

Effects of Crop Rotation, Fertilization and Biostimulants on Winter Wheat Yield and Soil Nutrient Dynamics in Southern Romania

Liviu Târșoagă^{1*}, Aida Păunescu², Roxana Horoiș¹, Leonard Ilie¹

¹University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd, District 1, Bucharest, Romania

²University of Craiova, 13 Alexandru Ioan Cuza Street, Craiova, Dolj County, Romania

*Corresponding author. E-mail: ltarsoaga@gmail.com

ABSTRACT

Optimizing winter wheat productivity while maintaining soil fertility is a key challenge for sustainable crop production. This study evaluated the individual and interactive effects of previous crop, fertilization strategy and biostimulant application on grain yield, soil pH, humus content and post-harvest macronutrient dynamics during the 2023-2024 and 2024-2025 growing seasons under the pedoclimatic conditions of southern Romania. A three-factor field experiment was established and the data were analysed using three-way analyses of variance (ANOVA) followed by Tukey's HSD test at $p < 0.05$. Crop rotation was the consistently factor controlling post-harvest soil fertility, while balanced fertilization combined with biostimulant application consistently increased grain yield and improved nutrient availability. Wheat cultivated after pea achieved the highest yields and maintained higher post-harvest levels of total nitrogen, available phosphorus and exchangeable potassium than wheat grown after rapeseed or sunflower. Soil pH remained relatively stable across treatments, whereas humus content was influenced primarily by experimental year and previous crop. Overall, the results demonstrate that integrating legume-based crop rotations with balanced fertilization and biostimulant application provides an effective strategy for improving winter wheat productivity while preserving soil fertility, supporting the implementation of sustainable crop management systems under the environmental conditions of southern Romania.

Keywords: *Triticum aestivum* L., crop rotation, soil fertility, fertilization strategy, biostimulants, macronutrients, sustainable agriculture.

INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops, providing a major source of food and contributing substantially to agricultural production in Europe. In Romania, winter wheat occupies a large proportion of arable land and plays a key role in ensuring both domestic grain supply and export potential (Burtan et al., 2026). Consequently, maintaining high and stable yields while preserving soil fertility has become a major objective of sustainable crop production (El-Akhdar et al., 2024; Cheema and Pires, 2025).

Modern wheat production is increasingly challenged by climate variability, soil degradation and the rising cost of agricultural inputs (Van Eynde et al., 2025). These constraints require integrated crop

management systems capable of improving productivity while maintaining long-term soil fertility and reducing environmental impacts (Alzweek and Salem, 2025).

Balanced fertilization is another essential component of sustainable wheat production because it directly influences crop nutrition and soil nutrient reserves (Xing et al., 2025). Recent advances in fertilizer technologies, including controlled-release nitrogen fertilizers, have improved nutrient use efficiency by synchronizing nutrient availability with crop demand and reducing nutrient losses (Walder et al., 2023; Sanchez-Mendez et al., 2025). At the same time, plant biostimulants have attracted increasing attention due to their ability to stimulate nutrient uptake, improve plant metabolism and enhance crop tolerance to environmental stresses (Maignan et al., 2021; Bo et al., 2025). Although numerous studies have investigated these practices

separately, few have evaluated their combined effects within an integrated crop management system (Kushwah et al., 2025).

The sustainability of agricultural systems cannot be assessed solely through grain yield. Changes in soil fertility indicators, including soil pH, humus content and the availability of nitrogen, phosphorus and potassium, provide essential information on nutrient cycling and the capacity of crop management practices to maintain soil productivity over time. Simultaneous evaluation of crop performance and post-harvest soil fertility therefore provides a more comprehensive assessment of management sustainability than yield measurements alone (Gholap et al., 2012; Singh et al., 2025).

The present study aimed to quantify the individual and interactive effects of crop rotation, fertilization strategy and biostimulant application on winter wheat productivity, soil fertility indicators and post-harvest macronutrient dynamics under the pedoclimatic conditions of southern Romania. It was hypothesized that integrating appropriate crop rotation with balanced fertilization and biostimulant application improves grain yield while maintaining soil fertility and reducing post-harvest nutrient depletion.

MATERIAL AND METHODS

Experimental site and climatic conditions

The field experiment was conducted during two consecutive growing seasons (2023-2024 and 2024-2025) at the Agricultural Research and Development Station (ARDS) Caracal, located in the southern part of Romania. The experimental area is characterized by a temperate continental climate with frequent drought episodes during spring and early summer, making efficient nutrient management essential for stable winter wheat production. Climatic data were recorded by the meteorological station located at ARDS Caracal. The experimental period was characterized by typical climatic conditions for the region, without the occurrence of extreme weather events.

Experimental design and crop management

The experiment was established as a three-factor factorial trial arranged in a randomized complete block design with three replications. Individual experimental plots measured 10 sqm.

The experimental factors were:

Factor Y - Years of research

- Y1: 2023-2024;
- Y2: 2024-2025.

Factor A - Previous crop

- A1: pea;
- A2: rapeseed;
- A3: sunflower.

Factor B - Fertilization strategy

- B1: unfertilized in autumn + 250 kg ha⁻¹ ammonium nitrate applied once in spring;
- B2: 250 kg ha⁻¹ NPK in autumn + 250 kg ha⁻¹ ammonium nitrate applied once in spring;
- B3: 250 kg ha⁻¹ NPK in autumn + 250 kg ha⁻¹ ammonium nitrate split into two spring applications;
- B4: 250 kg ha⁻¹ Nutri Top 80 in autumn + 250 kg ha⁻¹ controlled-release urea (UREE NG) applied in spring.

Factor C - Biostimulant application

- C1: untreated control;
- C2: Agrii Start PolyZN ST applied as seed treatment at 1 L t⁻¹;
- C3: Agrii Start PolyZN ST applied as seed treatment (1 L t⁻¹) and Quantis as foliar application (2 L ha⁻¹) at BBCH 40-45.

The selected fertilization strategies were designed to evaluate the combined effects of mineral fertilization and biostimulant application (B×C) on wheat productivity and selected indicators of soil fertility. The winter wheat variety Glosa, one of the most widely cultivated Romanian cultivars due to its high adaptability and stable productivity, was used throughout the experiment.

Soil sampling and chemical analyses

Soil samples were collected from the 0-30 cm layer after harvest each experimental year (typically, in August). They were collected separately from each experimental plot to

evaluate the influence of the applied technologies on soil chemical properties.

The following soil fertility indicators and nutrients were determined:

- soil pH;
- humus content (%);
- nitrogen (Nt%);
- available phosphorus (PAL%);
- exchangeable potassium (KAL%).

Soil samples collected after wheat harvest were air-dried, homogenized and passed through a 2-mm sieve prior to laboratory analysis. Soil pH was determined potentiometrically in a soil-to-water suspension (1:2.5, w/v). Humus content was determined according to the Walkley-Black wet oxidation method. Total nitrogen was determined using the Kjeldahl method, available phosphorus (PAL) by the Egner-Riehm-Domingo extraction method, and exchangeable potassium (KAL) by ammonium acetate extraction followed by flame photometry. All analyses were performed in the Soil Science Laboratory of the Agricultural Research and Development Station (ARDS) Caracal, according to the laboratory's accredited analytical procedures.

Grain yield determination

Winter wheat was harvested at physiological maturity from the entire useful plot area. Grain yield was determined for each experimental plot and expressed as $q\ ha^{-1}$ after standardization to commercial grain moisture (14%).

Statistical analysis

Statistical analyses were performed using JASP (version 0.19.3, JASP Team, University of Amsterdam, The Netherlands) and XLSTAT (Addinsoft, Paris, France).

The effects of experimental year (Y), previous crop (A), and fertilization strategy combined with biostimulant application ($B \times C$) on grain yield and soil fertility indicators were evaluated using a three-way factorial analysis of variance (ANOVA) based on Type III Sum of Squares. The significance of the main effects and interactions was assessed at $p < 0.05$.

Whenever significant differences among treatment means were detected, multiple comparisons were performed using Tukey's Honestly Significant Difference (HSD) test at the 5% probability level. The resulting compact letter display (CLD) was used to identify statistically homogeneous groups and is presented in the corresponding figures.

RESULTS AND DISCUSSION

Effects of crop rotation, fertilization strategy and biostimulant application on yield

Grain yield of winter wheat was significantly influenced by the combined effects of previous crop, fertilization strategy and biostimulant application, with consistently higher yields recorded during the 2024-2025 growing season (Table 1, Figure 1).

Table 1. Effect of experimental year, previous crop and fertilization strategy on winter wheat grain yield ($q\ ha^{-1}$)

Year (Y)	Previous crop (A)	Fertilization (B) × Biostimulant (C)											
		B1×C1	B1×C2	B1×C3	B2×C1	B2×C2	B2×C3	B3×C1	B3×C2	B3×C3	B4×C1	B4×C2	B4×C3
		yield (q ha ⁻¹)											
2023- 2024	Pea	49.38	52.22	54.96	48.10	47.62	51.82	49.09	51.70	54.18	56.07	54.39	57.21
	Rapeseed	42.91	45.54	46.19	50.46	48.38	55.36	54.75	53.47	57.03	54.92	52.25	56.49
	Sunflower	38.44	38.57	41.98	46.15	43.67	50.65	45.87	43.56	55.16	52.02	55.63	54.66
2024- 2025	Pea	61.03	60.17	65.95	57.82	60.90	63.38	59.14	65.45	63.59	64.83	67.66	68.29
	Rapeseed	55.57	54.08	57.30	61.51	60.18	64.24	63.83	65.00	66.59	63.27	66.05	65.30
	Sunflower	43.25	44.61	43.15	60.67	57.22	62.83	62.04	61.95	65.36	62.07	62.11	66.43

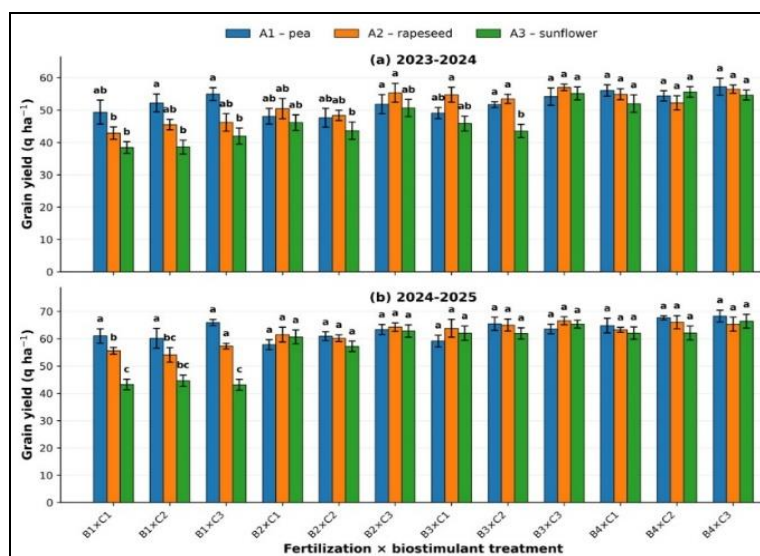


Figure 1. Grain yield of winter wheat as affected by previous crop, fertilization strategy and biostimulant application during the 2023-2024 (a) and 2024-2025 (b) growing seasons. Bars represent means $\pm$ SE ($n = 3$). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

Figure 1 shows a gradual increase in grain yield with increasing technological intensity. The highest yields were generally obtained under the B4 fertilization strategy combined with biostimulant application (C3), whereas the lowest values were associated with the basic fertilization treatments. The relatively small error bars demonstrate good experimental repeatability, while Tukey's HSD test identified significant differences mainly between low-input and high-input management systems.

Previous crop had a marked influence on wheat productivity. In both years, wheat grown after pea consistently produced the highest yields, followed by rapeseed, whereas sunflower represented the least favourable

predecessor. The beneficial effect of pea was particularly evident under reduced fertilization levels, reflecting the positive residual contribution of legume crops to soil fertility. In contrast, yield differences among previous crops became less pronounced under the most intensive fertilization treatments, suggesting that balanced nutrient supply partially compensated for differences in the initial fertility status.

The ANOVA results (Table 2) confirmed that experimental year, fertilization strategy and previous crop were the principal sources of variation, while the significant interaction between previous crop and fertilization demonstrated that fertilizer efficiency depended on crop rotation.

Table 2. Three-way ANOVA for winter wheat grain yield as affected by experimental year, previous crop and fertilization strategy

Source of variation*	df	Sum of squares (SS)	Mean square	F-value	p-value	Effect size (partial η^2)
Year (Y)	1	3383.84	3383.84	416.87	<0.001	0.743
Previous crop (A)	2	631.13	315.57	38.89	<0.001	0.351
Fertilization $\times$ Biostimulant (B $\times$ C)	11	2086.27	189.66	23.37	<0.001	0.641
Y $\times$ A	2	1.75	0.88	0.11	0.895	0.002
Y $\times$ (B $\times$ C)	11	113.31	10.30	1.28	0.243	0.089
A $\times$ (B $\times$ C)	22	874.92	39.77	4.90	<0.001	0.428
Y $\times$ A $\times$ (B $\times$ C)	22	181.26	8.24	1.02	0.450	0.134
Residual (Error)	144	1169.15	8.12	-	-	-

*Three-way factorial ANOVA evaluating the effects of experimental year (Y), previous crop (P), fertilization strategy combined with biostimulant application (F), and their interactions on winter wheat grain yield. Partial η^2 values indicate the proportion of explained variance attributable to each source of variation. Significant effects are reported at $p < 0.05$.

Conversely, interactions involving experimental year were not significant, indicating that treatment performance remained consistent across the two growing seasons. Overall, the results demonstrate that integrating an appropriate crop rotation with balanced fertilization and biostimulant application provides a reliable strategy for improving winter wheat productivity under the pedoclimatic conditions of southern Romania.

Effects of crop rotation, fertilization strategy and biostimulant application on soil fertility indicators

Soil reaction and humus content were evaluated to assess the effects of crop rotation, fertilization strategy and biostimulant application on short-term changes in soil fertility.

Soil reaction (pH)

The mean soil pH values obtained after winter wheat harvest are presented in Table 3, while their graphical distribution is shown in Figure 2. Across both growing seasons, soil pH varied within a relatively narrow interval, indicating that neither crop rotation nor fertilization induced substantial short-term changes in soil reaction.

A slight increase in pH was observed under the more intensive fertilization strategies, particularly B3 and B4, although these differences remained relatively small in absolute terms (Table 3, Figure 2). The limited dispersion of the error bars further confirmed the good repeatability of the experimental data.

Table 3. Effect of experimental year, previous crop, fertilization strategy and biostimulant application on soil pH

Year (Y)	Previous crop (A)	Fertilization (B) × Biostimulant (C)											
		B1×C1	B1×C2	B1×C3	B2×C1	B2×C2	B2×C3	B3×C1	B3×C2	B3×C3	B4×C1	B4×C2	B4×C3
		pH											
2023- 2024	Pea	5.81	5.84	6.02	5.87	5.90	5.98	5.96	5.97	6.07	5.96	5.95	6.12
	Rapeseed	5.75	5.79	5.92	5.81	5.94	5.95	5.89	6.04	6.04	6.00	5.92	5.97
	Sunflower	5.87	5.91	5.93	5.94	5.96	5.98	5.99	6.02	6.03	6.01	6.05	6.03
2024- 2025	Pea	5.78	5.82	5.98	5.84	5.90	6.01	5.99	6.05	6.10	6.02	6.03	6.08
	Rapeseed	5.84	5.87	5.89	5.89	5.92	6.06	5.94	5.97	6.06	5.96	6.00	6.16
	Sunflower	5.86	5.89	5.91	5.92	5.94	6.03	5.97	5.93	6.00	5.98	6.05	6.07

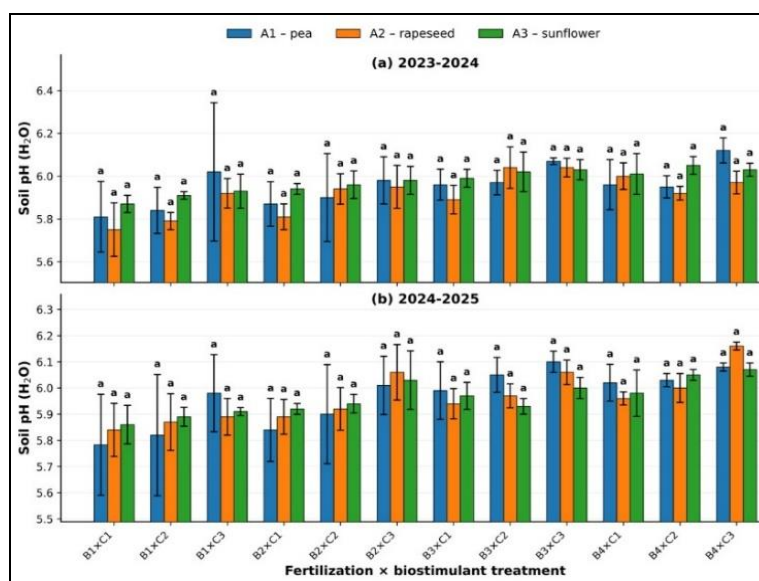


Figure 2. Soil pH measured after winter wheat harvest as affected by previous crop, fertilization strategy and biostimulant application during the 2023-2024 (a) and 2024-2025 (b) growing seasons. Values represent means ± standard error (SE). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

The analysis of variance (Table 4) identified fertilization strategy as the only significant source of variation ($P < 0.001$), whereas neither growing season nor previous crop significantly affected soil pH. Likewise, none of the interaction terms reached statistical significance, indicating that soil reaction remained relatively stable across the different crop rotations. Although several fertilization treatments differed according to

Tukey's HSD test, the overall variation among treatment means remained small and all measured values were maintained within the optimum range for winter wheat cultivation. These findings indicate that the investigated fertilization and biostimulant programmes preserved the chemical stability of the soil throughout the two-year experimental period.

Table 4. Three-way ANOVA for pH as affected by experimental year, previous crop and fertilization strategy

Source of variation*	df	Sum of squares (SS)	Mean square	F-value	p-value	Effect size (partial η^2)
Year (Y)	1	0.0116	0.0116	0.451	0.503	0.003
Previous crop (A)	2	0.0456	0.0228	0.884	0.415	0.011
Fertilization $\times$ Biostimulant (B $\times$ C)	12	1.1770	0.0980	3.799	<0.001	0.226
Y $\times$ A	2	0.0340	0.0170	0.658	0.519	0.008
Y $\times$ (B $\times$ C)	12	0.0504	0.0042	0.163	0.999	0.012
A $\times$ (B $\times$ C)	24	0.1915	0.0080	0.309	0.999	0.045
Y $\times$ A $\times$ (B $\times$ C)	24	0.1129	0.0047	0.182	1.000	0.027
Residual (Error)	156	4.0260	0.0258	-	-	-

*Three-way factorial ANOVA evaluating the effects of experimental year (Y), previous crop (A), fertilization strategy combined with biostimulant application (B $\times$ C), and their interactions on soil pH. Partial η^2 values indicate the proportion of variance explained by each source of variation. Significant effects were considered at $p < 0.05$.

Humus content

Compared with soil pH, humus showed a clearer response to the experimental conditions, particularly between growing seasons and previous crops. In both years, wheat cultivated after pea generally maintained higher humus contents than the other crop rotations, whereas

sunflower tended to be associated with lower values. Differences among fertilization strategies were comparatively small, indicating that changes in soil organic matter occurred gradually during the experimental period (Table 5, Figure 3).

Table 5. Effect of experimental year, previous crop, fertilization strategy and biostimulant application on humus (%)

Year (Y)	Previous crop (A)	Fertilization (B) × Biostimulant (C)											
		B1×C1	B1×C2	B1×C3	B2×C1	B2×C2	B2×C3	B3×C1	B3×C2	B3×C3	B4×C1	B4×C2	B4×C3
		%											
2023- 2024	Pea	3.10	3.12	3.16	3.14	3.16	3.21	3.18	3.24	3.23	3.20	3.26	3.34
	Rapeseed	3.14	3.39	3.22	3.17	3.20	3.25	3.22	3.26	3.28	3.22	3.35	3.29
	Sunflower	3.11	3.13	3.15	3.15	3.17	3.22	3.20	3.23	3.29	3.21	3.27	3.31
2024- 2025	Pea	3.36	3.39	3.40	3.41	3.44	3.45	3.46	3.50	3.50	3.47	3.56	3.53
	Rapeseed	3.27	3.29	3.33	3.31	3.34	3.38	3.36	3.25	3.41	3.45	3.44	3.48
	Sunflower	3.08	3.10	3.15	3.11	3.14	3.18	3.16	3.20	3.27	3.21	3.24	3.26

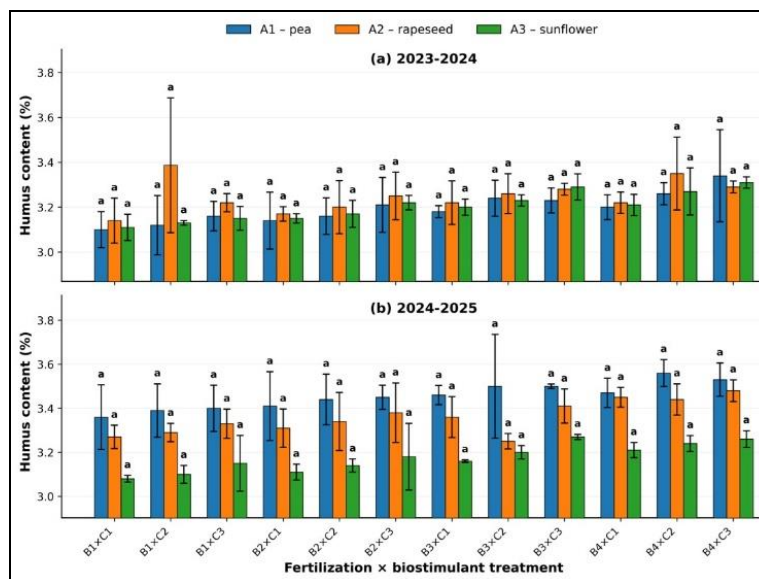


Figure 3. Humus (%) measured after winter wheat harvest as affected by previous crop, fertilization strategy and biostimulant application during the 2023–2024 (a) and 2024–2025 (b) growing seasons. Values represent means $\pm$ standard error (SE). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

According to the ANOVA (Table 6), both growing season and previous crop significantly affected humus content ($P < 0.001$), whereas fertilization strategy had no significant effect ($P = 0.069$). A significant

interaction between growing season and previous crop further demonstrated that the influence of crop rotation differed slightly between the two experimental years.

Table 6. Three-way ANOVA for humus content (%) as affected by experimental year, previous crop and fertilization strategy

Source of variation*	df	Sum of squares (SS)	Mean square	F-value	p-value	Effect size (partial η^2)
Year (Y)	1	0.7323	0.7323	23.170	<0.001	0.129
Previous crop (A)	2	0.8689	0.4344	13.750	<0.001	0.150
Fertilization $\times$ Biostimulant (B $\times$ C)	12	0.6489	0.0541	1.711	0.069	0.116
Y $\times$ A	2	0.7771	0.3885	12.300	<0.001	0.136
Y $\times$ (B $\times$ C)	12	0.0471	0.0039	0.124	1.000	0.009
A $\times$ (B $\times$ C)	24	0.1062	0.0044	0.140	1.000	0.021
Y $\times$ A $\times$ (B $\times$ C)	24	0.1076	0.0045	0.142	1.000	0.021
Residual (Error)	156	4.9290	0.0316	-	-	-

* Three-way factorial ANOVA evaluating the effects of experimental year (Y), previous crop (A), fertilization strategy combined with biostimulant application (B $\times$ C), and their interactions on humus content. Partial η^2 values indicate the proportion of variance explained by each source of variation. Significant effects were considered at $p < 0.05$.

Overall, these results indicate that short-term changes in soil organic matter were driven primarily by crop rotation and seasonal conditions rather than by modifications in fertilization strategy. Consequently, the inclusion of leguminous crops within the rotation appears to represent the most effective management practice for maintaining soil organic matter under the conditions of southern Romania.

Macronutrient dynamics

In the present study, total nitrogen, available phosphorus and exchangeable potassium were evaluated after harvest to assess the effects of crop rotation, fertilization strategy and biostimulant application on short-term nutrient dynamics.

Total nitrogen

Compared with soil pH and humus content,

total nitrogen showed a much stronger response to the experimental treatments. Across both growing seasons, residual nitrogen concentrations consistently increased with fertilization intensity, while clear differences were also observed among previous crops (Table 7, Figure 4). In nearly all fertilization strategies, wheat cultivated after pea (A1) maintained the highest post-harvest nitrogen concentrations, followed by rapeseed (A2), whereas sunflower (A3) resulted in the lowest residual nitrogen levels.

This pattern agrees with the beneficial contribution of legumes to soil nitrogen reserves through biological nitrogen fixation and residue mineralization.

The positive effect of fertilization became progressively more evident from B1 to B4. The highest nitrogen concentrations were generally recorded under the B4 fertilization strategy combined with biostimulant application, whereas the lowest values occurred under the basic fertilization programme (B1).

Table 7. Effect of experimental year, previous crop, fertilization strategy and biostimulant application on total nitrogen (%)

Year (Y)	Previous crop (A)	Fertilization (B) × Biostimulant (C)											
		B1×C1	B1×C2	B1×C3	B2×C1	B2×C2	B2×C3	B3×C1	B3×C2	B3×C3	B4×C1	B4×C2	B4×C3
		%											
2023-2024	Pea	0.186	0.197	0.199	0.191	0.202	0.205	0.205	0.210	0.211	0.207	0.215	0.213
	Rapeseed	0.169	0.177	0.182	0.175	0.189	0.190	0.172	0.189	0.196	0.184	0.198	0.195
	Sunflower	0.156	0.158	0.166	0.159	0.161	0.174	0.165	0.169	0.190	0.168	0.173	0.194
2024-2025	Pea	0.165	0.167	0.179	0.157	0.172	0.188	0.175	0.177	0.180	0.176	0.183	0.194
	Rapeseed	0.163	0.165	0.173	0.167	0.179	0.177	0.179	0.175	0.181	0.175	0.184	0.184
	Sunflower	0.131	0.129	0.142	0.134	0.146	0.145	0.139	0.144	0.145	0.141	0.149	0.140

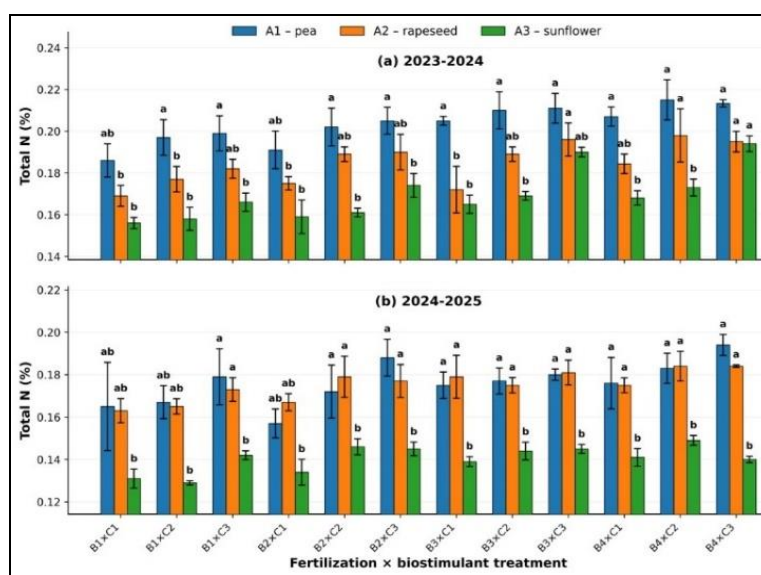


Figure 4. Total nitrogen (%) measured after winter wheat harvest as affected by previous crop, fertilization strategy and biostimulant application during the 2023–2024 (a) and 2024–2025 (b) growing seasons. Values represent means $\pm$ standard error (SE). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

The magnitude of the fertilization response depended on the previous crop. The increase in soil nitrogen was particularly pronounced after sunflower, indicating that intensive nutrient management partially compensated for the lower residual fertility associated with this crop. The graphical representation (Figure 4) further supports

these observations. Error bars indicate good experimental repeatability, while Tukey's HSD test clearly separated several treatment groups. Distinct statistical differences were particularly evident between pea and sunflower, whereas rapeseed generally showed an intermediate response. Differences among fertilization strategies

became more pronounced under the intensive management systems, especially during the 2024-2025 growing season.

The ANOVA confirmed that experimental year, previous crop and fertilization strategy significantly affected soil total nitrogen ($p < 0.001$, Table 8). Previous crop explained the largest proportion of the observed variability (partial $\eta^2 = 0.711$), followed by experimental year (partial $\eta^2 = 0.562$) and fertilization strategy

(partial $\eta^2 = 0.376$). Among the interaction terms, only the interaction between year and previous crop was significant ($p < 0.001$), whereas interactions involving fertilization strategy were not significant. These results indicate that crop rotation represented the principal driver of nitrogen dynamics, while fertilization mainly influenced the magnitude of nitrogen accumulation without altering the overall response pattern.

Table 8. Three-way ANOVA for total nitrogen (%) as affected by experimental year, previous crop and fertilization strategy

Source of variation*	df	Sum of squares (SS)	Mean square	F-value	p-value	Effect size (partial η^2)
Year (Y)	1	0.0276	0.0276	199.800	<0.001	0.562
Previous crop (A)	2	0.0531	0.0266	191.900	<0.001	0.711
Fertilization $\times$ Biostimulant (B $\times$ C)	12	0.0130	0.0011	7.818	<0.001	0.376
Y $\times$ A	2	0.0043	0.0021	15.420	<0.001	0.165
Y $\times$ (B $\times$ C)	12	0.0010	0.0001	0.584	0.853	0.043
A $\times$ (B $\times$ C)	24	0.0016	0.0001	0.479	0.981	0.069
Y $\times$ A $\times$ (B $\times$ C)	24	0.0021	0.0001	0.637	0.902	0.089
Residual (Error)	156	0.0216	0.0001	-	-	-

* Three-way ANOVA was performed using Type III Sum of Squares to evaluate the effects of experimental year (Y), previous crop (A), fertilization strategy $\times$ biostimulant application (B $\times$ C), and their interactions on post-harvest soil total nitrogen. Significant effects were considered at $p < 0.05$.

Overall, the results demonstrate that integrating leguminous crops into the rotation, together with balanced fertilization and biostimulant application, represents an effective strategy for maintaining higher post-harvest soil nitrogen reserves and improving soil fertility under the conditions of southern Romania.

Available phosphorus

Available phosphorus exhibited pronounced differences among previous crops and

fertilization strategies in both experimental years. During the 2023-2024 season, wheat grown after pea (A1) generally maintained the highest levels of available phosphorus, whereas sunflower (A3) consistently resulted in the lowest concentrations (Table 9, Figure 5). In contrast, during 2024-2025 the highest phosphorus values were recorded predominantly after rapeseed (A2), indicating that the influence of the previous crop varied between years.

Table 9. Effect of experimental year, previous crop, fertilization strategy and biostimulant application on available phosphorus (mg kg⁻¹)

Year (Y)	Previous crop (A)	Fertilization (B) × Biostimulant (C)											
		B1×C1	B1×C2	B1×C3	B2×C1	B2×C2	B2×C3	B3×C1	B3×C2	B3×C3	B4×C1	B4×C2	B4×C3
		mg kg ⁻¹											
2023- 2024	Pea	48.75	51.79	52.59	50.32	53.39	54.18	53.45	58.31	56.84	55.24	58.43	55.90
	Rapeseed	46.20	46.93	47.65	47.17	48.37	49.09	49.33	51.02	52.52	50.06	52.94	52.46
	Sunflower	34.35	35.08	35.62	35.44	38.16	36.70	34.88	38.15	38.33	37.43	39.41	39.05
2024- 2025	Pea	42.60	44.26	45.93	43.70	45.81	44.48	45.70	49.48	47.70	46.37	50.25	49.81
	Rapeseed	52.43	53.52	54.34	54.06	57.16	55.98	56.25	59.43	58.98	57.34	61.62	60.07
	Sunflower	31.43	32.09	33.75	32.42	33.25	36.74	33.91	35.06	38.48	34.40	36.39	38.06

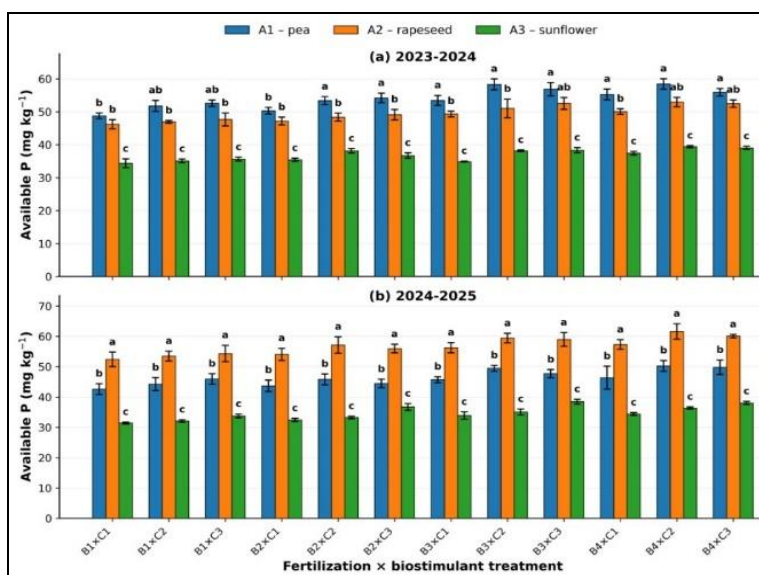


Figure 5. Available phosphorus (mg kg^{-1}) measured after winter wheat harvest as affected by previous crop, fertilization strategy and biostimulant application during the 2023–2024 (a) and 2024–2025 (b) growing seasons.

Values represent means $\pm$ standard error (SE). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

Across both growing seasons, available phosphorus generally increased with fertilization intensity. The lowest concentrations were associated with the basic fertilization strategy (B1), while the highest values were recorded under the integrated fertilization programmes (B3 and B4), particularly when combined with biostimulant application. This response was most evident for the pea and rapeseed rotations, whereas wheat following sunflower maintained comparatively lower phosphorus concentrations regardless of fertilization level.

The graphical representation (Figure 5) confirms these trends. The separation of Tukey's HSD groups demonstrates significant differences among previous crops, with sunflower forming the lowest statistical group in most treatment combinations. Error

bars were generally small, indicating good repeatability of the experimental data and supporting the consistency of the observed treatment effects.

The analysis of variance (Table 10) confirmed that available phosphorus was significantly affected by experimental year, previous crop and fertilization strategy ($p \leq 0.007$). Previous crop represented the dominant source of variation (partial $\eta^2 = 0.927$), followed by the interaction between year and previous crop (partial $\eta^2 = 0.674$) and fertilization strategy (partial $\eta^2 = 0.509$), whereas interactions involving fertilization were not statistically significant. These findings indicate that phosphorus availability was determined primarily by crop rotation, while fertilization mainly enhanced phosphorus availability without modifying the overall response pattern.

Table 10. Three-way ANOVA for available phosphorus (mg kg^{-1}) as affected by experimental year, previous crop and fertilization strategy

Source of variation*	df	Sum of squares (SS)	Mean square	F-value	p-value	Effect size (partial η^2)
Year (Y)	1	51.6100	51.6100	7.517	0.007	0.046
Previous crop (A)	2	13600.0000	6802.0000	990.800	<0.001	0.927
Fertilization $\times$ Biostimulant (B $\times$ C)	12	1110.0000	92.5000	13.470	<0.001	0.509
Y $\times$ A	2	2210.0000	1105.0000	160.900	<0.001	0.674
Y $\times$ (B $\times$ C)	12	10.7300	0.8945	0.130	1.000	0.010
A $\times$ (B $\times$ C)	24	95.2200	3.9670	0.578	0.942	0.082
Y $\times$ A $\times$ (B $\times$ C)	24	81.7800	3.4070	0.496	0.977	0.071
Residual (Error)	156	1071.0000	6.8650	-	-	-

* Three-way ANOVA was performed using Type III Sum of Squares to evaluate the effects of experimental year (Y), previous crop (A), fertilization strategy $\times$ biostimulant application (B $\times$ C), and their interactions on post-harvest soil phosphorus. Significant effects were considered at $p < 0.05$.

Overall, the results demonstrate that the combination of an appropriate crop rotation and balanced fertilization is essential for maintaining adequate levels of available phosphorus in the soil. The strong influence of the previous crop highlights the importance of rotational planning for improving phosphorus availability and sustaining soil fertility under the conditions of south Romania.

Exchangeable potassium

Exchangeable potassium showed significant differences among previous crops, fertilization strategies and experimental years. Across both growing seasons, wheat cultivated after pea (A1) consistently maintained the highest post-harvest potassium concentrations, whereas sunflower (A3) resulted in the lowest values regardless of fertilization strategy (Table 11, Figure 6).

Table 11. Effect of experimental year, previous crop, fertilization strategy and biostimulant application on exchangeable potassium (mg kg^{-1})

Year (Y)	Previous crop (A)	Fertilization (B) × Biostimulant (C)											
		B1×C1	B1×C2	B1×C3	B2×C1	B2×C2	B2×C3	B3×C1	B3×C2	B3×C3	B4×C1	B4×C2	B4×C3
		mg kg ⁻¹											
2023- 2024	Pea	196	200	203	202	206	208	209	216	217	212	222	223
	Rapeseed	188	190	193	192	196	198	199	206	207	203	197	210
	Sunflower	153	157	154	156	159	167	155	167	174	164	172	177
2024- 2025	Pea	245	249	251	251	255	259	260	269	271	264	277	273
	Rapeseed	181	183	186	185	189	198	192	198	202	194	204	202
	Sunflower	172	176	174	175	179	184	176	183	183	184	193	191

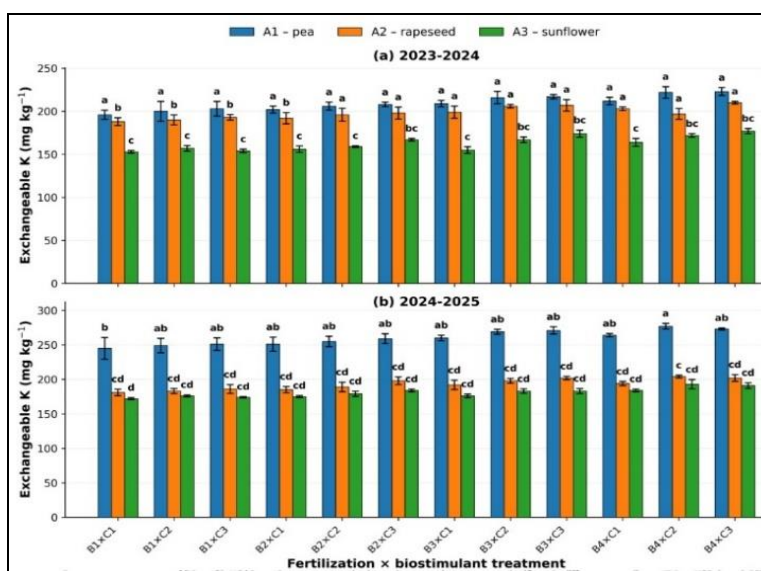


Figure 6. Exchangeable potassium (mg kg^{-1}) measured after winter wheat harvest as affected by previous crop, fertilization strategy and biostimulant application during the 2023–2024 (a) and 2024–2025 (b) growing seasons.

Values represent means $\pm$ standard error (SE). Different letters indicate significant differences according to Tukey's HSD test ($p < 0.05$).

A progressive increase in exchangeable potassium was generally observed with increasing fertilization intensity. The lowest concentrations were associated with the basic fertilization strategy (B1), while the highest values were recorded under the integrated fertilization programmes (B3 and B4), especially when biostimulants were included. This response was most pronounced after pea, where exchangeable potassium increased steadily from the basic to the intensive fertilization treatments. In contrast, wheat following sunflower maintained comparatively lower potassium concentrations, although improved fertilization partially reduced these differences.

The ANOVA results (Table 12) confirmed that experimental year, previous crop and fertilization strategy significantly affected exchangeable potassium ($P < 0.001$). Previous crop explained the largest proportion of the total variability (partial $\eta^2 = 0.920$), followed by the interaction between year and previous crop (partial $\eta^2 = 0.693$), experimental year (partial $\eta^2 = 0.652$) and fertilization strategy (partial $\eta^2 = 0.476$). In contrast, the interactions involving fertilization strategy were not statistically significant, indicating that although fertilization consistently increased soil potassium availability, its overall effect remained similar across years and previous crops.

Table 12. Three-way ANOVA for exchangeable potassium (mg kg^{-1}) as affected by experimental year, previous crop and fertilization strategy

Source of variation*	df	Sum of squares (SS)	Mean square	F-value	p-value	Effect size (partial η^2)
Year (Y)	1	25860.0000	25860.0000	292.500	<0.001	0.652
Previous crop (A)	2	157440.0000	78720.0000	890.400	<0.001	0.920
Fertilization $\times$ Biostimulant (B $\times$ C)	12	12550.0000	1046.0000	11.830	<0.001	0.476
Y $\times$ A	2	31180.0000	15590.0000	176.300	<0.001	0.693
Y $\times$ (B $\times$ C)	12	266.5000	22.2100	0.251	0.995	0.019
A $\times$ (B $\times$ C)	24	835.5000	34.8100	0.394	0.995	0.057
Y $\times$ A $\times$ (B $\times$ C)	24	372.3000	15.5100	0.176	1.000	0.026
Residual (Error)	156	13790.0000	88.4100	-	-	-

* Three-way ANOVA was performed using Type III Sum of Squares to evaluate the effects of experimental year (Y), previous crop (A), fertilization strategy $\times$ biostimulant application (B $\times$ C), and their interactions on post-harvest soil potassium. Significant effects were considered at $p < 0.05$.

These findings demonstrate that crop rotation was the primary determinant of post-harvest exchangeable potassium, while balanced fertilization combined with biostimulant application contributed to maintaining higher potassium reserves throughout the cropping system. The consistently superior performance of wheat following pea further emphasizes the importance of integrating leguminous crops into crop rotations to improve nutrient availability and sustain soil fertility under the pedoclimatic conditions of southern Romania.

CONCLUSIONS

This study demonstrated that previous crop, fertilization strategy and biostimulant application influenced winter wheat productivity and selected indicators of post-harvest soil fertility under the pedoclimatic conditions of southern Romania. Among the investigated factors, previous crop explained the largest proportion of the observed variability in soil nutrient status, while balanced fertilization and biostimulant application contributed to improved crop performance and nutrient availability.

Winter wheat grown after pea consistently achieved the highest grain yields and generally maintained superior post-harvest reserves of total nitrogen, available phosphorus and exchangeable potassium compared with wheat grown after rapeseed or sunflower. Soil pH remained relatively stable across treatments, whereas humus content was affected primarily by experimental year and previous crop. These results underline the importance of selecting appropriate preceding crops when aiming to maintain soil nutrient status and sustain winter wheat productivity.

The factorial ANOVA confirmed that previous crop explained the largest proportion of the variability observed for the three macronutrients, while fertilization strategy had a significant effect on nutrient availability. These findings highlight the complementary roles of crop rotation and

balanced fertilization in improving nutrient use efficiency and sustaining soil fertility. Integrating legume-based crop rotations with balanced fertilization and biostimulant application represents an effective agronomic strategy for simultaneously increasing winter wheat productivity and maintaining long-term soil fertility under the environmental conditions of southern Romania.

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