Co-fertilization of sulfur and struvite-phosphorus in a slow-release fertilizer improves soybean cultivation

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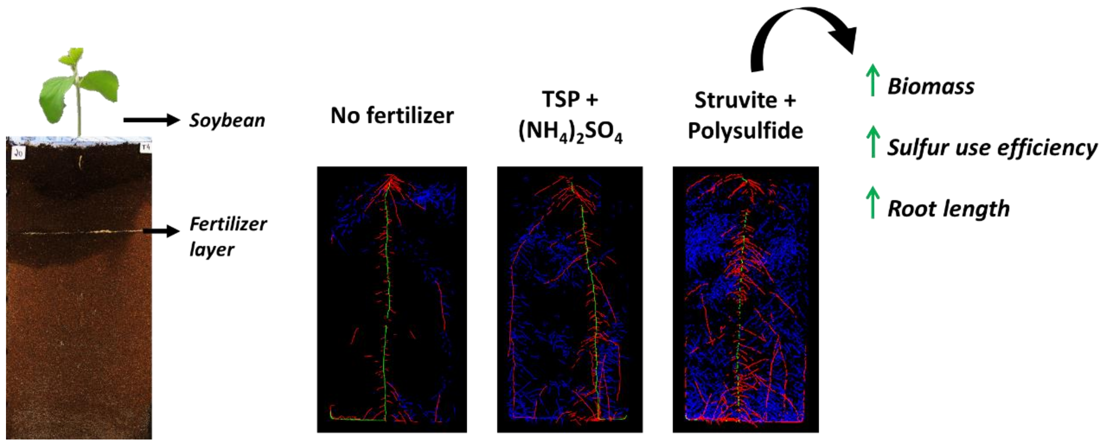
Abstract. In face of the alarming world population growth predictions and its threat to food security, the development of sustainable fertilizer alternatives is urgent. Moreover, fertilizer performance should be assessed not only in terms of yield but also root system development, as it impacts soil fertility and crop productivity. Fertilizers containing a polysulfide matrix (PS) with dispersed struvite (St) were studied for S and P nutrition due to their controlled-release behavior. Soybean cultivation with St/PS composites provided superior biomass compared to a reference of triple superphosphate (TSP) with ammonium sulfate (AS), with up to 3 and 10 times higher mass of shoots and roots, respectively. Additionally, St/PS achieved a 22% sulfur use efficiency against only 8% from TSP/AS. Root system architectural changes may explain these results, with higher proliferation of second order lateral roots in response to struvite ongoing P delivery. Overall, the composites showed great potential as efficient controlled-release fertilizers for enhanced soybean productivity.

Abstract Graphic.



1.Introduction

Phosphorus (P) is vital for plant nutrition and growth, and one of the most limiting elements for crop production. Agriculture represents nearly 90% of P use worldwide, yet, its current consumption rate has been unsustainable and incompatible with the element natural cycle, as phosphate rocks are non-renewable resources.[1–3] Moreover, the efficiency of P fertilizers is significantly restricted by soil immobilization processes of sorption and precipitation.[4] Conventional P fertilizers are readily soluble and thus release P faster than plants can uptake, contributing to soil fixation. These sources are also highly susceptible to runoff losses, causing eutrophication of water bodies and associated environmental damages.[5,6]

Sustainable solutions for phosphorus fertilization are, therefore, an urgent concern facing food security. Struvite ($MgNH_4PO_4 \cdot 6H_2O$) is a promising alternative, recovered from municipal wastewater streams, which could reduce the P cycle gap.[4,7–11] In addition, it serves as a source of nitrogen (N) and magnesium (Mg), essential macronutrients for plant development.[7,11] Moreover, struvite is considered as a slow-release fertilizer due to its low water solubility, which leads to reduced losses and a prolonged residual value to crops.[9] Nevertheless, low solubility may also result in an inadequate supply. Struvite dissolution can be significantly improved in acidic conditions and is highly affected by particle size, being much slower in granular form than as a powder.[12–15] For field application, however, fertilizers are usually managed as granules or pellets, which are easier for handling and storing.[16]

Therefore, by controlling local acidity and particle size, struvite can provide P fertilization more efficiently and safely. Recently, our research group accomplished both of these criteria with the development of fertilizer composites based on a polysulfide matrix containing dispersed ground struvite.[17] Matrices are strategic for getting around the particle size problem, as they can be processed as granules while, simultaneously, keeping small P particles from agglomerating.[18] At the same time, the matrix acts as a barrier, preventing a fast P delivery.[19] The studied polysulfide is an especially interesting material as it can provide sulfur to plants, an important macronutrient for plant growth that is frequently unavailable in agricultural soils.[20–22] The polysulfide structure contains polymeric sulfur chains, obtained by inverse vulcanization of elemental sulfur (S_8), a residue from oil industry.[23–27] For plant uptake, both the polysulfide and pure S_8 have to be oxidized in soil to sulfate, a slow rate process promoted by soil microorganisms.[28,29] The polysulfides from our previous studies displayed superior oxidation compared to S_8, especially when combined with struvite. Additionally, sulfate formation lowered the local pH, assisting struvite dissolution.[17,20]

Despite its potential as an environmentally friendly fertilizer, the struvite-polysulfide effect on plants is still unknown, and its dynamics in a soil-plant system should be further investigated. Most importantly, we were interested in understanding the fertilizer influence on root development and spatial distribution of roots in the growth medium, as an indicative of how the fertilizer can be accessed by plants. In the current work we investigated the effect of struvite-polysulfide fertilizers on nutrient uptake, biomass formation, and root system architecture. Soybean (*Glycine max* L.) was selected for the study, as a plant with high protein content and high S demand.[30,31] We hypothesized that soybean would respond differently to the struvite-polysulfide composites compared to a soluble reference, due to the controlled delivery of P. In addition, we hypothesized that the S chemical structure from the fertilizers would affect S supply and soybean root system traits, as polysulfides need to be biologically converted to sulfate.

2. Materials and Methods

2.1. Preparation of Composites

Composite fertilizers containing a polysulfide matrix and dispersed struvite particles were prepared as described by Valle et al. (2021),[17] illustrated in **Figure 1**. The polysulfide structure was obtained using the inverse vulcanization between elemental sulfur (S_8; Synth, Brazil) and soybean oil (Liza, Brazil), each at 50 wt%. This method is solvent-free and has no byproduct formation. The reaction was conducted in the presence of ground struvite (Ostara Crystal Green®, UK), with different mass ratios (25, 50, and 75 wt% of struvite in relation to the composite). All compounds were mixed in a flask, and the system was kept under constant agitation and heat, using a mechanical stirrer and oil bath. Temperature was kept at approximately 165ºC, allowing the ring-opening polymerization (ROP) of S_8, followed by the reaction between bi-radical polymeric sulfur chains and unsaturated bonds from soybean oil, until a light brown material was obtained.

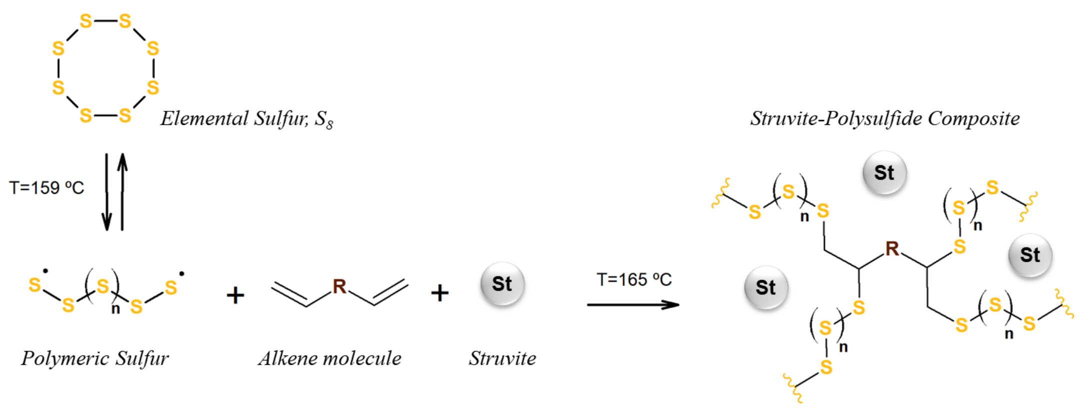


Figure 1. Preparation of the Struvite-Polysulfide fertilizer composite (generic structure). Elemental sulfur undergoes ROP and reacts with alkene molecules (in this work, soybean oil), in the presence of ground struvite, producing the polysulfide matrix with dispersed phosphate particles.

2.2. Greenhouse Experiment

To test the agronomic efficiency of the St/PS composite fertilizers and their effect on root and shoot soybean plant performance, an experiment was conducted at controlled greenhouse conditions at the Institute of Bio- and Geosciences, IBG-2: Plant Sciences, *Forschungszentrum Jülich GmbH*, Germany (50°54′36″N, 6°24′49″E), from May to July 2020. An average temperature of 23ºC and air humidity of 48% were maintained at the greenhouse over this period.

In order to evaluate the combined effect of struvite and the polysulfide, the following treatments were applied: no fertilizer (control); a positive reference with the highly soluble sources triple superphosphate for P and ammonium sulfate for S (TSP/AS); mixed pure struvite and elemental sulfur powder (St/S8); and ground fertilizer composites with different mass ratios of struvite and polysulfide – St 25/PS, St 50/PS, and St 75/PS (respectively with 25, 50, and 75 wt% of struvite). A fixed ratio of 50 g of S per kg of soil was established to all fertilized treatments. To achieve a P concentration of 200 mg per kg of soil, additional struvite was supplied with the composite treatments. Nitrogen was supplemented with ammonium nitrate in all fertilized treatments to complete 300 mg of N/kg of soil. Potassium, zinc, and copper were also supplemented to achieve concentrations of 200 mg/kg, 5 mg/kg, and 1.5 mg/kg, respectively, using a nutrient solution containing KCl, ZnCl, and $CuSO_4$. Detailed information on nutrient content and supply can be found in **Table S1**.

Peat substrate ("Nullerde", Einheitserde/Patzer Erden, Germany) was selected as growth medium due to an assumed high microbial activity of organic-rich environments, which is necessary to promote S oxidation. The substrate consisted of a mixture of 30% clay and 70% white peat, with no prior addition of fertilizers. Detailed substrate characterization can be seen in **Table S2**. Before the experiment, the substrate was shredded and sieved (< 0.7 cm) to remove coarse particles. Flat rhizotrons (60 x 30 x 2 cm)[32] were filled with 2 kg of substrate (approximately 3.36 dm^3), with 10 replicates per treatment. Fertilizers were added eight days before sowing, placed on a fixed layer at 40 cm from the bottom of the rhizotron (at approximately 16 cm from the substrate surface, 20 cm from the rhizotron top), as illustrated in **Figure 2a**. After completely filling up the rhizotrons, 100 mL of tap water was added to moisten the medium and allow initial solubilization of the fertilizers.

Soybean seeds (*Glycine max* L., Eiko cultivar; Asgrow, USA) were pre-germinated in Petri dishes with moistened filter paper. The Petri dishes were sealed and covered with aluminum foil, and kept incubated for 48 hours in the greenhouse. Seedlings with equal radical sizes were then selected and transplanted, one seedling per rhizotron. The seedlings were placed in a centralized position close to the transparent plate of the rhizotrons, at a depth of approximately 2 cm from the substrate surface. The rhizotrons were kept at 45º inclination in a fixed randomized position, with the transparent plates facing downwards, covered by black plastic sheets, as shown in **Figure 2b**.

The growth medium was moistened throughout the experiment with 100 mL water supply two times per week. All plants were treated against downy mildew contamination with Ortiva® (Syngenta, Germany), applied at 19 days from sowing. Images of the visible root system were recorded two to three times a week, along with measurements of the number of leaves and plant height. Harvest was conducted after 40 days of cultivation in the rhizotrons. Prior to shoot harvest, SPAD values were measured from trifoliate leaves at the uppermost node with a Chlorophyll Meter SPAD-502Plus (Konica Minolta). The growth medium and the roots were collected in layers, cut as illustrated in **Figure 2c**: A (top layer, between 0-10 cm depth), B (middle layer, between 10-30 cm depth), and C (bottom layer, below 30 cm depth). Roots were separated from the substrate samples with a sieve (9 x 5 mm mesh holes).

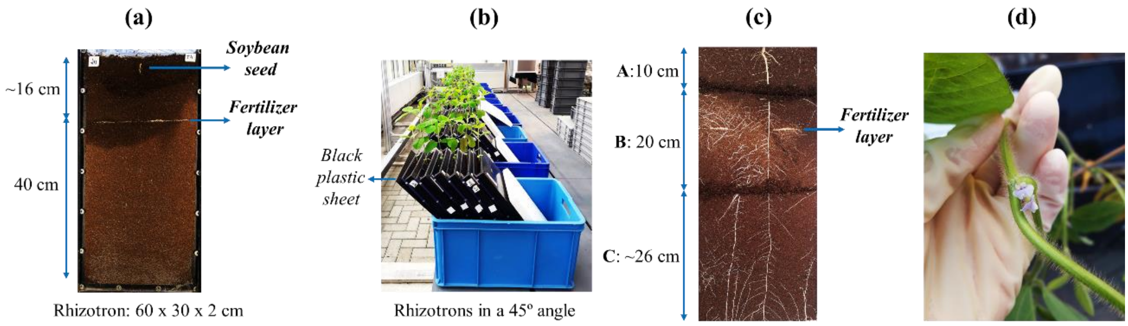


Figure 2. (a) Rhizotron with a fixed layer of fertilizer and pre-germinated soybean seedling; (b) Rhizotrons during cultivation; (c) Substrate and root sampling in layers A (top layer, 10 cm), B (middle layer, 20 cm, including the fertilizer layer), and C (bottom layer, ~26 cm); (d) Flower bloom 30 days after sowing.

2.3. Post-Harvest Analysis

After harvesting, leaf area was determined with a leaf area meter (LI-3100, LI-COR) and, subsequently, the shoots were dried in an oven at 60°C until constant weight to determine total dry biomass. Roots were immediately stored in flasks containing 50% v/v ethanol solution and kept in a dark cooling chamber at 4ºC until further analysis. Roots were carefully washed and scanned (Epson Expression 10000 XL) for measurements of total root length, average root diameter, and root surface area, using WinRHIZO Pro V.2009 2020a software, followed by drying in the same conditions as the shoots. Dry biomass of shoots and roots were measured, and shoot:root-ratio based on biomass was calculated.

Chemical analysis of the ground biomass was determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Thermo Scientific iCAP6500) for P, S, Mg, and K, and via CHN elemental analysis (Leco TCH 600) for N. Based on the elemental analysis results, N:S ratio was calculated. Sulfur and phosphorus use efficiency (SUE and PUE, respectively) were estimated using the following equations:[33]

$$Uptake\ (g/pot) = Shoot\ Biomass\ (g/pot) \times \frac{Nutrient\ Concentration\ (\%)}{100} \quad (1)$$

$$SUE\ (\%) = \frac{S\ uptake\ (fertilized) - S\ uptake\ (control)\ (g/pot)}{S\ applied\ (g/pot)} \times 100 \quad (2)$$

$$PUE\ (\%) = \frac{P\ uptake\ (fertilized) - P\ uptake\ (control)\ (g/pot)}{P\ applied\ (g/pot)} \times 100 \quad (3)$$

Homogenized substrate samples from each layer were analyzed to determine nutrient concentrations. Available S (in sulfate form) was extracted with mono-calcium phosphate and the concentration was determined turbidimetrically with an UV-Vis spectrophotometer (Femto 600plus).[34] Available P (phosphate in soil solution) was extracted with water and anionic resin, as proposed by Quaggio and Raij, and quantified in UV-Vis spectrophotometer (Femto 600plus).[35] Mg was extracted using a cationic resin and estimated with atomic absorption spectrophotometer (Perkin Elmer 2380). Nitrogen (total) was determined by CHN elemental analysis with a Perkin Elmer 2400 analyzer.

2.4. Rhizotron Image Analysis

Rhizotron images were analyzed using the software *GrowScreen-Root*, according to Nagel et al. (2012).[32] The roots were manually marked as primary roots or as first and second order lateral roots, labeled in green, red, or blue, respectively (**Figures S2-S7**). The length of each root type, total root length, root length density, root system depth (representing the maximal vertical distribution of a root system), and convex hull area (representing the surface area of a rhizotron covered by the whole root system) were determined.

2.5. Statistical Analysis

All results were submitted to one-way statistical analysis (ANOVA) with Tukey's test at the significance level $p < 0.05$.

3. Results and Discussion

Fertilizer composites with a controlled-release dynamic were obtained as sustainable alternatives to P and S fertilization, consisting of a polysulfide matrix (PS) as support to dispersed struvite particles (St). The fertilizers were produced with different mass ratios of each component, namely 25, 50, and 75 wt% of the phosphate source. The same materials were studied in a previous work from our group, displaying a controlled-release behavior for phosphate in citric acid solution and a synergistic dynamic between S and P in soil.[17] Sulfur is partially polymerized in the composite, with a fraction remaining unreacted as re-crystallized elemental sulfur (S_8).[17,20] Nevertheless, the achieved polysulfide formation sufficiently provides functionality to the material, as an easily processible matrix to support struvite. Chemical characterizations of the materials in Valle et al. (2021) also revealed that, during the preparation of the composites, struvite crystalline phase is converted to dittmarite ($Mg(NH_4)(PO_4)\cdot H_2O$), losing structural water.[17] This phase transition does not significantly impact the fertilizer's properties and, most importantly, it does not reduce the efficiency. Dittmarite has a similar P release profile to struvite, as it tends to rapidly re-hydrate when in solution, returning to struvite crystalline phase.[36] Dittmarite is more thermally stable than struvite, which could be favorable for processing purposes.[37] Moreover, dittmarite presents a higher nutrient concentration, which is more interesting for agronomic purposes.

Effect of different treatments on soybean development and root system architecture

Soybean was cultivated in rhizotrons with different sources of S and P over 40 days. Plants grown with no additional fertilizer (control treatment) remained relatively small and did not evolve significantly over time, unlike the fertilized treatments (**Figure S1**). It was possible to observe a rapid development after around 30 days of plant growth for TSP/AS, St/S8, and the St/PS composites, corresponding to the appearance of flowers (**Figure 2d**). As the reproductive stage starts, soybean tends to rapidly accumulate biomass to complete the vegetative development.[38]

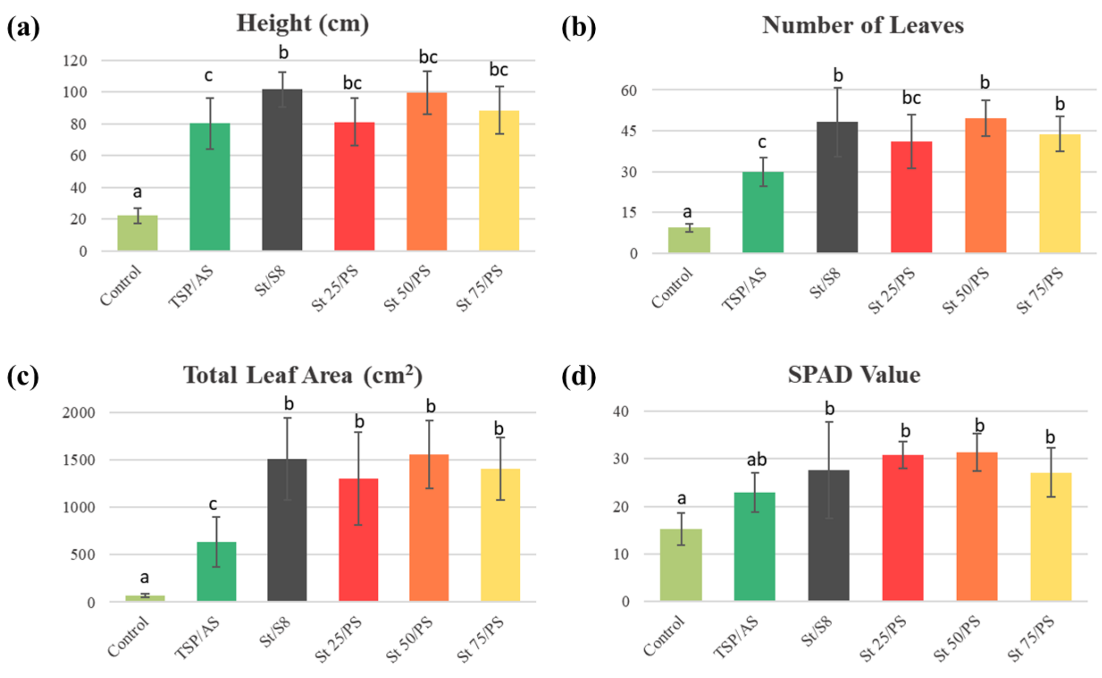


Figure 3. Average plant (a) height, (b) number of leaves, (c) total leaf area, and (d) SPAD value, measured before harvest, 40 days after sowing. Bars show mean values ± standard deviations. Indexes a, b, and c indicate significant differences between treatments ($p < 0.05$).

On the harvest day, measurements were carried out for the final plant height, number of leaves, total leaf area, and SPAD values (**Figure 3**). Plants under the unfertilized control achieved a significantly lower performance than the others in all measurements. It is interesting to notice that the treatments containing struvite (with S_8 or PS) were statistically superior to the positive control (TSP/AS), reaching more than double the leaf area, for instance. While TSP/AS featured on average 30 leaves per plant, St/S8 and St 50/PS displayed nearly 50 leaves. The SPAD values, which estimate the chlorophyll content of leaves, were less divergent among fertilized treatments, as expected by their development. The results indicate an increased development of soybean in the presence of struvite, demonstrating that phosphate can be efficiently provided to plants in this form. The results might also be related to the co-management of struvite with sulfur (in S^0 oxidation state) or to the additional Mg supply. Moreover, the relatively higher application of NH_4NO_3 with water-soluble sources in TSP/AS probably elevated soil salinity, which is limiting to plant growth.

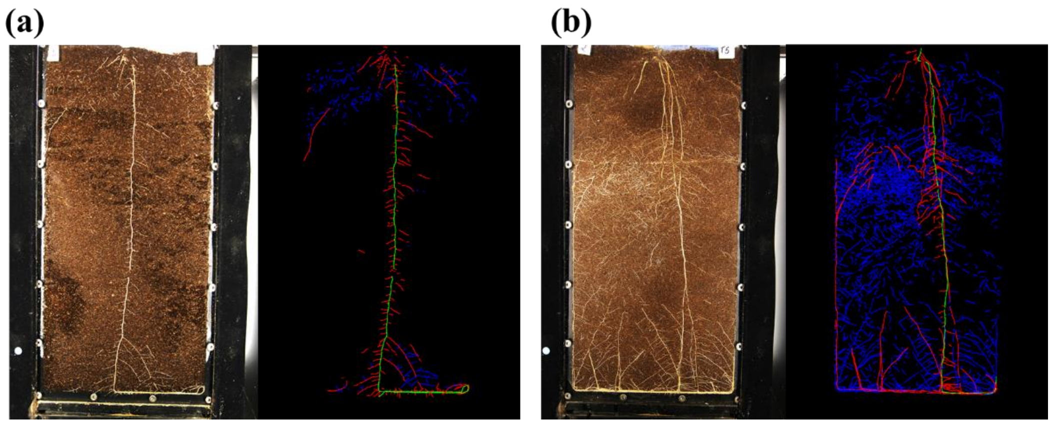


Figure 4. Original and analyzed color coded rhizotron images of (a) control with no fertilizer and (b) St 50/PS treatment, 40 days after sowing. Primary roots and first and second order lateral roots are represented by the colors green, red, and blue, respectively.

Root system architecture of unfertilized control plants strongly differed from the fertilized treatments, which presented pronounced second order lateral root development

(**Figure 4**). Representative rhizotron images of all treatments over time can be found in SI (**Figures S2-S7**). Plants that showed greater vegetative development (i.e., struvite treatments) also featured greater presence of thinner roots and a more homogeneous distribution throughout the substrate volume. It is known that lateral roots contribute the most to the absorption of water and nutrients by plants, due to their activity and capillarity in soil.

Visible root measurements from plants at 40 days of cultivation can be found in **Table S3**. While the final primary root length was similar among treatments, lateral root development was more affected by the fertilizer source. St 50/PS featured the largest first and second order lateral roots, with respectively 565 cm and 1400 cm, which were significantly superior to TSP/AS (368 cm and 549 cm, respectively) and the unfertilized control (203 and 202, respectively). Moreover, struvite treatments achieved in general higher total root length than TSP/AS and control.

Plant response to nutrient availability or deficiency can be indicated by the differences in growth and in spatial distribution of roots within the soil. In some plants, like common wallcress (*Arabidopsis thaliana*) and alfalfa (*Medicago sativa*), S deficiency has relatively little effect on root morphology and affects more negatively shoot biomass production, decreasing shoot:root ratio.[39,40] Nevertheless, soybean plants treated with S_8 in Zhao et al. (2008) displayed an increase in lateral roots compared to a control with no S supply.[30] Phosphorus effect on root system architecture patterns is often more species-dependent. Gruber et al. (2013) reported that *A. thaliana* plants present shallower and branched root systems under insufficient P, for instance.[40] According to López-Bucio et al. (2003), their root system senses and responds to P deprivation locally.[41] Robles-Aguilar et al. (2019) found that lupine (*Lupinus angustifolius* L.), a leguminous plant like soybean, increased primary root elongation in unfertilized treatments, compared to struvite fertilization.[42] On the other hand, in a study with soybean cultivation by Milton et al. (1991), P supply promoted an increase in total root length.[43] In Watt & Evans (2003), soybean produced more branched roots with P addition, which grew more concentrated around the area where the fertilizer was applied.[44]

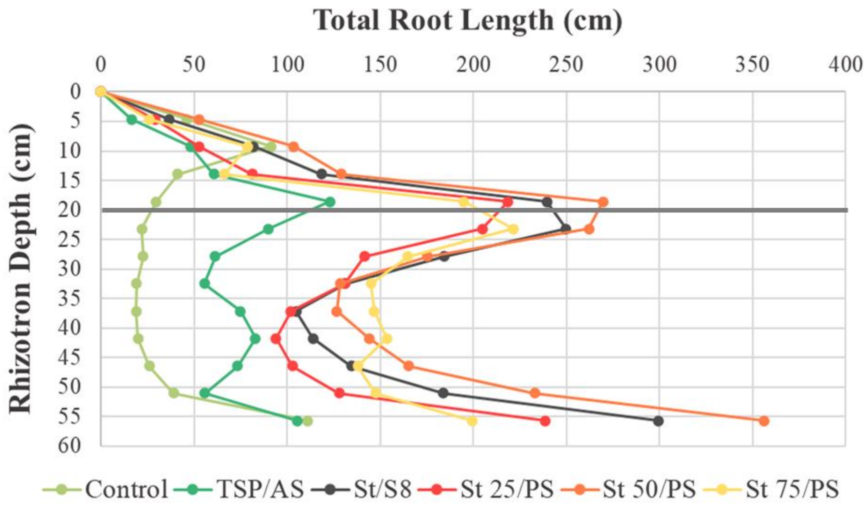


Figure 5. Effect of treatments on visible total root length. Trends of root length density over the rhizotron depth are shown at harvest time point (40 days after sowing). The applied fertilizer layer is at a depth of 20 cm from top (marked with the grey line).

Figure 5 illustrates the visible root length density profiles, indicating quite some variation in spatial root distributions across the different fertilizer treatments. A pronounced root development can be found in the region around the fertilizer layer (at 20 cm from the top), except for the unfertilized control, highlighting the relation between root growth and the presence of nutrients, also noticed by Watt & Evans (2003).[44] It should be noted that all treatments displayed an increased root length in the lowest 10 cm of the rhizotrons. Roots started to reach the bottom of the rhizotrons 10 days after sowing and, thereafter, an enhanced root development could be found along the bottom part of the rhizotrons as a consequence of the experimental design.

The lowest root length density is observed in the unfertilized control, compatible to its inferior shoot development. Unlike other treatments, the control presents a relatively larger root production closer to the substrate surface, which might be a response to P deficiency, as reported for *A. thaliana* plants.[40] Struvite treatments achieved a higher apparent root accumulation than TSP/AS over the rhizotron volume, especially composite St 50/PS. While the results clearly differed between struvite and TSP, plant behavior did not vary between S_8 and PS, indicating that soybean root distribution might be more strongly related to P supply than to the S source.

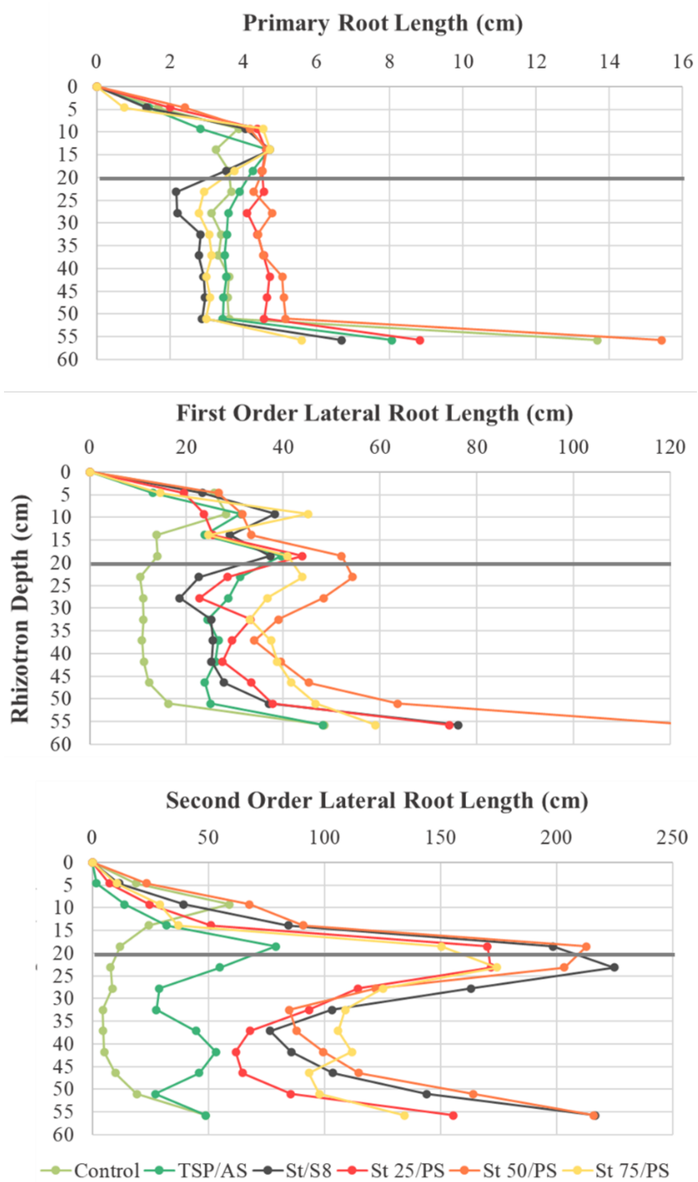


Figure 6. Effect of treatments on different root types: primary roots and first and second order lateral roots. Trends of root length density over the rhizotron depth are shown at harvest time point (40 days after sowing). The applied fertilizer layer is at a depth of 20 cm from top (marked with the grey line).

Root production around the fertilizer layer corresponded mainly to second order lateral roots, as can be seen in **Figure 6**. Primary root growth pattern was similar in all treatments, contributing less to the total root length density results. First order lateral roots showed a maximum around the fertilizer layer and a smaller peak of accumulation in the upper layer, probably from plant anchoring. Second order lateral roots occupied the largest volume of the rhizotron and could be found mainly in the fertilized region. The profiles were consistent with the data found in **Table S3**, with a superior second order lateral root production in struvite-treated plants than TSP/AS.

Watt & Evans (2003) correlated soybean's high development of thinner branched roots to plant P uptake. The continuous root growth across the soil volume allows the interception of labile P from soil solution before it becomes soil-bound.[44] The different outcomes from TSP and struvite treatments could be related to their distinct phosphate release profiles. TSP has a fast initial release of P and, therefore, phosphate was probably highly available during the first days of soybean cultivation, before undergoing immobilization processes in the substrate. In contrast, struvite is a slow-release fertilizer with an ongoing dissolution. Phosphate from struvite treatments is delivered more steadily, and may be accessed by roots over a longer period of time. The increased development of thinner lateral roots in struvite treatments, highly concentrated around the fertilizer layer, are strong indications that roots continued to grow and occupy the rhizotron as a response to phosphate prolonged delivery.

It is interesting to notice that St/S8 had a comparable second order lateral root length to St 50/PS, but its first order lateral root was inferior to all polysulfide treatments (**Table S3** and **Figure 6**). This could be related to the differences in S structure. Zhao et al. (2008) showed that S supply to soybean as S_8 not only increased lateral root development, but also the amount of soil microorganisms and enzyme activity.[30] Both PS and S_8 require biological activity to be oxidized to sulfate, and roots may contribute to this by releasing organic compounds that stimulate soil microorganisms.[45] Therefore, even though P supply appeared to contribute more significantly to soybean root system distribution, the S sources probably played a role in root traits as well.

The dynamic trend of root development over time revealed an increased rate of second order lateral root growth after 30 days of cultivation (**Figure S8**). This result goes along with the enhanced plant height and number of leaves at the same period of time (**Figure 3**), corresponding to soybean reproductive period. Trends of root system depth and convex hull area can be found in Supplementary Information (**Figure S9**).

Since rhizotron images only provide information regarding visible roots, the complete root systems were measured after harvest by washing and scanning the roots (**Table 1**). It should be noted that the data corresponds mostly to primary and first order lateral roots. The sampling method was not adequate to collect thinner roots, as a considerable portion of the second order lateral roots was not separated from the soil during sieving, hence not contributing to the root measurements. Following the same trend from rhizotron images, St 50/PS achieved the largest total root length (4291 cm) and root surface area (593 cm^2, **Table 1**). The lowest values, however, were from TSP/AS, instead of unfertilized control plants, which could be attributed to the loss of second order lateral roots, more prominent in the fertilized treatments (**Table S3**).

Control plants with no fertilizer displayed a smaller average root diameter than struvite treatments (**Table 1**), which goes along with the reduced root and shoot development and biomass accumulation. Root diameter was also analyzed in the three different layers (**Table S4**). The average root diameter of unfertilized control plants was constant in all layers (in the range of 0.33-0.35 mm). In contrast to the control, plants grown in fertilized treatments produced thicker roots in the top layer (top layer: 0.58-0.72 mm vs. bottom layer: 0.34-0.38 mm), possibly to support the higher biomass production. Plants under all treatments exhibited the highest proportion of roots in the root diameter class 0.2 and 0.3 mm (**Table S5**; around 30% of the total root length). In addition, plants treated with struvite had a high proportion of thicker roots (> 0.5 mm) which is less pronounced in control plants, reflecting the average results from **Table 1**. Nevertheless, thinner roots could be underestimated, especially in struvite treatments, which had a high second order lateral root development.

Table 1. Effect of treatment on average total root length, root diameter, and surface area. Indexes a and b signal significant differences between treatments ($p < 0.05$).

Root Measurements			
Treatment	Total Length (cm)	Diameter (mm)	Surface Area (cm^2)
Control	1592.2 *ab*	0.34 *a*	167.0 *a*
TSP/AS	982.2 *a*	0.42 *ab*	118.3 *a*
St/S8	1571.9 *ab*	0.50 *b*	215.4 *ab*
St 25/PS	1942.0 *ab*	0.48 *b*	256.1 *ab*
St 50/PS	4290.6 *b*	0.49 *b*	592.6 *b*
St 75/PS	3674.8 *ab*	0.48 *b*	481.5 *ab*

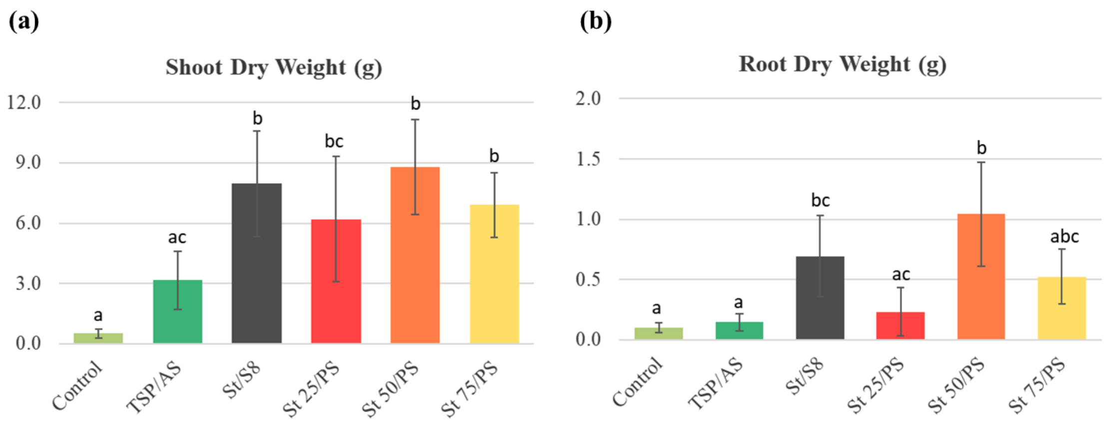


Figure 7. Effect of treatments on biomass from (a) shoots and (b) roots. For shoots, n=9 (Control and St 50/PS), n=8 (St/S8, St 25/PS, and St 75/PS), and n=7 (TSP/AS). For roots, n=6 (Control and St/S8) and n=5 (TSP/AS, St 25/PS, St 50/PS, and St 75/PS). Bars show mean values ± standard deviations. Indexes a, b, and c indicate significant differences between treatments ($p < 0.05$).

Dry biomass was measured both for shoots and roots (**Figure 7**). Shoot biomass was higher in treatments with struvite and significantly lower in the unfertilized control. Regarding root biomass, both plants under no fertilizer and TSP/AS treatments achieved inferior results. Plants treated with St 50/PS reached 10 times the root dry matter of TSP/AS grown plants, for instance. The fertilized treatments had comparable shoot:root ratios, superior to the unfertilized plants (**Figure S10**). The relation shows that plant biomass production was predominantly directed to shoot development when additional nutrients were supplied, indicating that struvite and polysulfide were able to properly provide P and S.

Soybean cultivation with the struvite-polysulfide composites not only displayed a significant biomass production, superior to the treatment with TSP and ammonium sulfate, but also a larger root proliferation. The intense root growth could be a response to the prolonged availability of phosphate due to struvite slow-release character.

Enhanced root growth can significantly benefit crop production, improving soil microstructure, soil porosity, and bulk density, among an overall enrichment of organic carbon in the soil. Most importantly, it implicates in an increased soil rhizosphere, with a more diverse microbial community and better nutrient mobility and bioavailability. In field conditions this is especially favorable, benefiting the following crop cultivations.

Nutrient availability and uptake

For a more accurate understanding of the relationship between plant development and the fertilizers, it is essential to determine the nutrient recovery, as well as P and S final concentrations in the substrate. The control plants with no fertilizer displayed a lower relative concentration of all elements in shoots compared to the other treatments, except for sulfur **(Table S6)**. Sulfur uptake by control plants was probably obtained from mineralization of organic S, promoted by enhanced root growth.[45] S plays a central role in the synthesis of proteins in plants, and also in symbiotic N_2 fixation, a process which soybean uses to assimilate nitrogen when this nutrient is deficient in soil.[46] However, nodule formation on roots was not observed, suggesting the unfertilized control plants did not fixate nitrogen. In addition, N uptake achieved by the control plant was critically low (0.74 wt%, **Table S6**), possibly due to low availability of N and other essential nutrients.[47,48] Furthermore, the results indicate P deficiency in the unfertilized treatment (**Table S6**). Triple superphosphate provided the highest relative P concentration in shoots (1.15 wt%), although it did not outperform the other fertilized treatments for other elements. Root elemental analysis of the complete root system and from the three rhizotron layers can be found in the SI (**Table S6-7**).

Table 2. Nutrient uptake efficiency parameters from plant biomass: average N:S ratio, sulfur use efficiency (SUE, %), and phosphorus use efficiency (PUE, %). Nutrient concentration in the substrate after soybean harvest: available phosphate (mg/dm^3), available sulfate (mg/dm^3), total nitrogen (mg/dm^3), and magnesium (mg/dm^3). Indexes a, b, c, and d indicate significant differences between treatments ($p < 0.05$).

	Nutrient Uptake Efficiency			**Nutrient Concentration in Soil**			
Treatment	N:S	SUE (%)	PUE (%)	P available (mg/dm^3)	S available (mg/dm^3)	N total (mg/dm^3)	Mg (mg/dm^3)
Control	2.2 *a*	-	-	16.5 *a*	14.3 *a*	2790.4 *a*	211.5 *ac*
TSP/AS	15.5 *b*	8.1 *a*	10.7 *a*	74.5 *b*	53.1 *b*	3949.5 *a*	177.9 *a*
St/S8	16.2 *b*	16.0 *ab*	11.4 *a*	95.7 *b*	37.4 *c*	3647.7 *a*	255.7 *b*
St 25/PS	15.2 *b*	11.8 *a*	11.5 *a*	85.5 *b*	39.4 *cd*	3128.7 *a*	232.0 *bc*
St 50/PS	15.8 *b*	22.0 *b*	14.1 *a*	93.9 *b*	51.3 *b*	2588.9 *a*	214.3 *ac*
St 75/PS	16.2 *b*	16.2 *ab*	13.6 *a*	86.4 *b*	47.7 *bd*	3125.9 *a*	241.8 *bc*

All fertilized treatments resulted in adequate N:S ratios (**Table 2**), essential for protein synthesis and for crop yields.[31] The control plants with no fertilizer presented a low N:S relation due to insufficient nitrogen uptake. The highest sulfur use efficiency (SUE) was achieved by St 50/PS (22%), while the lowest efficiency was from the soluble form TSP/AS (8%). Furthermore, the triple superphosphate treatment featured the lowest phosphorus use efficiency (PUE), although at $p < 0.05$ it was comparable to the other treatments. The results indicate an efficient S oxidation from the polysulfide and sufficient struvite solubilization.

The concentration of available phosphate in the rhizotron was statistically similar between the different fertilized treatments, ranging from 75 to 96 mg/dm^3 (**Table 2**). Considering that TSP/AS is readily soluble, this result indicates the immobilization or loss of P from this source, reducing the expected fertilizer efficiency. Struvite treatments, on the other hand, have a controlled-release behavior, and may have not fully solubilized up to that point. In a long-term assessment with ryegrass, Bogdan et al. (2021) found that significant struvite dissolution and phosphate release was only observed after four months of cultivation.[49]

In the unfertilized control, available P presented no distinction between the three soil layers (**Table S8**). This shows that phosphate mobilization from the substrate by root exudates occurred equally over the rhizotron profile, as root length was relatively similar in all layers of the unfertilized control. In contrast, the middle layer (B) from TSP/AS and struvite treatments featured a significantly higher available P concentration, ranging from 164 to 237 mg/dm^3, while values from the top and bottom layers (A and C) were closer to the unfertilized control (around 20 mg/dm^3). This result shows the typical low mobility and diffusion of phosphate, observed in agricultural soils in general. Furthermore, it is consistent with the assumption that root proliferation in the middle layer (**Figures 5 and 6**) was associated to struvite ongoing dissolution.

The highest available sulfur concentration in the substrate was from TSP/AS and St 50/PS, while St/S8 achieved the lowest (**Table 2**). Since phosphate presence tends to block soil SO_4^{2-} adsorption sites, this explains why sulfate from the soluble source (AS) remains highly available.[22] The results also reveal that S oxidation into sulfate was more effective from the composites with higher PS content (St 50/PS and St 75/PS) than from S_8, which is compatible to the hypothesis that S_8 and PS different S forms could have altered effects on the substrate microbial activity and plant growth dynamics. Sulfate concentration in the unfertilized control indicates S mineralization by root exudates, as discussed in the shoot recovery results. Contrary to phosphate, the middle and bottom layers have similar soil S contents (**Table S8**), indicating sulfate had a better transportation over the substrate depth.

High N values reveal a low incidence of N volatilization and high organic N content (**Table 2**). St/S_8 achieved a superior Mg concentration by the end, which was expected from struvite composition. The other treatments displayed significant Mg concentrations, including the unfertilized control and TSP/AS, indicating a great mobilization from the organic fraction of the substrate. Moreover, this suggests Mg content in struvite was not decisive for the better performance and vegetative development of St/S_8 and St/PS treatments. Based on these results, the lower Mg and N uptake by the unfertilized control plant was mostly related to insufficient P on the substrate.

4.Conclusion

The elucidation of plant-soil dynamics and roots growth patterns under struvite-polysulfide fertilization is important to understand and validate the agronomic efficiency of this new class of controlled-release fertilizers. Hence, sustainable fertilizers with a polysulfide matrix and dispersed struvite (containing 25, 50, or 75 wt% of struvite) were prepared, using the simple and green method of inverse vulcanization. The effect of P and S supply from this system on soybean cultivation was compared both to the co-management of soluble commercial sources (TSP and $(NH_4)_2SO_4$) and to pure struvite mixed with S_8. The results revealed a superior performance due to the combined application of struvite with S^0 sources (polysulfide or S_8), featuring a significantly higher biomass production than TSP/$(NH_4)_2SO_4$ treatment. Struvite achieved a similar phosphorus use efficiency as the TSP reference, proving its controlled-release behavior can properly provide P to plants in the studied conditions. The composite St 50/PS displayed the greatest sulfur use efficiency, superior to the fine particles from S_8 powder and to ammonium sulfate, which reached the lowest SUE. Root system architecture analysis using rhizotrons revealed an intense accumulation of second order lateral roots around the fertilizer layer, especially in struvite treatments. The higher development of thinner roots was attributed to the controlled-release and continuous availability of phosphate from struvite, in contrast to TSP quick solubilization and P losses. Although root traits were more significantly influenced by the P source, differences in first order lateral root lengths from PS and S_8 could be related to the S structure and its influence in the local microbial activity. The final concentration of sulfate in the growth medium also indicated a superior oxidation of S from the polysulfide than S_8. In summary, the controlled-release struvite-polysulfide composites proved to be efficient fertilizer alternatives to soluble commercial sources, and beneficial to soybean development.

ASSOCIATED CONTENT

Supporting Information. Fertilizers contents, substrate characterization, rhizotron images over time, and data from root and substrate measurements in layers (PDF).

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References

1. Cordell, D., Drangert, J. & White, S. The story of phosphorus: Global food security and food for thought. *Glob. Environ. Chang.* **19**, 292–305 (2009).
2. Chowdhury, R. B., Moore, G. A., Weatherley, A. J. & Arora, M. Key sustainability challenges for the global phosphorus resource, their implications for global food security, and options for mitigation. *J. Clean. Prod.* **140**, 945–963 (2017).
3. Scholz, R. W., Ulrich, A. E., Eilittä, M. & Roy, A. Sustainable use of phosphorus: A finite resource. *Sci. Total Environ.* **461**–**462**, 799–803 (2013).
4. Rech, I., Withers, P. J. A., Jones, D. L. & Pavinato, P. S. Solubility, diffusion and crop uptake of phosphorus in three different struvites. *Sustain.* **11**, (2018).
5. Chien, S. H., Prochnow, L. I., Tu, S. & Snyder, C. S. Agronomic and environmental aspects of phosphate fertilizers varying in source and solubility: An update review. *Nutr. Cycl. Agroecosystems* **89**, 229–255 (2011).
6. International Plant Nutrition Institute (IPNI). *Better Crops With Plant Food*. **103**, (2019).

7. Rahman, M. *et al.* Production of slow release crystal fertilizer from wastewaters through struvite crystallization – A review. *Arab. J. Chem.* **7**, 139–155 (2014).
8. Yetilmezsoy, K., Ilhan, F., Kocak, E. & Akbin, H. M. Feasibility of struvite recovery process for fertilizer industry: A study of financial and economic analysis. *J. Clean. Prod.* **152**, 88–102 (2017).
9. Talboys, P. J. *et al.* Struvite: a slow-release fertiliser for sustainable phosphorus management? *Plant Soil* **401**, 109–123 (2016).
10. Mehta, C. M., Hunter, M. N., Leong, G. & Batstone, D. J. The Value of Wastewater Derived Struvite as a Source of Phosphorus Fertilizer. *Clean - Soil, Air, Water* **46**, (2018).
11. Kataki, S., West, H., Clarke, M. & Baruah, D. C. Phosphorus recovery as struvite : Recent concerns for use of seed , alternative Mg source , nitrogen conservation and fertilizer potential. *Resour. Conserv. Recycl.* **107**, 142–156 (2016).
12. Tansel, B. & Monje, O. Struvite formation and decomposition characteristics for ammonia and phosphorus recovery: A review of magnesium-ammonia- phosphate interactions. *Chemosphere* **194**, 504–514 (2018).
13. Hertzberger, A. J., Cusick, R. D. & Margenot, A. J. A review and meta-analysis of the agricultural potential of struvite as a phosphorus fertilizer. *Soil Sci. Soc. Am. J.* **84**, 653–671 (2020).
14. Robles-Aguilar, A. A. *et al.* The effect of pH on morphological and physiological root traits of Lupinus angustifolius treated with struvite as a recycled phosphorus source. *Plant Soil* **434**, 65–78 (2019).
15. Degryse, F., Baird, R., Silva, R. C. & Mclaughlin, M. J. Dissolution rate and agronomic effectiveness of struvite fertilizers – effect of soil pH, granulation and base excess. *Plant Soil* 139–152 (2017). doi:10.1007/s11104-016-2990-2

16. Giroto, A. S. *et al.* Tailoring efficient materials for NPK all-in-one granular fertilization. *Ind. Eng. Chem. Res.* **59**, 18387–18395 (2020).

17. Valle, S. F. do, Giroto, A. S., Reis, H. P. G., Guimaraes, G. G. F. & Ribeiro, C. Synergy of Phosphate-Controlled Release and Sulfur Oxidation in Novel Polysulfide Composites for Sustainable Fertilization. *J. Agric. Food Chem.* **69**, 2392–2402 (2021).

18. Ribeiro, C. & Carmo, M. Why nonconventional materials are answers for sustainable agriculture. *MRS Energy Sustain. A Rev. J.* **6**, 1–15 (2019).

19. Mann, M. *et al.* Sulfur polymer composites as controlled-release fertilisers. *Org. Biomol. Chem.* **17**, 1929–1936 (2019).

20. Valle, S. F., Giroto, A. S., Klaic, R., Guimarães, G. G. F. & Ribeiro, C. Sulfur fertilizer based on inverse vulcanization process with soybean oil. *Polym. Degrad. Stab.* **162**, 102–105 (2019).

21. Lucheta, A. R. & Lambais, M. R. Sulfur in Agriculture. *Rev. Bras. Ciência do Solo* **36**, 1369–1379 (2012).

22. Scherer, H. W. Sulphur in crop production. *Eur. J. Agron.* **14**, 81–111 (2001).

23. Chung, W. J. *et al.* The use of elemental sulfur as an alternative feedstock for polymeric materials. *Nat. Chem.* **5**, 518–524 (2013).

24. Chalker, J. M., Worthington, M. J. H., Lundquist, N. A. & Esdaile, L. J. Synthesis and Applications of Polymers Made by Inverse Vulcanization. *Top. Curr. Chem.* **377**, 1–27 (2019).

25. Abbasi, A., Nasef, M. M. & Yahya, W. Z. N. Copolymerization of vegetable oils and bio-based monomers with elemental sulfur: A new promising route for bio-based polymers. *Sustain. Chem. Pharm.* **13**, 100158 (2019).

26. Zhang, Y., Glass, R. S., Char, K. & Pyun, J. Recent advances in the polymerization

of elemental sulphur, inverse vulcanization and methods to obtain functional Chalcogenide Hybrid Inorganic/Organic Polymers (CHIPs). *Polym. Chem.* **10**, 4078–4105 (2019).

27. Park, K. W. & Leitao, E. M. The link to polysulfides and their applications. *Chem. Commun.* **57**, 3190–3202 (2021).

28. Germida, J. J. & Janzen, H. H. Factors affecting the oxidation of elemental sulfur in soils. *Fertil. Res.* **35**, 101–114 (1993).

29. Degryse, F., Ajiboye, B., Baird, R., Silva, R. C. & Mclaughlin, M. J. Oxidation of Elemental Sulfur in Granular Fertilizers Depends on the Soil-Exposed Surface Area. *Soil Sci. Soc. Am. J.* **80**, 294–305 (2016).

30. Zhao, Y., Xiao, X., Bi, D. & Hu, F. Effects of sulfur fertilization on soybean root and leaf traits, and soil microbial activity. *J. Plant Nutr.* **31**, 473–483 (2008).

31. Ibañez, T. B. *et al.* Sulfur modulates yield and storage proteins in soybean grains. *Sci. Agric.* **78**, 1–9 (2020).

32. Nagel, K. A. *et al.* GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous measurements of root and shoot growth for plants grown in soil-filled rhizotrons. *Funct. Plant Biol.* **39**, 891–904 (2012).

33. Chowdhury, M. A. H. *et al.* Sulphur fertilization enhanced yield, its uptake, use efficiency and economic returns of Aloe vera L. *Heliyon* **6**, e05726 (2020).

34. Raij, B. V., Andrade, J. C. DE, Cantarella, H. & Quaggio, J. A. *Análise Química para Avaliação da Fertilidade de Solos Tropicais*. (2001).

35. van Raij, B., Quaggio, J. A. & da Silva, N. M. Extraction of phosphorus, potassium, calcium, and magnesium from soils by an ion-exchange resin procedure. *Commun. Soil Sci. Plant Anal.* **17**, 547–566 (1986).

36. Massey, M. S., Davis, J. G., Ippolito, J. A. & Sheffield, R. E. Effectiveness of

recovered magnesium phosphates as fertilizers in neutral and slightly alkaline soils. *Agron. J.* **101**, 323–329 (2009).

37. Farhana, S. Thermal Decomposition Of Struvite: A Novel Approach To Recover Ammonia From Wastewater Using Struvite Decomposition Products. **Thesis**, (2015).

38. McWilliams, D. A., Berglund, D. R. & Endres, G. J. Soybean Growth and Management. *NDSu Extension Circular* 1–8 (1999).

39. Wang, Y. F. *et al.* Effects of sulphur supply on the morphology of shoots and roots of alfalfa (Medicago sativa L.). *Grass Forage Sci.* **58**, 160–167 (2003).

40. Gruber, B. D., Giehl, R. F. H., Friedel, S. & von Wirén, N. Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant Physiol.* **163**, 161–179 (2013).

41. López-Bucio, J., Cruz-Ramírez, A. & Herrera-Estrella, L. The role of nutrient availability in regulating root architecture. *Curr. Opin. Plant Biol.* **6**, 280–287 (2003).

42. Robles-Aguilar, A. A., Schrey, S. D., Postma, J. A., Temperton, V. M. & Jablonowski, N. D. Phosphorus uptake from struvite is modulated by the nitrogen form applied. *J. Plant Nutr. Soil Sci.* **183**, 80–90 (2020).

43. Milton, N. M., Eiswerth, B. A. & Ager, C. M. Effect of phosphorus deficiency on spectral reflectance and morphology of soybean plants. *Remote Sens. Environ.* **36**, 121–127 (1991).

44. Watt, M. & Evans, J. R. Phosphorus acquisition from soil by white lupin (Lupinus albus L.) and soybean (Glycine max L.), species with contrasting root development. *Plant Soil* **248**, 271–283 (2003).

45. van Veelen, A. *et al.* Root-induced soil deformation influences Fe, S and P:

rhizosphere chemistry investigated using synchrotron XRF and XANES. *New Phytol.* **225**, 1476–1490 (2020).

46. Becana, M., Wienkoop, S. & Matamoros, M. A. Sulfur transport and metabolism in legume root nodules. *Front. Plant Sci.* **9**, 1–10 (2018).

47. Robles-Aguilar, A. A. *et al.* Effect of Applying Struvite and Organic N as Recovered Fertilizers on the Rhizosphere Dynamics and Cultivation of Lupine (Lupinus angustifolius). *Front. Plant Sci.* **11**, 1–17 (2020).

48. IFA, WFO & GACSA. Nutrient Management Handbook. (2016).

49. Bogdan, A. *et al.* Impact of time and phosphorus application rate on phosphorus bioavailability and efficiency of secondary fertilizers recovered from municipal wastewater. *Chemosphere* **282**, 2–11 (2021).