EFFECT OF NPK FERTILIZATION ON ORGANIC MATTER AND MINERAL ELEMENT CONTENT IN SOYBEAN (*GLYCINE MAX* L.) ROOTS AND NODULES

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(Received 19th Jul 2025; accepted 2nd Oct 2025)

Abstract. The study analyzed the variation in the organic matter and mineral elements content in roots and nodules of soybean plants after NPK mineral fertilization. Phosphorus (P) and potassium (K) were applied in four level, 0, 60, 100 and 140 kg ha^{-1} a.s. (a.s. – active substance). At each PK level, nitrogen was applied, 0, 50, and 100 kg ha^{-1} a.s. Twelve experimental variants resulted (T1 to T12), organized into four replications. At the beginning of flowering, plant samples from the experimental variants were randomly taken. The organic matter content in roots and nodules (OM, %), the content of total nitrogen (TN, %), phosphorus (P, %), and potassium (K, %) in roots, and the content of microelements (Fe, Mn, Cu, and Zn, mg kg^{-1}) in roots (r) and nodules (n) were determined. The independent variables (N, and PK from fertilizers) showed variable influence on the content of organic matter, macroelements and microelements in roots and nodules. Very strong and strong interdependence was recorded between the content of microelements in nodules and the content of organic matter and mineral elements in roots. Mann-Whitney test (post-hoc step) confirmed the differences between mean values for organic matter and microelement content in soybean roots and nodules.
Keywords: *Bartlett´s sphericity test, copper, iron, KMO, manganese, mineral fertilizers, models, nodule/root ratio, PCA, soybean, zinc*

Introduction

Soybean is a crop of great importance worldwide, primarily for the production of vegetable proteins and oil, with use in the food industry and animal feed (Qin et al., 2022; Borbélyné et al., 2023; Ihsan et al., 2024; Popescu, 2024). Soybean (*Glycine max* L.) is a plant with multiple agronomic, ecosystemic, economic and social values (Rotundo et al., 2024; Yan et al., 2024; Yang et al., 2024; Freitas et al., 2025). It is a crop suitable for the circular economy (Karim et al., 2025) and also a plant with important agronomic and ecosystem effects for the sustainable use of agricultural land and farm areas (Dreoni et al., 2022; Nuraini et al., 2024; Gai et al., 2025).

In the context of climate change, the response to drought was evaluated in soybean through specific genetic and physiological tests in order to decipher the genetic control of drought tolerance (Luo et al., 2024). Under different water stress-induced conditions, the drought tolerance of plants, the relationship of soybean plants with soil nutrients, and the response of plants at the level of physiological and biochemical indices were evaluated (Li, 2025; Wang et al., 2025). Soybean yield was analyzed in relation to different genotypes, environmental conditions, crop systems and technologies, water regime, fertilizers, stress factors (Anda et al., 2020; Ding et al., 2024). The relationship

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2306_1105311072

of soybean plants with different types of fertilizers was evaluated based on physiological indices, agronomic parameters, yield and quality indices (Epie et al., 2022; Nget et al., 2022; Egribas Endes, 2024).

In soybean plants, the nodulation process and symbiotic nitrogen fixation are relevant (Carter and Tegeder, 2016; Ciampitti et al., 2021; Lyu et al., 2022). With compatible microorganisms, soybean establishes a functional relationship with nodule formation and symbiotic molecular nitrogen fixation (Zhao et al., 2025). The nodulation and nitrogen fixation processes are dependent on the plant genotype, the specificity of microorganisms (virulence, compatibility), the "plant × microorganisms" interaction, environmental factors, and technological factors (Wang et al., 2020; Bender et al., 2022; Shen and Bisseling, 2025; Thingujam et al., 2025).

Mineral elements differentially influence the nodulation process and nitrogen fixation in soybeans (Wang et al., 2020; Abeje et al., 2021; Shen and Bisseling, 2025). According to some studies, certain mineral elements, phosphorus (P), copper (Cu), zinc (Zn) and molybdenum (Mo) have a positive influence on the symbiotic nitrogen fixation process in soybean (Liu et al., 2022). In general, high amounts of mineral nitrogen in the soil negatively affect the nodulation process, leading to its inhibition and a reduction in symbiotic nitrogen fixation (Xu et al., 2024; Zhou et al., 2024).

Soybean plants use nitrogen from different sources (fertilizers, nodules) in relation to the vegetation stage, and the level of phosphorus supply positively influenced nitrogen fixation (Li et al., 2022). The low level of phosphorus available in the soil for plant nutrition has been recorded as a major constraint for the nodulation and nitrogen fixation process in soybean (Qin et al., 2022). Similar results regarding the reduction of nodulation in relation to low phosphorus levels have been reported in experimental conditions on nutrient solutions (Miao et al., 2007). Experiments with phosphorus and potassium showed a linear increase in nodule mass in soybean in relation to phosphorus dose at a given P rate, and combined application of phosphorus and potassium (PK) significantly modified nodule weight (Lynd et al., 1984). Potassium (K^+) is the mineral element with the highest percentage in the plant organism, and the deficit of accessible potassium in the soil reduced the efficiency of symbiotic nitrogen fixation in soybeans (Liu et al., 2022). Potassium supplementation in the form of humic products (K-humate) led to an increase in the number of nodules in soybeans (number and weight of nodules) in conditions of statistical safety, $p<0.05$ (de Andrade da Silva et al., 2021).

Calcium plays an important role in plant nutrition and in the process of nodulation and symbiotic nitrogen fixation in soybeans (Zartdinova and Nikitin, 2023). According to the authors, disruption of calcium homeostasis in nodule cells can trigger nodule senescence, and priming seeds with calcium sources led to increased efficacy of the symbiosis (Zartdinova and Nikitin, 2023). Root morphology, nodule formation and growth, nitrogen fixation, plant growth, and yield were significantly affected by sulfur deficiency (Hu et al., 2023). Sulfur supplementation associated with inoculation of rhizobium microorganisms in soybeans led to improved yield (Bi et al., 2024).

Microelements have a differentiated functional role in the nodulation and symbiotic nitrogen fixation process in soybean, and high intake has been highlighted in the case of certain microelements, e.g. Fe, Cu, Zn, Mo, Co (Zahoor et al., 2013; Liu et al., 2022).

The objective of the study was to evaluate the variation in the content of organic matter and mineral elements in soybean roots and nodules in relation to NPK mineral fertilization, and used regression analysis, multivariate analysis and comparative analysis to explain the interdependence and distribution of the results.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Materials and methods

The soybean field experiment was located at SC Minear Laboratoare SRL (RENAR accredited laboratory), Arad-Zadareni km 6, Arad County, Romania (*Fig. 1*). The experiment was conducted between 2011 and 2013. A soybean cultivar from Caussade Semences was cultivated. The soybean seeds were not bacterized, the nodulation and nodules were formed based on free microorganisms from the soil.

Figure 1. *Experimental location (63)*

Inoculation was not considered to observe the natural process of nodule formation and development, against the background of differentiated fertilization. The sowing was done at the optimal time, first decade of April.

Soil conditions

The soil in the crop plot was of the cambic chernozem type, gleyed, characterized by: moderate acidity, pH_{KCl} = 4.62±0.015, humus content at the level of H = 2.04 ±0.03%, very good phosphorus and potassium supply (P_2O_5 = 155.58±11.92 ppm; K_2O = 230.83±8.05 ppm), calcium supply at the level of Ca = 0.22±0.004 %, magnesium at the level of Mg = 430.58±7.23 ppm, and microelements of different content (Zn = 1.91±0.09 ppm, Cu = 8.88±0.17 ppm, Fe and Mn > 200 ppm). The soil samples were determined at Vizsgéló Laboratórum (MINERÁG Fejlesztési, Termelési és Kereskedelmi Kft. LABORCONTROL LABORATÓRIUM (NAT-1-1207/2006); (NAT-1-1207/2010, accredited laboratory, Hungary).

Climatic conditions

The Western Plain of Romania has macroclimatic particularities determined by its geographical position on the European Continent. The climatic conditions in terms of thermal and pluviometric regime, during the study period and multiannual mean, are presented in graphic format (*Fig. 2*).

Experimental variants

The experimental variants were created by fertilization with mineral fertilizers. Phosphorus and potassium (PK) were provided in four levels, in doses of 0, 60, 100 and 140 kg ha^{-1} a.s. (active substance). Nitrogen was provided in three levels, in doses of 0, 50 and 100 kg ha^{-1} a.s. on each PK level. 12 experimental variants resulted (T1 to T12).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Plot size for each experimental variant was 50 m^2 (10 m length, and 5 m width). Complex fertilizers 0:10:28, concentrated superphosphate and ammonium nitrate were used. P and K fertilizers were applied and incorporated into the soil with the basic soil works. Nitrogen was applied during the preparation of the seedbed and incorporated with the combiner. The land was prepared by plowing, disc harrow and combinator.

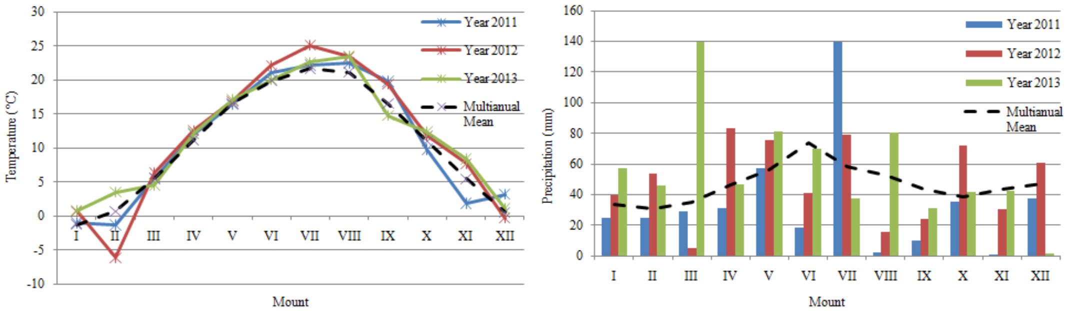

Figure 2. *Climatic parameters in the study area*

Plant sampling and determined indicators

Plant sampling was done randomly, on each experimental variant, at the age of 60 days of soybean plants (July 20), 60-62 BBCH Code, Flowering, Main shoot (Meier, 2001). Plant samples were taken by pulling plants from the soil, randomly, from ten points for each experimental variant. The plants were placed in transport bags, per experimental variant. The soil attached to the root was removed with a brush, and the roots were subsequently washed and dried in the laboratory. The roots of the plants from each sample were detached, and the nodules were removed from the roots (*Fig. 3*).

Figure 3. *Soybean plant samples; (a) – root with nodules, (b) – section through nodules (original photographs, by Florin Sala)*

The organic matter content (OM, %) and the mineral element content (total nitrogen – TN, P, K, %; Fe, Mn, Cu, Zn, mg kg-1) were determined in root and nodule samples of soybean plants, in the experimental variants. Based on the recorded values, the ratio between the content in nodules (n) and the content in roots (r) was calculated for microelements, Fe n/r, Mn n/r, Cu n/r, Zn n/r. The determination of the samples was made within the OSPA Timisoara Laboratory, a RENAR accredited laboratory.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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Statistical analysis of data

The experimental results were analyzed to assess the statistical reliability and the presence of variance in the data set (ANOVA Test). The variation of the determined parameters (OM, mineral element content) in relation to the applied fertilizers (N, PK) was evaluated by quadratic regression analysis. The interdependence between the determined parameters in the root and nodules was evaluated by correlation analysis (Pearson's r). The variation of organic matter and trace element content in nodules in relation to the content in the roots of soybean plants was evaluated by quadratic regression analysis. Multivariate analysis (PCA – Principal Component Analysis) was used to explain the position of the determined parameters in relation to the experimental variants. The Kruskal-Wallis test, followed by Mann-Whitney test (post-hoc step), were applied to compare the medians and means. The calculation module in EXCEL, the PAST software v.4.17 (Hammer et al., 2001), and the JASP software v.0.16.4 (2002) were used for data analysis and graph generation.

Results and discussions

Organic matter and mineral elements content in plant samples

Analysis of samples from soybean plants led to the values of the content of organic matter and mineral elements in roots (*Table 1*), respectively in nodules (*Table 2*).

Table 1. *Content of organic matter and mineral elements in the roots samples of soybean plants*

Fertilization Variants	Trial	OM	TN	P	K	Fe	Mn	Cu	Zn
		(%)				(mg kg^{-1})			
P0K0N0	T1	95.97	0.514	0.019	0.456	185.52	20.71	8.55	3.56
P0K0N50	T2	94.29	0.562	0.027	0.531	241.55	31.14	9.67	6.25
P0K0N100	T3	93.49	0.652	0.027	0.564	260.98	41.20	11.06	7.64
P60K60N0	T4	93.40	0.563	0.020	0.456	269.77	28.99	8.96	5.55
P60K60N50	T5	92.62	0.659	0.031	0.614	275.18	39.37	11.82	10.76
P60K60N100	T6	93.27	0.702	0.029	0.655	331.55	38.02	13.96	13.91
P100K100N0	T7	94.46	0.460	0.038	0.506	236.28	28.92	11.14	5.95
P100K100N50	T8	93.73	0.594	0.048	0.755	263.03	35.30	13.00	7.85
P100K100N100	T9	93.19	0.653	0.039	0.904	632.00	41.39	16.05	14.95
P140K140N0	T10	93.95	0.575	0.013	0.547	634.00	43.41	15.96	12.26
P140K140N50	T11	94.14	0.672	0.069	0.821	574.00	34.69	15.47	13.82
P140K140N100	T12	95.49	0.615	0.041	0.622	570.00	28.95	13.24	12.62
SE		±0.28	±0.02	±0.004	±0.041	±50.16	±1.95	±0.76	±1.12

In root samples, organic matter showed values of OM = 92.62 – 95.97±0.28%. In the case of macroelements, total nitrogen showed values of TN = 0.46 – 0.70±0.020%, phosphorus showed values of P = 0.01 – 0.07±0.004%, and potassium showed values of K = 0.46 – 0.90±0.041%. In the case of microelements, different values were recorded, Fe = 185.52 – 634.00±50.15 mg kg^{-1}, Mn = 20.71 – 43.41±1.95 mg kg^{-1}, Cu = 8.55 – 16.05±0.76 mg kg^{-1}, and Zn = 3.56 – 14.95±1.12 mg kg^{-1}.

In the nodule samples, the organic matter presented values of OM = 87.70 – 94.38±0.57%. In the case of microelements, values of: Fe = 172.29 – 358.20±19.52 mg

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kg^{-1}, Mn = 14.90 – 53.22±3.55 mg kg^{-1}, Cu = 5.84 – 8.74±0.26 mg kg^{-1}, and Zn = 20.90 – 30.62±1.02 mg kg^{-1} were recorded (*Table 2*). Compared to the content in roots, higher values were recorded in nodules for Fe (T3, T6, T7 and T8 trials), for Mn (T2 and T3 trials) and for Zn (all trials).

Table 2. *Content of organic matter and microelement in nodule samples of soybean plants*

Fertilization Variants	Trial	OM	Fe	Mn	Cu	Zn
		(%)	(mg kg^{-1})			
P0K0N0	T1	90.81	172.29	18.76	6.26	22.20
P0K0N50	T2	93.86	229.12	35.04	7.30	24.29
P0K0N100	T3	87.70	357.67	53.22	6.83	30.25
P60K60N0	T4	92.49	202.88	14.90	6.91	23.10
P60K60N50	T5	92.27	221.95	31.04	7.97	28.53
P60K60N100	T6	94.38	332.45	31.88	7.84	28.82
P100K100N0	T7	92.56	257.59	18.73	6.59	20.90
P100K100N50	T8	93.21	291.76	22.43	7.98	23.46
P100K100N100	T9	93.72	358.20	28.47	8.74	30.62
P140K140N0	T10	93.26	204.45	20.98	6.92	24.37
P140K140N50	T11	93.65	273.26	17.08	5.84	24.62
P140K140N100	T12	-	-	-	-	-
SE		±0.57	±19.52	±3.35	±0.26	±1.02

Note: In the T12 trial, no nodules were found on the roots of soybean plants

Based on the recorded values, the ratio of the content of microelements in nodules (n) and roots (r) was calculated, Fe n/r, Fe n/r, Mn n/r, Cu n/r, and Zn n/r, respectively (*Table 3*). In the case of iron, the resulting values of the ratio were Fe n/r = 0.322 – 1.370±0.093. In the case of manganese, the resulting values of the ratio were Mn n/r = 0.483 – 1.292±0.079. In the case of copper, the resulting values of the ratio were Cu n/r = 0.378 – 0.771±0.038.

Table 3. *Values of the ratio between the content of microelements in nodules and roots in soybean plants on experimental plots*

Fertilization Variants	Trial	Microelement content ratio values			
		Fe n/r	Mn n/r	Cu n/r	Zn n/r
P0K0N0	T1	0.929	0.906	0.732	6.236
P0K0N50	T2	0.949	1.125	0.755	3.886
P0K0N100	T3	1.370	1.292	0.618	3.959
P60K60N0	T4	0.752	0.514	0.771	4.162
P60K60N50	T5	0.807	0.788	0.674	2.651
P60K60N100	T6	1.003	0.839	0.562	2.072
P100K100N0	T7	1.090	0.648	0.592	3.513
P100K100N50	T8	1.109	0.635	0.614	2.989
P100K100N100	T9	0.567	0.688	0.545	2.048
P140K140N0	T10	0.322	0.483	0.434	1.988
P140K140N50	T11	0.476	0.492	0.378	1.781
P140K140N100	T12	-	-	-	-
SE		±0.093	±0.079	±0.038	±0.401

Note: n/r – indicates the nodule/root ratio for micronutrients; In the T12 trial, nodules were not found on the roots of soybean plants, and the ratio was not calculated

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In the case of zinc, the resulting values of the ratio were Zn n/r = 1.781 – 6.236±0.401. Subunit values of the ratio indicated higher content of microelements in the root compared to nodules, and superunit values indicated higher values for the content of microelements in nodules.

Statistical analysis of the entire experimental data package confirmed the presence of variance, and the statistical reliability of the recorded results (*Table 4*).

Table 4. *ANOVA test results*

Statistical Parameters	Sum of sqrs	df	Mean square	F	p (same)
Between groups	1.88E+06	12	157033	57.61	1.20E-47
Within groups	376168	138	2725.86		
Total	2.26E+06	150	1.00E-05		
Components of variance (only for random effects)					
Var(group)	13286.6	Var(error)	2725.86	ICC:	0.82976
omega2	0.8181				
Levene´s test for homogeneity of variance, from means	p (same)	6.64E-44			
Levene´s test, from medians	p (same)	5.02E-12			
Welch F test in the case of unequal variances	F=1.046E04; df=49.69; p=4.002E-80				
Bayes factor	2.534E44 (decisive evidence for unequal means)				

Interdependence level between organic matter and mineral elements

Correlation analysis showed different levels of interdependence between organic matter and mineral elements in roots and nodules in soybean plants (*Fig. 4*).

Root organic matter (OM_r) showed negative correlation with mineral elements in roots and nodules, with a strong, negative correlation with root manganese, Mn_r (r = -0.758**). Nodule organic matter (OM_n) showed low correlation values with mineral elements in roots and nodules.

Very strong, positive correlation was recorded between Cu_r and Zn_r (r = 0.919***), Strong positive correlation was recorded between Cu_r and Fe_r (r = 0.8887***), between Zn_r and Fe_r (r = 0.813**). Strong positive correlation was recorded between TN_r and Zn_r (r = 0.765**), between P_r and K_r (r = 0.729*), between K_r and Cu_r (r = 0.774**), between K_r and Zn_r (r = 0.769**), between Mn_r and Cu_r (r = 0.741**), and between Mn_r and Zn_r (r = 0.758**). Also, weak correlations were recorded between certain mineral elements in plant roots. These correlations may indicate the positive interaction of the respective mineral elements for absorption in soybean plant roots, in direct relation to the level of intensity of the recorded correlation.

At the nodules level, a moderate, positive correlation was identified between Fe_n and Zn_n (r = 0.708*) and between Mn_n and Zn_n (r = 0.72*). Other positive or negative correlations of varying intensity were also identified between the mineral elements at the nodules level. At the level of the calculated ratios, a positive or negative correlation, of variable intensity, was identified with OM or mineral elements in the root or nodules. The level of correlation of the ratios with microelements in the root or nodules varied, and indicated the participation weight of the microelements in the ratio value.

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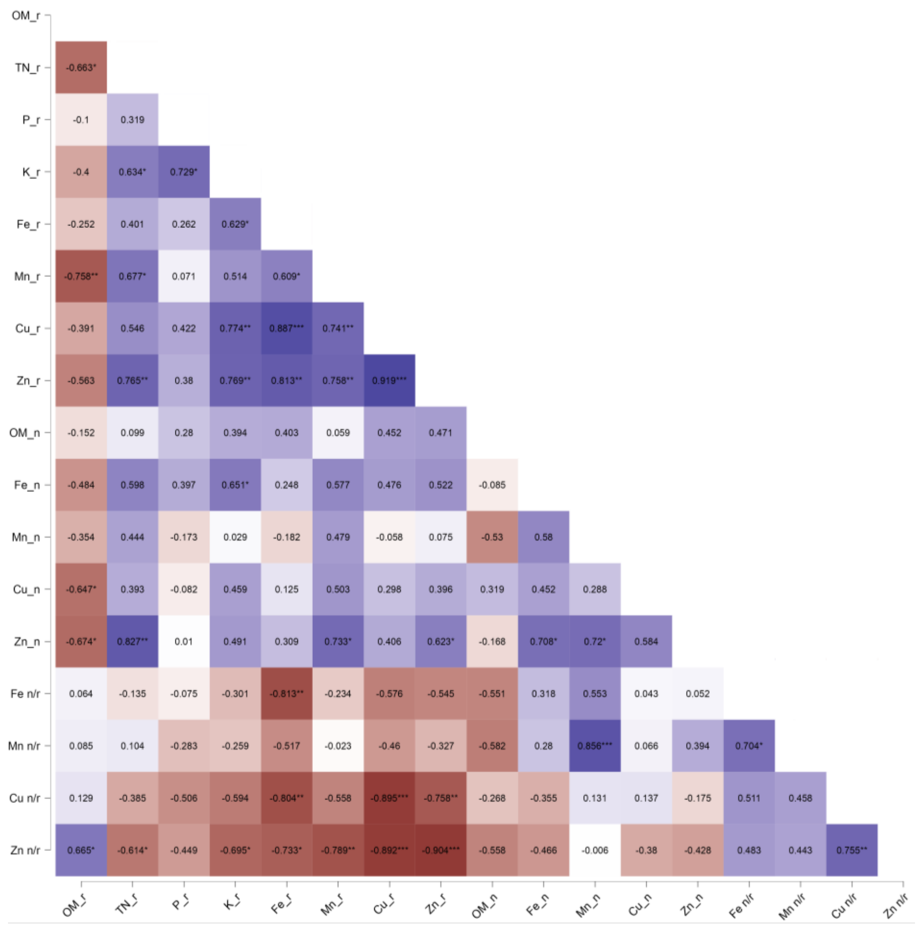

Figure 4. *Correlations between organic matter and mineral elements in roots and nodules in soybean; "_r" indicates root; "_n" indicates nodules; n/r indicates nodule/root ratio*

Fertilizers contribution to the variation of organic matter and mineral elements

The applied fertilizers differentially influenced the organic matter content and the mineral element content in the root and nodules of soybean plants. Regression analysis was applied to obtain polynomial models that described the dependent variables (organic matter, mineral elements) in relation to the independent variables (N, and PK).

The variation of organic matter content in roots (OM_r) and in nodules (OM_n), in relation to the independent variables (N, PK) was described by *Equation 1* with $R^2 = 0.821$, $p = 0.029$ for roots, and by *Equation 2* with $R^2 = 0.514$, $p = 0.475$, without statistical certainty in the case of nodules. The results may indicate that the organic matter in the nodules was not directly influenced by the applied fertilizers, but rather by the plant metabolism.

$$OM\_r = 95.878495 - 0.042115x - 0.024118y + 0.000183x^2 + 0.000066y^2 + 0.000120xy \quad \text{(Eq.1)}$$

$$OM\_n = 91.541119 + 0.013258x + 0.013056y - 0.000325x^2 - 0.000037y^2 + 0.000149xy \quad \text{(Eq.2)}$$

where: x –N doses (kg ha^{-1} a.s.); y – PK doses (kg ha^{-1} a.s.).

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The variation of macroelement content in the root of soybean plants, in relation to the independent variables (N, and PK), was described by *Equation 3* in the case of total nitrogen (TN), with $R^2 = 0.676$, $p = 0.146$, by *Equation 4* in the case of phosphorus (P), with $R^2 = 0.631$, $p = 0.202$, and by *Equation 5* in the case of potassium (K), with $R^2 = 0.697$, $p = 0.123$.

$$TN = 0.492624 + 0.002826x + 0.000389y - 0.000012x^2 - 6.94\,E{-}07y^2 - 2.341\,E{-}06xy \quad (Eq.3)$$

$$P = 0.01634 + 0.00065x + 3.181\,E{-}05y - 0.000006x^2 + 4.1787\,E{-}08y^2 - 5.1869\,E{-}07xy \quad (Eq.4)$$

$$K = 0.39144 + 0.005347x + 0.001120y - 0.000036x^2 - 2.056\,E{-}06y^2 + 1.7477\,E{-}06xy \quad (Eq.5)$$

where: x –N doses (kg ha^{-1} a.s.); y – PK doses (kg ha^{-1} a.s.).

The variation of the content of microelements in plant roots, in relation to the independent variables (N, PK) was described by equation (6) in the case of iron (Fe_r), with $R^2 = 0.779$, $p = 0.053$, by *Equation 7* in the case of manganese (Mn_r), with $R^2 = 0.773$, $p = 0.058$, by *Equation 8* in the case of copper (Cu_r), with $R^2 = 0.793$, $p = 0.045$, and respectively by *Equation 9* in the case of zinc (Zn_r), with $R^2 = 0.771$, $p = 0.059$.

$$Fe\_r = 188.451558 - 0.830696x - 0286032y + 0.020629x^2 + 0.005597y^2 - 0.000398xy \quad (Eq.6)$$

$$Mn\_r = 19.242595 + 0.277114x + 0.097475y - 0.00047x^2 - 0.000101y^2 - 0.001074xy \quad (Eq.7)$$

$$Cu\_r = 7.364294 + 0.051490x + 0.022299y - 0.000049x^2 + 0.000013y^2 - 0.000148xy \quad (Eq.8)$$

$$Zn\_r = 2.648789 + 0.071927x + 0.030639y - 0.000046x^2 - 0.000012y^2 - 0.000085xy \quad (Eq.9)$$

where: x –N doses (kg ha^{-1} a.s.); y – PK doses (kg ha^{-1} a.s.).

The variation of the content of microelements in nodules, in relation to the independent variables (N and PK), was described by *Equation 10* in the case of iron (Fe_n), with $R^2 = 0.887$, $p = 0.020$, by *Equation 11* in the case of manganese (Mn_n), with $R^2 = 0.959$, $p = 0.0017$, by *Equation 12* in the case of copper (Cu_n), with $R^2 = 0.611$, $p = 0.315$, and respectively by *Equation 13* in the case of zinc (Zn_n), with $R^2 = 0.800$, $p = 0.077$. Based on the results, it was observed that the variation of Fe and Mn in nodules was influenced in statistically safety conditions by the applied fertilizers.

$$Fe\_n = 173.889952 + 0.640134x + 0.315421y + 0.010289x^2 - 0.000358y^2 - 0.001734xy \quad (Eq.10)$$

$$Mn\_n = 17.974217 + 0.381786x - 0.014410y - 0.000379x^2 + 0.000076y^2 - 0.0013446xy \quad (Eq.11)$$

$$Cu\_n = 6.295782 + 0.012635x + 0.013822y - 0.000040x^2 - 0.000051y^2 + 9.584\,E{-}05xy \quad (Eq.12)$$

$$Zn\_n = 22.066236 + 0.042320x + 0.007114y + 0.000379x^2 - 0.000014y^2 - 6.423\,E{-}05xy \quad (Eq.13)$$

where: x –N doses (kg ha^{-1} a.s.); y – PK doses (kg ha^{-1} a.s.).

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DOI: http://dx.doi.org/10.15666/aeer/2306_1105311072

Variation of mineral elements in nodules in relation to organic matter and mineral elements in roots

The variation of microelement content in nodules was analyzed in relation to organic matter (OM) and mineral elements in the roots of soybean plants.

In the case of Fe content in nodules (Fe_n), the regression analysis led to a model with statistical certainty, based on OM_r and Mn_r, *Equation 14*. According to the regression coefficient (R^2 = 0.903), a very high fit was recorded between the mathematical model and the real values, with p = 0.014.

$$\mathrm{Fe\_r} = -1883740.19 + 39085.164x + 3077.624y - 202.5924x^2 - 0.1956y^2 - 32.6628xy \quad \text{(Eq.14)}$$

where: x – OM in root (%), y – Mn content in root (mg kg^{-1}).

The regression coefficient values (R^2) indicated moderate and low level of fit and no statistical safety (p>0.05) between the mathematical models and the real values for Fe content in nodules (Fe_n), in relation to OM and/or other mineral elements in roots, in different combinations.

In the case of Mn content in nodules (Mn_n), regression analysis conducted to variation models in relation to root parameters in different combinations (OM, mineral elements), without statistical safety (p>0.05).

The variation of copper content in nodules (Cu_n) was described in statistical safety conditions in relation to OM_r and P_r in roots, *Equation 15*, with R^2 = 0.932, p = 0.0060, in relation to OM and K in roots, *Equation 16*, with R^2 = 0.863, p = 0.0032, in relation to OM and Cu in roots, *Equation 17*, with R^2 = 0.857, p = 0.035, in relation to OM and Zn in roots, *Equation 18*, with R^2 = 0.837, p = 0.0482, in relation to P and K in roots, *Equation 19*, with R^2 = 0.913, p = 0.0110, and in relation to K and Mn in roots according to *Equation 20*, with R^2 = 0.838, p = 0.0477.

$$\mathrm{Cu\_n} = -3761.9507 + 77.6911x + 10489.524y - 0.40004x^2 - 821.3477y^2 - 110.9353xy \quad \text{(Eq.15)}$$

where: x – OM content in root (OM_r); y – P content in root (P_r).

$$\mathrm{Cu\_n} = -3249.0346 + 64.9135x + 8383327.524y - 0.3224x^2 - 9.1733y^2 - 8.8063xy \quad \text{(Eq.16)}$$

where: x – OM content in root (OM_r); y – K content in root (K_r).

$$\mathrm{Cu\_n} = -4403.2094 + 89.3541x + 45.3541y - 0.4507x^2 - 0.0130y^2 - 0.4801xy \quad \text{(Eq.17)}$$

where: x – OM content in root (OM_r); y – Cu content in root (Cu_r).

$$\mathrm{Cu\_n} = -6772.161 + 141.8532x + 37.0015y - 0.7418x^2 - 0.0128y^2 - 0.3921xy \quad \text{(Eq.18)}$$

where: x – OM content in root (OM_r); y – Zn content in root (Zn_r).

$$\mathrm{Cu\_n} = 1.262550 + 38.246514x + 13.379043y - 2502.576279x^2 - 10.292853y^2 + 173.8354xy \quad \text{(Eq.19)}$$

where: x –P content in root (P_r); y – K content in root (K_r).

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$$\text{Cu\_n} = 12.312084 - 11.823026x - 0.234112y - 39.537427x^2 - 0.010536y^2 + 1.794287xy \quad \text{(Eq.20)}$$

where: x – K content in root (K_r); y – Mn content in root (Mn_r).

The variation of zinc content in nodules (Zn_n) in relation to organic matter and mineral elements in soybean plant roots presented the most models with statistical safety. Regression analysis generated models of Zn_n variation with statistical safety in relation to OM_r and TN_r, *Equation 21*, with $R^2 = 0.862$, $p = 0.0330$, in relation to OM and Mn from roots, *Equation 22*, with $R^2 = 0.864$, $p = 0.0317$, in relation to TN and P from roots, *Equation 23* with $R^2 = 0.862$, $p = 0.0326$; in relation to TN and K from roots, *Equation 24* with $R^2 = 0.847$, $p = 0.0413$; in relation to TN and Mn from roots, *Equation 25* with $R^2 = 0.962$, $p = 0.0014$; in relation to P and Mn from roots, *Equation 26* with $R^2 = 0.879$, $p = 0.023$; in relation to P and Zn in roots, *Equation 27* with $R^2 = 0.850$, $p = 0.039$.

$$\text{Zn\_n} = -25660.219 + 500.3199x + 7438.9238y - 2.4286x^2 - 238.2302y^2 - 75.2907xy \quad \text{(Eq.21)}$$

where: x – OM content in root (OM_r); y – total nitrogen content in root (TN_r).

$$\text{Zn\_n} = -39849.166 + 822.05506x + 85.34456y - 4.23278x^2 + 0.00027y^2 - 0.90771xy \quad \text{(Eq.22)}$$

where: x – OM content in root (OM_r); y – Mn content in root (Mn_r).

$$\text{Zn\_n} = 15.73051 - 5.02212x - 41.76257y + 22.19833x^2 - 5181.6099y^2 + 639.99444xy \quad \text{(Eq.23)}$$

where: x – TN content in root (TN_r); y – phosphorus content in root (P_r).

$$\text{Zn\_n} = 21.33527 - 119.76927x + 94.16857y + 399.76388x^2 + 164.92587y^2 - 515.32843xy \quad \text{(Eq.24)}$$

where: x – TN content in root (TN_r); y – potassium content in root (K_r).

$$\text{Zn\_n} = 68.35185 + 22.51977x - 3.66928y - 261.71616x^2 - 0.01909y^2 + 9.0044xy \quad \text{(Eq.25)}$$

where: x – TN content in root (TN_r); y – manganesse content in root (Mn_r).

$$\text{Zn\_n} = 36.96536 - 1104.95x - 0.2852y - 994.70536x^2 - 0.00189y^2 + 33.44221xy \quad \text{(Eq.26)}$$

where: x – phosphorus content in root (P_r); y – manganesse content in root (Mn_r).

$$\text{Zn\_n} = 14.65214 + 25.17727x + 2.16502y - 5457.7331x^2 - 0.16505y^2 + 55.67749xy \quad \text{(Eq.27)}$$

where: x – phosphorus content in root (P_r); y – zinc content in root (Zn_r).

Based on the other combinations of organic matter and mineral elements in the roots, the regression analysis led to models in which the degree of fit between the estimated and actual values was low, and without statistical certainty ($p>0.05$).

Multivariate component analysis (PCA) explained the variance of the experimental data in roots and nodules, respectively, based on the microelement content. Thus, in the case of the microelement content in soybean roots, PC1 explained 80.505% of the variance, and PC2 explained 14.1% of the variance (*Fig. 5a*). In the case of the

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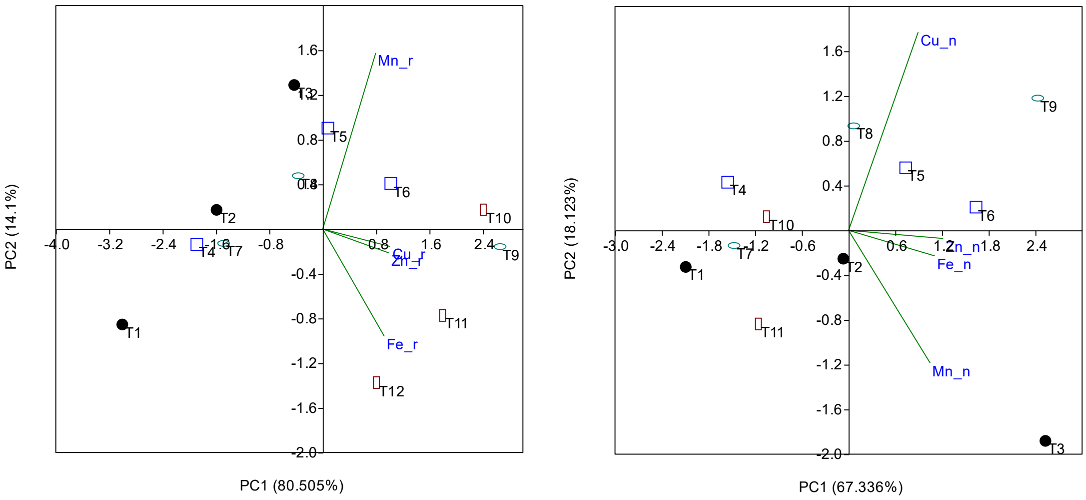
DOI: http://dx.doi.org/10.15666/aeer/2306_1105311072

microelement content in nodules, PC1 explained 67.336% of the variance, and PC2 explained 18.123% of the variance (*Fig. 5b*).



(a) (b)

Figure 5. *PCA diagram with the distribution of variants in relation to the content of microelements in roots (a) and in nodules (b)*

The experimental variants were positioned differently in the two diagrams, depending on the correlation with the determined microelements. The parameter loading values showed to what extent the variables contributed to the components (*Table 5*).

Table 5. *Parameter loadings in the principal components*

Root level					Nodule level				
	PC 1	**PC 2**	**PC 3**	**PC 4**		**PC 1**	**PC 2**	**PC 3**	**PC 4**
Fe_r	0.888	-0.386	0.234	0.086	Fe_n	0.847	-0.090	-0.516	0.089
Mn_r	0.764	0.635	0.103	0.045	Mn_n	0.802	-0.470	0.308	0.201
Cu_r	0.971	-0.062	-0.028	-0.228	Cu_n	0.684	0.703	0.144	0.131
Zn_r	0.951	-0.085	-0.272	0.115	Zn_n	0.931	-0.028	0.098	-0.351

The values obtained showed the correlation between a variable (microelement in the root, or nodules) and the PC score. In the case of roots, a positive correlation of microelements was observed in relation to PC1, of very strong intensity for Cu_r (r = 0.971), and for Zn_r (r = 0.951), strong intensity for Fe_r (r = 0.888), and moderate intensity for Mn_r (r = 0.764). In relation to PC2, a positive correlation of weak intensity was recorded for Mn_r (r = 0.635). In the case of nodules, a positive correlation of microelements was observed in relation to PC1, of very strong intensity for Zn_n (r = 0.931), of strong intensity for Fe_n (r = 0.847) and for Mn_n (r = 0.802), and of weak intensity for Cu_n (r = 0.684). In relation to PC2, a positive correlation of moderate intensity was recorded for Cu_n (r = 0.703). In the other cases, at the level of roots and nodules, a weak correlation, positive or negative, was recorded.

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DOI: http://dx.doi.org/10.15666/aeer/2306_1105311072

In the multivariate analysis, the test of sphericity (Bartlett, 1951; Hammer, 2025) was applied to test the null hypothesis. According to the chi^2 test values and the statistical safety parameter values ($p<0.05$), a good significance was obtained for both the root microelement content and the nodule microelement content (*Table 6*). Within the PCA analysis, on the correlation matrix, the Kaiser-Meyer-Olkin (KMO) measure was applied (Kaiser, 1970), *Equation 28*. The result was KMO = 0.7647 $p<0.001$ for the root level data, respectively KMO = 0.7087 with p = 0.0195 for the nodule level data (*Table 6*); $0.7 \leq KMO < 0.8$, indicated "good" significance.

$$\chi^2 = -\left(M - 1 - \frac{1}{6}(2N+5)\right)\ln\det R \qquad \text{(Eq.28)}$$

where: M - points number; N - variables number; R - correlation matrix.

Table 6. *Bartlett´s sphericity test*

Test and parameters	Root level	Nodule level
Bartlett´s sphericity test		
Determinant:	0.0192	0.1448
chi^2:	34.9170	15.1360
df:	6	6
p (spherical):	4.47E-06	0.0192
Kaiser-Meyer-Olkin measure		
KMO: value	0.7647	0.7087
KMO: significance	good	good

Kruskal-Wallis test indicated significant differences between the median values of organic matter and microelement content in roots and nodules in soybean plants, H (chi^2) = 108.2, H_c (tie corrected) = 108.2, $p < 0.001$. Based on statistically confirmed differences between median values (Kruskal-Wallis test), paired Mann-Whitney test (post-hoc step) confirmed the differences between mean values for organic matter and microelement content in soybean roots and nodules (*Table 7*).

Table 7. *Mann-Whitney post-hoc test for organic matter and microelement content in roots and nodules*

	OM_r	**OM_n**	**Fe_r**	**Fe_n**	**Mn_r**	**Mn_n**	**Cu_r**	**Cu_n**	**Zn_r**	**Zn_n**
OM_r		0.0247	3.66E-05	5.55E-05	3.66E-05	5.55E-05	3.66E-05	5.55E-05	3.66E-05	5.55E-05
OM_n	0.0247		5.55E-05	8.15E-05	5.55E-05	8.15E-05	5.55E-05	8.15E-05	5.55E-05	8.15E-05
Fe_r	3.66E-05	5.55E-05		0.1858	3.66E-05	5.55E-05	3.66E-05	5.55E-05	3.66E-05	5.55E-05
Fe_n	5.55E-05	8.15E-05	0.1858		5.55E-05	8.15E-05	5.55E-05	8.15E-05	5.55E-05	8.15E-05
Mn_r	3.66E-05	5.55E-05	3.66E-05	5.55E-05		0.0289	3.66E-05	5.55E-05	3.66E-05	0.002836
Mn_n	5.55E-05	8.15E-05	5.55E-05	8.15E-05	0.0289		0.0001	8.15E-05	7.20E-05	0.6936
Cu_r	3.66E-05	5.55E-05	3.66E-05	5.55E-05	3.66E-05	0.0001		7.20E-05	0.0690	5.55E-05
Cu_n	5.55E-05	8.15E-05	5.55E-05	8.15E-05	5.55E-05	8.15E-05	7.20E-05		0.3099	8.15E-05
Zn_r	3.66E-05	5.55E-05	3.66E-05	5.55E-05	3.66E-05	7.20E-05	0.0690	0.3099		5.55E-05
Zn_n	5.55E-05	8.15E-05	5.55E-05	8.15E-05	0.0028	0.6936	5.55E-05	8.15E-05	5.55E-05	

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DOI: http://dx.doi.org/10.15666/aeer/2306_1105311072

Discussion

To evaluate the influence of NPK mineral fertilization, as independent variables (N, PK) on the organic matter content and mineral element content in soybean roots and nodules, the results were analyzed on several levels.

First, the direct influence of the fertilizer variants (N, and PK) on the determined parameters was analyzed. The strong influence of the independent variables (N, PK) was recorded in the variation of MO at the root level and the weak influence in the variation of MO at the nodule level, according to R^2, in *Equation 1*, and *Equation 2*. This may suggest the direct dependence of roots on soil nutrients and those provided by fertilization, and in the case of nodules the dependence is stronger on the host plant, on the plant metabolism in the "root system - nodules" relationship. Such "endosymbiotic mutualistic relationships" have been reported in legumes (Suzaki, 2023; Yeremko et al., 2025).

Under the study conditions, given by soil fertility (Soil conditions, in Material and Methods), the regression coefficient (R^2) values indicated a low level of fit between the mathematical models, *Equations 3*, *4*, and *5* and the real values of TN, P and K in soybean plant roots, in relation to the independent variables (N, PK). For the content of microelements in the roots of soybean plants, the values of the regression coefficients (R^2) indicated a moderate level of fit between the mathematical models and the real values, with statistical safety in the case of copper ($p = 0.045$), and without statistical certainty in the case of iron, manganese and zinc, *Equations (6) – (9)*. In the case of variant T12 (N100, PK 140) no nodules were recorded on the roots of the plants.

The relationship between mineral fertilizers (NPK), soil nitrogen and soybean plant parameters, the nodulation process, and the content of mineral elements in soybean roots has been confirmed in other studies. In experiments with variable N and PK rates, in experimental variants with high N doses, associated with high PK fertilization, no nodules were identified on soybean roots, and nitrogen was associated with inhibition of the nodulation process (Lyu et al., 2022). High nitrogen availability in the soil negatively influenced nodule formation and symbiotic nitrogen fixation in soybean plants (Wang et al., 2024). High values for root weight and N and K content in soybean plants were recorded with experimental fertilization with DAP (Egribas Endes, 2024). High values for root length in soybean were recorded when applying microbial fertilizer, and high content of mineral elements (e.g. Cu, Fe, Mn, Zn) were recorded when complex fertilization with different types of fertilizers (Egribas Endes, 2024).

The regression coefficient values (R^2) indicated a high level of fit between the mathematical models and the real values, in relation to the independent variables (N and PK), in the case of iron in nodules (Fe_n), *Equation 10*, $p<0.05$, and a very high level of fit in the case of manganese in nodules (Mn_n), *Equation 11*, $p<0.01$. In the case of copper in nodules (Cu_n), *Equation 12*, the value of the regression coefficient indicated a low level of fit, and in the case of zinc in nodules (Zn_n), *Equation 13*, a strong level of fit between the models and the real values was recorded, but without statistical safety ($p>0.05$).

The variation of microelement content in nodules in relation to the content of organic matter and mineral elements in roots was described by *Equations 14 – 27*, with variable levels of fit between theoretical and real values, in relation to root-level variables. The regression coefficient (R^2) values indicated a very high level of fit for Fe_n in relation to OM_r and Mn_r, and a low level of fit for the other independent variables at the root level. In the case of Mn_n, statistical safety was not found, according to R^2 and p (p>0.05). In the case of copper in nodules (Cu_n), six models with statistical certainty were obtained, *Equation 15* to *Equation 20*, for which the regression coefficient values (R^2) indicated different levels of fit with the real values, in relation to OM and mineral elements in the root. In the case of zinc in nodules (Zn_n), the regression coefficient values (R^2) indicated different levels of fit to the real values, in the case of seven models, *Equation 21* to *Equation 27*, in relation to OM and mineral elements in the root.

The root growth process, based on specific characteristics, and the distribution of nodules on soybean roots have been analyzed and described through certain theoretical models (Arima et al., 2000). A study on samples of flowering soybean plants showed a decrease in differences in vertical root distribution as phosphorus rates increased (Li et al., 2017). The interaction effect of some macroelements (N, P, Mg) was analyzed in relation to the root system architecture, the nodulation process and the N concentration in the root tissues of soybean (Egamberdieva et al., 2018). Quantitative evaluations were made to study the functional structure and nodulation process in soybean (Nakhforoosh et al., 2024). Iron is important for the nodulation process, and the presence of “iron storage reservoirs” in the peribacterioid space zone has been reported (LeVier et al., 1996). Iron is important and necessary in the symbiotic nitrogen fixation system in soybean (e.g. for leghemoglobin, nitrogenase, cytochromes), and iron deficiency in plant nutrition affects nodule initiation and formation (Brear et al., 2013). Iron nutrition in soybean has often been approached in a segmented manner, in relation to soil, plant genetics and physiology and microorganisms. An integrated, systemic approach to these aspects, regarding phenotyping, genetics, iron physiology in soybean plants, on the flow “soil – root/nodules – plant – seeds” would provide relevant information regarding the role and distribution of iron in the soybean plant. An important role of iron is associated with the “soybean - rhizobia” relationship at the root system level (Merry et al., 2022).

In experiments on nutrient solutions, with increasing concentrations of Mn and contrasting concentrations of Fe, variable values of Mn and Fe were recorded in two soybean genotypes, antagonistic relationships between the concentrations of the two microelements, and some changes in the nodulation process at high concentrations of Mn (Izaguirre-Mayoral and Sinclair, 2005). At normal concentrations, no effects were recorded on the mass of roots, nodules and shoots in soybean plants, or on the variation of some compounds in nodules and leaves. No interdependence relationships were recorded between Mn, Fe and the content of P, Cu, Zn in soybean plants, which showed the differentiated importance of micronutrients in soybean (Izaguirre-Mayoral and Sinclair, 2005). In experiments on soybean plants in pots with variable manganese (Mn) application rates, critical limits for manganese content of 3.55 to 3.60 $\mu g\ g^{-1}$ were found (Rashed et al., 2023). According to the authors, manganese supplementation (1 $\mu g\ g^{-1}$ Mn in soil) led to an increase in the root weight of soybean plants.

Copper is important for the nodulation process and nitrogen synthesis in soybean, and experiments with variable concentrations of Cu identified an optimal value (20 μM Cu) that stimulated the nodulation process and symbiotic nitrogen fixation (Tortosa et al., 2020). Different types of copper compounds, applied at variable concentration rates, generated variations in root architecture in soybean plants, in relation to the type of product, concentration and their interaction (Yusefi-Tanha et al., 2020).

The assortment of zinc compounds, their concentration and interaction significantly influenced the absorption and accumulation of zinc in the root and aerial organs of

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soybean plants (Yusefi-Tanha et al., 2022). Nonlinear and linear models were obtained that described zinc accumulation in relation to different tissues analyzed.

The correlation matrix confirmed certain interdependencies between organic matter and mineral elements in the root and nodules, against the background of NPK mineral fertilization.

According to PCA, experimental variants were identified that positioned independently (e.g. T1, T2, and T3) in relation to root microelements, and variants that were associated with certain microelements (e.g. T11 and T12 with Fe_r; T9 with Cu_r and Zn_r). In the case of nodules, according to PCA, certain variants were positioned independently (e.g. T1, T4, T7, T10), and other variants were positioned in association with certain microelements (e.g. T3 with Mn_n; T6 with Zn_n and Fe_n). In the case of roots, Cu_r and Zn_r were associated (as biplot), and in the case of nodules, Fe_n and Zn_n were associated (as biplot). These associations may indicate the share of microelements in roots, respectively nodules, and respectively the synergistic relationship of these microelements in the two plant components, roots, respectively nodules. The loading values of the parameters in relation to the principal components (*Table 5*) confirmed the important position of copper and zinc in the roots of soybean plants in relation to PC1 ($r = 0.971$ for Cu_r, respectively $r = 0.951$ for Zn_r), in the case of nodules. The parameter loading values indicated the high importance of zinc and iron in relation to PC1 ($r = 0.931$ for Zn_n, respectively $r = 0.847$ for Fe_n). The sphericity test (Bartlett´s sphericity test) and the KMO measure within the test confirmed good significance in the case of microelements in the root and nodules of soybean plants.

Conclusions

NPK mineral fertilization, with variable application rates for N and PK, generated differentiated effects on organic matter content and mineral element content in soybean roots and nodules. The independent variables N and PK showed strong influence on the variation of organic matter in roots ($R^2 = 0.821$, $p = 0.029$), and weak influence on TN, P and K in roots and on organic matter in nodules ($p>0.05$). Moderate influence was recorded on microelements in roots, and at the level of nodules there was a very strong influence on the variation of manganese (Mn_n), strong in the variation of iron and zinc (Fe_n and Zn_n), respectively moderate influence on the variation of copper (Cu_n).

The content of microelements in nodules showed a very strong and strong interdependence with the content of organic matter and mineral elements in roots. Zinc showed higher values in nodules compared to the content in roots, and copper showed higher values in roots compared to the content in nodules, in all experimental variants. In the case of Fe and Mn, higher values prevailed in roots, but in some variants there were higher concentrations in nodules.

Quadratic polynomial mathematical models described the variation of organic matter and mineral element content in roots and nodules in relation to the independent variables (N, and PK). Also in the case of microelements in nodules, mathematical models described the variation in relation to organic matter and mineral element content in roots. Multivariate analysis explained 94.605% of the total variance for microelement content in plant roots and 85.459% of the total variance for microelement content in nodules. Bartlett´s sphericity test, and Kaiser-Meyer-Olkin measure, confirmed the reliability of the data for microelements in soybean roots and nodules.

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DOI: http://dx.doi.org/10.15666/aeer/2306_1105311072

The Kruskal-Wallis and Mann-Whitney tests (post-hoc step) confirmed the statistical safety in the comparative analysis of the medians and means of the data series, for the considered parameters.

For the ecological benefits of soybean crop, moderate nitrogen application rates, based on soil analysis, are recommended.

Acknowledgments. The authors thank the SC MinearLaboratoare SRL, and University of Life Sciences "King Mihai I" from Timisoara for facilitating this study. This paper is published from the own funds of the University of Life Sciences "King Mihai I" from Timisoara.

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