Phosphorus uptake from struvite is modulated by the nitrogen form applied

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Abstract

Next to nitrogen, phosphorus (P) is the most limiting nutrient for plant production worldwide. To secure food production, new nutrient management strategies using alternative P sources instead of mined P fertilizers need to be implemented. Struvite (MgNH₄PO₄ · 6 H₂O) is a promising example of a recycled mineral P fertilizer. Besides positive agronomic results regarding crop yields, further investigations are required to improve the use efficiency of the product and thereby increase its value. Using an automated plant phenotyping platform, we investigated the dynamic response to struvite by two plant species (lupine and maize) with diverse P acquisition strategies in an acidic sandy substrate. Although at three weeks after germination both maize and lupine had reduced leaf area in the struvite treatments compared to the commercial triple superphosphate (TSP), from week four onwards struvite plants grew larger than the TSP-treated plants, indicating a slow release fertilizing effect. Greater P uptake efficiency (g / root length), but reduced root length were observed in the combined treatment of struvite and ammonium, in comparison to struvite and nitrate. We propose that rhizosphere acidification in response to ammonium uptake may enhance P recovery from struvite. A possible additional acidification effect by lupine root exudation might explain the higher P uptake efficiency in this species compared to maize. We conclude that struvite combined with ammonium can be used as a sustainable slow-release P fertilizer on acidic sandy soils.

Key words: ammonium / nitrate / recycled phosphorus / root morphology modification / slow-release fertilizer / struvite

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1 Introduction

Phosphorus (P) is an essential macro-element that plays a key role in many essential plant processes (Missoum et al., 2005; Jouhet et al., 2007). Consequently, the growing demand for food and feed production resulted in the need to apply P in the form of fertilizers (Vance et al., 2003). The main source of P comes from rock phosphate mines, a non-renewable resource predicted to be depleted within a few hundred years (Fixen and Johnston, 2012).

To foster a more sustainable use of P, the European Union has recently proposed to implement different strategies, including the recovery of P from waste (Bonvini et al., 2015; Stutter et al., 2015; Witters et al., 2015). A promising example is struvite (MgNH₄PO₄ · 6 H₂O) that can be recovered from wastewater (Batstone et al., 2015) or manure (Zarebska et al., 2015). As reviewed by Zarebska et al. (2015), numerous experiments have examined which technologies are the most efficient for struvite recovery regarding the source of input. Within the larger question to which extent struvite can replace unsustainable rock phosphate-based fertilizers, less research has been conducted on how to apply struvite most efficiently as a fertilizer and how well plants can metabolize it.

Struvite solubility is low in water. Nevertheless, it is generally accepted that struvite is a good candidate to be used as a P source for plants. As reviewed by Kataki et al. (2016), struvite fertilization results in similar crop yields as mineral fertilization on different plant species such as Lolium perenne (Johnston and Richards, 2003), Zea mays (Antonini et al., 2012) or Triticum aestivum (Massey et al., 2009). Furthermore, struvite has been proposed as a slow-release fertilizer, with a steady nutrient supply for plants (Li and Zhao, 2003; Antonini et al., 2012). Most of these studies show comparable or even higher effectiveness of struvite than the water-soluble fertilizers at the end of the experiments (Möller et al., 2018).

If we assume that struvite releases P slower than highly soluble fertilizers, low P solubility might be observed in the early plant growth stage after using struvite as fertilizer. The low P solubility can be overcome by some plant species with specif-
ic root traits to enhance P acquisition (Clarkson, 1981; Talboys et al., 2016). Species can differ strongly in their P acquisition strategies, as reflected in their differences in various root traits (Lynch, 1995; Abdolzadeh et al., 2009). Here we compare Zea mays (maize) and Lupinus angustifolius (narrow-leaf lupine), which differ in plant root physiology and morphology. Narrow-leaf lupine has high physiological root plasticity, related to exudation of large amounts of organic acids (Pang et al., 2010a, 2010b). Besides that, roots of nitrogen-fixing legumes like lupine further acidify the rhizosphere, enhancing P mobilization in soil and additional subsequent P acquisition (Hinsinger, 2001). Contrary to lupine, maize is known to exhibit extensive root morphological plasticity in response to P fertilization, but does not seem to rely on carboxylate exudation for mobilizing insoluble P (Wen et al., 2017). To allow us to investigate the struvite-P uptake on low exudative and non-nitrogen fixing species, maize was used as a control.

Studying possible effects of the applied P fertilizers on the root morphology should help to identify those traits that are related to an increase in P uptake. As P concentrations can act as growth regulators, the applied P sources (that might have different phosphorus release rates into the rhizosphere) can significantly alter root system architecture. For example, it was previously shown that root morphological responses of maize (i.e., specific root length and proportion of fine roots) increased with decreasing shoot P concentration (Wen et al., 2017). Similarly, the nitrogen source applied can also act as a growth regulator. Sattelmacher et al. (1993) reported ammonium concentration-dependent stimulatory effects on root growth. In addition, the nutrient source applied can change the rhizosphere pH (Gahoonia et al., 1992), which might also influence root morphology as previously described for the total root length of Lupinus angustifolius (Tang et al., 1992; Robson and Tang, 1998).

Few studies have addressed these multiple interactions between struvite and other fertilizers on plant growth (Talboys et al., 2016). In the present study, we analyzed the effect of the N and P sources applied on root morphology and P uptake. Struvite decomposition is a proton consuming process that increases the soil pH. Therefore, we tested if the assumed acidification in the rhizosphere induced by the application of ammonium could neutralize this pH increase and facilitate the struvite solubility. We hypothesized that the application of N in the form of ammonium rather than nitrate will increase P uptake when applied together with the struvite.

In order to confirm the suggested positive effects of the slow P and N release from struvite on plant performance, an alternative approach rather than only chemical analyses is now necessary. Due to major progress in non-invasive shoot phenotyping achieved with imaging sensors (Fiorani and Schurr, 2013), we could investigate plant leaf area accumulation during the growth period using an automated shoot-imaging platform and dynamically observe the effect of the slow vs. quick P release at different plant growth stages.

We performed a greenhouse study, subdivided into experiment A and B to determine the P fertilizing effect of struvite. Maize and lupine were investigated using an acidic, nutrient poor sandy substrate (hereafter referred to as a “marginal substrate”), where struvite is more soluble than in alkaline or neutral substrates. In experiment A we investigated: (1) struvite P fertilizing effect on plant biomass; (2) effect of N source applied on root architecture; (3) whether the nitrogen source applied modified the P uptake efficiency from struvite, described as the P uptake per unit of root length, and whether it might be plant species dependent. In experiment B we investigated: (1) the continuous effect of struvite as a slow-release fertilizer on plant biomass in lupine and maize; (2) if, besides the known exudation capacity, lupine also relies on additional changes in root architecture to increase P uptake efficiency from struvite in comparison with TSP, and whether it might be modified by applying nitrogen as NH₄⁺ or NO₃⁻.

With this study we are aiming to first, identify those root traits that are related to an increase in struvite-P uptake efficiency, and second, analyze the fertilizing effects of struvite over time. We hypothesize that: (1) P uptake per unit root length from lupine and maize will be similar; however, the morphological root changes in maize in response to ammonium will be greater than in lupine that rely more on other strategies such as exudation of carboxylates; (2) we further hypothesize that lupine is a more suitable crop to use when fertilized with struvite-P together with ammonium, comparable with the effect of the highly available TSP, that will not be affected by the N source jointly applied. Once we understand to what extent species and growth conditions influence P recovery from struvite used as a fertilizer, proper management advice for its application can be provided.

2 Material and methods

2.1 Experimental set-up

Experiment A followed a three-factorial (P-fertilizer, N-fertilizer, and plant species) completely randomized design with 10 replications. Fertilizer treatments comprised of struvite and an unfertilized control (No P), and each of them was combined with either ammonium or nitrate as the N form applied. The two different plant species in this study were lupine (Lupinus angustifolius L. subsp. angustifolius, cultivar: blau "Boregine", Kiepenkerl, Germany) and maize (Zea mays, “Badischer Gelber” Kiepenkerl, Germany). Lupine and maize plants were grown during six weeks in 3.5-L pots and were continuously monitored in the automatic shoot phenotyping platform ScreenHouse (Nakhforoosh et al., 2016) at controlled greenhouse conditions at the Forschungszentrum in Jülich, IBG-2: Plant Sciences, Germany (50.89942°N 6.39211°E).

Experiment B was conducted subsequently and followed Experiment A with minor modifications in which the No P treatment was replaced with a positive control: fertilization with highly available triple superphosphate (TSP). In Experiment B, the number of replicates was reduced to five based on the level of variability we found in Experiment A. Lupine and maize plants were grown for a period of approximately eight weeks.
2.2 Phosphorus sources, fertilization, and substrate used

The struvite used in this study was provided by Lequia (Girona, Spain). It was recovered from pig manure after anaerobic digestion and solid–liquid separation before biological nitrogen removal. The struvite contained 131.7 mg g⁻¹ P, 5.15 mg g⁻¹ N, and 10.5 mg g⁻¹ Mg; particle size: 113 μm. The product used in this study was previously characterized by X-ray diffraction (XRD) analysis as pure struvite crystals, showing no presence of amorphous substances, contrasted with optic microscope observations, as previously described (Tarragó et al., 2016). For the positive control, we used the highly soluble mineral P fertilizer triple superphosphate (TSP), containing 45% P₂O₅, equaling 197 g kg⁻¹ P (Van Loon Hoeven, The Netherlands).

The different P sources were applied to the marginal sandy substrate and were thoroughly mixed in an end-over-end mixer for 10 min to allow an even distribution of the fertilizers. The recycled P as solid struvite powder or highly soluble mineral P in the form of solid powder TSP were applied at a rate equivalent to 36 mg P per plant (7.2 mg kg⁻¹ substrate) due to short growth period. The fertilized sand was left undisturbed in containers for three days before setting up the experiment allowing for equilibration and nutrient distribution. Pots with no addition of extra P were set up as the control (No P). Subsequently, each pot (3.5-L volume) was filled with 5 kg of the mixed sand.

Nutrients other than P were supplied through fertigation. As the application of struvite accounted for 8 mg of ammonium, the amount of N applied through fertigation was reduced for the application of struvite accounted for 8 mg of ammonium, ensuring an equal total N supply in the substrate. The final N concentration of 72 mg kg⁻¹ dry substrate. During the time of final harvest, i.e., 40 d after sowing in experiment A and 50 d after sowing in experiment B, the heights of the plants were measured. Shoots were separated into leaves and stems directly after harvesting, and fresh weights were determined. Subsequently, the leaf area was measured using a leaf area meter (Li-3100, Li-cor, Nebraska, USA). Rolled up maize blades were unwound before scanning, but leaf sheaths were considered as stems.

All root samples were carefully washed to remove any attached substrate. The washed root samples were stored in 50% (v/v) ethanol/water solution until root scanning (Epson Expression Scan 1680, WinRHIZO STD 1680, Long Beach, Canada). Data for several root traits, such as total root length, root surface area, root diameter, and diameter class length (DCL, root length within a diameter class), were obtained using WinRHIZO V.2009 software (Regent Instruments Inc., Quebec, Canada). In experiment A, roots of lupine and maize were analyzed. In experiment B, only lupine roots were analyzed. The root length was partitioned into 11 diameter classes: from < 0.25 to > 3.5 in 0.5-mm increments from root images for each root section. The following parameters were based on observed and/or computed data: root-to-shoot mass ratio (root dry mass / shoot dry mass), specific root length (SRL) = root length / root dry mass (g g⁻¹), and relative diameter class length (rDCL) = DCL / root length (yielding a proportion of root length to normalize disparity between plants of different sizes).

2.3 Plant growing conditions

Plants were grown under natural light during the day; additional assimilation lighting was supplied by mercury lamps (SON–T AGRO 400, Phillips) whenever natural light intensity was below 400 μmol s⁻¹ m⁻², providing a total daily light period of 16 h. Average temperature during the experiment was 19°C during the day and 17°C at night, with a relative humidity of 60% during the day and 50% at night. All seeds were pre-germinated on filter paper at staggered times between the two species according to their pre-determined germination time. Two seedlings of each species were transplanted into each pot at a depth of 2 cm. After one week one seedling was removed. A water system was established in both setups (custom-made, Hellmuth Bahrs GmbH & Co. KG, Brüggen-Bracht, Germany) to maintain the substrate water content at 50% of its water holding capacity calculated by pot weight every second day. Plants were harvested after 40 d of growth in Experiment A and 50 d of growth in Experiment B. Automated randomization of pot position was conducted three times per week.

2.4 Data collection on plant performance, nutrient uptake, and pH

For each plant, data of plant growth based on projected leaf area were recorded three times per week non-invasively during the whole experiment using the automatic shoot phenotyping platform ScreenHouse [for detailed description see Nakhtoroosh et al. (2016)].
After the shoot and root measurements, all samples were dried at 65°C in a forced-draft oven until dry weights were constant and subsequently homogeneously ground. Hundred mg of the powdered and homogenized plant sample were digested with 3 mL HNO₃ and 2 mL H₂O₂ in the microwave and made up to a total volume of 14 mL. The samples were then analyzed for total Mg, P, and K contents by ICP-OES. The N content was determined by elemental analysis (VarioELcube, Elementar). P uptake efficiency of the various P sources was calculated with modifications as described by Hammond et al. (2009) who defined PUE as the increase of plant total P content per unit of added P fertilizer (g P plant⁻¹ g⁻¹ P fertilizer). We modified the formula, normalizing the P uptake in the vegetative parts per unit of root length instead of per amount of P applied, renaming the term into “root P uptake efficiency” (PUEr: mg P plant⁻¹ cm root). The sand pH was analyzed in a solution of 0.01 M CaCl₂ at the end of the experiment.

2.5 Statistical analysis

Statistical analysis was performed using the statistical program R 2.12.2 (R Core Team, 2012) and biomass yield, leaf area, nutrient uptake, and root morphological traits were measured and were compared with three-way analysis of variance (ANOVA), using plant species, P fertilizer treatment, and N source applied as factors. Tukey’s HSD post-hoc test after ANOVAs at $p = 0.05$ was used to check which level of a factor differed from one another. Data were calculated as arithmetic means ± standard error of the mean of $n = 10$ or 5 replicates for experiment A and B, respectively.

3 Results

3.1 Plant growth

Plants subjected to the struvite treatment in Experiment A produced greater shoot biomass than control plants without any P application (Tab. 1). In Experiment B, lupine plants treated with struvite or TSP showed no significant differences in biomass production. In contrast, maize plants subjected to struvite created significantly greater biomass ($p < 0.05$) than maize treated with TSP (Tab. 1).

We did not observe significantly higher biomass in maize ($p = 0.78$) or lupine plants grown with ammonium and struvite compared with nitrate and struvite. In Experiment B, the biomass of lupine plants grown with struvite was even lower with ammonium as opposed to nitrate as the N-form. This could raise concerns about N being limiting, however, the total amount of N supplied at the start of the experiment in all treat-

| Species    | P source | N source | Shoot P content (mg P plant⁻¹) | Shoot P concentration (mg P g plant⁻¹) | Shoot N content (mg N plant⁻¹) | Shoot Mg content (mg Mg plant⁻¹) | Shoot biomass (g) |
|------------|----------|----------|-------------------------------|----------------------------------------|-------------------------------|---------------------------------|-------------------|
| Experiment A Maize struvite NH₄⁺ 5.1a 1.5b 83a 15.2a 3.2 ± 0.31a
| NO₃⁻        4.4a 1.3c 99a 15a 3.2 ± 0.42a
| No P NH₄⁺ 0.5d 0.8d 20b 1.4b 0.6 ± 0.081c
| NO₃⁻        0.4d 0.9c 20b 2.1b 0.5 ± 0.11c
| Lupine struvite NH₄⁺ 3.5b 1.9a 68a 7.9ab 1.9 ± 0.45b
| NO₃⁻        2.5c 1.5bc 63a 8.1ab 1.7 ± 0.21b
| No P NH₄⁺ 0.3d 0.9d 16b 1.1b 0.3 ± 0.06d
| NO₃⁻        0.4d 0.7d 27b 2.1b 0.5 ± 0.14c
| Experiment B Maize struvite NH₄⁺ 8.1a 1.1c 98a 32ab 9.7 ± 0.84a
| NO₃⁻        9.3ab 1.2c 88abc 34a 9.7 ± 0.7a
| TSP NH₄⁺ 6.9ab 1.2cc 78bc 27abc 7.8 ± 0.82b
| NO₃⁻        6.9ab 1.2cc 82abc 31ab 7.9 ± 0.74b
| Lupine struvite NH₄⁺ 4.9b 2.3a 61d 12c 2 ± 0.3d
| NO₃⁻        5.3b 1.9b 93ab 16bc 2.7 ± 0.14c
| TSP NH₄⁺ 5.5b 2.1ab 58d 13c 2.5 ± 0.63c
| NO₃⁻        4.9b 1.8b 73cd 15c 2.4 ± 0.28c

Table 1: Influence of P fertilizer and N source applied on shoot biomass, shoot P content (P uptake), and shoot P, N, and Mg concentration of maize and lupine plants growing in acidic sand for Experiment A and B. Values represent the mean of $n = 10$ for Experiment A, and the mean of $n = 5$ for Experiment B. Different letters indicate significant differences at $p < 0.05$. Mean values with the same letter within the same shoot parameter and Experiment (A or B) are not significantly different.
ments was 360 mg N per plant, which is nearly 4 times the maximum amount of N taken up, which was 99 mg per plant (Tab. 1). We conclude that N supply was more than sufficient to support plant growth and avoid N deficiencies. This conclusion is supported by the observation of the plants which did not show any signs of chlorosis. Rather, in the No P treatments nutrient deficiency symptoms were according to typical nutrient deficiency symptoms for P-deficiency: dark green and small leaves and slight anthocyanin production in the leaf sheath of maize (Supplementary material, Figs. S1–S3). Plant growth also responded strongly to struvite P-fertilization (Experiment A; Tab. 1).

3.2 Non-invasive measurements of leaf area

The total leaf area was estimated from the projected leaf area using a calibration curve for which 60 plants were measured with the cameras immediately before, and with a leaf area meter (Li-3100, Li-cor, Nebraska, USA) after harvest. The obtained calibration curve was linear with a $R^2$ of 0.95. From hereon we present total leaf area as estimated based on this calibration.

As observed with the biomass (Tab. 1; Experiment B), struvite treated maize plants had greater leaf area at the end of the experiment compared to TSP (Fig. 1). For lupine we found no significant differences in leaf area between struvite- or TSP-fertilized plants until 44 days after sowing (Fig. 1). Between 9 to 21 DAS, however, lupine plants had greater leaf area when fertilized with TSP compared to struvite. This difference was greatest at 20 DAS when TSP-fertilized plants had 14% greater leaf area. From 21 DAS onwards the leaf area of lupine plants treated with TSP did not differ from those treated with struvite (TSP, 263 cm$^2$; struvite, 285 cm$^2$; $p < 0.001$ as an example for 44 DAS). In maize, a very similar pattern was observed. However, the greater leaf area of TSP fertilized plants during early growth stages was not as strong (1.6%) and occurred later at 24 DAS. From 26 DAS, leaf area increased more rapidly in the struvite fertilized plants, which had at the end of the experiment 9.5% more leaf area compared to those fertilized with TSP.

3.3 Shoot nutrient content

We used shoot P content (mg P plant$^{-1}$) as an approximate measure of total P uptake by the plant. Although ammonium+struvite fertilized plants did not have greater biomass than nitrate+struvite fertilized plants, we observed greater P uptake by the ammonium+struvite fertilized lupine in Experiment A. Even so, the ANOVA results suggest that the N treatment effect on P uptake from struvite is independent of the plant species studied (Tab. 1).

This response to N form found in Experiment A was not observed in Experiment B. Here, uptake of P from struvite applied with nitrate was higher than uptake of P from struvite when applied with ammonium. In Experiment B we observed significant differences in P uptake between the plant species. The P uptake was higher in maize than in lupine, as maize is a faster-growing plant that accumulates more biomass and therefore more total P in the shoots (Tab. 1).

In order to compare both species irrespective of plant size, the shoot P concentration (mg P g plant$^{-1}$) was analyzed.

![Figure 1: Leaf area (cm²) of lupine (A) and maize (B) treated with struvite or TSP, calculated at different plant stages [Days after sowing (DAS) from 7 to 44]. The differences between ammonium and nitrate treatments are not shown. Struvite treated plants had higher leaf area than TSP at the end of the experiment; this effect is significant for maize plants (as of 33 DAS). The graph shows the typical growth curve for higher plants with an initial slow growth (lag phase), until 25 DAS approximately, then a rapid period of growth (exponential phase) where maximum growth is seen and the last phase where growth is slow; however, the plants did not reach a steady phase. Points are averages of $n = 5 \pm$ standard error of the mean. *significant differences between struvite and TSP fertilization.](image-url)
The species had a significant effect on P concentrations that were on average greater in lupine than in maize in both experiments. However, P and N fertilization had a much greater effect on shoot P concentrations. In Experiment A, shoot P concentration was most strongly affected by P fertilization, and within the struvite treatments, ammonium fertilization resulted in significantly greater P concentrations compared with nitrate. A similar pattern was observed in Experiment B. However, the N effect on shoot P concentration (TSP or struvite) was only observed in lupine (Tab. 1).

In experiment B, lupine plants treated with struvite-nitrate had a greater N uptake than those treated with ammonium. No significant differences were observed in the shoot N and Mg content in any other treatment.

3.4 Root architecture analyzes

In Experiment A, both lupine and maize plants fertilized with struvite had greater root length and root surface area compared to those that were unfertilized. We observed an increased root length and root surface area when plants were fertilized with nitrate, although the magnitude of the effect differed among species and P treatments (Tab. 2). As hypothesized, the largest effect was observed in struvite-fertilized maize, which had 78% greater root length when N was applied as nitrate.

In Experiment B, we ask if the observed effect of the applied N source on root morphology (i.e., increased root length and root surface area when plants were fertilized with nitrate) was also observed with the highly soluble TSP. For the root analyzes, necessary for calculation of root P uptake efficiency, we focused on lupine plants since we asked if lupine, besides the known physiological root traits that improve the P mobilization making it more suitable for struvite fertilization, might also modify root morphology to further increase struvite-P uptake. No significant differences were observed for root length irrespective of the P form applied.

The observed increase in root length of nitrate-fertilized plants was accompanied by greater specific root length (SRL, cm g\(^{-1}\)). Similar to the total root length, this effect was only significant in Experiment A, and an especially large (2-fold) increase in SRL was observed in struvite-fertilized maize.

Roots of lupine plants showed nodulation in most cases (approx. 80%) when P was added. The nodulation was higher in plants treated with nitrate than those treated with ammonium. However, the differences were not significant.

3.5 P uptake efficiency

Greater root length may result in greater P uptake. However, total P uptake is also affected by the root P uptake efficiency (PUEr: shoot P content, taken as a proxy of total P uptake, normalized for the total root length, given as µg cm\(^{-1}\)). The root P uptake efficiency was affected by the N and P source applied in our study. In Experiment A, PUEr from struvite was two (lupine) and three (maize) times higher (\(p < 0.05\)) when combined with ammonium than with nitrate (Fig. 2A). We observed a similar trend in Experiment B for struvite (Fig. 2B). For TSP there was no effect of N source on PUEr.

Table 2: Root morphological traits (total root length, root surface area, average root diameter, specific root length, and root biomass) of lupine and maize treated with struvite and affected by the N source applied (\(\text{NH}_4^+\) and \(\text{NO}_3^-\)) compared with the No P application (control) in lupine and maize in Experiment A, and with TSP in lupine in Experiment B. Values are mean (\(n = 10/5\)) ± SEM. Mean values with the same letter within the same root trait and Experiment (A or B) are not significantly different.

|                        | P source | N source | Total root length (cm) | Root surface area (cm\(^2\)) | Average root diameter (mm) | Specific root length (cm mg\(^{-1}\)) | Root biomass (g) |
|------------------------|----------|----------|------------------------|-------------------------------|-----------------------------|----------------------------------------|-----------------|
| **Experiment A**       | Lupine   | Struvite | \(\text{NH}_4^+\) 2167 ± 743a | 462.7 ± 150cd | 0.7 ± 0.04a | 4.6 ± 1.03b | 0.5 ± 0.1a |
|                        |          |          | \(\text{NO}_3^-\) 2688 ± 512a | 611.2 ± 139b | 0.7 ± 0.02a | 5.7 ± 0.7b | 0.4 ± 0.1a |
|                        |          | No P     | \(\text{NH}_4^+\) 522 ± 181b | 123.5 ± 42f | 0.7 ± 0.05a | 5.1 ± 0.9b | 0.1 ± 0.03a |
|                        |          |          | \(\text{NO}_3^-\) 995 ± 248b | 229.8 ± 49ef | 0.7 ± 0.06a | 5.0 ± 0.9b | 0.1 ± 0.03a |
|                        | Maize    | Struvite | \(\text{NH}_4^+\) 4104 ± 599d | 492.4 ± 68bc | 0.31 ± 0.04b | 8.1 ± 3.08b | 0.5a ± 0.06a |
|                        |          |          | \(\text{NO}_3^-\) 11430 ± 1371c | 895.8 ± 59a | 0.3 ± 0.03b | 17.5 ± 1.75a | 0.6a ± 0.1a |
|                        |          | No P     | \(\text{NH}_4^+\) 2949 ± 609e | 259.9 ± 62e | 0.3 ± 0.07b | 14.7 ± 4.1a | 0.2 ± 0.05a |
|                        |          |          | \(\text{NO}_3^-\) 3092 ± 882de | 338.6 ± 59de | 0.3 ± 0.09b | 18.2 ± 4.6a | 0.2 ± 0.04a |
| **Experiment B**       | Lupine   | Struvite | \(\text{NH}_4^+\) 10244.5 ± 3535a | 5721.4 ± 2086a | 1.7 ± 0.06b | 17.2 ± 3.1a | 0.6 ± 0.1a |
|                        |          |          | \(\text{NO}_3^-\) 12217.5 ± 1220a | 7034.3 ± 549a | 1.8 ± 0.09ab | 16.1 ± 2.6a | 0.8 ± 0.08a |
|                        |          | TSP      | \(\text{NH}_4^+\) 12877.9 ± 3232a | 7562.1 ± 1929a | 1.8 ± 0.1ab | 16.4 ± 1.4a | 0.7 ± 0.1a |
|                        |          |          | \(\text{NO}_3^-\) 10295.2 ± 3240a | 6370 ± 1953a | 1.9 ± 0.03a | 13.3 ± 2.7a | 0.7 ± 0.1a |
3.6 Substrate pH

The pH of the substrate in Experiment A was significantly influenced by the N, P, and species treatments. On average, the maize substrate had slightly lower pH, nitrate application resulted in higher pH compared to ammonium, and struvite increased pH compared to No P. Lowest pH was observed in struvite-ammonium fertilized maize and lupine plants (pH = 4.4), whereas the highest pH was measured in the struvite+nitrate fertilized plants (5.8–6.0 for maize and lupine, respectively). The modifications of the pH in the sand were not significant in Experiment B, in which all treatments had pH values close to 6.0. We suggest that the longer duration (ten more days) of Experiment B might have caused other processes to have a neutralizing effect on pH.

4 Discussion

4.1 Struvite is a sustainable replacement of TSP as a slow-release fertilizer

Our results suggest that struvite has the potential to replace highly soluble P sources like the commercial TSP. Ours and previous studies confirm that struvite is a good candidate to be used as a P source for crops or potted plants based on biomass production (Johnston and Richards, 2003; Massey et al., 2009; Antonini et al., 2012). However, a higher phosphorus uptake efficiency (see discussion below), was not always reflected in higher biomass production in lupine. This result is in accordance with previous findings in relation to N uptake (Temperton et al., 2007). Here a grass species managed to translate higher leaf N when growing near legumes into higher biomass, whereas a forb did not. This highlights how species-specific the parameter-dependent effects can be.

So far, most of the studies that analyze the slow release properties of struvite are based on analyzes of the chemical and physical properties of the product itself, not the efficiency of plant uptake over time (Rahman et al., 2011; Yetilmezsoy et al., 2013). The new techniques used in our study, in which we monitored the fertilizer effect on leaf area development during the full experimental growth period, revealed that initially TSP treatment led to significantly higher leaf areas, not observed at the end of the trial. As demonstrated earlier, different P concentrations supplied to the soil significantly influenced leaf area and overall shoot biomass (Pang et al., 2010a, 2010b). Therefore, we explain our observation by the faster initial release of P from TSP compared with struvite, since all other nutrients were equally and sufficiently provided in all setups via nutrient solution. For our experimental substrate and plant parameters, measurements might indicate that the slower nutrient release from struvite (Rahman et al., 2014) could ensure a
steady nutrient supply according to the needs of the plants, improving fertilizer efficiency after three weeks.

Slow-release of nutrients can contribute to a more sustainable P fertilization. Environments such as those in, for example, Western Australia, with low P retention in acidic sands (Summers et al., 1993), fertilizers like struvite with a slow release of P can potentially be used as a nutrient management strategy.

4.2 Morphological root adaptations in response to P and N source

In our experiments, we provide evidence that struvite treatment might limit the P availability during the initial period of plant growth, triggering a distinct root growth response in comparison to the quick release P fertilizers like TSP. Unexpectedly, the P source applied, i.e., struvite or TSP, did not show a significant effect on the root morphology in Experiment B (Tab. 2).

Results in the literature about the effect of P on roots show a range of different outcomes that are often species-specific. For maize, some studies have found that limited P has no significant effect on root elongation or lateral root density, but does have negative effects on emergence of new axial roots (Hajabbasi and Schumacher, 1994; Mollier and Pellerin, 1999). The decrease in the total root length (TRL) observed in Experiment A for maize without added P might be due to a reduction in the emergence of new roots (Tab. 2). In our study, P starvation had no effect on lateral root density in maize, resulting in the SRL remaining similar to the SRL of struvite-treated plants in Experiment A. Under P starvation, lupine modified its root morphology by increasing primary root elongation. A similar observation was made by Wang et al. (2008), who also described a large number of first-order lateral roots with probably large amounts of root hairs developed in lupine grown under low P conditions. This could explain why in our study the SRL of lupines without added P increased in comparison with struvite-treated lupines.

The N-source-dependent changes in the root morphology were similar for both plant species (Tab. 2) and were greater than those resulting from the form of applied P. In Experiment A, struvite-fertilized plants increased the total root length and root surface area when they were grown with NO\textsubscript{3}-N. Nitrate application has been reported to increase primary root growth, which can be highly correlated in some species with increased total root length (López-Bucio et al., 2003; Gruber et al., 2013). We expect that this might be the case in lupine, as it has a typical legume root system consisting of a dominant taproot with a relatively large number of primary lateral roots and few secondary roots (Clements et al., 1993).

It is known that an increase in root diameter does not correlate with an increase in the uptake capacity of NO\textsubscript{3} or NH\textsubscript{4} (Garnett et al., 2009). In our study, nitrate treated maize increased the SRL in comparison with ammonium in contrast to lupine. This effect might be explained by an increase in the number and the average length of lateral roots after nitrate application, as previously reported for maize plants (Schortemeier et al., 1993). Lupine treated with struvite+ammonium probably invested more in thin roots than those treated with struvite+nitrate, even if it was not translated into a significant reduction of the average root diameter.

A number of previous studies have shown that the availability of different nutrients can distinctively affect different root morphological parameters and vice versa. Postma et al. (2014) concluded that there is a root architectural trade-off for the acquisition of nitrate and phosphorus, suggesting that roots might modify the morphology depending on the relative availability of both nutrients. In this study the P form did not fundamentally alter the responses of root morphology. However, the N form had a large effect. Furthermore, the level of the effect was plant species-specific. Maize showed greater root morphological plasticity than lupine. This is in line with our hypothesis relating to lupine possibly relying more on the release of carboxylates than maize, as previously described (Robles-Aguilar et al., 2019), with maize relying more on morphological changes.

Besides the P and N effect on root morphology, struvite or TSP application induce different pH changes in the soil as well other possible interactions with nutrients, e.g., more Ca provided by TSP versus Mg provided by struvite that explains the differences in nutrient availability.

The interactive effects of P and N fertilization in this experiment might be confounded by the fact that the struvite+nitrate treatments contained a small (8 out of 360 mg per plant) amount of ammonium from the struvite. We mitigated this effect by ensuring that the total amount of N in all treatments was the same. However, we could not avoid that the N form in the nitrate treatments was not 100% pure nitrate. Although we regard this effect as small, we cannot fully exclude that the small amount of ammonium in the struvite+nitrate treatment had no significant effects on growth or root architecture.

4.3 Struvite-PUEr is modulated by the nitrogen source applied but not the TSP-P

We hypothesized that struvite-P availability would be enhanced through rhizosphere acidification, which can be triggered by fertilizing with ammonium (Gahoonia and Nelson, 1992; Marschner, 2011). Consequently, ammonium fertilization might result in greater P uptake per unit root length (so called root P uptake efficiency). In the literature there is no consensus on whether the struvite P fertilizer effectiveness is dependent on the soil or substrate pH. While lower yields have been reported for struvite-treated plants in alkaline substrates [e.g., Brassica napus (Ackerman et al., 2013)], other studies showed high struvite P fertilizer efficiency in both acidic and calcareous soils (Möller et al., 2018).

In our study, in agreement with a previous report on maize (Jing et al., 2010), lupine and maize treated with struvite + ammonium had high P uptake efficiency despite a reduced total root length (Tab. 2), compared with those treated with struvite + nitrate (Fig. 2). We observed a decrease of pH when ammonium was applied and an increase of pH when nitrate was applied in experiment A. However, modifications of the pH were not significant in Experiment B. As reported earlier (Talboys et al., 2016), initial P dissolution rates from struvite decreased significantly.

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with increasing pH. However, the slow dissolution of struvite increased the solution pH itself over time (Talboys et al., 2016). This additional increase of pH might be the reason why struvite+nitrate induced a higher pH change compared with the addition of ammonium. Furthermore, previous studies have reported that increased P uptake efficiency by maize with ammonium application was not only related to a decrease in the soil pH (Hoffmann et al., 1994). This could mean that besides acidification other mechanisms might play a role, such as stimulating effects of ammonium on the formation of root hairs or other changes in root morphology. Overall, this could explain why in experiment B an increase in PUEr was observed when struvite+ammonium was applied (Fig. 2B), where no pH changes were measured.

As was previously discussed, N source applied in our experiment had a greater effect on root morphology than P source. In agreement with previous studies (e.g., Ma et al., 2013), we propose that besides the effect on pH, the enhanced P uptake by maize, when the P fertilizer was applied together with ammonium, could be explained by differences in the root spatial distribution. Maize plants, due to a greater root length in the nitrate treatment, had a greater P uptake in comparison than those treated with ammonium. However, the higher root length of maize in the nitrate treatment resulted in a lower rate of P uptake cm⁻¹ root (PUEr) in comparison with ammonium treatment. Therefore, nitrate application might decrease the PUEr in comparison with ammonium.

It is known that the root morphological and physiological responses to P supply or N form applied depend on the plant species (Tranbarger et al., 2003; Hammond et al., 2004; White et al., 2005). The comparison between species in Experiment A (Fig. 2A) shows that N source applied modified the struvite effect in both analyzed species. However, even though in both cases the ammonium increased the struvite PUEr, lupine showed greater P uptake efficiency compared with maize. A reason why lupine showed higher PUEr can be explained by exudation of organic acids that occurs in some species (Hinsinger, 2001) such as in the lupine used in our study. The exudates, i.e., organic acids among root-induced pH acidification, can displace the phosphate from the soil matrix by ligand exchange increasing the P availability (Lambers et al., 2006). As shown in a previous experiment on P recovery from struvite, lupine exudates include significant amounts of carboxylates, mostly citrate (Robles-Aguilar et al., 2019). Among the exudation of carboxylates in lupine, the biological nitrogen fixation indicated by successful nodulation and its associated soil acidification (Jensen and Hauggaard-Nielsen, 2003), could be an additional reason for the higher PUEr in lupine compared with maize (Fig. 2). These abilities make lupine a more suitable crop for use of struvite as a P fertilizer compared to maize. As observed in Experiment B, the use of struvite together with ammonium had a comparable fertilizing effect to TSP in lupine, with efficiency unaffected by the co-applied N source.

Our study provides further support for the need for fertilizer management practices in marginal substrates to use appropriate N forms to enhance P-use efficiency. It is likely that changes we observed in the root morphology and PUEr in the current greenhouse study might deviate from field studies. Especially, the greenhouse studies conducted here only observed growth and development during the early vegetative stage (i.e., 5 weeks), whereas effects on yield will have to be tested in the field on longer time scales. However, our approach in this experiment was to describe the influence of the N source applied on the P release from struvite and the differences between the two plant species used (lupine and maize) in a marginal acidic substrate. We chose the acidic substrate in order to facilitate the struvite-P release, i.e., starting from a condition that should already be optimal for struvite dissolution and resulting potential plant uptake/availability. Besides the acidic pH, we chose a marginal mineral sand as the substrate in order to control in which form and amount the nutrients were given to the plants.

Thomas and Rengel (2002) showed that banding of P and ammonium fertilizers improved P fertilizer use efficiency in P fixing soils in comparison with TSP fertilizers. Besides struvite being an ammonium-phosphorus fertilizer, it still needs to be applied with extra N to totally fulfill a crop’s nutrient demand due to its low N content. The present study indicates that the use of ammonium-N together with the struvite may facilitate P acquisition also in acidic P-deficient marginal substrates. This was not observed for other highly soluble P sources like TSP, where the plant P use efficiency was not affected by the N source applied. The use of highly exudating species might increase the struvite use efficiency, as deduced by the higher PUEr of lupine compared with maize.

5 Conclusions

Different approaches are needed in order to increase the fertilizer efficiency of struvite or any other recycled P products. Our study shows that non-invasive phenotyping methods can be instrumental for dynamically tracking plant responses to recycled fertilizers at different plant phenological growth stages. Phenotyping of shoot growth of lupine and maize showed that, compared to triple super phosphate (TSP), struvite-fertilized plants had initially lower leaf area, but plants were able to catch up at later stages of the experiment resulting in similar biomass for lupine or even greater biomass for maize. To our knowledge, this is the first time a study has been performed in such temporal detail to prove the beneficial slow release properties of struvite on plant performance.

The hypothesis that the slower rate of P release from struvite may improve the efficiency of plant P uptake was also supported by the higher PUEr from struvite than from TSP. The ammonium application compared with the nitrate, increased the PUEr of struvite in lupine and maize, an effect not observed with the quick available P source TSP. The high PUEr with ammonium might be explained by rhizosphere acidification due to ammonium uptake that would favor the struvite solubilization and the N-dependent modifications in the root morphology. A greater root morphological plasticity was observed in maize than in lupine. It is known that the exuded organic acids by some species such as lupine can displace the phosphate from the matrix by ligand exchange increasing the P availability.

The use of struvite together with ammonium is recommended to increase the use efficiency of the recycled P. This might not be
related to an increase in the biomass production in the first stage of the plant growth, but the increase in the P use efficiency might equal plant yields at a later stage compared to those reached with quick-release mineral fertilizers. Thus, we hope that the use of struvite as an alternative and sustainable fertilizer will increase, resulting in a higher acceptance and thus a contribution to the recycling of nutrients as well as to a circular bio-economy.

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