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The effect of broadcast struvite fertilization on element soil content and microbial activity changes in winter wheat cultivation in southwest Poland

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This study aimed to evaluate the potential of struvite as a phosphorus fertilizer in winter wheat cultivation in southwest Poland. The field experiment was conducted at Wroclaw University of Environmental and Life Sciences in Swojec in 2022/2023. The studied factors were two cultivars of winter wheat (Activus and Chevignon) and two phosphorus fertilization methods (traditional superphosphate and struvite). Aditionally, the study attempted to develop a reasonable phosphorus testing method to analyze phosphorus content in soil. Three phosphorus extraction methods were used to determine P, Mg and K content where Mehlich 3 and Yanai seem to be most appropriate. The following traits in the study were estimated: grain yield, content of P, Mg and K in the grain, as well as soil microbial activity expressed as enzyme activity (acid and alkaline phosphatase, dehydrogenase), metabolic activity (Biolog EcoPlates™) and phosphate solubilizing bacteria (PSB) abundance. An increase in the Mg content of wheat grain under the influence of struvite application was found. Phosphorus content in the soil depended primarily on the date of soil sampling followed by fertilization method and varieties based on the Egner–Rhiem method as well as Mehlich 3. Soil enzymatic activity depended mainly on the sampling date and then on fertilization. In the case of PSB, the dominant factor was the wheat cultivars. Biolog EcoPlate analysis showed that the most metabolically active microbial communities were recorded in samples collected at the second time of sampling (end of winter wheat vegetation) To see how the phosphorus content develops after the application of struvite under field conditions as well as its fraction, a long-term experiment should be conducted.

Keywords Winter wheat, Mehlich 3 test, Yanai test, Egner–Rhiem test, Yield, Microbial activity

The abundance of plant-available phosphorus in Polish soils according to studies by Chemical and Agricultural Stations[1] requires constant monitoring, since as much as 40% of soils show a very low or low phosphorus content[2]. The amount of phosphorus available depends not only on soil conditions but also on analytical factors. In Poland, the Egner–Riehm DL method has traditionally been used to determine the abundance of plant-available phosphorus and potassium in soils[3,4]. The Egner–Riehm method presents many analytical difficulties due to the specific nature of the reagents used. A method that is gaining popularity in Poland is the Mehlich 3 method, which is a basic element of fertilizer recommendations for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and some micronutrients for optimal yield[3–6]. Another method, not as popular because

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of its lack of a scale for element abundance in soils, is the Yanai method[7,8]. A comparison of the three analytical methods will allow us to visualize the amount of available phosphorus from struvite one year after its application in field conditions.

The phosphorus fertilizer that was used in the experiment with winter wheat cultivation using struvite. Struvite (chemical name of the compound) contains mainly P and N, but also contains Mg; it is poorly soluble in water, highly soluble in organic acids, pathogen-free, non-toxic and can leach nutrients very slowly over a long period of time compared to highly soluble mineral fertilizers, which leach nutrients very quickly[9,10].

The content of phosphorus in the soil is also strictly dependent on the microbial and biochemical activity of the soil, and consequently the transformation and availability of other nutrients[11]. The effect of struvite on microbial community and enzymatic activities is not known; neither is how acid and alkaline phosphatase enzyme activities seasonally fluctuate under struvite fertilization. Therefore, we examined the impact of the effects of struvite on the yield of winter wheat (*Triticum aestivum* L.) and on the soil enzymatic and metabolic activities and phosphorus bacteria number. To the best of our knowledge, there is little published research on this topic without phosphatase activity under struvite fertilization[12–14]. This study is the first to examine the impact of struvite on soil microbiological changes in field crops, particularly in Poland. Microorganism activity, is studied under controlled laboratory conditions rather than in field. By considering these changes, this research provides new insights into how struvite can influence soil quality and fertility. This study provides the first indication of the short-term effects of struvite on alkaline sandy soil, focusing on key factors influencing soil fertility and qulaty, such as enzyme activity, total abundance of cultivable phosphorus-solubilizing bacteria (PSB), and community-level physiological profiling.

The course of the process is catalyzed by soil enzymes, including phosphatases[11]. The term phosphatase refers to a broad group of enzymes that catalyze the hydrolysis of phosphoric acid (V) esters and anhydrides. In the soil environment, the main sources of phosphatases are microorganisms, as well as plant roots and soil fauna, and their activity depends on and is influenced by both abiotic and biotic factors[15]. Phosphatases can be a good indicator of the phosphorus mineralization potential of organic compounds and the biological activity of the soil[16]. The intensity of the secretion of phosphatases by crops and microorganisms is in close dependence with the phosphorus requirements of the crop. The nutritional needs of young crops are high, and as a result of the rate of mineralization of soil organic phosphorus pools by the enzymatic pathway is intensified during this period[17]. Moreover, a microbiological parameter that is widely used in the analysis of the soil environment status is the Biolog Ecoplate™ method which is adopted at determining community level physiological profiles (CLPP)[18,19]. Metabolic diversity is a mirror of the ecological traits of microrganisms and all disturbances and changes in environmental ecosystems and plays a key role as an indicator for gauging soil health and fertility[20].

Microorganisms and their enzymes play a vital role in maintaining soil quality, health, and carbon sequestration. Their various metabolic processes are fundamental to biogeochemical cycles. However, there is a lack of a of knowledge how struvite effect on microbiological changes on the specific regions soil conditions. Soil microorganisms contribute significantly to agricultural productivity by facilitating the provision of vital nutrients to plants, supporting soil structure and stability[21], and playing a crucial role in the cycling of carbon and nutrients[22].

As our working hypothesis, we assumed that fertilization with struvite in one-year field study with winter wheat would be more effective than superphosphate and that the pool of available phosphorus and magnesium in the soil would be increased (using the Mehlich 3 method) as would enzymatic and metabolic activities.

The aim of the study was to determine the effect of fertilization with struvite compared to superphosphate on the content of P, Mg and K in the soil as determined by three analytical methods Moreover, the aim of the work was also to determine the activity of three soil enzymes (acid and alkaline phosphatase, dehydrogenases), metabolic activity and phosphate solubilizing bacteria (PSB) abundance. We aim to investigate whether struvite affects the growth of phosphorus-solubilizing bacteria, which could contribute to an increased availability of phosphorus from struvite compared to traditional phosphorus fertilizer. The comparison of struvite with superphosphate is significant as it allows us to evaluate whether struvite can effectively replace traditional fertilizers while minimizing negative impacts on the soil microbiome and its long-term quality.

Methods

Experiment design and soil conditions

The field experiment with struvite and wheat was established at the Teaching and Research Station (Wrocław University of Environmental and Life Sciences) in 2022 with two variable factors. The first factor was the wheat variety: Chevignon (a mid-early, awnless wheat cultivar of high yield and good quality) and Activus (a cultivar of wheat belonging to class A; due to its high degree of tillering, it is more competitive in relation to weed; it is characterized by the ability to survive in conditions of water deficit and is recommended for cultivation on drought-prone soils; the early stage of ripening and the significant level of protein content make it an attractive component in the context of nutrition)[23]. The new cultivars were selected for the study, especially the Activus cultivar, which is a hulled variety with high resistance to diseases (including Fusarium) and unfavorable, making it an interesting cultivar for testing under varying environmental conditions. These are cultivars that were used for the first time in experiments with struvite. Additionally, Activus cultivar is known for its high protein content. This characteristic suggests it may have potential in animal nutrition, making it an important factor to consider in our research.

The second factor was the differentiated phosphate fertilizers. Two phosphate fertilizers were used in the experiment: traditional triple superphosphate (SUP), commonly used in winter wheat cultivation, and Crystal Green (CG). The fertilizers were applied broadcast. The experiment examined the effect of a fertilizer produced on the basis of sewage sludge with the trade name Crystal Green (manufactured by Ostara Nutrient Technologies) in comparison with a traditional fertilizer, triple superphosphate. The white struvite granules measured about

1–2 mm in diameter. Struvite contains N (2%), P (24%) and Mg (10%) and is characterized by a low content of heavy metals compared to triple superphosphate. From a chemical point of view, it is not pure struvite.

The abundance of the soil in the experiment examined with different methods is as follows:

- Egner–Rhiem: P – 53.8 mg kg^{-1} of the soil (low), K – 79.1 mg kg^{-1} (low) of the soil, Mg – 197 mg kg^{-1} (high). pH was slightly acidic and neutral.
- Mehlich 3: P – 73.16 (low), K – 110.1 (medium), Mg – 96 mg kg^{-1} of the soil . (medium)
- Yanai: P – 25. 95, K – 107.4, Mg – 160.7 mg kg^{-1} of the soil (no optimal numbers) with pH 5.7–6.

The experiment was established using the split-plot method. The total number of experimental plots was 24 with 4 repetitions with each variant. The area of a single plot was 1.5 m × 10 m = 15 m^2, and the whole experiment area was ca 0.05 ha. Winter rape constituted the forecrop. Winter wheat was sown at the optimum date for Lower Silesia (October 15/10/ 22) at a sowing depth of ca 3 cm. Sowing rate amounted to 260 seeds per m^2 at a spacing of 12.5 cm. Sowing rate for Activus accounted for 108,52 kg ha^{-1} , and for Chevignon 117,8 kg ha^{-1}. Before setting up the experiment, mineral fertilizers were applied. Nitrogen was applicated on all plots: nitrogen in two doses – first dose 40 kg N/ha (BBCH 31) and 60 kg N/ha (BBCH 42, ammonium nitrate 34%). Nitrogen doses were applied at the following stages: start of vegetation and stem shooting. Phosphorus fertilizers were also applied broadcast before sowing at doses of 70 kg ha^{-1} P_2O_5 in the form of SUP and CG as well as potassium fertilizer in the amount of 100 kg K_2O ha^{-1} (potassium salt 60%).

After sowing, on 12 November 2022, weed control was performed by spraying Trinity 590 SC at a dose of 2.5 dm^3/300 dm^3 of water, at the BBCH 12 stage. The main active substances in this preparation are chlorotoluron, diflufenican, and pendimethalin. A fungicide treatment was also carried out on 10 April 2023 using AsPik EC 0.9 dm^3/300 dm^3 water, at the BBCH 30 stage. The active subatances in this preparation are prothioconazole and tebuconazole. A growth regulator treatment was conducted on 20 April 2023; this was aimed at preventing lodging. CCC 750 SL 1.5 dm3/300 dm^3 of water containing chlormequat chloride was used for this, at the BBCH 31 stage.

Chemical analysis of plants

Grain yield was determined, with the results converted into 15% moisture content. The material for qualitative determinations were grain samples, average for the experimental combinations per cutlivar x fertilization. Grains were minced for chemical analysis. The contents of phosphorus, magnesium, and potassium in the plant material were determined colorimetrically: P using ammonium vanadomolybdate, Mg using the titanium yellow method, and K colorimetrically.

Soil sampling and chemical analysis

Soil samples (n = 221) were collected according to PNR-04031:1997 from the plough layer (0 to 30 cm) using a standard soil-sampling auger. Soil samples for chemical analysis was taken twice during the study: before vegetation start (September 2022, I term) and at the end of vegetation period (July 2023, II term). We also sampled soil from a deeper layer: 30–50 cm. Soils were air-dried, ground, and sieved through a 2 mm plastic mesh before analyses. The determination of soil particle size composition was performed using the laser diffraction method standard. The pH was measured potentiometrically in 1:2.5 (m/V) suspension of soil in 1 mol L-1 KCl solution (ISO 10,390 2005). The pH of the soil was measured with a glass pH electrode (1:5 soil:deionized water, measurements after 30 min) and the conductivity was assessed with a conductivity meter (conductivity method). The total contents of phosphorus, potassium, and magnesium were determined after microwave mineralization, while the available forms were determined by the methods of Egner–Riehm, Mehlich 3 and Yanai, as described in Jama-Rodzeńska et al.[8].

Weather conditions

Agro-meteorological conditions during the experiment were developed on the basis of data obtained from the "Wroclaw-Swojec" Departmental Agro- and Hydrometeorological Observatory. Weather conditions had a significant impact on the implementation of the experiment and the yield of winter wheat. The warm and very dry October was not conducive to winter wheat sowing, but it was preceded by September, which was characterized by 64 percentage points higher precipitation, which helped create sufficient water resources for crop emergence in October (Fig. 1). Air temperatures during the winter period were quite high, allowing the grain to overwinter well. Temperatures and precipitation in March and April were similar to multi-year levels, allowing spring vegetation to resume properly. In May, more than twice as much rain fell as in the 1968–2021 period average.

Enzyme activity

The determination of the activities of acid (AcP) and alkaline phosphatase (AlP) were analyzed using 1 g of soil incubated for 1 h (37 ∘C) with PNP (p-nitrophenyl phosphate) at their optimum pHs of 6.5 and 11, respectively, using the colorimetric method described by Tabatabai and Bremner[24]. Soil dehydrogenase activity (DHA) was measured in 6 g soil by colorimetric measurement of the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) solution to triphenylformazan (TPF) after incubation at 37 ∘C for 24 h, according to the method of Casida et al.[25]. Each measurement was performed in triplicate.

Total abundance of cultivable phosphorus-solubilizing bacteria (PSB)

Cultivable microbial quantification was performed by plate-count techniques using selective medium containing insoluble P[26]. The number of bacterial colony forming units (CFU) was calculated per gram of dry soil.

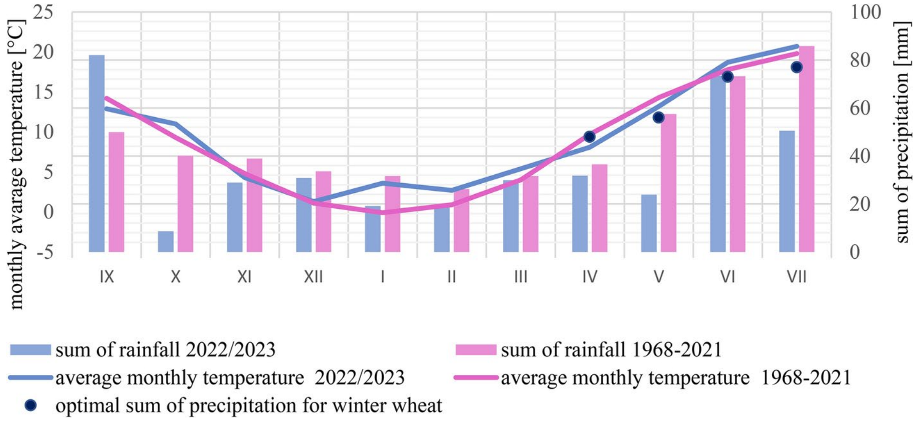

Fig. 1. Weather conditions in the year of experiment lasting (2022/2023).

Community-level physiological profiling

The Biolog EcoPlate™ method was used to perform a seven-day dynamic monitoring on the functional diversity of soil microbial community, and the average well-color development (AWCD) values of carbon-source utilization were collected. The inoculation procedure was based on the original EcoPlate method (Biolog™) according to the manufacturer's protocol. Moreover, for Biolog data analysis, heatmaps were generated to reflect the overall functional diversity of soil microbes[18].

Statistical analysis

Data from chemical and microbiological analyses were subjected to statistical analysis in Statistica software (version 13.1 StatSoft, Poland). The level of significance was $\alpha = 0.05$. One-way and two-way analyses of averages were performed to determine the effects of P fertilizer on chemical analyses of soil and plant. Due to the lack of normal distribution of variables, correlations and nonparametric tests were used. The relationships between the contents of elements/components in the soil and the analyzed factors were identified using a decision tree generated by the recursively partitioning C&RT method[27]. The binary random tree method consists of iteratively dividing the dataset into (here two) parts in such a way that the heterogeneity in the resulting subsets is as small as possible. The C&RT exhaustive search method searches all possible splits according to all available variables. The splits occur according to the value of one of the predictors. In other words, new sets of similar elements, in terms of the characteristic under study, are created. Then, for each of the resulting subsets, the procedure is repeated until the process reaches a stop condition. In all random tree graphs Id means node identification number, N—node abundance, Ave—average value for the analysed variable e.g. soil phosphorus content, Var—variance of the variable e.g. soil phosphorus content. Downwards, successive subdivision subsets are graphically represented. The subdivision variable is indicated on the green horizontal line and its values for each subset are shown above the node block.

In addition, multivariate statistical assessment using Spearman's rank correlation coefficient determined the association among the measured properties for the different samples. Matrix correlations (correlation coefficient values) between the different variables are presented in the Supplementary Material.

Experimental research and field studies on plants (either cultivated or wild), including the collection of plant material are complying with relevant institutional, national, and international guidelines and legislation. I declare that the plant material used for our study was purchased from Oxytree Solution Poland. We did not use endangered plant species for the experiments.

Results

Effect of struvite fertilization on the yield and macroelement content in winter wheat grain

Wheat yield was not dependent on either wheat cultivar or phosphorus fertilization. The wheat cultivar had a significant effect on the phosphorus content of wheat grain. Significantly more of this element was found in the Activus. . Struvite fertilization caused a significant increase in magnesium content in the wheat grain. Phosphorus fertilization had a significant effect on potassium content in winter wheat grain. (Table 1).

Negative correlations were found between magnesium and potassium and potassium and magnesium in wheat grain (Table 2): with a decrease in the potassium content of the grain, a decrease in the magnesium content is also observed, and vice versa.

Effect of struvite fertilization on P, Mg, and K content determined by different methods

Effect of struvite fertilization on P, Mg, and K content determined by the Egner–Rhiem method

The most important factor affecting the potassium content of soil determined by the Egner–Rhiem method was the depth of soil sampling. More potassium was found in the shallower layer, i.e. 0–20 cm, and this variation was also observed irrespective of the date of soil sampling. At the second date of soil sampling, i.e. after wheat harvest, more potassium was found in the shallower soil layer. Another factor within the first term was the effect of phosphorus fertilization. However, here we can observe variation in potassium content; this is definitely

Total content	Grain yield t·ha^{-1}	P	Mg	K
Variety (A)				
Activus	8.59	2.21b	1.41	3.11
Chevignin	9.05	1.65a	1.45	3.21
P value	ns	**<0.001*****	ns	ns
Phosphorus fertilization (B)				
control	8.70	1.76	1.35ab	2.86a
SUP	9.33	2.10	1.12a	3.65b
STR	8.42	1.18	1.67b	3.12a
P value	n.s	ns	<0.05*	<0.01**
A X B	n.s	ns	ns	ns

Table 1. Grain yield and content of P, Mg, and K in grain of winter wheat (g kg^{-1} d.m). *Analysis of variance at Significance at $P<0.05$. **Analysis of variance at Significance at $P<0.01$. ***Analysis of variance at Significance at $P<0.001$ means for factors. Different letters indicate significant differences between factors (Tukey's multiple range test).

	Spearman's correlation (chemical composition of wheat grain) correlation coefficients are significant with $P<0.05$		
Variable	P	K	Mg
P	1.00	0.29	−0.13
K	0.29	1.00	−0.42
Mg	−0.13	−0.42	1.00

Table 2. Correlations of studied elements in wheat grain.

greater where struvite was applied compared to the control. On the other hand, at the second soil sampling term, another important factor affecting the potassium content of the soil was the wheat cultivar. In the case of the deeper layer, the next-order factor affecting the content of this element was the wheat variety. More potassium was found in the soil under the Activus cultivar. The potassium content under the crop of the second cultivar, i.e. Chevignon, was found to depend on the sampling date. At the second date of sampling, differentation was noted under phosphorus fertilization (Fig. 2).

In the case of phosphorus, the overriding factor affecting its content is, as with potassium, the timing of sampling. More phosphorus in the soil was found at the second sampling date, i.e. after the wheat harvest. At the second date, more phosphorus was found in the soil under the winter wheat cultivar Chevignon. A higher content of P under the cultivation of this cultivar was snoted at the shallower depth of the soil, especially with struvite fertilization. At the first term, diferences were observed under phosphorus fertilization with a higher content on those plots with SUP (Fig. 3).

In the case of magnesium, the factor with the most significant effect on the content of this element in the soil was phosphorus fertilization. More phosphorus was found in plots where SUP was applied. In the case of superphosphate, variation was observed in the content of this element between the wheat cultivars tested, to the benefit of Chevignon cultivation. In the Chevignon cultivar, variation in magnesium content was found depending on the depth of soil sampling. There was slightly more of this element in the shallower soil layer. Depending on the depth of soil sampling, other factors affected the content of this element. For shallower sampling, the timing of sampling played a major role; for deeper soil sampling, phosphorus fertilization played a major role (Fig. 4).

Effect of struvite fertilization on P, Mg, and K content determined by the Mehlich 3 method

Similar to the Egner–Rhiem method, in the Mehlich method 3, the depth of soil sampling was the most important factor influencing potassium content in the soil. In shallower sampling, phosphorus fertilization, especially with struvite, was a differentiating factor in the content of this element. The downstream factor within the superphosphate fertilization control was winter wheat varieties. For deeper sampling, the timing of sampling was the differentiating factor for potassium content, and within the first factor, wheat varieties (Fig. 5).

The first factor of importance for soil phosphorus content determined by the Mehlich 3 method is the timing of sampling, as with the Egner–Rhiem method. At the first sampling date, the factor affecting the content of this element was fertilization with superphosphate and control. With superphosphate fertilization, variety is the differentiating factor. In contrast, for the Activus variety, the depth of sampling was the differentiating factor. More phosphorus was found in the shallower soil layer. For the second term, superphosphate fertilization is also

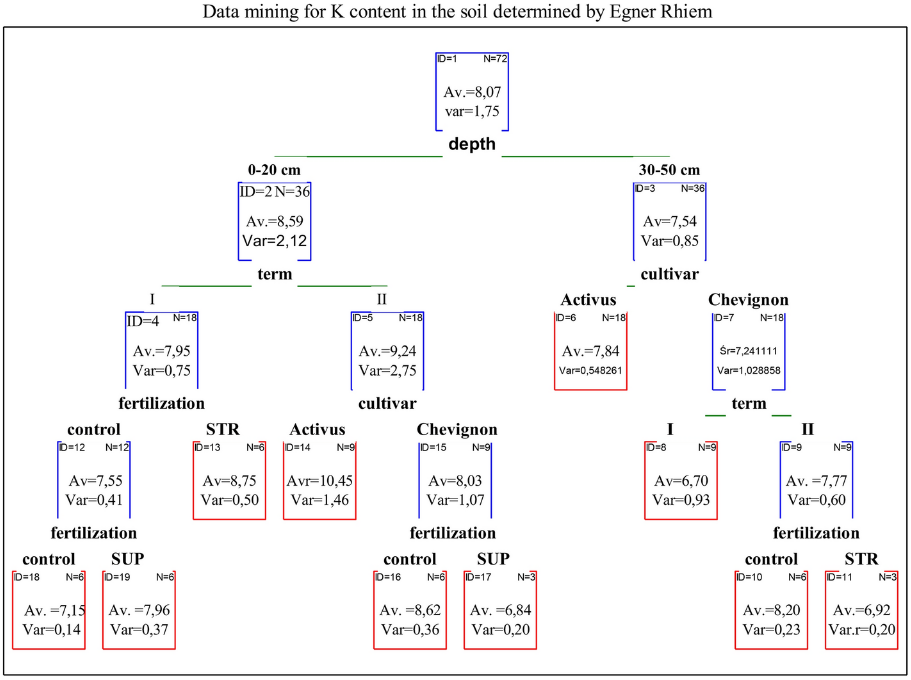

Fig. 2. Potassium content under struvite fertilization in the cultivation of winter wheat at two depths (P 100 g of soil).

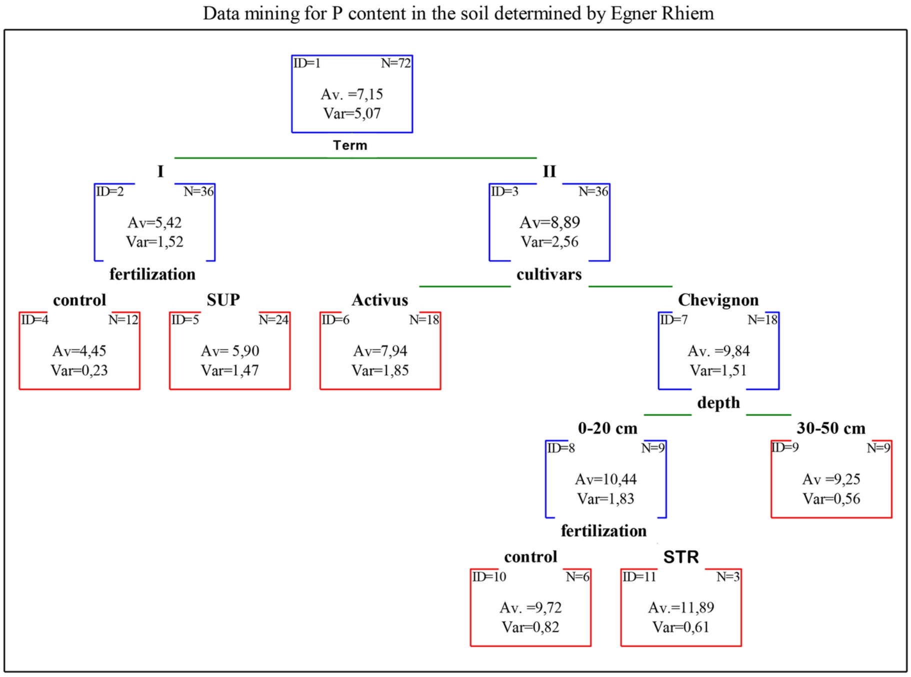

Fig. 3. Phosphorus content under struvite fertilization in the cultivation of winter wheat at two depths (P 100 g of soil).

a differentiating factor in phosphorus content. With superphosphate fertilization, on the other hand, the depth of soil sampling is the differentiating factor, and here the lower-ranking factor is variety (Fig. 6).

In the case of magnesium, a similar factor relationship was found to that in the Egner–Rhiem method. The first-order factor affecting magnesium content in the soil depended on phosphorus fertilization. In the case of the control object, the lower-order factor affecting the content of this element in the soil was the winter wheat variety. Within the Chevignon cultivar, the date of sampling was a differentiating factor. In the case of

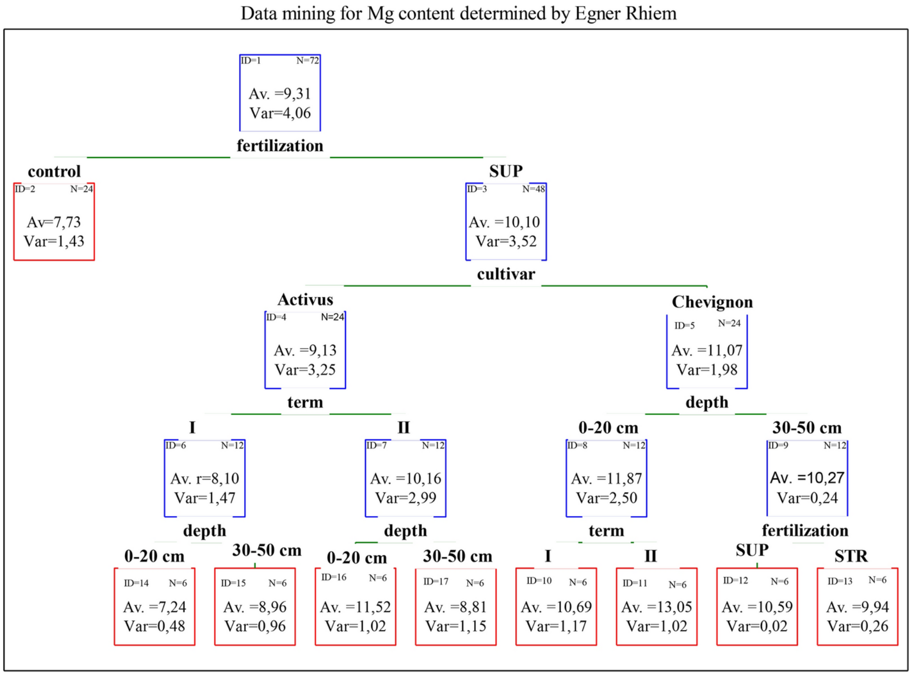

Fig. 4. Magnesium content under struvite fertilization in the cultivation of winter wheat at two depths (P 100 g of soil).

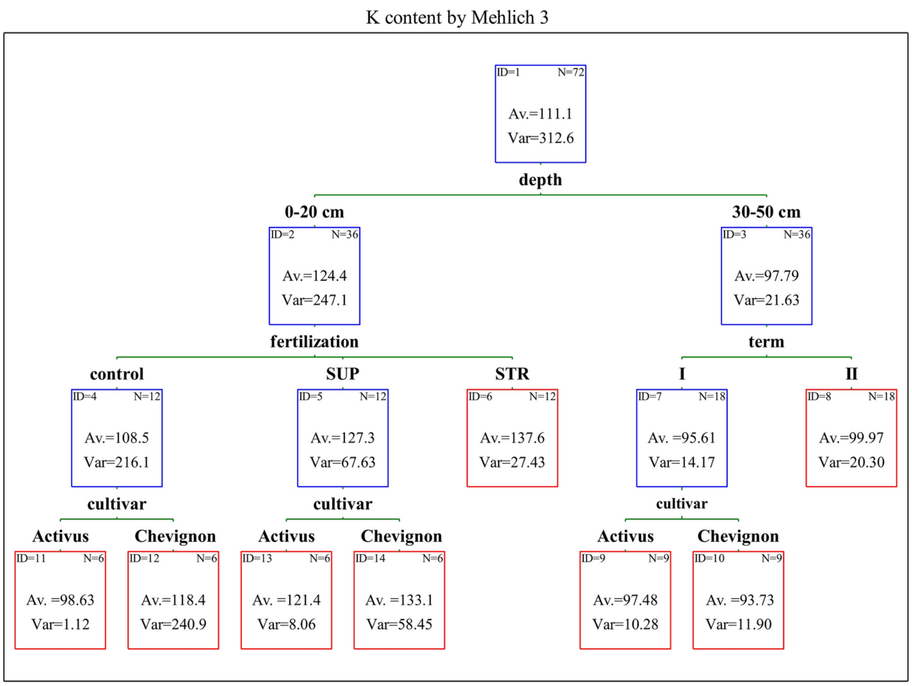

Fig. 5. Potassium content under struvite fertilization in the cultivation of winter wheat at two depths (mg kg^{-1} d.m of soil).

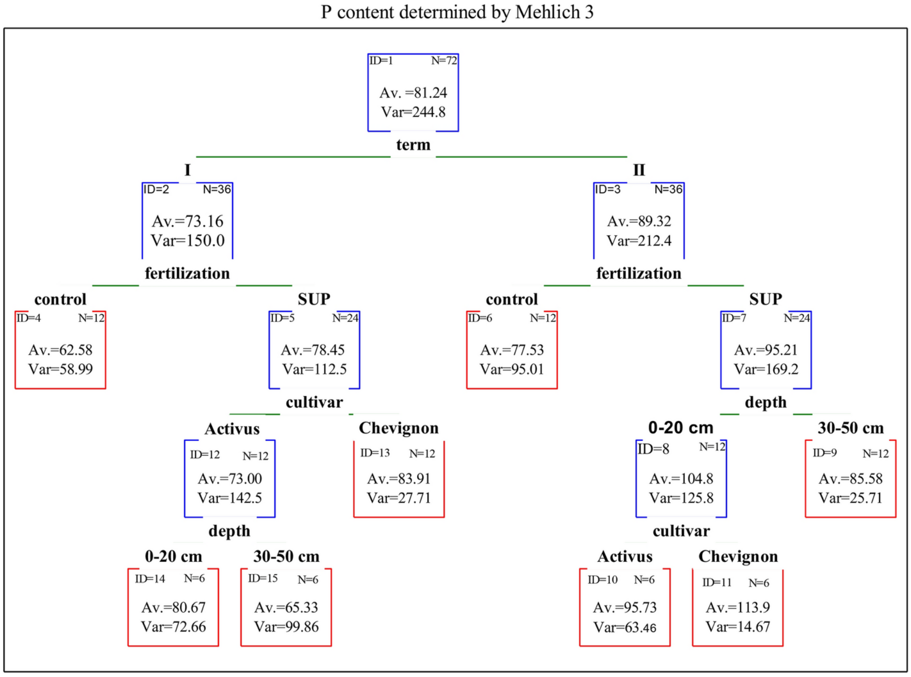

Fig. 6. Phosphorus content under struvite fertilization in the cultivation of winter wheat at two depths (mg kg^{-1} d.m of soil).

superphosphate fertilization, the depth of soil sampling was the next important factor affecting Mg content in the soil. For both depths, the differentiating factor for soil Mg content was the timing of soil sampling (Fig. 7).

Effect of struvite fertilization on P, Mg, and K content determined by the Yanai method

The potassium content in the soil determined by Yanai was dependent on the depth of the soil. The next factor affecting this element in the soil within the deeper level was cultivars. With the Chevignon cultivar the most important factor having an impact on potassium content in the soil was the samplong term (Fig. 8).

In the case of phosphorus, depth was the dominating factor affecting its content in the soil (Fig. 9).

In the case of an analysis of magnesium content in soil by Yanai, the most important factor affecting its content in the soil was phosphorus fertilization. For SUP fertilization, the factor having an impact on the content of this element in the soil was sampling term. Thereafter, depth differentiated the content of Mg content in the soil; at the shallower level, cultivar affecting Mg content (Fig. 10).

A significant positive correlation was found between two methods: Mehlich 3 and Egner–Rhiem; a negative correlation was found between Mehlich 3 and Yanai for soil potassium content. A higher content of potassium was found in soil with the Mehlich 3 method (Table 3).

In the case of P, there was a positive significant correlation between determining P with the Mehlich 3 and Egner–Rhiem methods. A higher content of phosphorus was found in the soil with the Mehlich 3 method (Table 4).

In terms of Mg, there was a positive significant correlation between determining Mg with the Yanai and Egner–Rhiem methods. A higher content of Mg was found in the soil with the Mehlich 3 method (Table 5).

Effect of struvite fertilization on pH

The change in soil pH from the initial value differed between terms, cultivars, phosphorus fertilization combinations, and the depth. The change in soil pH was not complex, but no clear trend was observed across terms, cultivars, and depths of the soil. Over the duration of the vegetation period, soil pH generally increased from the initial value. Taking into account fertilizer combinations the greatest pH was on the plots with struvite. As the depth increased, the pH decreased (Fig. 11).

Effect of struvite fertilization on enzymatic activities

The most significant factor influencing the activity of acidic phosphatase was the time of soil sampling. An increase in acidic phosphatase activity was observed with the duration of the experiment. During the entire experiment, the form of phosphorus fertilization affected the activity of this enzyme. At the first sampling date, the lowest acidic phosphatase activity was found in the soil fertilized with SUP, while subsequent sampling resulted in significantly higher activity of the soil fertilized with STR.

Another factor differentiating the activity of this enzyme at the first sampling date was the cultivar. In the case of most forms of phosphorus fertilization, lower acidic phosphatase activity was observed in soil where the Chevignon cultivar was grown. Moreover, for wheat varieties, acidic phosphatase was significantly differentiated

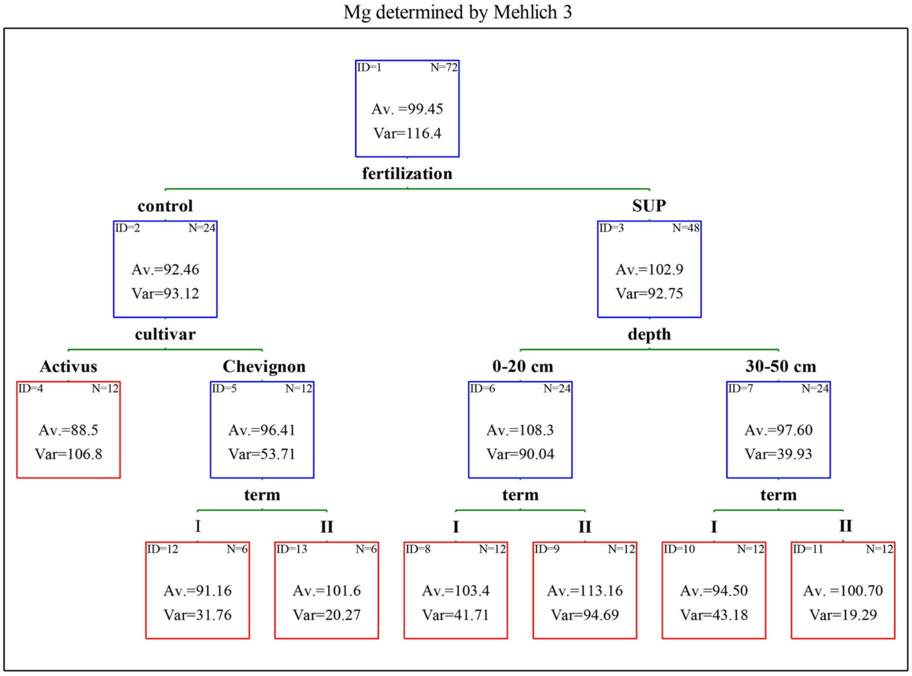

Fig. 7. Magnesium content under struvite fertilization in the cultivation of winter wheat at two depths (mg kg^{-1} d.m of soil).

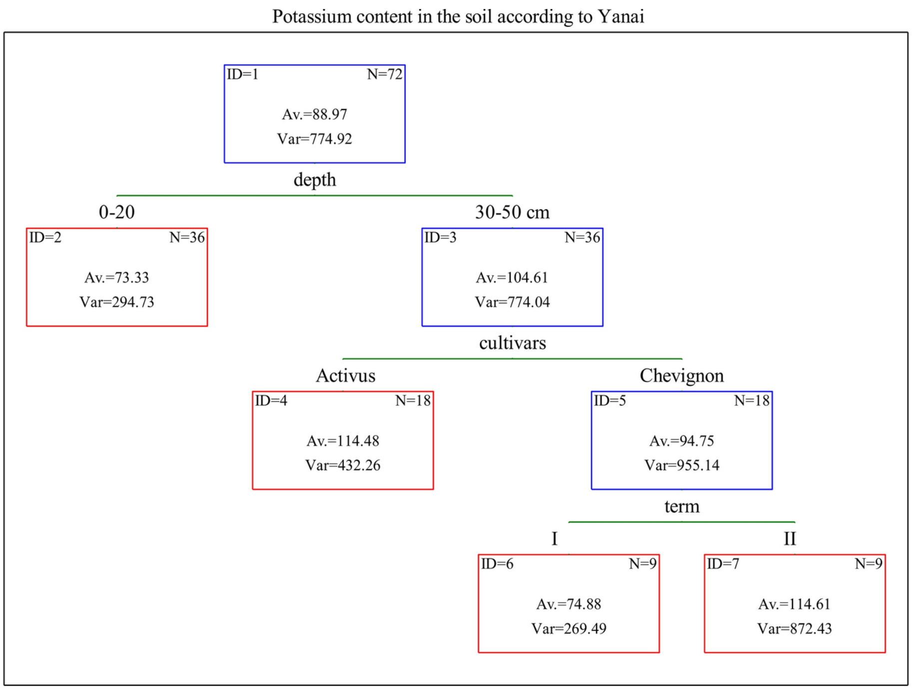

Fig. 8. Potassium content under struvite fertilization in the cultivation of winter wheat at two depths (mg kg^{-1} d.m of soil) according to the Yanai method.

by the form of fertilization. Regardless of the variety, acidic phosphatase activity was higher in the case of STR fertilization.

In soils sampled after harvest, the use of STR resulted in higher acidic phosphatase activity compared to other forms of fertilization. Another factor at later sampling was the cultivar. The Chevignon variety resulted in lower acidic phosphatase activity when the soil was fertilized with STR. In the case of other forms of fertilization,

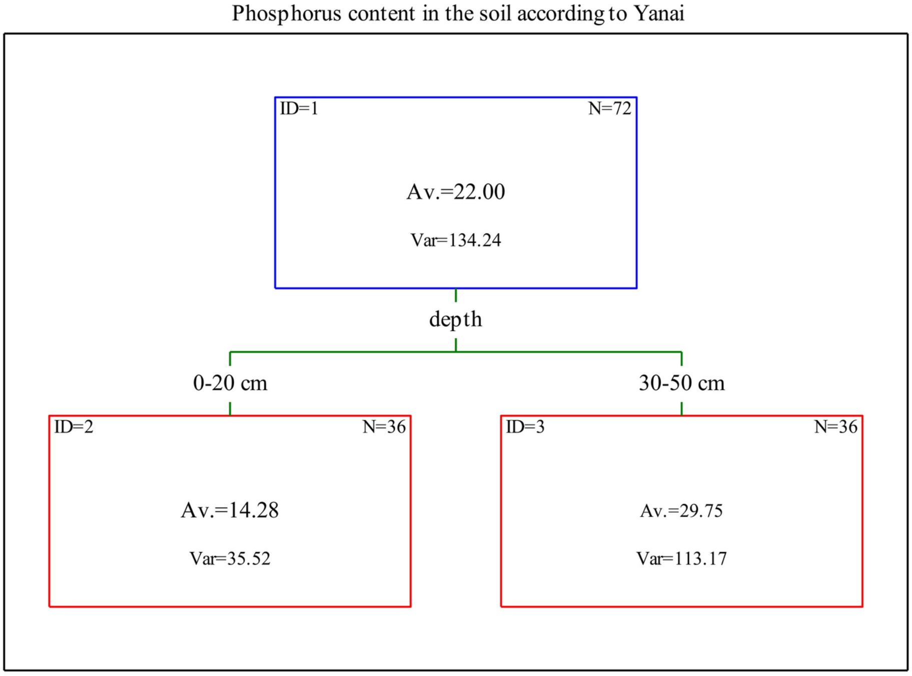

Fig. 9. Phosphorus content under struvite fertilization in the cultivation of winter wheat at two depths (mg kg^{-1} d.m of soil) according to the Yanai method.

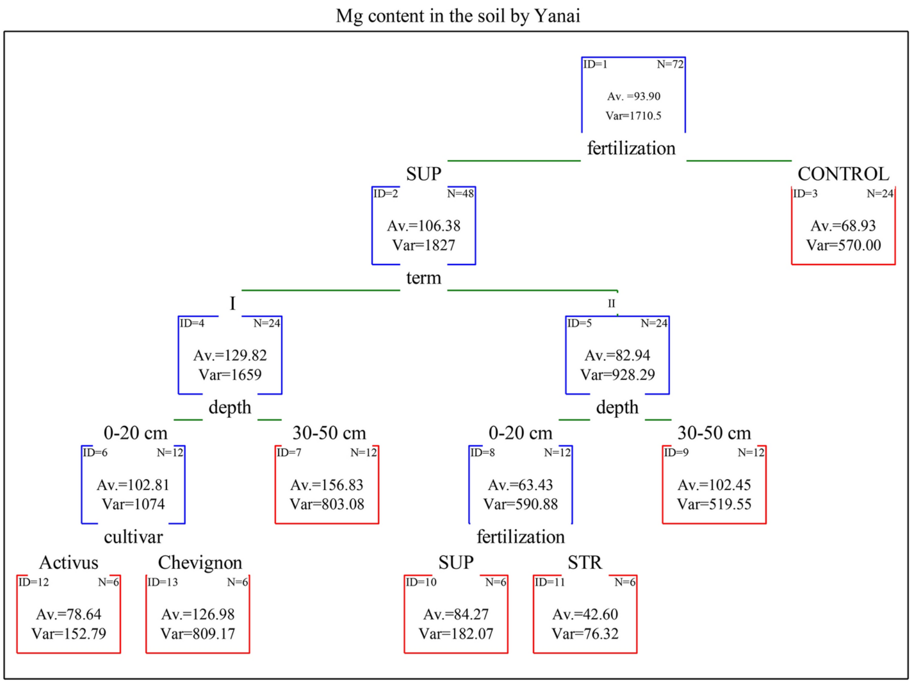

Fig. 10. Magnesium content under struvite fertilization in the cultivation of winter wheat at two depths (mg kg^{-1} d.m of soil) according to the Yanai method.

higher activity was measured under the Chevignon variety. The lowest-order factor affecting acidic phosphatase in later samplings was the fertilization form (Fig. 12).

The most significant factor influencing the activity of alkaline phosphatase was the soil sampling time. In this case, a higher level of alkaline phosphatase activity was observed for the second sampling. Another differentiating factor in the case of the second sampling was the type of fertilization. Lower alkaline phosphatase activity was found in soil fertilized with SUP. In the soil fertilized with both SUP and other forms of phosphorus,

Variable	Correlations determined by correlation coefficient when significant at $P < 0.05000$ N = 72				
	Average	Standard deviation	K Egner–Rhiem	K Mehlich 3	K Yanai
K Egner–Rhiem	8.04	1.33	1.00	0.39	− 0.22
K Mehlich 3	111.2	17.68	0.39	1.00	− 0.37
K Yanai	88.97	27.83	− 0.22	− 0.37	1.00

Table 3. Correlations between examined methods in terms of K content in the soil.

Variable	Correlations determined by correlation coefficient when significant with $P < 0.05000$ N = 72				
	Average	Standard deviation	P Egner–Rhiem	P Mehlich	P Yanai
P Egner–Rhiem	7.15	2.52	1.00	0.62	0.05
P Mehlich	81.24	15.64	0.62	1.00	− 0.07
P Yanai	22.00	11.58	0.05	− 0.07	1.00

Table 4. Correlations between examined methods in terms of P content in the soil.

Variable	Correlations determined by correlation coefficient when significant with $P < 0.05000$ N = 72				
	Average	Standard deviation	Mg Egner–Rhiem	Mg Mehlich 3	Mg Yanai
Mg Egner–Rhiem	7.65	2.86	1.00	− 0.19	0.62
Mg Mehlich 3	99.45	10.79	− 0.19	1.00	− 0.04
Mg Yanai	93.90	41.36	0.61	− 0.04	1.00

Table 5. Correlations between examined methods in terms of Mg content in the soil.

the enzymatic activity was lower under Chevignon. The effect of all fertilization forms on the activity of alkaline phosphatase was similar for this cultivar (Fig. 13).

At the first sampling term (other), the main factor shaping the activity of alkaline phosphatase was also the fertilization time. The highest enzymatic activity was observed after the use of STR. In the soil where STR fertilization was applied, the soil under the Chevignon cultivar was less active. At the same date, in plots with other forms of fertilization, sowing the Chevignon variety resulted in slightly higher activity.

The activity of dehydrogenases changed with the date of soil collection for microbiological tests. It was much higher at the first sampling date. Moreover, within the first soil sampling, a significant impact of fertilization type on the activity of dehydrogenases was observed. The activity was the lowest after using SUP. A comparison of STR fertilization with the control plots showed that the use of STR stimulated higher enzyme activity (Fig. 14).

In the second smpling time, the main differentiating factor was the cultivar. Lower dehydrogenase activity was observed in the soil under Chevignon. A minor factor for the Chevignon cultivar was the fertilization type: soils fertilized with SUP were characterized by lower dehydrogenase activity. The opposite effect was observed in the soil under the second variety.

In terms of PSB, the most important factor influencing their numbers was the winter wheat variety. For the Chevignon variety, the factor influencing PSB was fertilization, followed by the sampling date. For the other variety, the most important factor influencing PSB was the term of sampling, and the lower-order factor was fertilization (Fig. 15).

Bacterial community-level physiological profiling (CLPP)

To illustrate the Biolog EcoPlate results and bacterial community-level physiological profiling, a heatmap graph was generated. The comparisons of the use of the 31 carbon sources by soil microbial communities presented their metabolic diversity. The sampling date was the main factor influencing the metabolic activity of the bacterial community. On the second date, a higher metabolic activity of microorganisms was observed in the analyzed samples. On both the first and second dates, higher metabolic activity was noted in the wheat variety Activus fertilized with struvite, based on the AWCD index. However, no such correlation was noted for the variety Chevignon (Fig. 16).

Based on the utilisation patterns of 31 different carbon sources, microbial functional diversity in the soil samples was evaluated. Three-way ANOVA was performed for testing the interaction between the three independent variables, two phosphorus fertilization methods (traditional superphosphate and struvite), cultivars of winter wheat (Activus and Chevignon), and two term of sampling. The dependent variables were parameters of the biodiversity indices AWCD, H' and E. Table S2 summarises the three-way ANOVA. The biodiversity indices

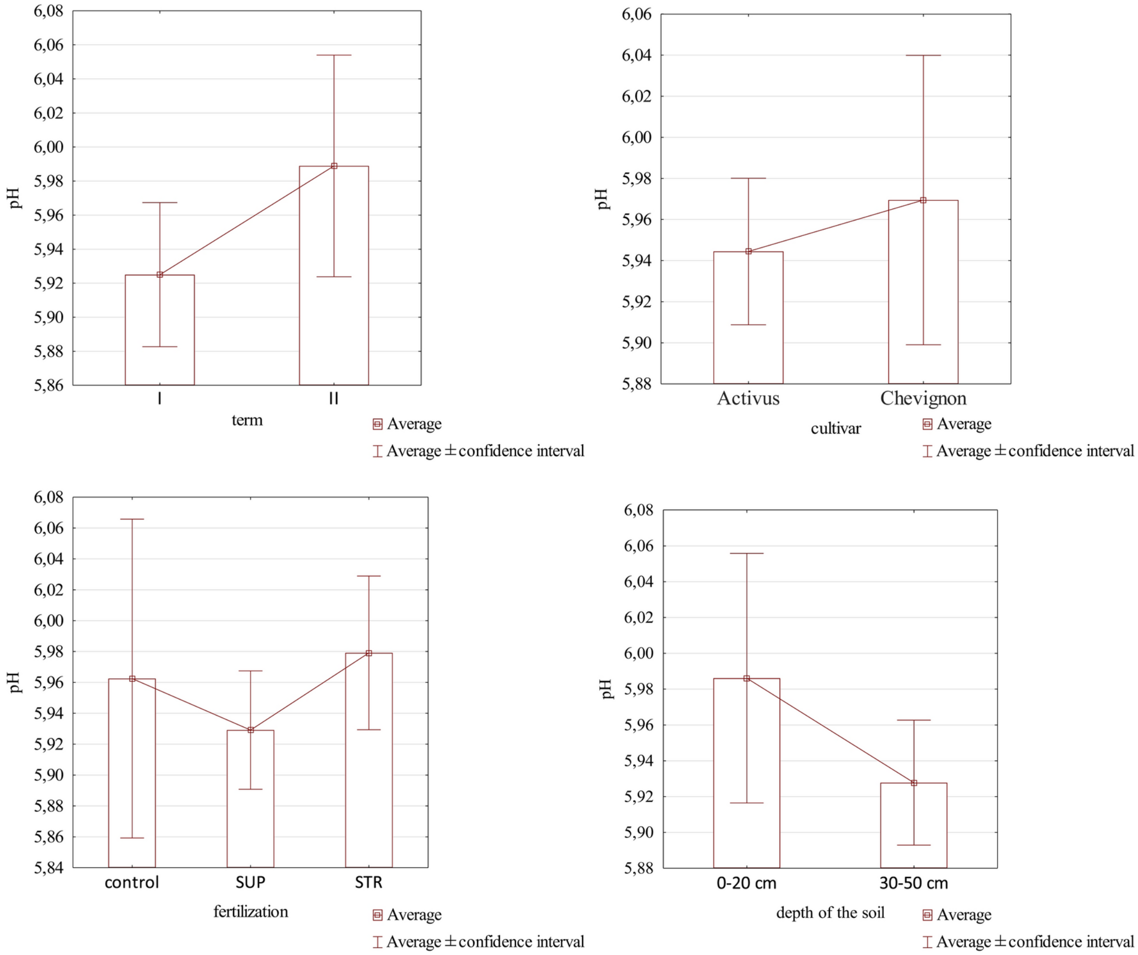

Fig. 11. Effect of date sampling, cultivar, phosphorus fertilization and depth of the soil on soil pH.

measured from the Biolog EcoPlates revealed differences between soil samples. The variation in functional diversity represented by all the indicators assessed was significantly influenced by the timing of sampling.

Table S1. Metabolic diversity indices of tested soil samples based on substrate utilization patterns for Biolog EcoPlates at 168 h.

Table S2. Summary of three-way analysis of variance (ANOVA) results testing the effects of cultivars of winter wheat (Activus and Chevignon), two phosphorus fertilization methods (traditional superphosphate and struvite), and two term of sampling, on basal biodiversity indices. Data shown represent F-value and significance levels for each factor and interaction.

Discussion

Effect of struvite application on the yield and content of elements

This study was undertaken to determine the effect of struvite fertilization on wheat grain P, Mg, and K content and yield. Magnesium content was dependent on phosphorus fertilization, with statistically higher content under struvite fertilization. In turn, potassium content was the greatest under SUP fertilization. No significant differences were observed for P content in the grain of winter wheat or the grain yield. Martens et al.[28] showed a mean wheat grain yield in different treatments and years ranging from 1.37 to 3.26 Mg ha^{-1}, while P concentration was from 2.9 to 4.0 mg g^{-1}. In turn, Turmel et al.[29] showed a P concentration from 3.8 to 4.3 mg g^{-1} observed in organic spring wheat in the same region. In Mertens et al.[28], grain yield and P accumulation were statistically greater under struvite fertilization. In the study by Uysal et al.[30], P uptakes by tomato plants under struvite application were greater for all dosages, whereas Mg uptake was higher compared with NPK application. In a study by Martens et al.[28], the dose of struvite had no significant effect on the P recovery efficiency of wheat. In fact, although not significantly different, the P recovery efficiency in wheat grain was numerically higher at higher doses of struvite application, further supporting our conclusion that P accumulation in wheat at 40 kg P ha^{-1} did not approach a maximum.

Effect of struvite application on the elements content in the soil

A study by Robles-Aquilar et al.[31] of cultivated substrate treated with struvite generally showed a 10–20 times higher P concentration compared to both organic fertilizer and no fertilizer. However, many studies have

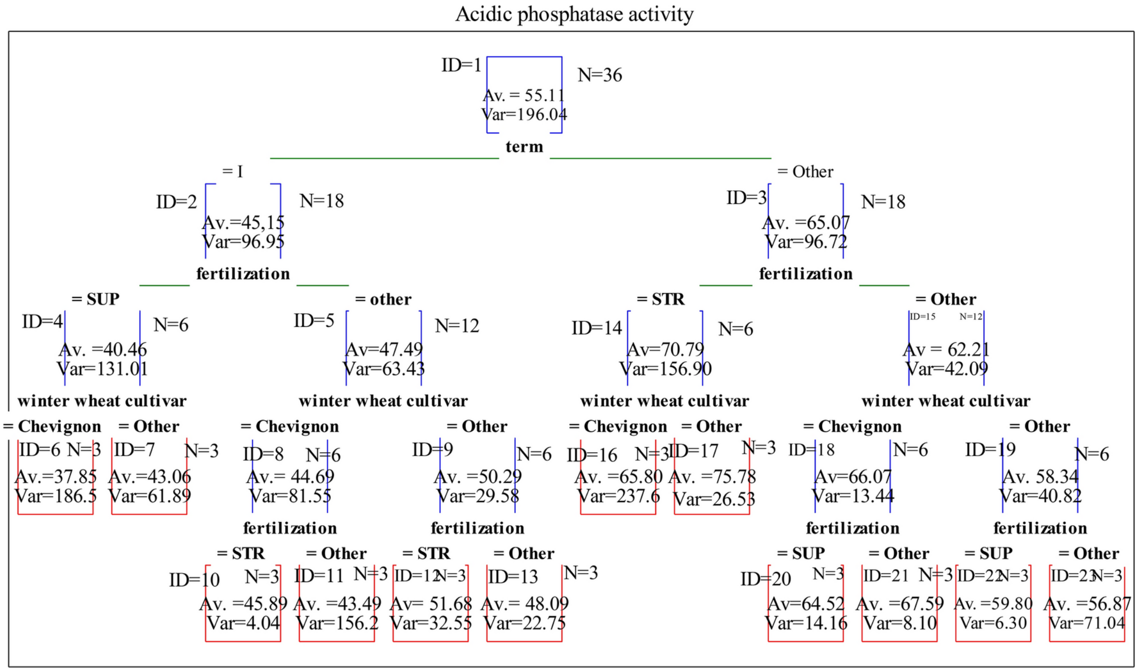

Fig. 12. Effect of phosphorus fertilization and winter wheat cultivar on acidic phosphatase activity (ug PNP/g d.m. soil /h).

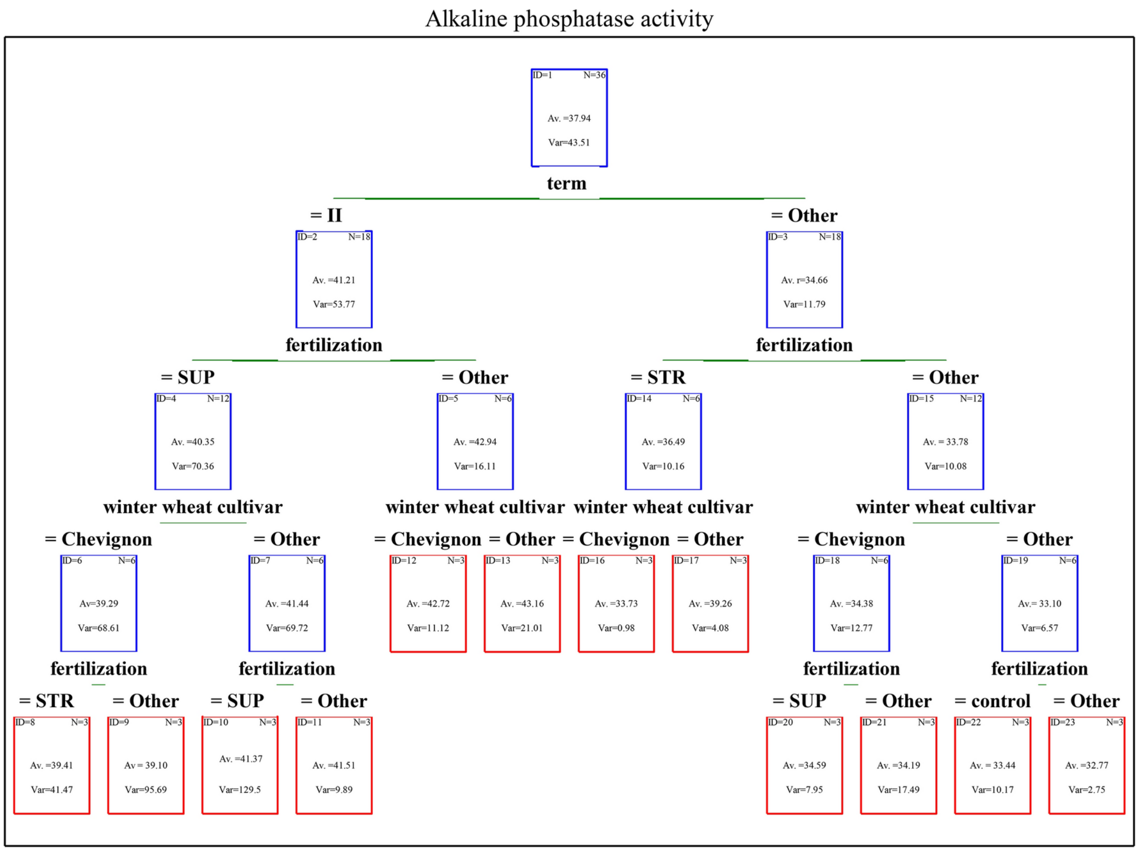

Fig. 13. Effect of phosphorus fertilization and winter wheat cultivar on alkaline phosphatase activity (ug PNP/g d.m. soil /h).

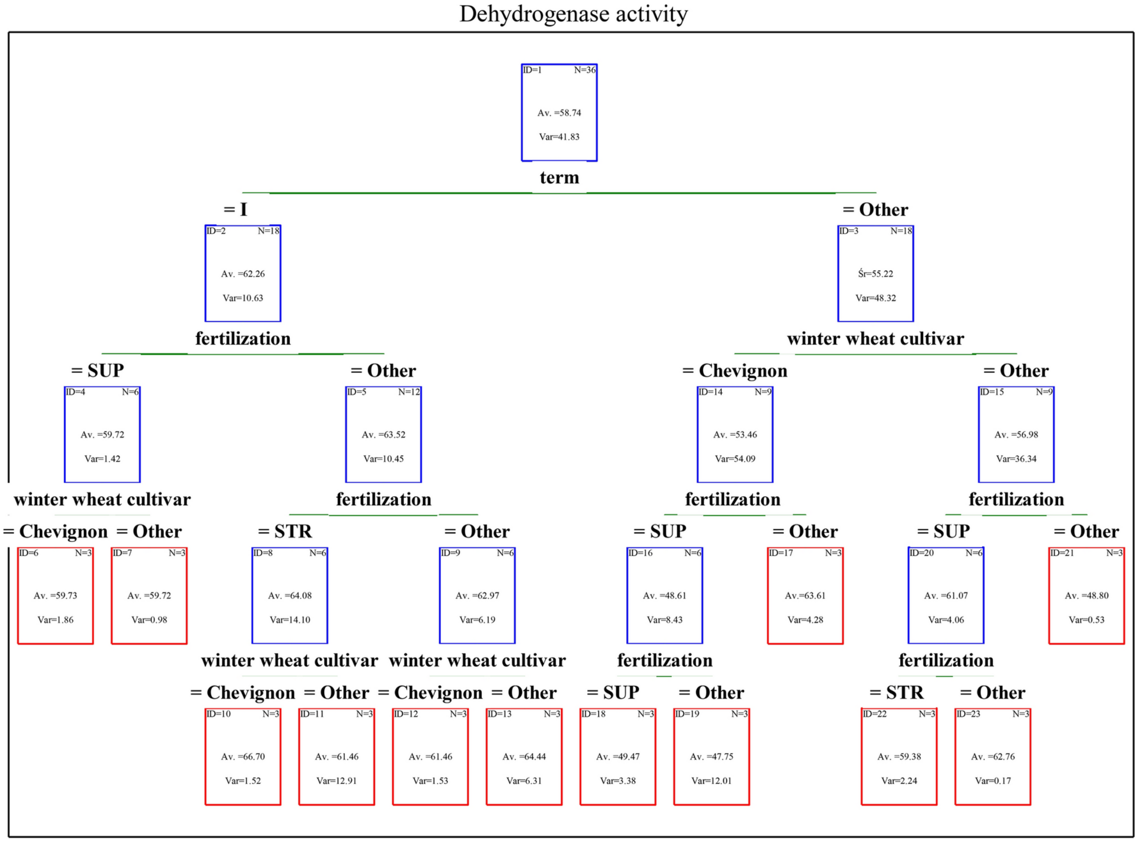

Fig. 14. Effect of phosphorus fertilization and winter wheat cultivar on dehydrogenase activity (ug PNP/g d.m. soil /h).

presented that phosphorus content in the soil under struvite fertilization relies on soil pH. Our studies are in agreement that in acidic soils, struvite is comparable to the commercial fertilizer used in our study. Many studies are largely consistent in that, in acidic soils, struvite is comparable or superior to commercial fertilizers, including ammonium phosphate, calcium phosphate, and mineral ammonium phosphate in a wide range of crops such as perennial ryegrass, fescue, buckwheat, horticultural crops, lettuce, corn, chickpea, and sorghum[32–36].

Bastida et al.[37] demonstrated that P availability was the greatest in struvite-enriched soil (St), followed by combined soil (Sl + St). P availability was lower after one month, but followed the same pattern, with St and Sl + St samples having the highest values. The decrease in P availability in their experiments over one month may have been caused by P precipitation in the form of Al, Fe, and Ca phosphates[38,39] and uptake by plants. Moreover, P availability is regulated by initial adsorption on the soil.

In the study of Anderson et al.[10], loam soil resulted in a large pH decrease in fertilizer treatments at different times in the incubation such that soil pH dropped below 6.0, which could have negatively affected nutrient availability, and thus negatively affected plant productivity. Low soil pH may also promote greater co-precipitation of P with Fe and minimize potential co-precipitation of P with Ca. In our study, pH increased from 5.92 to 5.98 and decreased between depths of the soil. This may be due to the fact that with the depth of the soil profile, there was a decrease in the pH of the soil. This process may be influenced by, among other factors, leaching of soluble salts and ions into the soil profile[40].

A study by Hilt et al.[41] found that struvite is comparable or better than MAP (magnesium ammonium phosphate) in acidic soils, but worse in alkaline soils. Based on their results with similar studies, this shows that the relationship between alkaline soils and struvite P availability is still not clearly understood; however, many other studies agree that struvite behaves well in acidic soils compared to many commercial fertilizers. Because of struvite's slower dissolution rate, P availability has been observed among struvite sources and MAP, DAP and TSP, and similar results have been reported in previous studies[36,42–44]. The performance of struvite in acidic soils is related to the solubility of struvite. Szymanska et al.[45] also proved that it is more effective as a fertilizer than commercial ammonium phosphate, because it releases nutrients in both the first and second year after fertilization, so its yield potential is maintained in the second growing season after application.

Effect of struvite on microbial activity in soil

Various fertilization methods are known to influence the activity and biodiversity of soil microorganisms. The introduction of diffrent substances into soil may change the composition of indigenous microbiota and modify processes occurring in the soil environment[46,47]. One of the most important functions of fertilizers is considered

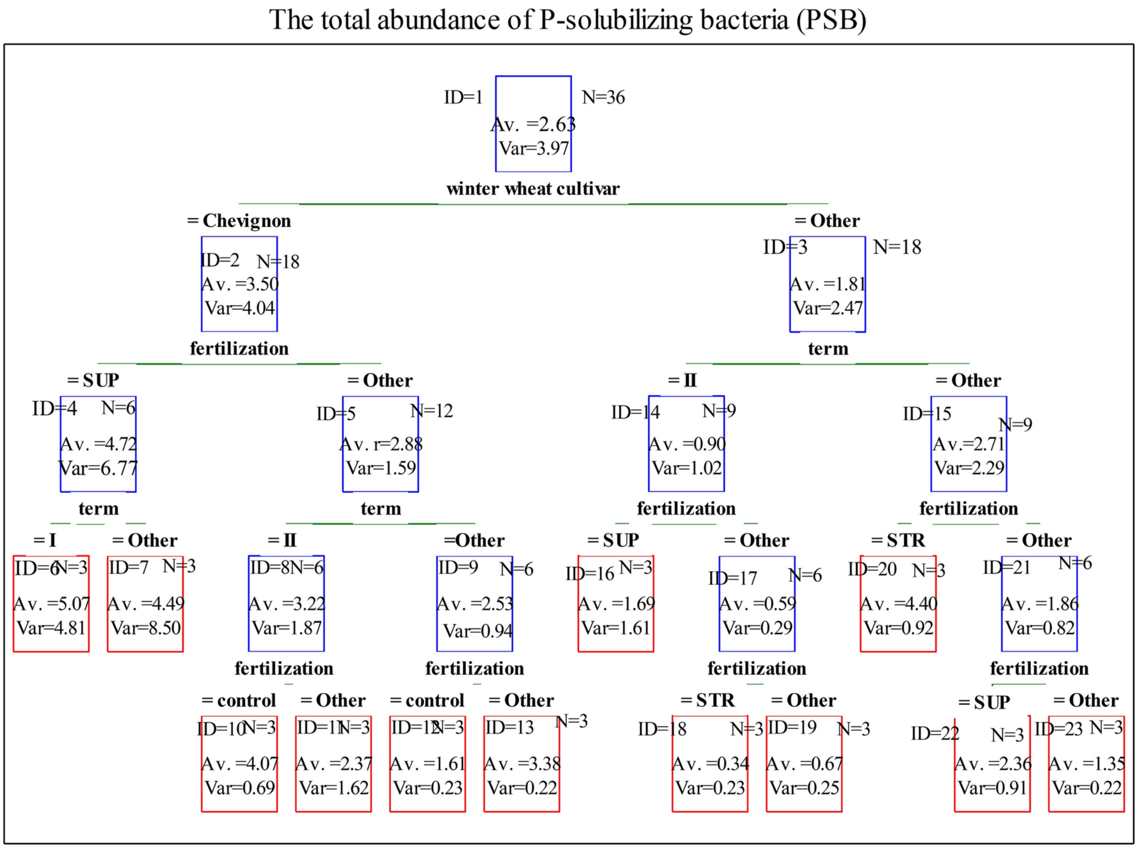

Fig. 15. Effect of phosphorus fertilization and winter wheat cultivar on the total abundance of P-solubilizing bacteria (PSB) (CFU g $^{-1}$ d.m soil).

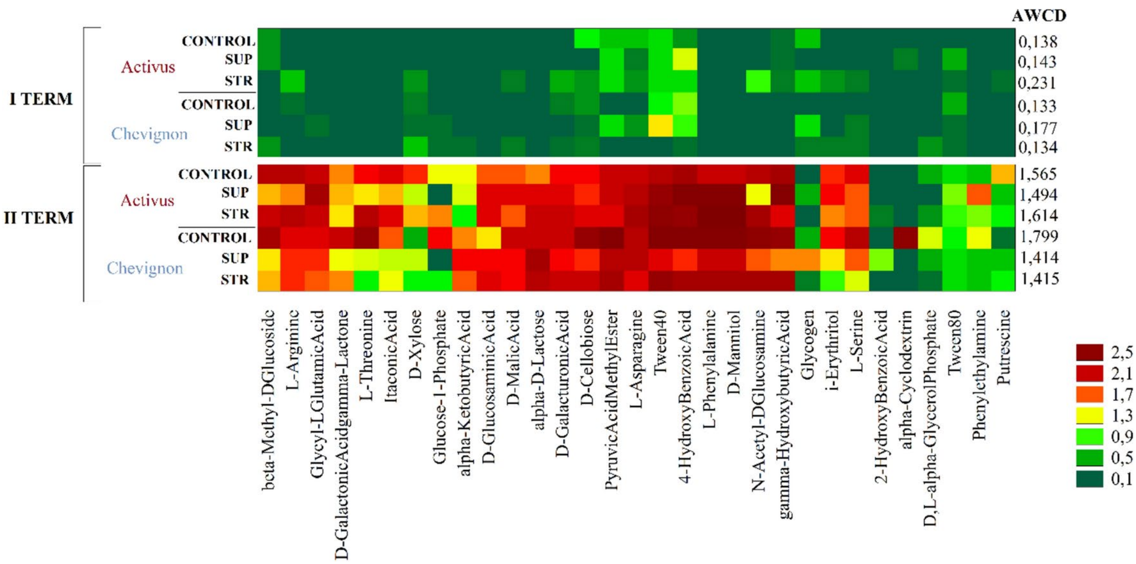

Fig. 16. Heatmap showing utilization of the 31 carbon sources by the soil microbial community in different samples.

to be providing plants with nutrients that are not readily available. Therefore, it is important to implement microbial-based techniques to improve phosphorus availability and uptake efficiency in fertilizer efficiency studies[48]. In this study, positive relationships were observed between acid phosphatase activity and the tested macronutrients (P and Mg, Supplementary material). According to the literature, soil enzymes are often involved as important indicators of microbiological and biochemical processes in the circulation and availability of nutrients, decomposition and synthesis of organic matter, and soil fertility and productivity[49]. Soil phosphatase enzymes are produced by plant roots and microorganisms and play a key role in the cycling of phosphorus (P). According to Margalef et al.[50] N fertilization leads to higher phosphatase activity, whereas P fertilization has the opposite effect. In Touhami et al.[51], long-term P fertilization decreased acid phosphatase activity, but increased alkaline phosphatase activity. These results showed that the application of stuvite did not negatively affect phosphatase activity. In the case of struvite treatments, there was a noticeable increase in enzymatic activity compared to the control soil. This indicates that native microorganisms can metabolize compounds intensively, thus providing nutrients for plant growth. The enhancement of enzymatic activity offers the possibility of reducing the dose of mineral fertilizer applied while having a beneficial effect on the intensity of biochemical processes occurring in the soil[52]. In the studies by Randall et al.[53] and Sun et al.[54]. P fertilization affected acidic and alkaline phosphatase enzyme activities more than soil moisture or temperature. Weather conditions have different effects on the activity of phosphateses. The occurrence of repeated drought episodes reduces the activity of soil phosphatases. However, their analyses showed no statistically significant effect of warming on soil phosphatase activity. In our study, we found that on the second date of soil sampling, struvite fertilization caused an increase in alkaline and acidic phosphatase activity. The observed stimulation of all enzymes measured after struvite application to soil is consistent with results published by other authors. Galamini et al.[55] observed increased activity of enzymes involved in N, C and P cycling, including phosphatases, when struvite was applied, especially when combined with zeolites. The authors attributed this shift in part to the increase in soil pH following this treatment. The struvite-induced pH increase was observed in our study, but to a lesser extent than in Galamini et al.[55] However, we can assume that any pH increase from the initial 5.9 can stimulate the enzymes. Also, other studies have shown that drought reduced acidic phosphatase activity, while high rainfall and prolonged watering reduced alkaline phosphatase enzyme activity due to anaerobic conditions[56–58]. Higher soil temperatures in spring and summer co-occurred with maximum acidic and alkaline phosphatase enzyme activity, while minimum acidic and alkaline phosphatase activity and plant growth were found in winter along with the highest levels of soil moisture. Touhami et al.[51] revealed that microbial biomass of P was similar in different seasons in the control but decreased in spring and autumn and increased in summer and winter under the influence of P fertilization. Acidic and alkaline phosphatase activity was significantly seasonally dependent and showed similar seasonal trends, reaching a maximum in summer and a minimum in winter, regardless of fertilization method. In our study, sesonal changes are also observed with higher activity in the second term of soil sampling, on the date of a harvest with a higher temperature. Correlation and principal component analysis in Touhami et al.[51] showed that acidic and alkaline phosphatase activity was significantly positively correlated with soil temperature and significantly negatively correlated with soil moisture. Rocobruna et al.'s[59] research also showed that the activity of phosphatases, which are directly related to the P cycle, are related with soil pH and moisture and also to factors related to biodiversity. In our study, we found a statistically significant correlation between pH and dehydrogenase activity. The results of some studies have revealed that soil pH influences enzyme activity and the soil microbial community[19,59]. It has been shown that the activity of soil enzymes increases with increasing soil pH[19,59,60]. It has also been reported that the main mechanism responsible for this is changes in the ionic form of the active sites of enzymes[60]. The microbiological parameter, which is widely used in assessing soil quality and fertility, is represented by community-level physiological profiles (CLPP)[18,61]. Microorganisms respond quickly to any environmental disturbance or stress, and changes in their metabolic profile can be a good and early indicator of changes in the ecosystem[19]. The BIOLOG system is widely used in environmental microbiology to assess the impact of different agricultural practices on the functional diversity of soil microbial communities[62–65]. In this study, different forms of phosphorus fertilization and the sampling date contributed to changes in the degree of C compound utilization, which is consistent with the results obtained in the enzymatic analysis of soils. Changes in the level of utilization of some carbon compounds reflect changes in the metabolic and catalytic abilities of soil microorganisms. The most important indicators in the evaluation of phosphorus fertilization are the utilization rates of D-glucose-1-phosphate and D, L-α-glycerol phosphate, which can be a source of phosphorus for soil microbiological communities. A higher level of D-glucose-1-phosphate metabolism was observed in samples fertilized with struvite on the second date for both wheat varieties compared to traditional superphosphate fertilization. On the other hand, D, L-α-glycerol phosphate is better metabolized only in the case of the Activus variety. Studies suggest that the introduction of various forms of fertilizers can improve the metabolic properties of microorganisms in terms of making essential nutrients available to both microorganisms and plants, such as phosphorus. It is also worth emphasizing that the increased rate of utilization of the above-mentioned compounds may be related to the increased activity of microorganisms involved in the biochemical conversion of nitrogen and phosphorus[52]. Based on the results of the EcoPlates bioassay, it was found that struvite fertilisation combined with the sampling date had a significant effect on the overall metabolic activity of microorganisms as expressed by the AWCD index and their metabolic diversity. Some studies have shown that proper fertiliser management, including the use of appropriate types and amounts of fertiliser, can increase the functional diversity of microorganisms to some extent, which is consistent with our findings[66]. Active and metabolically diverse microorganisms are a key element of sustainable agriculture, influencing ecosystem functioning and crop productivity[67]. It is also worth noting that the application of struvite to soil could reduce gaseous emissions from agricultural soils. As reported by (Galamini et al., 2025)[55] and (Yang et al., 2023)[68], struvite limited CO_2 and N_2O emissions from soil. This means that wider use of struvite-based fertilisers could facilitate more sustainable fertilisation strategies. Yang et al. 2023 observed that application of N-rich struvite resulted in lower gaseous N emissions than urea. In addition, N and P use efficiency by plants and yields were similar to conventional fertilisation. Therefore, struvite fertilisation can reduce the risk of N_20 emissions, which is a potent greenhouse gas, without reducing the economic performance of crop production[66]. Struvite as a secondary source of P and struvite-induced stimulation of phosphatases and dehydrogenases would promote more efficient use of phosphorus as a nutrient for crops, both through recycling of P from waste and stimulated release of P from soil organic matter.

Conclusions

The use of struvite can be an effective alternative to rock-P in the fertilizer industry and can represent a sustainable and renewable alternative to mineral fertilizers. Struvite is characterized by the potential to reduce global reliance on rock-phosphorus fertilizers and provides the potential to recycle P from sewage sludge. The results of this study provide evidence for positive aspects of struvite application. The highest amounts of extracted elements were found with the Mehlich 3 method, indicating that it is a method that should replace the Egner–Rhiem method in Polish chemical and agricultural stations. The results demonstrate that struvite had a positive impact on Mg content in the soil and an increase in soil pH and phosphatase activity. In our study, the term was the most important differentiating factor between dehydrogenase and phosphatase activity in the second term of soil sampling. With Biolog EcoPlate and enzyme measurements, it is possible to detect changes in the in the microbial community of agricultural soils, as well as evaluate methods of agricultural management and tillage applications. Despite the fact that these are one-year results, the soil microbiological analysis under wheat cultivation using struvite suggests a direction for microbial transformations. Future studies would be taken into account seasonal effects of struvite and its interactions with diverse soil microbial communities.

Data availability

All data generated or analysed during this study are included in this published article.

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R.R., A.J.R., M.W, S.S, A.SZ.T, B.G. —wrote the main manucript, R.R., B.G., AJ.R.- investigation, R.R.—literature; R.R., M.W—visualisation, J.K., M.W—statistical analysis, M.W., S.S.-microbiological analysis.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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