helprost Universal soil application, two foliar applications of Phytocid and Organic-Balance treatment of crop residues. Humus content is expressed as %, available nitrogen, phosphorus and potassium as mg/kg of soil, and pH is dimensionless. Values are presented as mean ± standard error based on four field replications.
*Significant difference from the control at $p < 0.05$.

Table 3. Microbiological indicators of soil activity after application of restoration treatments (2024).

Indicator	Control (K)	Variant B1	Variant B2	Variant B3	Variant B4	Variant B5
Total number of microorganisms, ×10^7 CFU/cm^3	2.7 ± 0.3	4.1 ± 0.4*	3.5 ± 0.3	4.9 ± 0.5*	6.2 ± 0.6*	8.7 ± 0.4*
Nitrogen-fixing bacteria, ×10^5 CFU/cm^3	0.8 ± 0.1	3.2 ± 0.3*	1.2 ± 0.1	4.5 ± 0.3*	2.1 ± 0.2*	5.1 ± 0.4*
Respiratory activity, mg $C\text{-}CO_2$ m^{-2} h^{-1}	40 ± 4	62 ± 5*	48 ± 4	75 ± 6*	88 ± 7*	112 ± 5*
Microbial diversity index	0.45 ± 0.04	0.58 ± 0.05*	0.52 ± 0.04	0.65 ± 0.06*	0.72 ± 0.06*	0.85 ± 0.07*

Note: K – legume monocultures without biopreparations; B1 – monocultures with Azotokhelp seed inoculation; B2 – mixed soybean–phacelia and chickpea–lucerne sowings without biopreparations; B3 – mixed sowings with Azotokhelp seed inoculation; B4 – mixed sowings with Helprost Universal soil application and Phytocid seed treatment; B5 – mixed sowings with Azotokhelp seed inoculation, Helprost Universal soil application, two foliar applications of Phytocid and Organic-Balance treatment of crop residues. Total microorganisms are expressed as ×10^7 CFU/g soil, nitrogen-fixing bacteria as ×10^5 CFU/g soil and respiratory activity as mg $C\text{-}CO_2$ m^{-2} h^{-1}; the microbial diversity index is dimensionless. Values are presented as mean ± standard error based on four field replications. All measurements were taken at the end of the 2024 vegetation period.
*Statistically significant differences compared with the control ($p < 0.05$).

The number of nitrogen-fixing bacteria of the genus *Azotobacter* showed a pronounced specific response to the application of the corresponding biopreparations. The highest values were recorded in variants B3 and B5, where the preparation 'Azotokhelp' was used: $4.5 \pm 0.3 \times 10^5$ CFU/cm^3 and $5.1 \pm 0.4 \times 10^5$ CFU/cm^3, respectively. These values significantly exceeded the control ($0.8 \pm 0.1 \times 10^5$ CFU/cm^3) and indicated successful colonisation of the soil by beneficial microorganisms. Variant B5 was characterised by synergistic interaction between different groups of microorganisms, which contributed to increased efficiency of nitrogen fixation processes.

Dehydrogenase activity, as a key enzyme of microbial metabolism, showed the most noticeable dynamics. In variant B5, the activity of this enzyme reached 485 ± 22 µg triphenylformazan/(g 24 h), which was 4.1 times higher than the control (118 ± 15 µg triphenylformazan/(g 24 h)) ($p < 0.001$). Intermediate values in variants B3 (215 ± 18 µg triphenylformazan/(g 24 h)) and B4 (305 ± 20 µg triphenylformazan/(g 24 h)) indicated a cumulative effect from the application of different biopreparations. These results testify to the intensification of metabolic processes in the soil environment and the increase of the energy exchange of the microbial community. Respiratory soil activity showed a close relationship with dehydrogenase activity (correlation coefficient $r = 0.94$). The CO_2 emission rate in variant B5 was 112 ± 5 mg C-CO_2 m^{-2} h^{-1}, which was 2.8 times higher than the control (40 ± 4 mg C-CO_2 m^{-2} h^{-1}) ($p < 0.01$). This indicates a significant increase in the metabolic activity of soil microbiota and intensification of organic matter transformation processes.

Additional studies revealed noticeable improvements in the structure of the microbial community. In the variants with the complex application of biopreparations, an increase in the proportion of beneficial microorganisms (nitrogen fixers, phosphorus mobilisers, cellulose degraders) and a simultaneous reduction in the number of potentially pathogenic forms were observed. The microbial diversity index increased by 35–40% in variant B5 compared with the control, which is an indicator of enhanced stability of the soil ecosystem. Seasonal dynamics of microbiological indicators revealed the most pronounced differences during the vegetation maximum (June–July), when the gap between variant B5 and the control reached its highest values. This confirms the importance of synchronising microbiological processes with the phenological phases of plant development.

The results show the high effectiveness of the complex application of biopreparations in combination with mixed sowings for restoring microbiological activity in degraded soils. This opens prospects for improving biological soil remediation technologies in the context of post-war restoration of agroecosystems.

INFLUENCE OF LEGUME CROPS AND BIOPREPARATIONS ON AGROCENOSIS PRODUCTIVITY AS AN INDICATOR OF SOIL FERTILITY RESTORATION

Plant productivity is an integral indicator of the effectiveness of agrocenosis functioning and directly reflects the level of soil fertility restoration. In the context of post-war recovery, yield indicators demonstrate the practical effectiveness of the proposed agrotechnological solutions for remediation of degraded soils. The research results revealed a statistically significant dependence of legume crop productivity on the applied methods of soil fertility restoration. The highest yield indicators were recorded in the variants with the complex application of biopreparations in combination with mixed sowings, which indicates a synergistic effect between microbiological activation of the soil and optimisation of agrocenosis structure.

Cultivated soybean showed the most pronounced positive response to the proposed measures. In variant B5 with the full treatment scheme, yield reached 2.8 ± 0.12 t/ha, which exceeded the control values (1.6 ± 0.09 t/ha; $p < 0.01$) by 75%. It was noted that improvement in the qualitative characteristics of the yield correlated with better nitrogen nutrition: the 1000-seed weight increased from 142 ± 3 g to 168 ± 4 g, and the protein content in seeds rose from 36.2% to 39.8%. The number of pods per plant increased by 40.7% (from 18.2 ± 1.2 to 25.6 ± 1.5), which is directly related to improved availability of mineral forms of nitrogen and phosphorus as a result of microbial transformation.

Chickpea showed high sensitivity to biopreparations. In variant B5, yield reached 2.3 ± 0.1 t/ha, which exceeded the control (1.4 ± 0.08 t/ha; $p < 0.05$) by 64%. Joint cultivation of chickpea with lucerne ensured optimisation of the microclimate and moisture regime, which is particularly important under insufficient moisture in the Southern Steppe. Protein content in seeds increased from 22.5% to 25.1%, which correlates with an increase in the intensity of symbiotic nitrogen fixation by 40–45%.

Sown lucerne showed substantial results in mixed sowings. The green mass yield of the first cut in variant B5 was 45.2 ± 1.8 t/ha, which exceeded the control value of 28.5 ± 1.5 t/ha by 58.6%. Over the vegetation period, the total lucerne productivity reached 112.3 ± 4.2 t/ha of green mass versus 72.6 ± 3.1 t/ha in the control. Crude protein content increased from 16.8% to 19.2%, which indicates improved nitrogen nutrition of plants.

The obtained productivity indicators demonstrate a close relationship with the results of microbiological and agrochemical studies. The 75% increase in soybean yield shows a high correlation ($r = 0.89$) with a tenfold increase in the number of nitrogen-fixing bacteria and a 40% increase in the content of readily available nitrogen forms. The 18% rise in 1000-seed weight also shows a significant correlation ($r = 0.82$) with an increase in available phosphorus forms by 20–25%, which is the result of the activity of phosphorus-mobilising microorganisms.

Thus, the obtained results prove the high effectiveness of the proposed complex approach to restoring the fertility of degraded chernozems. The combination of mixed sowings of legume crops with green manures and the sequential application of biopreparations with different mechanisms of action ('Azotokhelp', 'Phytocid', 'Helpros Universal', 'Organic-Balance') provided a synergistic effect, which led to: a significant increase in microbiological soil activity, acceleration of humification processes and accumulation of organic matter, improvement of the soil nitrogen balance due to biological fixation and a substantial increase in legume crop productivity. This technology is promising for regenerative agriculture and the restoration of agroecosystems in the conditions of post-war recovery of Ukraine.

Discussion

The obtained findings demonstrate the potential of the combined application of legume crops and biopreparations for restoring the fertility of degraded chernozems of the Southern Steppe of Ukraine. The data obtained confirm the strategic importance of integrating legume crops into the crop rotation system for stabilising agroecosystems. The study revealed that the use of legumes, in particular pea and lentil, contributed to the intensification of biological nitrogen accumulation processes. This was manifested in an increase in the concentration of available forms of nitrogen in the soil profile, which forms the basis for restoring the balance of nutrients without involving additional volumes of mineral fertilisers. The results of the study are consistent with the work of Drobitko *et al.* (2025), who indicated the key role of legume crops in ensuring the sustainable development of the agricultural sector through the ability for biological nitrogen fixation and improvement of soil structure.

An increase in humus content by 31% in the variant with complex treatment confirms the data of Bossio *et al.* (2020) on the key role of organic matter in the formation of stable soil aggregates and carbon sequestration. This result is important in the context of nature-based climate solutions, as the accumulation of soil organic carbon is one of the most effective mechanisms for climate change mitigation. The high effectiveness of the inoculant 'Azotokhelp' is consistent with the study of Gamayunova *et al.* (2025), who demonstrated an increase in soybean productivity by 15–25% due to the use of microbial inoculants. However, the present study demonstrated a substantially greater increase of 75%. This response may be associated with synergistic interactions among the different bacterial groups contained in the complex preparations. The results regarding the improvement of microbiological soil activity are confirmed by the studies of dos Reis *et al.* (2024), who indicated the ability of microbial inoculants to increase the number of beneficial microorganisms and the activity of soil enzymes. A notable result is the 4.1-fold increase in dehydrogenase activity, as this indicator is a sensitive measure of the metabolic activity of soil microbiota. The effectiveness of mixed sowings of legume crops is confirmed by the work of Shebanin *et al.* (2025), who demonstrated the positive impact of biologisation on the quality of agricultural products and environmental conservation. The combination of different types of legume crops made it possible to create diverse microbial communities, which increased the resilience of agroecosystems (Coutinho *et al*., 2016; Kerimkhulle *et al*., 2025).

The rapid increase in humus under treatment B5 was probably associated with several interacting processes. Higher crop productivity likely increased root biomass, rhizodeposition and the amount of plant residues entering the soil, while Organic-Balance may have accelerated residue transformation and the formation of partially stabilised organic matter. The simultaneous increase in microbial abundance and dehydrogenase activity suggests more intensive carbon processing, although microbial carbon use efficiency was not measured directly. Therefore, the relative contribution of root inputs, microbial assimilation and residue humification cannot be separated and requires further investigation using root biomass, microbial biomass carbon and soil organic carbon fraction measurements.

The large effects observed in variant B5 should be interpreted cautiously. The 75% increase in soybean yield partly reflects the low productivity of the untreated control and the combined action of mixed sowing, biological nitrogen fixation, nutrient mobilisation, microbial inoculation, foliar treatment and residue decomposition. The rapid rise in humus may have resulted from increased root biomass and plant-residue inputs, but it may also have been influenced by short-term organic matter accumulation and field variability (Kunakh *et al*., 2020; Fedoniuk *et al*., 2025). Moreover, the B5 treatment required several application stages, potentially increasing labour and production costs. Therefore, although B5 produced the strongest response, its long-term stability, economic feasibility and additional labour requirements should be evaluated before large-scale implementation.

The increase in protein content in legume seeds (by 10–14%) is consistent with the results of Kovalenko *et al.* (2024), who proved the effectiveness of legume crops in improving the quality of animal feed by increasing protein content and improving amino acid composition. The data on the improvement of phosphorus and potassium availability under the influence of the preparation 'Helpros' are confirmed by the studies of Singh and Gaur (2024), who described the mechanisms of microbial mobilisation of hardly accessible mineral compounds. Phosphorus-mobilising microorganisms are able to release organic acids and enzymes that convert phosphorus into forms available to plants (Paramonova *et al*., 2022; Biliavska *et al*., 2024). The results of the study are also consistent with the work of de Moraes Sá *et al.* (2024), who emphasised the key role of organic carbon in restoring soil health in no-till systems. The restoration of organic matter is fundamental for improving all aspects of soil fertility. The effectiveness of biopreparations under stress conditions caused by climate change is confirmed by the studies of Tukenova *et al.* (2021), who demonstrated the positive effect of biological preparations on crop productivity under stressful environmental conditions. The results obtained are important for developing climate change adaptation strategies, since legume crops with the deep root systems are capable of withstanding droughts, which are becoming increasingly frequent in the Southern Steppe region, as noted by Horodyska *et al.* (2021).

Recent field studies generally support the combined use of crop diversification and microbial preparations, but they also demonstrate that the effects of these practices are strongly dependent on crop composition and environmental conditions. Sui *et al.* (2025) found that legume living mulch increased dissolved organic carbon and available nitrogen and modified the microbial community during crop development. However, the legume treatment also reduced soil organic carbon and total nitrogen at the maturation stage, whereas a mixture of legume and grass crops maintained these indicators while improving nutrient availability. This comparison suggests that the favourable results obtained in variant B5 cannot be attributed exclusively to the presence of legume crops. The balance between the quantity and quality of plant residues, the composition of mixed sowings and the activity of the applied microorganisms probably determined the observed response.

The importance of selecting compatible crop combinations is further demonstrated by McAuley *et al.* (2025). In their study conducted under semi-arid conditions, some legume–oilseed intercrops showed productivity advantages, whereas cereal–legume combinations were less successful because the cereal component competed strongly with the legume. Moreover, increases in active carbon and soil protein were associated primarily with the presence of legumes rather than with crop diversity itself. These findings qualify the interpretation of the present results as a general synergistic effect of mixed sowings. The positive response of the soybean–phacelia and chickpea–lucerne combinations may therefore reflect the compatibility of the selected crops, but the experimental design does not allow the individual contribution of crop interaction to be separated completely from the effects of the multiple biopreparations included in B5.

The microbiological results also require a differentiated interpretation. Zhang *et al.* (2025) reported that Bacillus- and Trichoderma-based biofertilisers increased soybean yield by 17.48% and 15.32%, respectively, while simultaneously increasing nutrient availability and the stability of microbial co-occurrence networks. This supports the relationship observed in the present study between microbial activation, improved nutrient status and crop productivity. Nevertheless, higher microbial activity should not automatically be interpreted as an unequivocal improvement in soil functioning. Shrestha *et al.* (2025) found that diversified cover crops increased fungal and arbuscular mycorrhizal diversity, whereas the presence of legumes reduced microbial respiration. Therefore, the 2.8-fold increase in respiratory activity recorded in B5 may indicate accelerated organic matter transformation, but it may also represent increased carbon mineralisation. Its positive interpretation is supported by the simultaneous increase in humus content and dehydrogenase activity; however, longer-term measurements of soil carbon stocks and CO_2 losses are required to determine whether this balance remains favourable.

The magnitude of the yield response in the present study was higher than that reported in several recent field experiments. Wang *et al.* (2026) found that the combined inoculation of soybean with Bacillus and Rhizobium increased yield by 38.3–57.6% under an optimised planting density, while Khaitov *et al.* (2026) recorded a 34.6% increase in mungbean yield when conservation agriculture was combined with crop-specific microbial inoculation under arid conditions. By comparison, the 75% increase in soybean yield obtained in B5 may be related to the relatively low productivity of the untreated control, the simultaneous application of several microbial preparations and the contribution of mixed sowing.

Consequently, this result should be interpreted as the response to an integrated management system rather than as the effect of an individual biological preparation.

Recent evidence also indicates that microbial inoculation does not produce uniform results across farming systems. Lahijanian *et al.* (2025) showed that the effects of cover crops and microbial inoculants on microbial diversity and crop yield varied substantially among conventional, transitional and organic fields, with the farming system and initial soil properties exerting a stronger influence than inoculation alone. This context dependence limits the direct generalisation of the present results beyond the southern chernozems and climatic conditions of the Southern Steppe of Ukraine. Nevertheless, the consistency of the responses across agrochemical, microbiological and productivity indicators suggests that integrated biological management may be an effective strategy for degraded soils with low biological activity.

Overall, comparison with the most recent studies confirms the potential of combining legume crops, mixed sowings and microbial preparations, while also showing that the magnitude and stability of the response depend on crop compatibility, baseline soil fertility, inoculant composition and the broader management system. Further multi-site and longer-term experiments should therefore separate the effects of individual preparations, mixed sowing and their interaction, while also evaluating carbon balance, microbial community composition and economic efficiency.

From a practical perspective, the findings provide a basis for developing biologically oriented soil-restoration strategies for farms operating on degraded chernozems. The most effective approach combined mixed sowings of soybean with phacelia and chickpea with lucerne, with the integrated application of 'Azotokhelp', 'Helpros Universal', 'Phytocid' and 'Organic-Balance'. This treatment may be considered by agronomists when the simultaneous objectives are to improve soil nutrient availability, stimulate microbial activity and increase legume productivity. The proposed approach may be particularly relevant where access to mineral fertilisers is limited or where reducing dependence on intensive chemical inputs is a priority. However, implementation should be adapted to local soil properties, climatic conditions, crop rotations and available farm resources. Before large-scale adoption, field-scale testing and economic assessment are recommended to determine appropriate application rates, labour requirements and cost-effectiveness under specific production conditions. Thus, the study offers a practical framework for integrating legumes, green-manure crops and microbial preparations into regenerative and post-conflict agricultural recovery programmes.

CONCLUSIONS

The study demonstrated that the integrated use of mixed legume sowings and microbial biopreparations can substantially improve the fertility and biological functioning of degraded southern chernozems. The most effective treatment was variant B5, which combined soybean–phacelia and chickpea–lucerne sowings with the sequential application of Azotokhelp, Helpros Universal, Phytocid and Organic-Balance. This treatment increased humus content by 31%, readily available nitrogen by 40%, available phosphorus by 24.8% and exchangeable potassium by 22.2%.

The improvement in agrochemical properties was accompanied by a pronounced activation of the soil microbiome. Under the B5 treatment, the total number of microorganisms increased 3.2-fold, the number of nitrogen-fixing bacteria increased 6.4-fold and dehydrogenase activity increased 4.1-fold. These changes were closely associated with higher crop productivity: soybean yield increased by 75%, chickpea yield by 64% and lucerne green mass yield by 59%, while protein content and other yield-quality indicators also improved.

The findings indicate that the restoration of degraded soils is most effective when biological inoculation is combined with diversified cropping rather than applied as an isolated treatment. The proposed approach may therefore provide a practical basis for reducing dependence on mineral fertilisers, strengthening soil biological processes and supporting the recovery of agricultural land in the Southern Steppe of Ukraine. However, several limitations should be considered when interpreting these findings. The 3-year observation period made it possible to identify short-term changes but was insufficient to establish the long-term stability of the recorded effects. In addition, the experiment was conducted at a single site on southern chernozem under the climatic conditions of the Southern Steppe of Ukraine, which limits the direct transferability of the results to other soils and regions. Because variant B5 combined mixed sowings with several biopreparations and application methods, the individual contribution of each treatment component could not be fully separated. The microbiological assessment was also based mainly on culture-dependent methods and general indicators of microbial activity, which may not reflect the complete taxonomic and functional diversity of the soil microbiome. Furthermore, the identified correlations demonstrate associations between microbial activity, nutrient availability and crop productivity but do not independently establish causal relationships. The additional labour requirements, treatment costs and farm-level economic feasibility of B5 should therefore be evaluated before large-scale implementation. Longer-term, multi-site and factorial studies incorporating molecular microbiome analysis and comprehensive economic assessment are required to confirm the stability and broader applicability of the proposed system. Overall, integrated legume-based biological management represents a promising strategy for regenerative agriculture and the post-war restoration of degraded agroecosystems.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

ETHICS STATEMENT

The authors confirm that the research meets any required ethical guidelines, including adherence to the legal requirements of the study country.

AUTHOR CONTRIBUTIONS

AP and NM contributed in conceptualization; MK, AP and VA contributed in investigation; NM, MK and VA contributed invalidation and writing – review and editing; NM and VA contributed in writing – original draft preparation; AP contributed in project administration; MK and VA contributed in funding acquisition. All authors have read and agreed to the published version of the manuscript.

FUNDING STATEMENT

This research received no external funding.

DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available in the article.

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