Journal of Elementology ISSN 1644-2296

Ramut, R., Jama-Rodzeńska, A., Gębarowska, E., Gałka, B., Szuba-Trznadel, A.,
Wiliusz-Nogueira, M., Jarki, D., Kamińska, J. and Białkowska, M. (2024)
'Effect of struvite (Crystal Green) application on microbial activity and soybean yield –
a preliminary study',
Journal of Elementology, 29(2), 485-503,
available: https://doi.org/10.5601/jelem.2024.29.1.3275

RECEIVED: 1 March 2024
ACCEPTED: 10 June 2024

ORIGINAL PAPER

Effect of struvite (Crystal Green) application on microbial activity and soybean yield – a preliminary study*

Rafał Ramut[1], Anna Jama-Rodzeńska[2], Elżbieta Gębarowska[3], Bernard Gałka[1], Anna Szuba-Trznadel[4], Małgorzata Wilusz-Nogueira[1], Daniel Jarki[5], Joanna A. Kamińska[6], Monika Białkowska[2]

[1] Institute of Soil Science, Plant Nutrition and Environmental Protection
[2] Institute of Agroecology and Plant Production
[3] Division of Biogeochemistry and Environmental Microbiology
[4] Department of Animal Nutrition and Feed Management
[6] Department of Applied Mathematics
Wrocław, University of Environmental and Life Sciences, Wroclaw, Poland
[5] Saatbau Poland Sp. z o.o., Żytnia 1, 55-300 Środa Śląska, Poland

Abstract

In comparison to mineral commercial phosphorus fertilizers, struvite is considered to be a promising P fertilizer, which – in the face of dwindling phosphate resources – may replace phosphate fertilisers in the future. The fertilising value of struvite arises from its significant phosphorus content as well as magnesium and nitrogen content, enhanced by the slow release of the components. The purpose of this study was to explore the response of the soybean cultivar Abaca to struvite fertilization in terms of the plant's yield and chlorophyll content as well as changes in the soil's microbiological life. A pot experiment was set up at the Pawłowice Research and Education Station, on soil low in phosphorus, and with two variable factors: different application methods of phosphorus fertilizer (band and broadcast), and two phosphorus fertilisers (superphosphate and struvite). The method of phosphorus application had a significant effect on the plant's biometric traits, chlorophyll a content and the microbial activity of soil. Struvite fertilization caused significant changes in the chlorophyll and carotenoid content. Significantly higher content of chlorophyll a and carotenoids as well as a higher total chlorophyll content were noted under struvite fertilization. Fertilizer application techniques caused a significant effect on the chlorophyll a content. Both the application methods and the type of phosphorus fertilizer had a significant effect on microbiological activity at the two measurement dates. Total glomalins content was significantly higher only in the case of band application of phosphate fertilizers at the end of the experiment, and the dehydrogenase activity was significantly increased at the flowering time of soybean following the use of this fertilizer application method. Despite the lack of significant differences in the studied elements of yield structure, their values in many cases were comparable and even slightly higher; hence, a fertilizer that aligns with the assumptions of the circular economy is worth attention and further studies under field conditions. However, long-term studies are needed to test the cultivation of plants under field conditions and their reaction to struvite as well as the effect on the content of phosphatases, which stimulate the conversion of organic phosphorus compounds into inorganic phosphates, directly available to plants and soil organisms.

Keywords: carotenoids, circular economy, chlorophyll, dehydrogenase activity, phosphorus fertilizer

---

Bernard Gałka, PhD SDc, prof., Institute of Soil Science, Plant Nutrition and Environmental Protection, Faculty of Life Sciences and Technology, Wroclaw University of Environmental and Life Sciences, Grunwaldzka Street 53, 50-337 Wroclaw, Poland, e-mail: bernard.galka@upwr.edu.pl

* The source of funding National Centre of Science DEC-2022/06/X/ST10/00047.

INTRODUCTION

Adequate phosphorus fertilization is essential for efficient crop production in order to achieve optimal yields, and for the initiation of nodulation in legumes. Phosphorus deficiency will have a key impact on agricultural productivity in the coming years (Cordell, White 2011). Commercial mineral phosphate fertilizers are produced from limited resources of phosphate rock, which are being depleted by intensive exploitation stimulated by demographic and economic factors (Cordell et al. 2009, Antonkiewicz, Łabętowicz 2016, Withers et al. 2020). It is predicted that the natural source of phosphorus will be depleted by 2033 due to, among other things, the population growth causing an increase in food demand (Cordell et al. 2009, Withers et al. 2020). The predicted phosphorus supply constraints and the human population growth implicate the need to explore sustainable sources of phosphorus so as to maintain agricultural productivity (Desmidt et al. 2015). Additionally, phosphorus resources are mined in only a few locations across the world: Morocco, Iraq, China, Algeria, and Syria (Roy 2017). Taking into account environmental, social and economic factors, attention should be drawn to finding alternative sources of phosphorus in a circular reuse system (Kasprzyk, Gajewska 2019).

Produced from sewage sludge, struvite is an example of a source of phosphorus in agriculture whose use will close the phosphorus cycle. Struvite is regarded as an alternative source of phosphorus, nitrogen, and magnesium in the agricultural sector (Gilbert 2009). Struvite effectiveness has been studied in the cultivation of many plants, i.e. barley (Muys et al. 2021), perennial ryegrass (Cabeza et al. 2011), maize, *Lolium multiflorum*, and lettuce (Plaza et al. 2007, Talboys et al. 2016). Due to its high phosphorus content (similar to conventional phosphorus fertilizers), it can be an effective fertilizer, especially when applied to acidic soils (Szymańska et al. 2019, 2020, Muys et al. 2021,). Based on previous studies, struvite from sewage sludge seems to be as effective as superphosphate in promoting dry matter production and P uptake by plants (Stark et al. 2006; Uysal et al. 2010). The value of struvite as a fertilizer has recently been recognized, and it is now receiving increasing attention among scholars, but still needs further research (Huygens, Saveyn 2018, Muys et al. 2021, O'Donnell et al. 2022).

Soybean (*Glycine max* (L.) Merr.) is one of the most important legumes grown worldwide. The area under soybean cultivation in Poland in 2023 amounted to 44 621 ha (ARiMR 2023). It is an economically important legume that can be used in the feed industry, for livestock production, and in human nutrition. Its high nutritional value results from its unique amino acid composition, high digestibility due to its low fiber content, and high protein and fat content (an average of 40% protein and ca 20% fat in the dry weight of the seeds). The level of available phosphorus (P) critical for soybean production is 10-15 mg kg^{-1} soil (Aune, Lal 1997). Low soil phosphorus content is a major limiting factor for soybean growth and yield, which are also dependent on atmospheric nitrogen (Xue et al. 2014). Phosphorus deficiency in plants, including soybean, disrupts chlorophyll production, causing leaf chlorosis (Khan et al. 2023). Prolonged P deficiency can additionally cause anthocyanin accumulation, consequently leading to purple discoloration on the leaf surface (Rech et al. 2019).

The method of phosphorus application also has a significant impact on the yield, quality, and economic viability of a crop. P banding mainly results in phosphorus availability for plants (via early root growth) in a limited zone. Additionally, P diffusion to the root when fertilized with a band technique is high because of the large concentration gradient of phosphorus, which is low in broadcast (traditional) fertilization (Ticconi, Abel 2004). In soils with optimal or high phosphorus content, band fertilization is less favorable, and surface fertilization is usually better (Ticconi and Abel 2004).

Hu et al. (2019) suggested that proper application of mineral and organic nutrients, especially those containing phosphorus, has a positive effect on different soil parameters, including enzyme activity. The level of enzymatic activity in soil is a sensitive indicator of their fertility and fecundity, and provides information on ecological changes in the soil environment (Viégas et al. 2018). One of the bioindicator is the activity of dehydrogenase (DHA), the most relevant and sensitive soil enzyme relating to soil fertility and quality (Natywa, Ambroży 2010). The second one is glomalin, an extracellular protein produced by arbuscular mycorrhizal fungi (AMF), playing an important role in the formation and stabilization of soil aggregates. It was discovered in the 1990s by Wright and Upadhaya (1996). In recent years, studies involving determinations of the concentration of gomalins in soil activity have become increasingly common. The evaluation of the glomalin content as an indicator of changes in soil fertility quality is substantiated by its positive correlation with changes in the carbon content of soil organic matter (CSOM) – Wolińska (2008). It is also one of the possible indicators of soil condition and AMF activity (Księżak et al. 2018). However, the method of their extraction by autoclaving in sodium citrate buffer means they are glomalin-related soil proteins (GRSP) rather than pure proteins produced by mycorrhizal fungi. This is why the term “glomalin” is used for the purified protein gene product.

Research into P fertilization in plant production and its effect on the growth, development, photosynthesis, nitrogen fixation, and yield of soybean has been widely conducted. However, there have been few studies into the effect of struvite from sewage sludge on soybean. The working hypothesis was that the application of struvite would increase soybean seed yield and influence a change in the leaf chlorophyll content. The purpose of a pot and laboratory study was to determine the response of soybean (Abaca variety) to phosphorus fertilization and its effects on biometric traits, yield, microbial changes and chlorophyll content, and to assess the possibility of replacing superphosphate with struvite.

MATERIALS AND METHODS

Plant material

Seeds of Abaca soybean were sown into each pot on 14 May 2022. Abaca belongs to the “000” earliness group with resilient early vigor. It is characterized by high yield potential with high stability in subsequent crop years. Seeds were inoculated according to the Fix Fertig technology, which involves factory-coating seeds with dormant bacteria. The seeds were coated with *Bradyrhizobium japonicum* bacteria.

Soil

The soil used in the experiment was alluvial soil, very light, on loose sand and sandy gravel (V grade) (soil classification used in Poland) (Kabała et al. 2019). The soil originated from the Experimental Station of Wroclaw University of Environmental and Life Sciences (Pawłowice) (geographical location 17°7′ E and 51°08′ N) in the Lower Silesian Voivodship, Wrocław, Poland), and the following chemical parameters were determined by the Egner-Riehm method: P – 7.3 mg kg^{-1} of d.m., K – 22 mg kg^{-1} of d.m., Mg – 3.8 mg kg^{-1} of d.m. (K – high, P – low , Mg – low content), pH – 5.65-5.92 (slightly acidic), low conductivity – 33.18-136.01. Soil pH was measured with a glass pH electrode (1:5 soil:deionized water, measurements after 30 min), and conductivity was assessed using a conductivity meter (the conductivity method).

Experimental design

In 2022, a pot experiment was established at the Experimental Station (Pawlowice) of the Wroclaw University of Life Sciences, Wroclaw, Poland, using soybean (*Glycine max* (L.) Merr.) as an experimental plant. It was a two-factor experiment. The first factor was the different ways of applying phosphorus fertilizer: band A1, and broadcast A2. Broadcast fertilization consisted of random placement of fertilizers on the surface of the soil in a pot, while band application was carried out by placing fertilizer granules at a depth of about 5 cm below the sown soybean seed. The second factor was the type of fertilizer. Two phosphorus fertilizers were used: traditional triple superphosphate (SUP, B2), commonly used in soybean cultivation, and struvite (STR, B3), Crystal Green (Ostara, Canada) produced from sewage sludge. The experiment examined the effect of the fertilizer produced from sewage sludge, Crystal Green (produced by Ostara Nutrient Technologies), in comparison to a traditional fertilizer, such as triple superphosphate (Ostara, 2022). Struvite also contains N (2%), P (24%), and Mg (10%), and is characterized by a low content of heavy metals compared to triple superphosphate, which contains 40% P_2O_5. This fertilizer was in the form of granules with a *ca* 1–2 mm diameter. The experiment was conducted in six replications. The total number of pots was 36 (six combinations). The pots, with a diameter of 20 cm, were filled with soil and fertilizers. Fertilizer doses in the experiment were based on the optimum amounts for growing soybeans under field conditions, i.e. 70 kg ha^{-1} P_2O_5, 120 kg ha^{-1} K_2O, and a starting nitrogen dose of 30 kg ha^{-1} N. Only nitrogen and potassium were applied at that time. The following fertilizer doses per pot (converted) were applied: struvite – 0.76 g, superphosphate – 0.54 g, ammonium nitrate – 0.27 g, potassium salt – 1.25 g.

The control samples (B1) contained only ammonium nitrate (N) and potassium salt (P) without STR and SUP. Four Abaca soybean seeds were sown into each pot on 14 May 2022. During the growing season, observations were made for the presence of pests, diseases or weeds, and for the determination of developmental stages. The soybean was regularly watered.

Biometric measurements of soybean

Harvesting was carried out at full maturity (BBCH 99) on 15 September 2022. At full maturity, four plants were taken from each pot for biometric measurements and determination of yield. Each variant was repeated six times (one pot = one repetition), and four plants were taken for biometric measurements.

Seed yield was converted per hectare at 15% moisture content. The following traits were determined: the height of plant (cm), height of the 1st pod (cm), number of branches (pcs), stem weight after harvest (g), root weight after harvest (g), number of pods per plant (pcs), the weight of pods per plant (pcs), and number of seeds per plant (pcs). Leaves were sampled after soybean flowering to determine their chlorophyll content.

Chlorophyll content

A 0.4 g leaf sample was crushed using a clean pestle and mortar. 20 ml of 80% acetone and 0.5 g of powdered $MgCO_3$ were added to the homogenized leaf material. The contents were mixed, then shaken for three minutes, and automatically extracted with a pump. The supernatant was transferred to a 100 mL volumetric flask, and the volume was replenished by adding 80% acetone. The color absorbance of the solution was evaluated with a spectrophotometer at 645 and 663 nm.

Formula:

$$Chl\ a = 11.75 \times A662.6 - 2.35 \times A645.6$$

$$Chl\ b = 18.61 \times A645.6 - 3.96 \times A662.6$$

$$\text{carotenoids} = (1000 \times \text{A}470) - (3.27 \times Chl\ a) - (104 \times Chl\ b)/229$$

(Lichtenthaler 1987), where: *Chl a* and *Chl b* – chlorophyll *a* and chlorophyll *b*, *A* – absorbance. Chlorophyll content was given as mg of chlorophyll 100 g^{-1} fresh weight, and total chlorophyll was calculated as the sum of chlorophyll *a* and chlorophyll *b*, while carotenoids were given in μg 100 g^{-1} fm.

Dehydrogenase activity and total glomalin-related soil protein content

Soil samples for analysis were taken from randomly selected places from a pot at the time of soybean flowering (BBCH 60-69). 5 g of soil was weighed for dehydrogenase determination. Soil samples for microbiological analyses were taken on 26 July 2022. Soil dry matter content was also determined. Dehydrogenase activity (DHA) was analyzed according to PN-ISO 23753-1 (2008) – determination of dehydrogenase activity in soil using 2,3,5-triphenyltetrazolium hydrochloride (TTC). The TTC solution was added to the soil sample, and the mixture was incubated at 25°C for 16 h. The released triphenylformazane (TPF) was then extracted with acetone and determined photometrically at 485 nm.

Total glomalin-related soil proteins (total GRSP) from soil samples were extracted according to the method described by Wright and Upadhyaya (1999) with some modifications. The soil samples (10 g) were covered with 0.05M, pH 8.0 citrate buffers and autoclaved at 121°C for 60 min. Extraction was repeated several times until the total washing out of the organic fraction from the soil. After each autoclaving, the supernatant containing total GRSP was poured off and centrifuged at 10,000 rpm for 10 min. The pellet of soil sample was covered with a sterile buffer and autoclaved again. The extracts collected after each heating and centrifugation were combined and stored at 4°C until analysis. Total GRSP content in supernatants was quantified with the Bradford method using bovine serum albumin (Sigma-Aldrich, Inc., Saint Louis, USA) as a standard. The analysis was carried out twice: during the flowering of soybean (1) and at the end of the experiment (2).

Statistical analysis

The normality of the distribution of characteristics was tested using the Shapiro-Wilk normality test. All results were subjected to one-way and two-way statistical analyses (Anova/Manova) in Statistica software (Tibco 2017) and later the Tukey's test, with a significance level of $\alpha = 0.05$. The homogeneity of the groups was confirmed using a *post hoc* test (the Tukey's test at level $\alpha = 0.05$). Names of homogeneous groups were determined from the smallest to the largest value. Standard error (SE) was also added to all measured values.

RESULTS AND DISCUSSION

Effect of fertilization and struvite placement on biometric traits of soybeans

Phosphorus fertilizer placement significantly affected two biometric parameters of soybean, i.e. 1st pod set height and stem weight after harvest (Figure 1). Significantly higher pod height as well as stem weight were noted

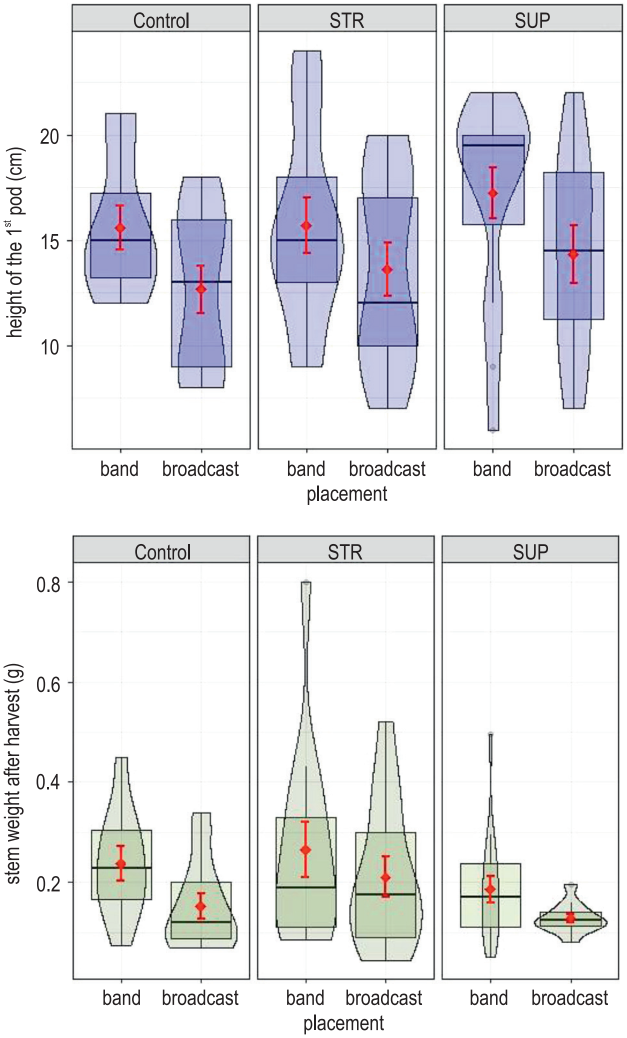

Fig 1. The effect of different phosphorus placement on height of the 1st pod and steam weight. Red dots – average, error bars – standard errors

when phosphorus fertilizer was placed according to the band method. The applied phosphorus fertilizers did not significantly affect any of the biometric traits studied or the interactions between factors (Figures 2 and 3). Soybean yield was not dependent on the examined traits (Figure 4). Among the many factors that can contribute to soybean success, phosphorus has a significant impact on growth and yield attributes.

Among the many factors that can contribute to the success of soybeans, phosphorus has an important influence on growth and yield attributes. Phosphate fertilization caused an increase in seed yield of common beans (Zucareli et al. 2006). Zabala et al. (2020) obtained not only an increased yield of soy-

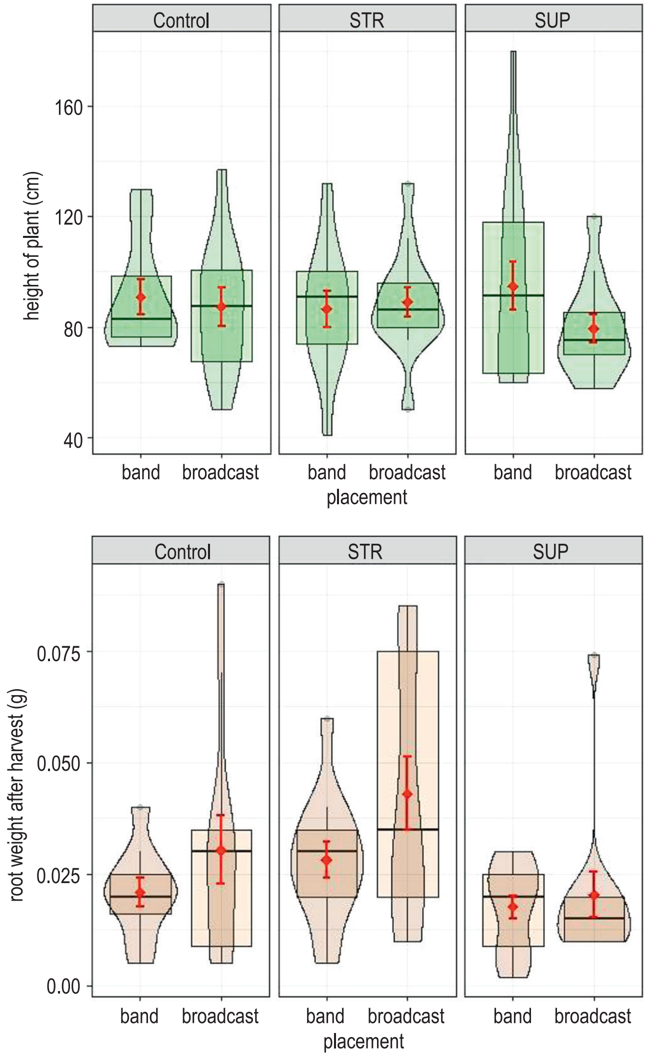

Fig 2. The effect of different phosphorus placement on plant height and root weight of soybean after harvest. Red dots – average, error bars – standard errors

bean seeds but also improved biometric traits and soybean yield components under phosphorus fertilization. Darwesh et al. (2013) also reported an increased yield of soybean seeds and a higher weight of 100 seeds under phosphorus fertilization. The average soybean seed yield obtained in an experiment ranged from 87 kg ha^{-1} to 217 kg ha^{-1}, as reported by Kamara et al. (2008). However, there was no consistent response to P by the number of pods per plant and seeds per pod. This was also observed in our study. In our research, phosphorus fertilization had no significant impact on the mass of seed or the number of pods per plant; however, a higher value was observed

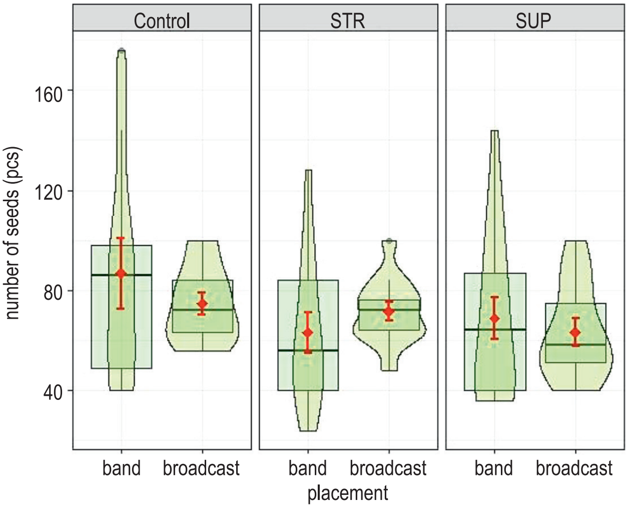

Fig 3. The effect of different phosphorus placement on number of seeds (pcs).
Red dots – average, error bars – standard errors

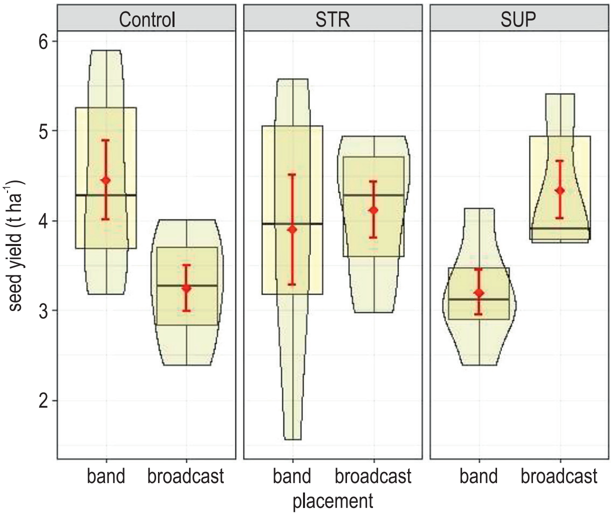

Fig 4. The effect of different phosphorus placement on seed yield.
Red dots – average, error bars – standard errors

under struvite fertilization. The effect of phosphorus fertilization on yield and biometric traits was also presented by Tarekegn (2017). A dose of phosphorus fertilizer at 46 kg ha^{-1} contributed not only to an increase in soybean seed yield but also to a higher number of seeds per plant, number of pods per plant, and weight of 100 seeds. These results contradicted those of our study. Chiezey (2001) found that low soil phosphorus levels reduced soybean yields, and the content of phosphorus in the soils in that experiment was from low to average (10,3 mg kg^{-1}d.m). The critical available phosphorus (P) in soil for soybean production has been estimated at 10 to 15 mg kg^{-1} soil (Aune and Lal 1997).

Hussain and Faridullah (2003) indicated that P is essential for healthy plant growth. An optimal phosphorus level has an impact on root growth and increases flowering and fruit preparation, which is reflected in increased seed productivity, an effect, however, that was not seen in our study. In our study, there was no significant impact of struvite fertilization on root system mass; however, the values under fertilization with Crystal Green are higher compared to control and superphosphate fertilization variants.

Effect of fertilization and struvite placement on chlorophyll content

The method of phosphorus fertilizer placement had a significant effect only on chlorophyll a content. Significantly more chlorophyll a was found when seeds were band placed. The source of phosphorus had a significant effect on the content of chlorophyll a, total chlorophyll, and carotenoids. The highest levels of these pigments were found when STR was applied. The interaction between factors had no significant effect on the content of chlorophylls and carotenoids (Table 1).

Table 1

Effect of fertilization and struvite placement on chlorophyll and carotenoid content

Experiment factor	Chlorophyll and carotenoid content			
	chlorophyll a (mg 100 g^{-1} f.m)	chlorophyll b (mg 100 g^{-1} f.m)	chlorophyll a+b (mg 100 g^{-1} f.m)	carotenoids (µg 100 g^{-1} f.m)
Phosphorus placement (A)				
Band	213.57*b*±11.86	76.69±4.03	290.27±15.02	593.11±26.33
Broadcast	176. 93*a*±11.86	67.86±4.03	244.79±15.02	565.28±26.33
P value	*0.05	ns	ns	ns
Phosphorus fertilization (B)				
Control	163.52*a*±13.07	64.62±4.68	228.15*a*±16.51	514.76*a*±25.31
SUP	198.55*ab*±13.07	72.16±4.68	270.71*ab*±16.51	585.12*ab*±25.31
STR	223.64*b*±13.07	80.06±4.68	303.74*b*±16.51	637.71*b*±25.31
P value	**0.01	ns	*0.05	**0.01
A x B	ns	ns	ns	ns

C – control, SUP – superphosphate, STR – struvite, ns — not significant. Means for factors within a column marked with the same letter do not differ significantly at the level $\alpha = 0.05$.

Numerous studies have shown that the chlorophyll content in plants increases owing to fertilization with macroelements, particularly nitrogen (Fritshi, Ray 2007, Zhang et al. 2013, 2021). Based on Siedliska et al. (2021), the results demonstrated that in sugar beet and strawberry plants, exceeding the recommended dose of phosphorus in the nutrient solution resulted in a decrease in the chlorophyll content for all measured chlorophyll types (chlorophyll a, chlorophyll b, and total chlorophyll). A similar trend, but a weaker one, was observed in celery plants. These differences are related to different effects of phosphorus on chlorophyll activity in different species, but this was confirmed by our results.

Deficient or excessive application of phosphorus can cause a decrease in chlorophyll and carotenoid concentrations, which may be related to leaf chlorosis. In our research, struvite caused an increase in chlorophyll a, chlorophyll a+b, and carotenoid content, which could be related to the additional content of nitrogen and magnesium in STR. Chlorophyll content depends on the amount of nitrogen fertilizer. Depending on the growth stage, the chlorophyll content in leaves increases in those variants with nitrogen fertilizer. The plant growth stage and feeding strategy have a significant ($p<0.05$) effect on the chlorophyll content in plant leaves (Li et al. 2018). Peyvast et al. (2008) show that chlorophyll levels in plants increase after organic fertilizer application.

Correlations between examined traits

There is a positive correlation between the mass of the stem, the number of branches, and vice versa, as well as the mass of the roots and the mass of the stem (Figure 5).

A positive and statistically significant correlation was noted between the mass of seeds and seed yields, the number of seeds, and the number of pods per pot.

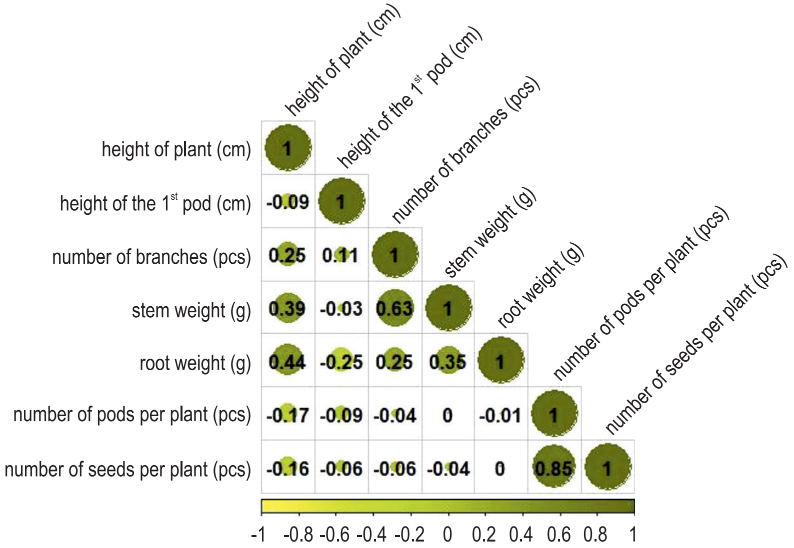

Fig 5. Correlations between biometric traits

In the case of the chlorophyll content, a positive correlation was noted between chlorophyll a and other chlorophylls and carotenoids, chlorophyll b and other chlorophylls and carotenoids, chlorophyll a+b and other chlorophylls and carotenoids, while carotenoids demonstrated a positive correlation with all chlorophylls (Table 2). Magnesium uptake (with average value 10.66 kg ha^{-1}) was not significantly positively correlated with chlorophyll or carotenoid content (Table 3).

Table 2

Correlations between chlorophyll and carotenoid content

Trait	Average	Standard error	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoids
Chlorophyll a	195.3	39.3	1.00	0.76	0.97	0.76
Chlorophyll b	72.3	12.6	0.76	1.00	0.86	0.94
Chlorophyll a+b	267.5	49.6	0.99	0.86	1.00	0.84
Carotenoids	579.2	78.0	0.76	0.95	0.84	1.00

Table 3

Dehydrogenase activity (DHA) and glomalin-related soil proteins (GRSP) content

Fertilizer application	Groups	DHA (μg TPF g^{-1} h^{-1})	Total GRSP (μg g^{-1})
Band 1	Control	4.75±0.1 *a*	1321.6±228.9 *b*
	SUP	8.41±0.3 *b*	1292.2±211.5 *b*
	STR	7.96±0.1 *b*	1200.1±7.8 *b*
Broadcast 1	Control	10.39±0.2 *c*	1260.8±88.3 *b*
	SUP	8.06±0.3 *b*	1284.4±126.7 *b*
	STR	10.33±0.4 *c*	1266.7±162.4 *b*
Band 2	Control	5.79±0.1 *a*	1112.0±62.2 *a*
	SUP	5.57±0.1 *a*	1380.3±236.9 *b*
	STR	5.61±0.1 *a*	1351.6±7.8 *b*
Broadcast 2	Control	5.81±0.2 *a*	1262.8±110.3 *b*
	SUP	4.99±0.0 *a*	1414.3±306.7 *b*
	STR	5.41±0.1 *a*	1325.5±445.2 *b*

Band – root fertilization, Broadcast – surface fertilization, control – soil with NK alone, SUP – triple superphosphate, STR – struvite, Crystal Green®, DHA – dehydrogenase activity expressed as the amount of μg triphenylformazan (TPF) released per hour per gram of soil, GRSP – glomalin-related soil protein content per gram of air-dried soil; ± – standard deviation; 1) – during the flowering of soybean; 2) – at the end of the experiment. Values are the means of four replicates of each sample. Values followed by different letters in columns indicate significant differences according to Tukey's test ($p<0.05$).

Effect of fertilization and struvite placement on dehydrogenase activity and glomalin-related protein content

The results for DHA and total GRSP content in fertilized samples are presented in Table 3. Based on the data presented, it can be concluded that the DHA values decreased depending on the date of soil sampling. Higher values for these indicators were found during the flowering period (1). Triple superphosphate and Struvite fertilizers significantly increased the activity of DHA, by 77% and 68% compared to control, respectively, on the first sampling date with band fertilization. In the case of broadcast fertilization, phosphorus fertilizers contributed to a decrease in DHA. On the second measurement date (2), after the soybean harvest, DHA values decreased compared to those obtained on the first date. Phosphorus fertilization lowered the content of this indicator compared to the control.

In the case of total GRSP, in the first analysis, carried out during the flowering of soybean (Band 1 and Broadcast 1), their content was not significantly ($p>0.05$) different from the corresponding controls. At the end of the experiment (2), total GRSP content was significantly higher ($p<0.05$), 24% and 21% compared to control, in soils fertilized with triple superphosphate and struvite, respectively, applied in the band method (Band 2). In the case of samples with surface (Broadcast 2) application, these values were 9.4% and 2.5% higher, respectively. However, the type of phosphate fertilizer did not affect the total GRSP content.

According to Natywa et al. (2010), dehydrogenase activity is strictly dependent on the season. High dehydrogenase activity during the summer may be related to more intense root secretion by plants, and at this time, phosphorous band fertilization is an essential factor for production of more root secretions. They are an excellent food source of nutrients for microorganisms, especially those that live in the rhizosphere. These authors reported that high doses of nitrogen fertilizer caused a decrease in dehydrogenase activity. A similar effect was observed in a study on field wheat and corn soil performed by Hu et al. (2014), when inorganic N fertilizer showed weaker effects on dehydrogenase activity than NP or organic manure treatment.

In our study, the type of phosphorous fertilizer had no significant effect on dehydrogenase activity. Organic fertilization is believed to have a more favorable effect on overall biological activity than mineral fertilization, which, while improving the physical and chemical properties of soil, can harm its enzymatic activity (Peywast et al. 2008). According to Sawicki et al. (2020), dehydrogenase activity changes with increasing nitrogen doses, and in sites with a 50 kg ha^{-1} dose of N, a significant increase in its activity has been observed. According to Pettersson and Bååth (2003), temperature is one of the most crucial environmental factors affecting the community of soil bacteria. Scherer et al. (2011) showed positive effects of manure on the abundance of microorganisms, which is also observed under struvite fertilization. In addition, the positive effect of mineral fertilizers and organic waste materials on the stimulation of enzymatic activity has been noted (Pettersson, Bååth 2003, Scherer et al. 2011). According to Sawicki et al. (2020), the activity of dehydrogenases changed with increasing nitrogen dose; a significant increase in their activity was observed in sites with a dose of 50 kg ha^{-1} N.

The usefulness of total or easily extractable GRSP content as another possible indicator of soil organic matter quality thus seems rather promising, and it has been increasingly taken into account in soil activity studies in recent years. At the end of our experiment, we observed a significantly higher content of total GRSP in soils with band fertilization, which was not recorded for the broadcast technique of fertilizer application. In this study, the type of fertilizer (struvite or superphosphate) did not lead to significant differences in the content of these proteins, while other authors recorded a more favorable effect of struvite compared to mono-ammonium phosphate, e.g. Tomassi et al. (2011). In their research into wild-type mycorrhizal tomato (*Solanum lycopersicum* L.), these authors observed increased N and P uptake by mycorrhizal tomatoes fertilized with struvite, suggesting that AM-associated plants could enhance the dissolution of this fertilizer and improve crop utilization of struvite-P. It should also be recalled that long-term experiments have a greater potential for positive results than smaller scale experimental studies. Changes in GRSP content caused by different fertilization treatments can be expected after a long time (min. 10 years). Wu et. al (2011) reported, after a multi-year experiment established in 1979 in China, that long-term fertilization had beneficial effects on GRSP content, fungal spore density, and AMF diversity, but a better effect was observed when mineral fertilizer was supplemented with manure and straw. The addition of manure and straw to soil under wheat cultivation also affected easily extractable glomalin content, as reported by Hu et al. (2014), significantly increasing it, whereas mineral fertilization caused no significant difference compared to the control. Glomalin content in the soil is the result of several factors. In addition to fertilization, for example, it is dependent on the crops grown (Wojewódzki, Ciesińska 2012). As reported by Wright and Anderson (2000) and Curaqueo et al. (2011), changes in the glomalin content are more dynamic due to improper soil cultivation or under drought conditions.

CONCLUSIONS

Summarizing our results and the literature review, it should be concluded that struvite can be a promising substitute for commercial phosphorus fertilizer. Although no significant statistical differences were determined in the elements of yield structure, seed yield as well as other morphological traits, in most traits the values of these elements under the influence of struvite fertilization are comparable or higher. Struvite application caused an increase in chlorophyll and carotenoid content due to the additive content of nitrogen and magnesium. Band phosphate fertilization improved soil condition as expressed by GRSP content growth over time. The higher DHA observed during the flowering time reflected the activity of different soil microbes, which seemed to be promoted by root phosphate fertilizer applied using the band method at this stage of plant development. The results indicate that struvite can be a substitute for superphosphate, as it does not contribute to lower yields, biometric traits, while capturing microbiological changes in such a short time is also promising and requires further research especially under field conditions. Therefore, long-term field experiments are needed as a way to measure sustainable phosphorus fertilization in agriculture, as they contribute to a better understanding of the effects of struvite on the availability of macro- and micronutrients, yields and their content in plant material.

Author contributions

R.R., A.J.R., E.G. – conceptualization, B.G., A.Sz-T. – data curation, A.Sz.-T.,M.W.-N., E.G. – formal analysis, R.R., A.J-R., D.J. – investigation, B.G., R.R. M.B. – methodology, J.K., E.G. – software, A.Sz.-T., M.B., M.W-N. – validation, A.J.R., E.G. – visualization, R.R., A.J.R. – writing – original draft, R.R. B.G., A.J.R. – writing – review & editing, J.K. – statistical analysis. All authors have read and agreed to publish the manuscript.

Conflicts of interest

The authors ensure that they have neither professional nor financial connections related to the manuscript sent to the Editorial Board.

The APC is financed by Wrocław University of Environmental and Life Sciences in Poland (Institute of Soil Science, Plant Nutrition and Environmental Protection).

REFERENCES

ARiMR (2023). ARiMR – Agencja Restrukturyzacji i Modernizacji Rolnictwa (Poland). Translation in English: The Agency for Restructuring and Modernisation of Agriculture (ARMA).

Antonkiewicz, J., Łabętowicz, J. (2016) ‘Chemical innovation in plant nutrition in a historical continuum from ancient Greece and Rome until modern times’, *Chemistry Didactics Ecology Metrology,* 21(1-2), 29-43. https://doi.org/https://doi.org/10.1515/cdem-2016-0002

Aune, J.B. and Lal R. (1997) ‘The tropical soil productivity calculator – a model for assessing effects of soil management on productivity’, In: Lal R, Stewart BA, editors. *Advances in Soil Science: Soil management: Experimental basis for sustainability and environment*, available: https://doi.org/10.1007/s10646-014-1329-0

Bastida, F., Jehmlich, N., Martínez-Navarro, J., Bayona, V., García, C., Moreno, J.L. (2019) ‘The effects of struvite and sewage sludge on plant yield and the microbial community of a semiarid Mediterranean soil’, *Geoderma,* 331, 1051-1067, available: https://doi.org/10.1016/j.geoderma.2018.10.046

Chiezey, U.F (2001) ‘Pod abortion and grain yield of soybean (Glycine max (L.) Merrill) as influenced by nitrogen and phosphorus nutrition in the northern guinea savannah zone of Nigeria’, *Tropical Oilseed Journal,* 6, 1-10.

COBORU 2023, website available at https://www.coboru.gov.pl/

Cordell, D. and White S. (2011) ‘Peak phosphorus: Clarifying the key issues of a vigorous debate about long-term phosphorus security’, *Sustainability*, 3(10), 747-758, available: https://doi.org/10.3390/su3102027.

Cordell, D., Drangert, J.O, White, S. (2009) ‘The story of phosphorus: Global food security and food for thought’, *Global Environmental Change*, 29(2), 292-305, available: https://doi.org/10.1016/j.gloenvcha.2008.10.009

Curaqueo, G., Barea, J.M., Acevedo, E., Rubio, R., Cornejo, P., Borie, F. (2011) ‘Effects of different tillage system on arbuscular mycorrhizal fungal propagules and physical properties in a Mediterranean agroecosystem in central Chile’, *Soil Tillage Research*, 113, 11-18, available: https://doi.org/10.1016/j.still.2011.02.004

Darwesh, D.A., Maulood, P.M., Amin, S.A. (2013) ‘Effect of phosphorus Fertilizers on growth and physiological phosphorus use efficiency of three soy bean cultivars’, IOSR *Journal of Agricultural Veterinary Science,* 3(6), 32-36.

Desmidt, E., Ghyselbrecht, K., Zhang, Y., Pinoy, L., Van Der Bruggen, B., Verstraete, W., Rabaey, K., Meesschaert, B. (2015) ‘Global phosphorus scarcity and full-scale P-recovery techniques: *A review’, Critical Reviews in Environmental Science and Technology*, 45(4), 336-384, available : https://doi.org/10.1080/10643389.2013.866531

Fritschi, F.B, Ray, J. D. (2007) ‘Soybean leaf nitrogen, chlorophyll content, and chlorophyll a/b ratio’, *Photosynthetica*, 45, 92-98, available: https://doi.org/10.1007/s11099-007-0014-4

Gilbert, N. (2009) ‘Environment: The disappearing nutrient’, *Nature Journal*, 461, 716-718, available: https://doi.org/10.1038/461716a

Hu, W., Jiao, Z., Wu, F., Liu, Y., Dong, M., Ma, X., Fan, T., An, L., Feng H. (2014) ‘Long-term effects of fertilizer on soil enzymatic activity of wheat field soil in Loess Plateau, China’, *Ecotoxicology,* 23, 2069-2080, available: https://doi.org/10.1007/s10646-014-1329-0

Hussain, M.A., and Faridullah S. (2003) ‘Effect of phosphorus on mustard and rapeseed in relation to grain filling period and yield potential’, *Journal of Agricultural Research*, 34, 59-369, available: https://doi.org/10.7176/JBAH/12-12-01

Huygens, D., Saveyn, H.G.M. (2018) ‘Agronomic efficiency of selected phosphorus fertilisers derived from secondary raw materials for European agriculture. A meta-analysis’, *Agronomy for Sustainable Development*, 38, 52, available: https://doi.org/10.1007/s13593-018-0527-1

Jama Rodzeńska, A., Chochura, P., Gałka, B., Szuba-Trznadel, A., Svecnjak, Z., Latkovic. D. (2021)’ Effect of various rates of p from alternative and traditional sources on butterhead lettuce (Lactuca sativa L.) grown on peat substrate’ *Agriculture*, 11(12), 1279, available: https://doi.org/10.3390/agriculture11121279

Jama-Rodzeńska A. (2022) ‘The Effect of Phosgreen Fertilization on the Growth and Phosphorus Uptake of Lettuce (Lactuca sativa)’, *International Journal of Agriculture and Biology*, 27, 1-7, available: https://doi.org/10.17957/IJAB/15.1892

Kabała, C., Charzyński, P., Chodorowski, J., Drewnik, M., Glina, B., Greinert, A., Hulisz, P., Jankowski, M., Jonczak, J., Łabaz, B., Łachacz, A., Marzec, M., Mendyk, Ł., Musiał, P., Musielok, Ł., Smreczak, B., Sowiński, P., Świtoniak, M., Uzarowicz, Ł., Waroszewski, J. 2019. ‘Polish Soil Classification, 6th edition – Principles, classification scheme and correlations’, *Soil Science Annual*, 70(2), 71-97.

Kamara, A.Y., Kwari, J., Ekeleme, Omoigui, L, Abaidoo, R. (2008) ‘Effect of phosphorus application and soybean cultivar on grain and dry matter yield of subsequent maize in the tropical savannas of north-eastern Nigeria’, *AJB Policies*, 7(15), 2593-2599.

Kasprzyk, M., Gajewska, M. (2019) ‘Phosphorus removal by application of natural and semi-natural materials for possible recovery according to assumptions of circular economy and closed circuit of P’, *Science Total Environmental*, 650(1): 249-256, available: https://doi.org/10.1016/j.scitotenv.2018.09.034

Khan F., Siddique A.B., Shabala. S, Zhou M., Zhao C. ‘Phosphorus plays key roles in regulating plants' physiological responses to abiotic stresses', *Plants*, 3, 12(15), 2861, available: https://doi.org/10.3390/plants12152861

Li, Y., He, N., Hou, J., Xu, L., Liu, C., Zhang, J., Wang, Q., Zhang, X., Wu, X. (2018) 'Factors influencing leaf chlorophyll content in natural forests at the biome scale', *Frontier in Ecology*, available at https://www.frontiersin.org/articles/10.3389/fevo.2018.00064/full

Lichtenthaler, H.K (1987) 'Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods of Enzymology', *Plant Cell Membranes, Pacekr & Douce*, 148, 350-582.

Muys, M., Phukan, R., Brader, G., Samad, A., Moretti, M., Haiden, B., Pluchon, S., Roest, K., Vlaeminck, S.E., Spiller, M. (2021) 'A systematic comparison of commercially produced struvite: Quantities, qualities and soil-maize phosphorus availability', *Science in Total Environment*, 756, 143726, available: http://dx.doi.org/ 10.1016/j.scitotenv.2020.143726

Natywa, M. and Ambroży, K.A.S. (2010) 'Changes of the number of selected groups of microorganisms under maize cultivation depending on the developmental phase of plant and sprinkling irrigation', *Nauka Przyroda Technologie,* 4, 88.

Niyi, S., Omidire, K.R, Brye, L., English, L.**,** Kekedy-Nagy, L. Greenlen L., Popp, J. and Trenton, L.R. (2023) 'Soybean growth and production as affected by struvite as a phosphorus source in eastern Arkansas', *Crop Science*, 63(1), 320-335, available: https://doi.org/10.1002/csc2.20852

O'Donnell, C., Barnett, D., Harrington, J. and Power, N. (2022) 'The extended effect of top-dressed recovered struvite fertiliser on residual irish grassland soil phosphorus levels compared to commercial phosphorus fertiliser', *Agronomy*, 12(1), 8, available: https://doi.org/10.3390/agronomy12010008

Ostara Nutrients, 2023 available at https://ostara.com/

Pettersson, M. and Bååth, E. (2003) 'Temperature-dependent changes in the soil bacterial community in limed and unlimed soil', *FEMS Microbiological Ecology*, 45, 13-21, available: https://doi.org/10.1016/S0168-6496(03)00106-5

Peyvast, G., Olfati, J.A., Madeni, S., Forghani, A. and Samizadeh, H. (2008) 'Vermicompost as a soil supplement to improve growth and yield of parsley', *Journal of Vegetable Science*, 14(1), 82-92.

Plaza, C., Sanz, R., Clemente, C., Fernández, .Jm., González, R., Polo, A. and Colmenarejo, M.F. (2007) 'Greenhouse evaluation of struvite and sludges from municipal wastewater treatment works as phosphorus sources for plants', *Journal of Agriculture and Food Chemistry*, 55(20), 8206-8212, available: https://doi.org/10.1021/jf071563y.

Rech, I., Withers, P.J.A., Jones, D.B..L and Pavinat P. (2019) 'Solubility, diffusion and crop uptake of phosphorus in three different', *Sustainability*, 11, 134-149, available: https://doi.org/10.3390/su11010134

Roy, E.D (2017) 'Phosphorus recovery and recycling with ecological engineering: A review', *Ecological Engineering*, 97, 213-277, available: https://doi.org/10.1016/j.ecoleng.2016.10.076

Sawicka, B., Krochmal-Marczak, B., Pszczółkowski, P., Bielińska, E.J., Wójcikowska-Kapusta, A., Barbaś, P. and Skiba, D. (2020) 'Effect of differentiated nitrogen fertilization on the enzymatic activity of the soil for sweet potato (Ipomoea batatas l. [Lam.]) cultivation', *Agronomy*, 10(12), 1970, available: https://doi.org/10.3390/agronomy10121970

Scherer, H.W., Metker, D.J. and Welp, G. (2011) 'Effect of long-term organic amendments on chemical and microbial properties of a luvisol' *Plant Soil and Environment*, 57, 513-518, available: https://doi.org/10.17221/3283-PSE

Siedliska, A., Baranowski, P., Pastuszka-Woźniak, J., Zubik, M. and Krzyszczak, J. (2021) 'Identification of plant leaf phosphorus content at different growth stages based on hyperspectral reflectance', *BMC Plant Biology*, 21, 28, available: https://doi.org/10.1186/s12870-020-02807-4

Szymańska, M., Sosulski, T., Bożętka, A., Dawidowicz, U., Wąs, A., Szara, E., Malak-Rawlikowska, A., Sulewski, P., Van Pruissen, G.W.P. and Cornelissen, R.L. (2020) 'Evaluating the struvite recovered from anaerobic digestate in a farm bio-refinery as a slow-release fertiliser', *Energies*, 13(20), 5342, available: https://doi.org/10.3390/en13205342

Szymanska, M., Szara, E., Was, A., Sosulski, T., Van Pruissen, G.W.P. and Cornelissen, R.L (2019) 'Struvite – an innovative fertilizer from anaerobic digestate produced in a bio-refinery' *Energies*, 12(2), 296, available: https://doi.org/10.3390/en12020296

Tarekegn, M.A.K.K. (2012) 'Effects of Rhizobium, nitrogen and phosphorus fertilizers on growth, nodulation, yield and yield attributes of soybean at pawe Northwestern Ethiopia', *WSN*, 67(2), 201-218.

TIBCO Software Inc. (2017). Statistica (Data Analysis Software System), Version 13.3.

Ticconi, C.A. and Abel, S. (2004) 'Short on phosphate: plant surveillance and countermeasures', *Trends in Plant Science*, 9, 548-55, available: https://doi.org/10.1016/j.tplants.2004.09.003

Tomassi, I.D., Chatterjee, N., Barrios-Masias, F.H, Zhou, Q., Gu, C. and Margenot, A.J (2021)'Arbuscular mycorrhizae increase biomass and nutrient uptake of tomato fertilized with struvite compared to monoammonium phosphate', *Plant Soil*, 464, 321-333, available: https://doi.org/10.1007/s11104-021-04957-2

Uysal, A., Yilmazel, Y.D. and Demire,R G. N. (2010) 'The determination of fertilizer quality of the formed struvite from effluent of a sewage sludge anaerobic digester', *Journal of Hazardous Materials*, 81(1-3), 248-54, available: https://doi.org/1016/j.jhazmat.2010.05.004.

Viégas, I. De J.M, Cordeiro, R.A.M., Almeida, G.M, De Silva D.A.S., Silva, B.C., Da Okumura, R.S, Da Silva S.P and Nunes De Freitas J.M. (2018) 'Growth and visual symptoms of nutrients deficiency in Mangosteens (Garcinia mangostana L.)', *American Journal of Plant Sciences*, 9, 1014-1028, available: https://doi.org/10.4236/ajps.2018.95078

Withers, P.J.A., Forber, K.G., Lyon, C., Rothwell, S., Doody, D.G, Jarvie, H.P., Martin-Ortega, J., Jacobs, B., Cordell, D., Patto, M., Camagro-Valero, M.A. and Cassidy, R. (2020) 'Towards resolving the phosphorus chaos created by food systems', *Ambio*, 49, 1076-1089, available: https://doi.org/10.1007/s13280-019-01255-1

Wojewódzki, P. and Cieścińska, B. (2012) 'Effect of crop rotation and long term fertilization on the carbon and glomalin content in the soil', *JCEA*, 13, 14-821, available: https://doi.org/10.5513/JCEA01/13.4.1134

Wolińska, A. (2010) 'Dehydrogenase activity of soil microorganisms and oxygen availability during reoxidation process of selected mineral soils from Poland', *Acta Agrophisica,* 3(180): 1-88.

Wright, S.F. and Anderson R. L. (2000) 'Aggregate stability and glomalin in alternative crop rotations for the central Great Plains', *Biology and Fertility of Soils*, 31, 249-253, available: https://doi.org/10.1007/s003740050653

Wright, S.F. and Upadhyaya, A (1999) 'Quantification of arbuscular mycorrhizal fungi activity by the glomalin concentration on hyphal traps' *Mycorrhiza*, 8, 283-285, available: https://doi.org/10.1007/s005720050247

Wu, F., Dong, M., Liu, Y., Ma, X., An, L. and Feng, H. (2011) 'Effects of long-term fertilization on AM fungal community structure and Glomalin-related soil protein in the Loess Plateau of China', *Plant Soil*, 342, 233-247, available: https://doi.org/10.1007/s11104-010-0688-4

Xue, A., Xiao, H.G., Qian, Z., Hui-Jun, Z., Hai-Ying, W., Zhao-Hui, M., Xiao-Ri, H., Ming-Hui, Z. and Fu,T X. (2014) 'Effect of phosphorus fertilization to P uptake and dry matter accumulation in soybean with different P', *Journal of Integrative Agriculture*, 13, 13(2), 326-334, available: https://doi.org/10.1016/S2095-3119(13)60390-1

Zabala, J.E (2020) 'Performance of soybean (Glycine Max L.) varieties in response to levels of phosphorus on lahar-laden condition', available at SSRN: https://ssrn.com/abstract=4134799 or https://doi.org/10.2139/ssrn.4134799

Zhang, H., Zhao, Q., Wang, Z., Wang, L., Li, X., Fan, Z., Zhang, Y., Li, J., Gao, X., Shi, J. and Chen, F. (2021) 'Effects of nitrogen fertilizer on photosynthetic characteristics, biomass, and yield of wheat under different shading conditions', *Agronomy*, 11(100), 1989, available: https://doi.org/10.3390/agronomy11101989

Zhang, X., Huang, G., Bian, X. and Zhao, Q. (2013) 'Effects of root interaction and nitrogen fertilization on the chlorophyll content, root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere', *Plant, Soil and Environment*, 59(2), 80-88, available: https://doi.org/10.17221/613/2012-PSE

Zucareli, C., Ramos, Júnior, E., Barreiro, A., Nakagawa, J. and Cavariani, C. (2006) 'Adubação fosfatada, componentes de produção, produtividade e qualidade fisiológica em sementes de feijão', *Revista Brasileira de Sementes*, 28, 9-15.