ORIGINAL PAPER

Wastewater-Derived Struvite has the Potential to Substitute for Soluble Phosphorus Fertiliser for Growth of Chickpea and Wheat

Manish Sharma[1,3,4] · Jiayin Pang[2,3] · Bede S. Mickan[1,3,4] · Megan H. Ryan[1,3,4] · Sasha N. Jenkins[1,3] · Kadambot H. M. Siddique[1,3]

Abstract

Phosphorus (P) recycling from wastewater can reduce the reliance on the primary source for P fertilisers—rock phosphate reserves. In light of this, we compared the effects of struvite (ST), a sparingly soluble P product derived from wastewater, and readily soluble KH_2PO_4 (KP) on chickpea (*Cicer arietinum* L.) and wheat (*Triticum aestivum* L.) growth and P acquisition. Plants were grown in a temperature-controlled glasshouse in plastic pots containing a soil mixture with low P availability, with ST or KP applied at five P rates (7, 14, 28, 56 and 112 µg P g^{-1} dry soil). Plants were harvested 56 days after sowing when there were visible differences in above-ground growth. Chickpea and wheat growth positively responded to increasing P rates of KP and ST. At low and medium P rates (7, 14, 28 and 56 µg P g^{-1} dry soil) of KP and ST, the plants acquired similar amounts of P, while at a higher P rate (112 µg P g^{-1} dry soil), plants supplied with ST, accumulated 1.2- to ~2.0- fold higher P than KP. Rhizosheath soil pH increased by ~0.1–0.7 units under ST than KP for both species. Wheat produced greater total root length but lower amount of rhizosheath carboxylates than chickpea under KP and ST, and wheat accumulated more P than chickpea under KP and ST. Chickpea and wheat can well access P from ST, indicating the great potential of ST as an alternative P fertiliser.

Keywords Carboxylates · Phosphorus acquisition · Recycled phosphorus · Root morphology · Slow-release fertiliser

1 Introduction

Phosphorus (P) is an essential element required for many vital biological processes in all forms of life (i.e., plants, human, animals and microbes) (Ashley et al. 2011; Jupp et al. 2021). Agriculture depends on P to maintain food production at required levels (Edixhoven et al. 2014), with P deficiency often limiting crop productivity worldwide (Vance et al. 2003). The primary source for P fertilisers—rock phosphate reserves—is a non-renewable and finite resource with no widely used substitute available (Cordell and White 2013; Hao et al. 2013). Due to increasing P demand, phosphate rock reserves will likely be exhausted in the next few centuries (300–450 years) (Fixen and Johnston 2012; Weatherald 2023). The P cycle for agriculture is not closed with large amounts of P applied as fertiliser being transported into waterways, leading to eutrophication (Desmidt et al. 2015; Smith and Schindler 2009). Therefore, sustainable P sources are urgently needed to increase food production for the increasing global human population (Childers et al. 2011; Cordell et al. 2009) meanwhile reducing eutrophication (Daniel et al. 1998; Smith and Schindler 2009).

Struvite, a crystal of magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) formed during wastewater treatment of human and animal waste (Doyle and Parsons 2002; Münch and Barr 2001), is gaining attention as an alternative P

✉ Manish Sharma
  manish.sharma@research.uwa.edu.au

✉ Sasha N. Jenkins
  sasha.jenkins@uwa.edu.au

1   UWA School of Agriculture and Environment, The University of Western Australia, 6001 Perth, WA, Australia

2   UWA School of Biological Sciences, The University of Western Australia, 6001 Perth, WA, Australia

3   The UWA Institute of Agriculture, The University of Western Australia, 6001 Perth, WA, Australia

4   Australian Research Council Training Centre for the Transformation of Australia's Biosolids Resource, 3083 Bundoora, VIC, Australia

fertiliser. ST contains significant amounts of macronutrients (i.e., ~ 12% P, ~ 6% N, ~ 10% Mg) and small amounts of micronutrients (Latifian et al. 2012) that can vary depending on the waste source and recovery process. ST has low solubility (< 1–5%) in water and alkaline soils (pH 9–11) compared to commonly used soluble P fertilisers (Antonini et al. 2012; Cabeza et al. 2011), but its solubility increases in acidic and neutral soils (pH < 8) and in the presence of organic acids (citrate, acetate, malate, and oxalate) (Rech et al. 2018; Talboys et al. 2016). However, its solubility can vary depending on particle size as well. Ground ST dissolves much quicker than granulated ST when mixed through soil (Degryse et al. 2017). Previous studies have shown ST to increase plant growth and P uptake in crop species, including maize (*Zea mays*) (Gell et al. 2011), potato (*Solanum tuberosum*) (Benjannet et al. 2020), lettuce (*Lactuca sativa*) (Jama-Rodzeńska et al. 2021), Chinese cabbage (*Brassica rapa*), chili pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*) (Min et al. 2019), narrow-leaf lupin (*Lupinus angustifolius* L.) (Robles-Aguilar et al. 2019) and many others. Furthermore, ST is characterised as a slow-release fertiliser due to its relatively low solubility and significant P content, and therefore it provides plants with P for longer than other readily soluble P fertilisers when applied at sowing (Hertzberger et al. 2020; Rech et al. 2018).

Struvite, as a slow-release fertiliser may provide a low amount P at early plant growth stage compared to readily soluble fertilisers (Talboys et al. 2016). However, plant species have developed a range of below-ground mechanisms to enhance P acquisition, such as changes in root morphological traits, e.g., increased root length, root surface area, specific root length (SRL) and root mass ratio (RMR) and decreased root diameter, to increase root exploration of soil volume (Haling et al. 2016; Lyu et al. 2016; Vance et al. 2003) and P-mining strategies via physiological adaptations to enhance P mobilisation from sparingly available sources through carboxylates (organic acids) exudation (Guyonnet et al. 2018; Lambers et al. 2006; Richardson et al. 2011). Large interspecific variation in root morphological and physiological traits associated with P acquisition exists among crop species (Kidd et al. 2016; Lyu et al. 2016; Schwerdtner et al. 2022; Wen et al. 2019). In the present study, we compared wheat and chickpea, which differs in root morphological and physiological traits. Wheat more depends on root morphological traits than physiological traits to acquire P (Li et al. 2014; Lyu et al. 2016). In contrast, chickpea, relies on greater carboxylate exudation, a root physiological trait to mobilise sparingly soluble soil P (Kidd et al. 2016; Nobile et al. 2019). For instance, Wen et al. (2019) observed that the thinner root species (wheat) exhibited increased root branching and SRL, whereas thicker root species (chickpea) enhanced the amount of rhizosheath carboxylates to

facilitate plant P acquisition in response to variable P supply, and Lyu et al. (2016) reported wheat displayed greater total root length and SRL coupled with a reduced mean root diameter to enhance P acquisition in response to varying P supply, while chickpea increased carboxylate exudation to facilitate P uptake. Furthermore, carboxylates (e.g., citrate, malate, malonate and oxalate) greatly increase ST solubility (Rech et al. 2018; Talboys et al. 2016) by creating acidic environment in the soil rhizosphere (Robles-Aguilar et al. 2020; Zhu et al. 2002). Moreover, plant species that exude higher amounts of carboxylates are more efficient in P acquisition from ST (Robles-Aguilar et al. 2019; Talboys et al. 2016).

Most agricultural soils in Western Australia (WA) are acidic sands (Gazey et al. 2014), characterised by poor nutrient retention, particularly P (Summers et al. 1993; Weaver and Wong 2011), which often leads to elevated surface runoff and leaching losses (Ozanne et al. 1961; Ritchie and Weaver 1993). The slow-release properties of ST in these soils offer a promising solution, providing consistent supply of P that can mitigate P losses and eutrophication (Molinos-Senante et al. 2011). Moreover, ST steadily increases the soil pH in acidic soils (Rahman et al. 2011).

While the fertilisation potential of ST compared to commercial soluble fertilisers has been investigated in various crop and grass species globally (Ahmed et al. 2018; Hertzberger et al. 2020; Kataki et al. 2016). However, the majority of these studies have been conducted in Europe and Central Asia (Hertzberger et al. 2020). Limited research has been undertaken on Australian soils, with studies including wheat grown on different soil types such as loamy sand, sandy loam, sandy and clay soils (Degryse et al. 2017; Everaert et al. 2017), perennial ryegrass (*Lolium perenne* L.) on loamy sand (Everaert et al. 2018), maize on sandy clay (Mehta et al. 2018), and only one study conducted in WA, focusing narrow-leaf lupin grown on washed river sand (Robles-Aguilar et al. 2019). Additionally, few studies have compared the effect of ST on high- and low-carboxylate-exuding crop species, with only one study conducted on chickpea fertilised with ST (Ghosh et al. 1996). Therefore, this study evaluated the agricultural potential of ST derived from wastewater as a P fertiliser compared to a highly soluble form of P, potassium dihydrogen phosphate (KH_2PO_4, KP). We evaluated chickpea (a high-carboxylate-exuding species) and wheat (a low-carboxylate-exuding species) in a slightly acidic (pH_{CaCl2} 5.7) and P-deficient sandy soil mixture. Our hypotheses were as follows: (1) adequate P availability from ST, matching that from KP for chickpea and wheat growth, is driven by the smaller particle size of ST and increased soil–fertiliser contact rather than its low solubility, (2) application of ST will increase rhizosheath soil pH for chickpea and wheat, and (3) higher carboxylate

exudation in chickpea will enhance P solubilisation from ST, leading to greater P recovery than wheat.

2 Materials and Methods

2.1 Phosphorus Source

Struvite derived from wastewater, a slow-release P form (Talboys et al. 2016) and KH_2PO_4, a readily soluble P form (del Pino et al. 1995) were used as P sources. ST was obtained from the Water Corporation (Perth, Western Australia) and KH_2PO_4 was purchased from ChemSupply Australia (Gillman, South Australia). Water Corporation, Perth had trialled a pilot plant at their water resource recovery facility and recovered ST granules by processing centrate (nutrient-rich effluent) from anaerobically digested sludge containing PO_4^{3-} nd NH_4^+ with concentrated brine containing Mg obtained from the Perth Seawater Desalination Plant. Note that for most previous studies (Degryse et al. 2017; Everaert et al. 2017; Robles-Aguilar et al. 2019) addressing the fertiliser potential of ST in Australia, ST was obtained either from overseas (e.g., crystal green™, SGN 240) or synthesised in the laboratory. This is the first study to use ST recovered commercially at a wastewater treatment facility in WA, Australia. Given the small amount of ST required for each pot, especially at the lowest P rates, we ground the ST granules to a rough powder (granule size ~ 1.00 mm) with a mortar and pestle. Prior to starting the experiment, the elemental composition of ST was determined in ground samples by acid digestion using concentrated HNO_3, followed by inductively coupled plasma optical emission spectrometry (ICP-OES). The struvite granules contained 12.35% P, 4.05% ammonium-N, 8.55% Mg, 0.34% Ca, 54.5 µg g^{-1} Fe, 9.5 µg g^{-1} Zn, 17.5 µg g^{-1} Al (Supplementary Table S1) (Lee 1995).

2.2 Phosphorus Source Solubility

Phosphorus source solubility was determined by mixing 2.0 g ST (247 mg P) with 200 mL deionised (DI) water (pH 6.7) or 2% citric acid (pH 2.1) and 1.08 g KP (247 mg P) with 200 mL DI water. The samples were mixed for 1 hour (h) and 1, 3, 5 and 7 days using an end-over-end tumbler at 25℃, then filtered through a 0.45 µm syringe filter and stored at 4℃ for further processing. The filtered samples were analysed for pH using a portable pH meter and P concentrations using an inductively coupled plasma-optical emission spectrometer (PerkinElmer Optima 5300DV ICP-OES).

2.3 Plant Material and Growth Conditions

Chickpea (cv. Neelam) and wheat (cv. Scepter) were selected for this study as both species are widely grown in Australia and worldwide. Chickpea is a dicotyledonous legume species, and wheat is a monocotyledonous cereal. These morphological distinctions affect many aspects of their growth and nutrition, including P requirements, carboxylate exudation and P-acquisition strategies. A loamy sandy soil was obtained from the top 10 cm layer of an unfertilised native vegetation site at The University of Western of Australia farm, Pingelly (32.51° S, 116.99° E). The field soil was a brownish loamy sand (87.4% sand, 4.0% silt and 8.5% clay). The initial field soil had an acidic pH ($CaCl_2$) of 5.0. It is well-established that chickpea and wheat grow well in soils with pH ($CaCl_2$) > 5.0, particularly > 5.5 (Gazey et al. 2014; Parker 2014). Therefore, we mixed the field soil with sterilised washed river sand in a 3:7 ratio, raising the soil pH ($CaCl_2$) to 5.7. This adjustment also facilitated the collection of rhizosheath carboxylates. The P-buffering index (PBI, a measure of a soil's tendency to adsorb P when P fertiliser is applied) was 46 (Allen and Jeffery 1990) for the field soil. Soils with very low PBI (36–70) bind relatively small amounts of P, leaving most available for plant uptake (Moody 2007). The field soil had 4.05 µg g^{-1} ammonium-N, 7.65 µg g^{-1} nitrate-N, 0.02% organic C (Rayment and Lyons 2011) and 2.55 µg g^{-1} Colwell-P (Colwell 1965) (Supplementary Table S2).

Plants were grown in rectangular plastic pots ($85 \times 85 \times 180$ mm) with drainage holes covered with perforated cloth to avoid soil loss. Each pot was filled with 1.2 kg of soil mixture, with a bulk density of 1.53 g cm^{-3}. There were five rates for each P source (KP and ST) —7 (P7), 14 (P14), 28 (P28), 56 (P56), and 112 (P112) µg P g^{-1} dry soil (equivalent to 16, 32, 64, 128, and 257 kg P ha^{-1}) — mixed with soil as a ground rough powder (ST) or applied as a nutrient solution (KP) before planting, together with a control treatment (P0) with no added P for each species. Each species × treatment combination was replicated four times yielding 40 pots, with an additional four pots with no added P for each species. In addition to providing P, ST was also a source of Mg and N: these were balanced in the KP treatments to match the elemental composition of ST (Table 1). The KP and ST treatments were supplemented with basal nutrients to ensure an adequate supply of other nutrients. The ST treatments received potassium (K) and sulfur (S) as K_2SO_4, while the KP treatments received N as $(NH_4)_2SO_4$, Mg as $MgSO_4 \cdot 7H_2O$ and $MgCl_2 \cdot 6H_2O$, and K as K_2SO_4 and KH_2PO_4. The KP and ST treatments received S, calcium (Ca), zinc (Zn), copper (Cu) and chloride (Cl) as $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and $CaCl_2$. Table 1 presents

Table 1 Nutrient composition for all P rates with struvite (ST) and KH$_2$PO$_4$ (KP)

P source	P rate (µg g^{-1} soil)	Nutrients (µg g^{-1} soil)							
		N	Mg	K	S	Ca	Zn	Cu	Cl
KP	0	0	0	90	38	30	1.8	0.2	53
	7	2.30	4.85	77	38	30	1.8	0.2	53
	14	4.59	9.69	64	38	30	1.8	0.2	53
	28	9.18	19.38	38	38	30	1.8	0.2	53
	56	18.36	38.77	110	38	30	1.8	0.2	166
	112	36.73	77.54	141	38	30	1.8	0.2	279
ST	0	0	0	90	38	30	1.8	0.2	53
	7	2.30	4.85	90	38	30	1.8	0.2	53
	14	4.59	9.69	90	38	30	1.8	0.2	53
	28	9.18	19.38	90	38	30	1.8	0.2	53
	56	18.36	38.77	90	38	30	1.8	0.2	53
	112	36.73	77.54	90	38	30	1.8	0.2	53

Note At 56 and 112 µg P g^{-1} soil of KH$_2$PO$_4$ (KP), K is not balanced completely due to an experimental artefact resulting from the need to balance P at those rates

the composition of nutrients added for all P rates in the KP and ST treatments.

Healthy and uniform seeds of each species were germinated in Petri dishes lined with moist filter paper at room temperature. After one week, three seedlings of each species were transplanted to each pot and chickpea seedlings inoculated with ~1 g pot^{-1} of peat-based Group N Rhizobium (New Edge Microbials, Albury, New South Wales, Australia). The seedlings were thinned to one plant per pot at seven days after transplanting and covered with ~7 mm layer of white plastic beads to minimise soil evaporation. Pots were watered every two days by weight to maintain 75–80% field capacity, and to prevent water leakage from the bottom of the pots. Shed leaves were collected promptly to avoid decay, oven-dried at 70°C and weighed.

The experiment was conducted in a naturally-lit temperature-controlled glasshouse from June to August 2021 at The University of Western Australia, Perth (31.57° S, 115.47° E), with a mean day and night temperature of 22°C and 12°C, respectively, and mean relative humidity of 72%.

2.4 Plant Harvest and Measurements

Plants were harvested 56 days after sowing, when significant differences in above-ground growth were apparent. Plant height was measured from the soil surface to the apical bud just before harvest. The number of branches (≥40 mm long) for chickpea and tillers for wheat, including the main stem, were counted. At harvest, the plants were removed carefully from the plastic pots by gently tapping each side of the pots. Roots were lifted gently from the soil to minimise the loss of fine roots and shaken gently to remove excess soil. The soil remaining attached to the roots was defined as rhizosheath soil (Pang et al. 2017). The roots and shoots were separated by dissecting the shoots just above the soil surface. The shoots were washed in water and blotted dry with paper towel. The shoots combined with senescent leaves were oven-dried at 70°C for 72 h and shoot dry weight (DW) recorded.

2.5 Rhizosheath Carboxylates

The entire root system was placed into a beaker containing a known volume of 0.2 mM CaCl$_2$, which varied depending on root system size. Roots were dipped into the solution and gently shaken for ~60 s to remove rhizosheath soil. To determine rhizosheath carboxylates, a subsample of rhizosheath extract was filtered through a 0.45 µm syringe filter into a 1 mL high-performance liquid chromatography (HPLC) vial. Each HPLC sample was acidified with a drop of orthophosphoric acid (H$_3$PO$_4$) and stored at –20°C until analysis. The protocol for carboxylate analysis followed Cawthray (2003). The HPLC analysis of carboxylates in the elution liquid was performed using a 600E pump and 717plus autosampler, 996 photodiode array detector (Waters Corp., Milford MA, USA). Organic acids were identified by comparing retention times and absorption spectra of samples on an Alltima C-18 reverse phase column (250×4.6 mm, Alltech, Deerfield, Illinois, USA) with known standards of malic, malonic, citric, lactic, *iso*-citric, *cis*-aconitic, succinic, fumaric, and *trans*-aconitic acids (ICN Biomedicals Inc., Aurora Ohio, USA).

2.6 Soil pH and Electrical Conductivity

The remaining rhizosheath soil extract from each pot was used to measure pH using a portable field pH meter, and electrical conductivity (EC) was measured on soil samples (1:5 dilution of soil:DI water) using a probe inserted in the soil suspension.

2.7 Root Morphological Traits

After extracting carboxylate samples, roots were washed with DI water carefully and kept at < 4°C until further processing within 2–3 days. Later, nodules were separated from roots and root samples were placed into a transparent plastic tray filled with water and scanned using a desktop scanner at 300 dpi (Epson Expression Scan 1680; Long Beach, California, USA) to obtain root images. The images were analysed using WinRHIZO software version 4.1c (Regent Instructions, Quebec, Canada) to measure total root length, root surface area and mean root diameter. The roots were dried at 70°C for 72 h to measure DW. The root mass ratio (RMR, ratio of root DW to total plant DW) and specific root length (SRL, ratio of total root length to root DW) were calculated.

2.8 Plant Nutrients

Dried shoot samples were ground to a fine powder using a portable coffee grinder to measure P, Mg and N concentration, with ~ 100 mg subsamples digested using a hot concentrated HNO_3–$HClO_4$ (v/v = 3:1) mixture. Shoot P and Mg concentrations were determined using an inductively coupled plasma-optical emission spectrometer (PerkinElmer Optima 5300DV ICP-OES). An Elementar Analyser (Vario Macro CNS; Elementar, Germany) was used to measure shoot N concentrations by combustion. Physiological P-use efficiency (PUE) was calculated using Eq. (1) (Hammond et al. 2009) and P-recovery efficiency (PRE) was calculated using Eq. (1):

$$Physiological PUE = \frac{ShootDW}{ShootPconcentration} \quad (1)$$

$$PRE = \left(\frac{ShootPcontent[\text{fertilised}] - ShootPcontent[\text{unfertilised}]}{Papplication} \right) \times 100 \quad (2)$$

2.9 Statistical Analysis

The experiment was a three-factorial (species × P source × P rate) randomised complete block design with four replicates. Prior to all statistical analysis, the Shapiro–Wilk test was performed to check the normal distribution of data. All statistical analysis was performed using RStudio software version 1.4.1717 (© 2009–2021 RStudio, PBC). When the species × P source × P rate interaction was significant, the least significant difference (LSD) at $P = 0.05$ ($LSD_{0.05}$) was presented. If the three-way interaction was not significant, $LSD_{0.05}$ was not presented.

3 Results

3.1 Solubility of Struvite

In DI water, the pH of ST was 9.4 after 1 h and 9.2 on day 1, remaining constant at 9.1 for days 3, 5, and 7. In the 2% citric acid solution, the pH of ST was 3.1 after 1 h, gradually increasing to 3.5 by day 7 (Table 2). Over the 7 days, ST dissolved only 1–2% P in water, while it dissolved 96–99% P in the 2% citric acid solution. Conversely, KP was 100% soluble within one h (Table 2).

3.2 Plant Growth

Chickpea and wheat growth steadily increased in response to increasing P rates supplied as KP or ST (Fig. 1). The number of branches/tillers and shoot DW showed no three-way interaction ($P > 0.05$), but rather showed one-way and two-way interactions of species, P source and P rate ($P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$; Fig. 2a and b). The branch/tiller number were constant at P0, P7, P14 in chickpea and at P0 in wheat, but increased linearly at other rates of KP and ST (Fig. 3a). The shoot DW increased linearly with increasing KP and ST rates (Fig. 3b). The root DW showed a species × P source × P rate interaction ($P \leq 0.05$; Fig. 2c). Chickpea root DW gradually decreased from 0.202 g plant^{-1} at P0 to 0.134 g plant^{-1} at P112 under KP, but decreased from P0 to P28 followed by a gradual increase at higher P rates under ST (Fig. 3c). Wheat root DW increased steadily from 0.182 g plant^{-1} at P0 to 0.518 g plant^{-1} at P112 under KP, with corresponding values from 0.182 to 0.692 g plant^{-1} under ST (Fig. 2c). Plant height also followed a similar trend to shoot DW for chickpea and wheat under KP and ST (Supplementary Table S3).

Table 2 pH and solubility of struvite (ST) and KH_2PO_4 (KP) in DI water and 2% citric acid solution

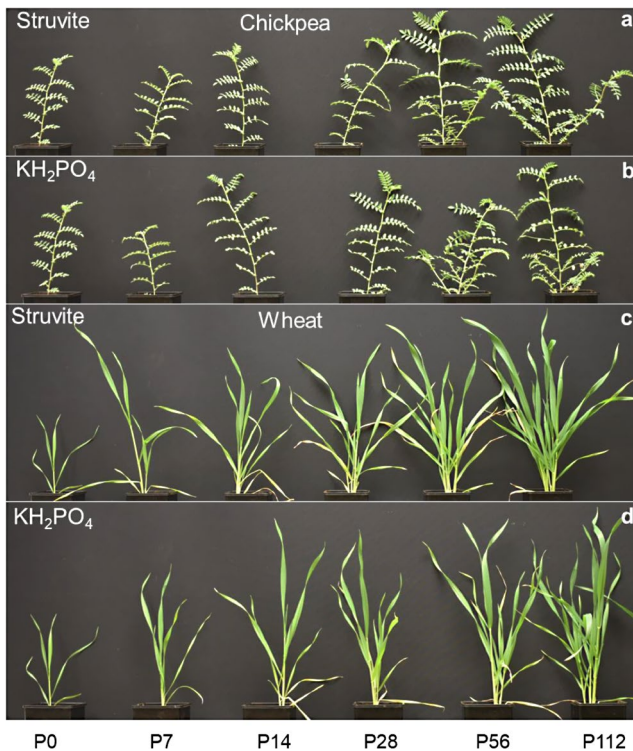| P source	Time	pH		P solubility (%)	
		Water	Citric acid	Water	Citric acid
ST	1 h	9.4 ± 0.02	3.1 ± 0.03	1.2 ± 0.03	97.4 ± 0.5
	Day 1	9.2 ± 0.02	3.3 ± 0.01	1.5 ± 0.00	99.0 ± 0.2
	Day 3	9.1 ± 0.03	3.4 ± 0.01	1.5 ± 0.01	96.6 ± 0.5
	Day 5	9.1 ± 0.01	3.4 ± 0.01	1.5 ± 0.03	98.0 ± 0.6
	Day 7	9.1 ± 0.03	3.5 ± 0.01	1.7 ± 0.01	96.6 ± 0.5
KP	1 h	6.5 ± 0.01	—	100	—

Fig. 1 Visual performance of (**a**), (**b**) chickpea and (**c**), (**d**) wheat in response to five phosphorus (P) rates as KP (KH_2PO_4) or ST (struvite) harvested at 50 days after transplanting

3.3 Plant Nutrient Content and Concentration

Shoot P contents and concentrations showed species × P source × P rate interactions ($P \leq 0.01$, $P > 0.05$; Fig. 3a and b). Shoot P contents in chickpea ranged from 0.37 to 4.02 mg plant^{-1} under KP and 0.37–7.20 mg plant^{-1} under ST, while shoot P contents in wheat ranged from 0.17 to 12.81 mg plant^{-1} under KP, and 0.17–21.35 mg plant^{-1} under ST. At the higher P rate (P112), both species acquired 1.7- to 1.8-fold more P under ST than KP. Overall wheat acquired significantly more P than chickpea under KP and ST (Fig. 3a). Shoot P concentrations in chickpea and wheat followed a similar trend to shoot P contents (Fig. 3b).

Shoot N contents showed a species × P source × P rate interaction ($P \leq 0.01$; Fig. 4a), while shoot Mg content did not ($P > 0.05$, Fig. 4b). Shoot N content in both species remained similar under KP and ST until P28, increased 1.2- to 1.9-fold at P56 and P112 under ST relative to KP (Fig. 4a). Shoot Mg content followed a similar trend to shoot P and N contents, although chickpea acquired significantly more Mg than wheat under KP and ST (Fig. 4b).

3.4 Physiological PUE and P-recovery Efficiency

Chickpea had the highest physiological PUE at P0, which gradually decreased with increasing P rates under KP and
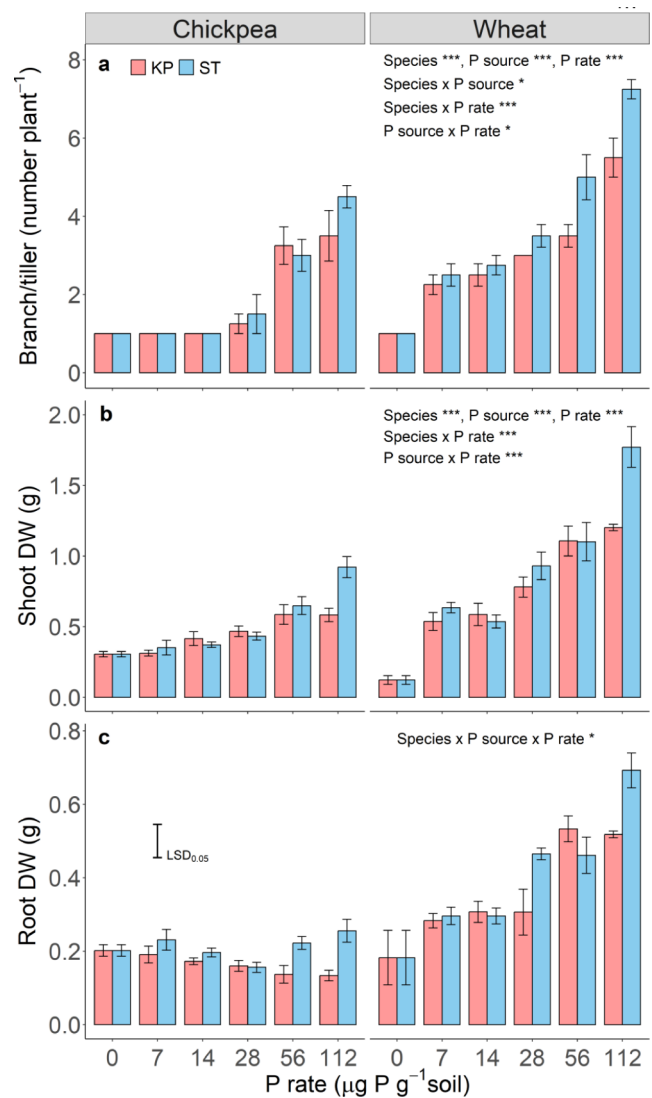
Fig. 2 (**a**) Branch/tiller numbers per plant, and (**b**) shoot and (**c**) root dry weight (DW) of chickpea and wheat in response to five phosphorus (P) rates as KP (KH_2PO_4) or ST (struvite). In (**a**) and (**b**), species ***, P source ***, P rate ***, species × P rate *** and P source × P rate *** represent the significant effects of species, P source and P rate and their two-way interactions at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$. Vertical bar in (**c**) represent the least significant difference at $P = 0.05$ for the species × P source × P rate interaction (* $P \leq 0.05$). Data are means ± standard error (SE) ($n = 4$). In (**a**), SE values are 0 for chickpea at 0, 7, 14 µg P g^{-1} soil and for wheat at 0 µg P g^{-1} soil

ST. Wheat had the highest physiological PUE at P7, varying among the other P rates under KP and ST, with no species × P source × P rate interaction ($P > 0.05$, Fig. 5a). In chickpea, PRE was highest at P56 under KP (~5%) and at P56 and P112 under ST (~6%) (Fig. 5b). PRE for wheat was highest at P7 under both KP (~23%) and ST (~26%), and then decreased linearly with increasing P rates under KP but stayed constant at the other P rates under ST (~19–20%). Overall, wheat accumulated more P at all P rates under ST than KP except at P14 and wheat also accumulated
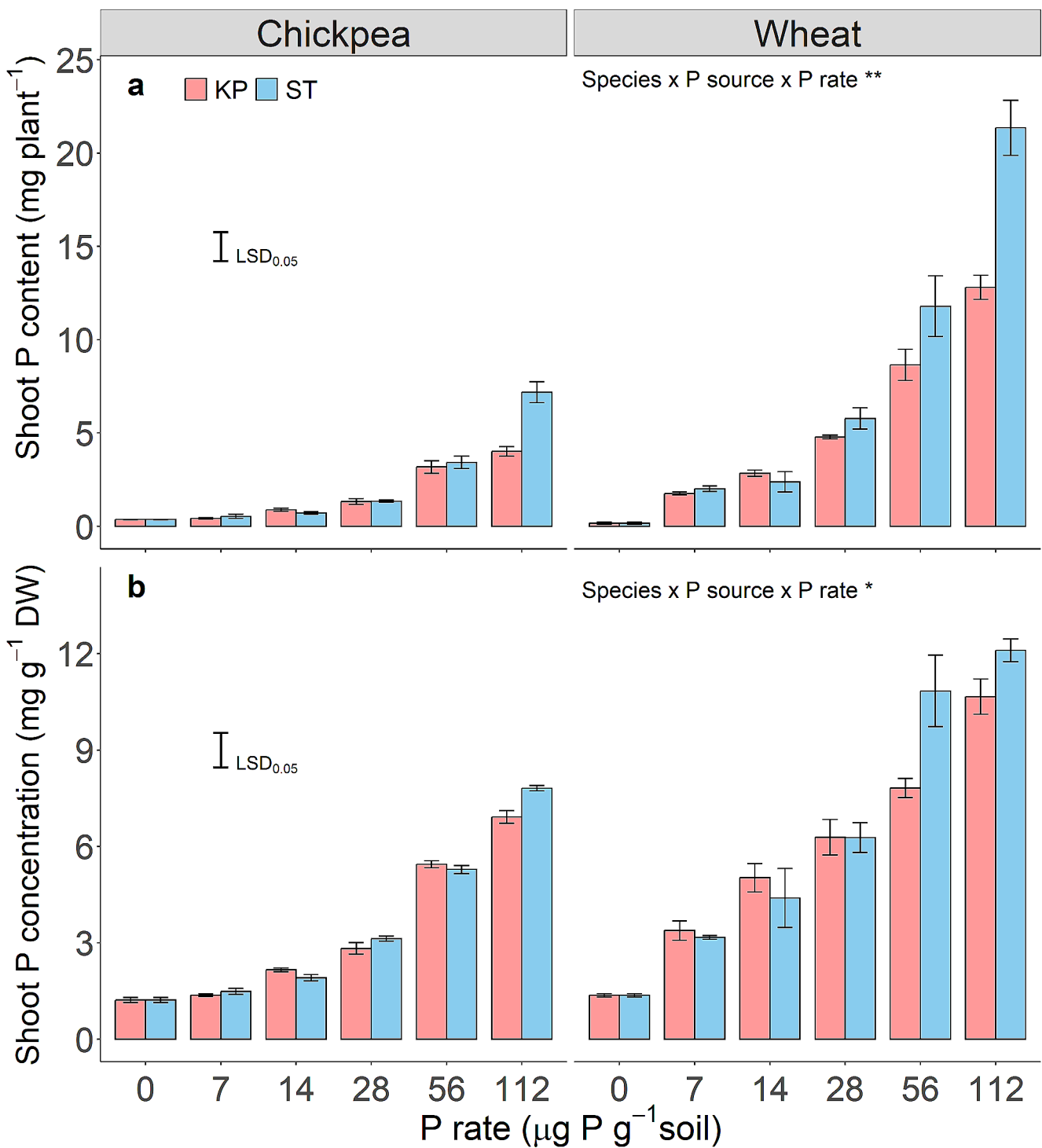
Fig. 3 Shoot P (**a**) contents and (**b**) concentrations of chickpea and wheat in response to five phosphorus (P) rates as KP (KH_2PO_4) or ST (struvite). Vertical bars in (**a**) and (**b**) represent the least significant dif-ference at $P=0.05$ for the species × P source × P rate interaction (** $P \leq 0.01$, * $P \leq 0.01$). Data are means ± standard error (SE) ($n=4$). In (**a**), SE values for chickpea and wheat at 0 μg P g^{-1} soil are 0
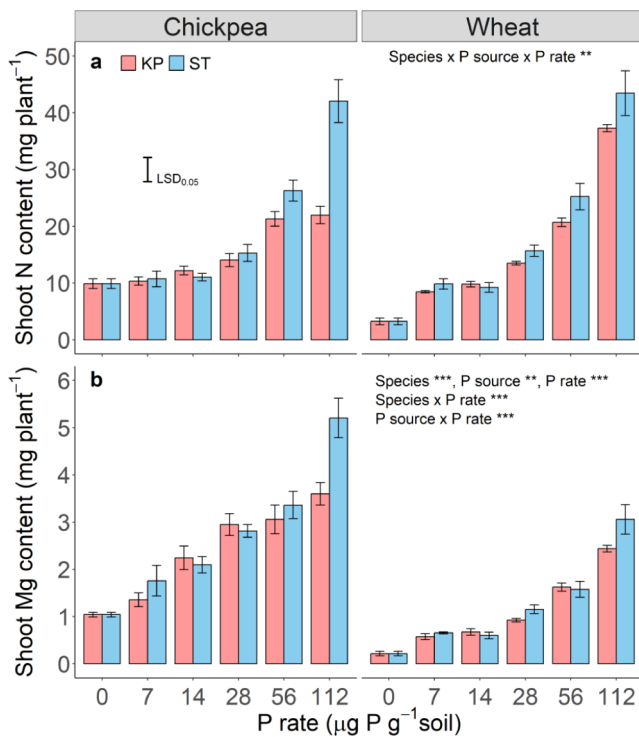
Fig. 4 Shoot (**a**) N, and (**b**) Mg contents of chickpea and wheat in response to five phosphorus (P) rates as KP (KH_2PO_4) or ST (struvite). Vertical bars in (**a**) represent the least significant difference at $P = 0.05$ for the species × P source × P rate interaction (** $P \leq 0.01$). In (**b**), species ***, P source **, P rate ***, species × P rate *** and P source × P rate *** represent the significant effects of species, P source and P rate and their two-way interactions at $P \leq 0.001$ and $P \leq 0.01$. Data are means ± standard error (SE) ($n = 4$)

Fig. 5 (**a**) Physiological P-use efficiency and (**b**) P-recovery efficiency of chickpea and wheat in response to five phosphorus (P) rates as KP (KH_2PO_4) or ST (struvite). Species ***, P rate ***, species × P rate * in (**a**) and species ***, P source ***, P rate *, species × P source **, species × P rate ***, P source × P rate * in (**b**) represent the significant effects of species, P source and P rate and their two-way interactions (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$). Data are means ± standard error (SE) ($n = 4$)

significantly more P than chickpea under KP and ST. There was no species × P source × P rate interaction for PRE ($P > 0.05$, Fig. 5b).

3.5 Root Morphological Traits

No species × P source × P rate interactions occurred for total root length and root surface area ($P > 0.05$; Fig. 6a and b). Total root length of chickpea remained constant from P0 to P28 under KP and ST, but increased by 1.3- to 2.1- fold at P56 and P112 under ST relative to KP (Fig. 6a). Total root length of wheat increased significantly with increasing P rates under KP and ST. At higher P rate (P112), total root length of wheat was 1.3-fold greater under ST than KP (Fig. 6a). Overall wheat had greater total root length than chickpea under KP and ST ($P \leq 0.001$). Root surface area followed a similar trend to total root length for chickpea and wheat under KP and ST (Fig. 6b).

No species × P source × P rate interactions occurred for RMR ($P > 0.05$; Fig. 6c). RMR declined with increasing P rates for chickpea and wheat under KP and ST. Chickpea had ~ 1.0- to 1.2-fold higher RMR under ST than KP. RMR
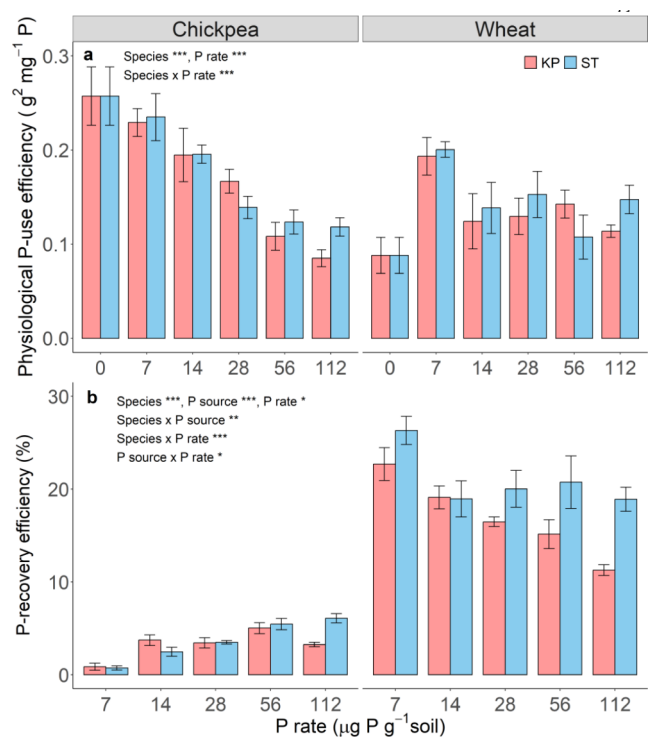
for wheat was highest at P0 under KP and ST, and varied little with increasing P rates (Fig. 6c). Mean root diameter and SRL showed no species × P source × P rate interactions ($P > 0.05$, data not shown). Chickpea had ~ 2.0- to ~ 3.0-fold higher mean root diameter, but 1.2- to 2.2-fold lower SRL, than wheat at all P rates of KP and ST (Supplementary Table S3).

3.6 Rhizosheath Carboxylates

There were no species × P source × P rate interaction for rhizosheath carboxylate amount relative to root DW and total rhizosheath carboxylates per plant (Fig. 7a and b). Rhizosheath carboxylate amount relative to root DW for chickpea ranged from 140 to 339 µmol g^{-1} root DW across all P rates of KP and ST, and increased with increasing P rates from P0 to P28, decreasing at higher P rates under KP and ST (Fig. 7a). In wheat, the rhizosheath carboxylate amount relative to root DW ranged from 17 to 30 µmol g^{-1} root DW under KP, much lower than chickpea, while it increased significantly with increasing P rate under ST, ranging from 24 to 69 µmol g^{-1} root DW (Fig. 7a). In both species, total

rhizosheath carboxylate amount per plant followed a similar trend to the rhizosheath carboxylate amount relative to root DW under KP and ST (Fig. 7b).

3.7 Soil pH and Electrical Conductivity

The rhizosheath soil pH showed a species $\times$ P source $\times$ P rate interaction ($P \leq 0.001$; Fig. 7c). Specifically, wheat had a higher rhizosheath soil pH than chickpea under KP and ST ($P \leq 0.001$). The rhizosheath soil pH of chickpea increased by ~ 0.1–0.5 units under all P rates of ST relative to KP. In wheat, rhizosheath soil pH increased by ~ 0.2–0.7 units at P28 to P112 under ST relative to KP, remaining constant at lower P rates of both P sources (Fig. 7c). The soil EC under all P rates for KP and ST was ≤ 0.14 dS m^{-1} (data not shown), classified as non-saline soil (Abrol et al. 1988; Richards 1954).

4 Discussion

The study revealed that chickpea and wheat had similar above- and below-ground dry matter, shoot nutrient contents, physiological PUE and PRE under low and medium P rates (P7, P14, P28 and P56) for both KP and ST, but at higher P rate (P112), both species had greater values under ST than KP after 56 days of plant growth. The underlying mechanisms of contributing to similar or higher growth of chickpea and wheat under ST compared to KP are discussed below.

The fertiliser performance of ST compared to readily soluble P fertilisers varies among previous studies. For instance, Bonvin et al. (2015) demonstrated that P and N from finely powdered ST were as readily available to plants as soluble mineral fertilisers in slightly-acidic sandy loam soil. Similarly, Plaza et al. (2007) found that ground (0.5 mm) ST mixed with soil was as effective as single superphosphate for growth and P uptake of ryegrass, while Cabeza et al. (2011) reported that finely ground (500 μm) ST was equally effective to triple super phosphate for growth and P uptake of maize in acidic and neutral soils. In contrast, ST was less effective than other soluble P fertilisers when used in granular form (2.0–3.5 mm) (Degryse et al. 2017; Hertzberger et al. 2021; Rech et al. 2018; Talboys et al. 2016). The discrepancy could be attributed to the size of ST granules and the increase in soil–fertiliser contact, leading to more rapid dissolution of P and its incorporation into the soil (Degryse et al. 2017). The physical composition particularly granule size and the surface area in contact with the soil significantly influence the P solubility of slow-release fertilisers (Chien and Menon 1995; Hefter and Tomkins 2003). For instance, finely ground (< 0.15 mm) ST fully solubilised in soil over 28 days, whereas only 50% solubilised when applied as 2.4 mm diameter granules (Degryse et al. 2017). Moreover, post-harvest soil Mehlich-3 P concentrations were higher for 1.5 mm than 3.0 mm diameter granules of ST (Hertzberger et al. 2021). In our study, roughly ground (~ 1.0 mm) ST was mixed thoroughly into the soil, facilitating greater interactions with soil, water, and root-exuded organic acids. These factors, coupled with soil acidity, accelerated ST dissolution, resulting in a P supply similar to that of KP applied at the same P rates (Degryse et al. 2017; Talboys et al. 2016). This finding supports our first hypothesis, suggesting that adequate P supply from ST, matching that from KP for chickpea and wheat growth, is driven by the smaller particle size of ST and increased soil–fertiliser contact rather than its low solubility.

The present study also showed that under both P sources the rhizosheath soil pH of chickpea was reduced by 0.2–0.5 units compared with the original soil mixture pH (5.7) while it increased by 0.1–0.3 units in wheat (except P56 and P112 rates of KP). Plant roots cause shifts in rhizosheath soil pH due to release of H$^+$ or OH$^-$/HCO$_3^-$ to balance a net excess of cations or anions entering the roots (Hinsinger 1998). The greater reduction in rhizosheath soil pH of chickpea compared with wheat might be related to rhizosphere acidification due to carboxylate exudation (Hinsinger 2001; Pang et al. 2015), and/or N$_2$ fixation (Hinsinger 2001; Nyatsanga and Pierre 1973). Chickpea, as a legume that acquires most of the N through N$_2$ fixation, would have taken up more cations than anions, thus releasing H$^+$ and acidifying the rhizosphere (McLay et al. 1997; Tang et al. 1997). Further reduction in soil pH of chickpea under KP (0.1–0.4 units) was likely due to the rapid release of H$^+$ from NH$_4^+$ (the N source in KP treatments) after dissolution (Hinsinger 2001; Wang et al. 2023), in contrast to the slow-release of NH$_4^+$ from ST (Rahman et al. 2011). The increase in rhizosheath soil pH of wheat under ST was likely due to the slow-release of nutrients (Rahman et al. 2011) and the release of OH$^-$ during ST dissolution (Wang et al. 2023). An increase in soil pH due to ST dissolution was also evident from pH measured during solubility tests. Degryse et al. (2017) and Everaert et al. (2017) similarly reported an increase in soil pH near the application site of ST compared to mono-ammonium phosphate in an incubation experiment which was associated with the consumption of protons during ST dissolution. In a field study in China, ST application increased the final soil pH by 0.24 units compared with soluble fertilisers, indicating the slow-release dissolution of ST accompanied by OH$^-$ release and a lack of reactive NH$_4^+$-N for nitrification (Wang et al. 2023). Overall, the changes in rhizosheath soil pH support our second hypothesis that the application of slow-release ST would increase rhizosheath soil pH for chickpea and wheat.
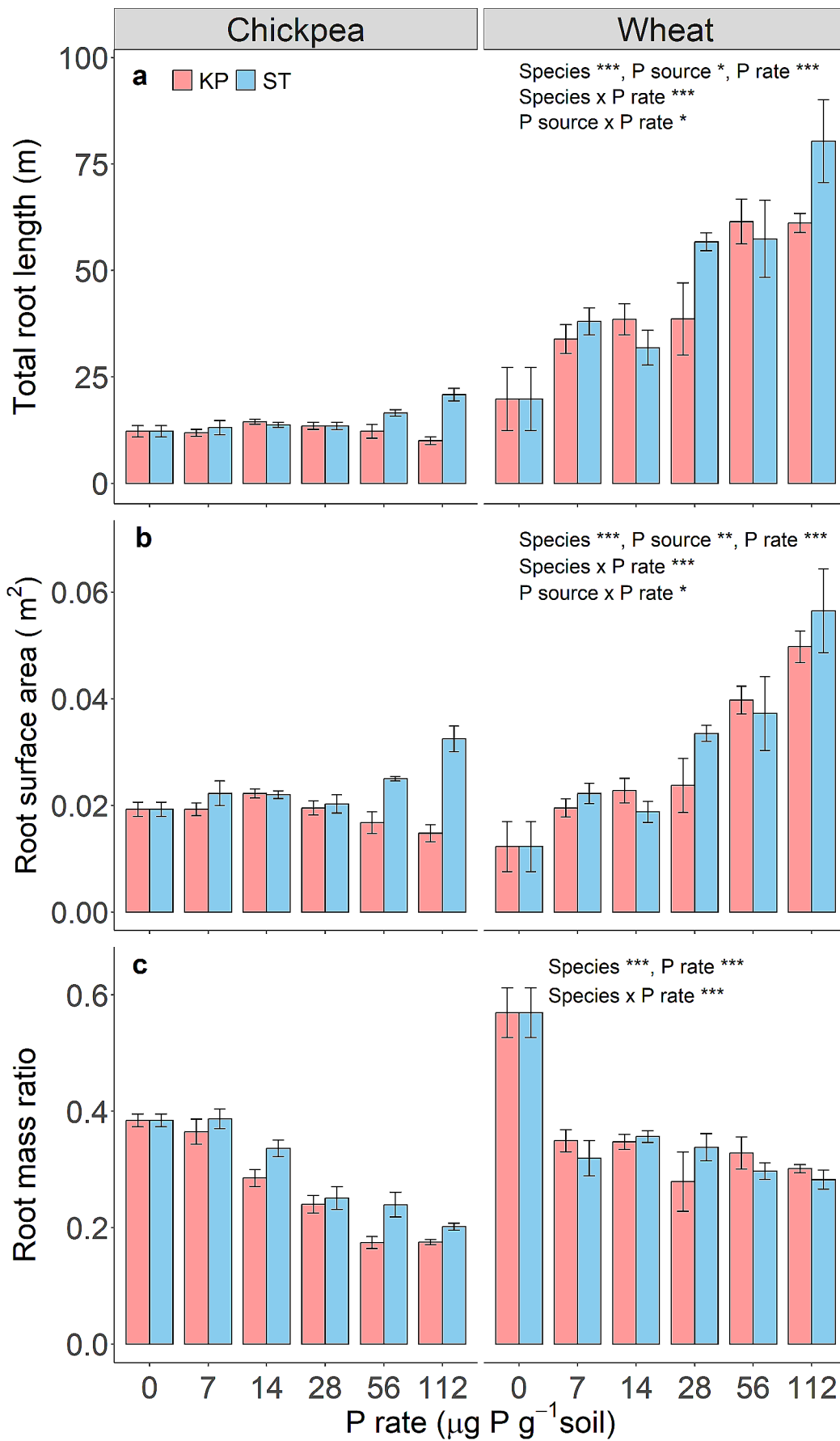
Fig. 6 (**a**) Total root length, (**b**) root surface area, and (**c**) root mass ratio of chickpea and wheat in response to five phosphorus (P) rates as KP (KH_2PO_4) or ST (struvite). Species ***, P source *, P rate ***, species × P rate *** and P source × P rate * in (**a**), (**b**) and species ***, P rate ***, and species × P rate *** in (**c**) represent the significant effects of species, P source and P rate and their two-way interactions (*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$). Data are means ± standard error (SE) ($n = 4$)

It should be noted that ST is a good source of N and Mg, with shoot N and Mg contents in chickpea and wheat increased linearly with increasing P rates under KP and ST. Similarly, N content in broad bean (*Vicia faba*) increased with increasing P rate under ST and was higher than commercial NPK fertiliser (El Diwani et al. 2007). Jama-Rodzeńska et al. (2021) also reported that ST application at high rates (50% higher than recommended) contributed to increased P and nitrate-N content in lettuce leaves relative to triple superphosphate when grown with peat substrate in a pot experiment. The increase in N content at higher P rates of ST could be related to the slow-release of ST which could result in uptake most of N without any leaching losses (Rahman et al. 2014). Hong-Duck et al. (2012) reported that ST released ammonium-N more slowly than complex fertiliser in a column experiment, improving lettuce growth and nutrient uptake when grown in pots. Wang et al. (2023) reported that the slow-release pattern of N from ST was able to meet the crop requirement without any leaching loss of N, resulting in comparable crop yield to soluble fertilisers.

Chickpea and wheat had different shoot and root response patterns in the present study. Wheat acquired more above- and below-ground biomass and had a higher shoot P and N content, but lower Mg content and carboxylate amounts than chickpea due to different P-acquisition strategies. Wheat, with its fibrous-root system, depends more on external P supply for P uptake than rhizosphere P mobilisation from residual soil P with relatively low availability. In contrast, chickpea with its tap-root system relies more on rhizosphere P mobilisation from residual soil P (Lyu et al. 2016; Wen et al. 2019). In the present study, wheat had longer roots and larger root surface area and RMR than chickpea under KP and ST, suggesting that wheat allocated proportionally more biomass to roots than chickpea, improving plant growth and nutrient uptake. Wheat also had relatively thinner roots and higher SRL than chickpea, an efficient and economical mean of increasing P acquisition (Eissenstat 1992), indicating a resource-acquisitive strategy (Ávila-Lovera et al. 2021; Ma et al. 2018). In contrast, chickpea had a smaller root system with lower root surface area, SRL and RMR, but relatively higher rhizosheath carboxylate amount than wheat, potentially mobilising P from KP and ST via high carboxylate exudation (Lyu et al. 2016; Veneklaas et al. 2003), suggesting a resource-conservative strategy (Ávila-Lovera et al. 2021; Han and Zhu 2021). Robles-Aguilar

et al. (2019) reported that ST recovered from municipal wastewater increased the rhizosheath carboxylate amount of narrow-leaf lupin grown in neutral soil pH compared to KP, resulting in efficient mobilisation of P from ST. Talboys et al. (2016) also reported that crop species that exude large amount of carboxylates have a significant impact on P solubility from ST and are much more effective at taking up P than other species. Our third hypothesis that higher carboxylate exudation in chickpea would enhance P solubilisation from ST, leading to greater P recovery than wheat, was not supported. Wheat had a significantly higher P-recovery efficiency than chickpea under KP and ST. Both species recovered similar or more P under ST than KP, possibly due to differences in their P-acquisition strategies. A resource-acquisitive strategy of wheat with thinner roots coupled with relatively higher SRL may have created a larger surface area of contact between roots and soil to explore a greater soil volume efficiently to enhance P acquisition (Liu et al. 2015; Ma et al. 2018), which may resulted in grater P recovery for wheat than chickpea under KP and ST. The low P recovery of wheat under KP could be due to the high solubility of KP, potentially causing P to move downwards in the pot away from the roots, especially during the early stages of plant growth when roots are not fully developed.

Nutrients serve specific functions individually and their interaction can synergistic or antagonistic effect plant growth (Tak et al. 2013). Imbalances in one or more nutrients e.g., K, Mg or Ca can alter the cation composition of the soil, leading to cation competition and antagonistic effects (Fageria 2016; Gransee and Führs 2013). These effects can restrict nutrient uptake and limit plant growth (Huber and Jones 2013). In the present study, the P112 rate of KP supplied 141 µg K g^{-1} soil, potentially may have caused a nutritional imbalance in K, Mg or Ca within the soil. Consequently, this imbalance may have affected chickpea and wheat growth, resulting in reduced or similar plant growth at P112 relative to P56.

5 Conclusions

This study demonstrated that struvite recovered from wastewater has the potential to supply adequate phosphorus to sustain chickpea and wheat growth as effectively as soluble fertilisers. This effectiveness was attributed to the smaller particle size of struvite and increased soil–fertiliser contact in the rhizosphere. Moreover, the slow-release of NH_4^+ from struvite and the release of OH^- during its dissolution increased the rhizosheath soil pH for both species. In summary, the findings suggest that wastewater-recovered struvite holds promise as an alternative P fertiliser,
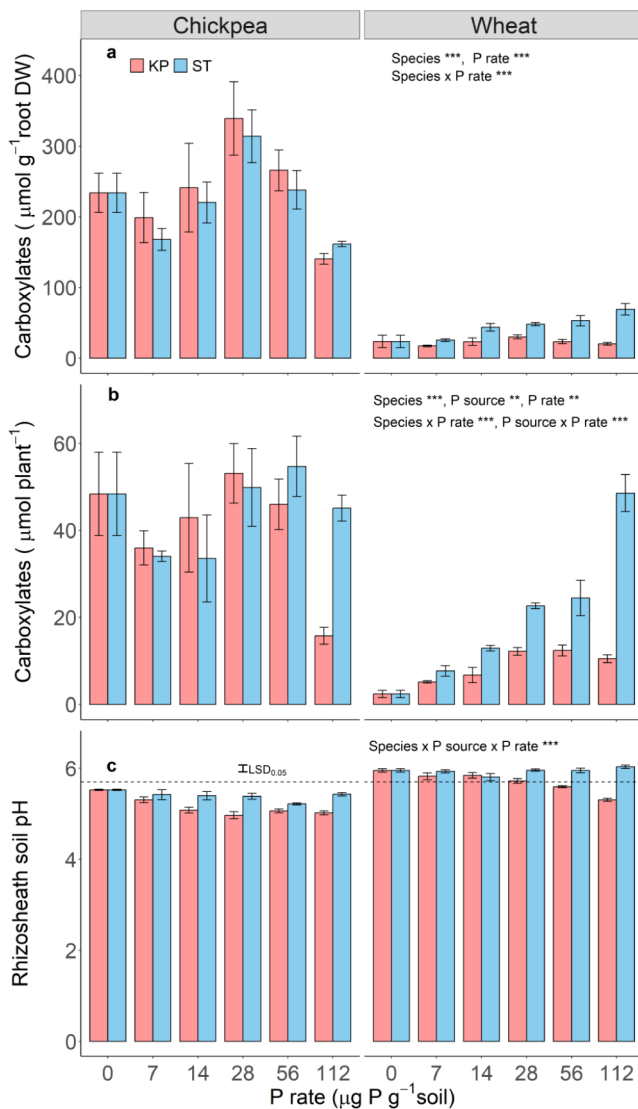
Fig. 7 (**a**) Amount of total carboxylates in rhizosheath soil per gram root dry weight (DW), (**b**) amount of total carboxylates per plant in rhizosheath soil, and (**c**) rhizosheath soil pH of chickpea and wheat in response to five phosphorus (P) rates as KP (KH_2PO_4) or ST (struvite). Species ***, P rate *** and species $\times$ P rate *** in (**a**) and species ***, P source **, P rate **, species $\times$ P rate *** and P source $\times$ P rate *** in (**b**) represent the significant effects of species, P source and P rate and their interactions (*** $P \leq 0.001$ and ** $P \leq 0.01$). Vertical bar in (**c**) represent the least significant difference at $P = 0.05$ for the species $\times$ P source $\times$ P rate interaction (*** $P \leq 0.001$). Horizontal dotted line in (**c**) represent the original pH (5.7) of soil mixture. Data are means $\pm$ standard error (SE) ($n = 4$)

offering comparable or even superior effects to readily soluble fertiliser.

Declarations

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