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RESEARCH ARTICLE

Heterogeneous co-supplies of phosphorus, sulfur and carbon enhance phosphorus availability by improving rhizosphere processes in a calcareous soil

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Abstract

Background and aims Phosphorus (P) availability in calcareous soils is limited. Acidifying the soil by adding sulfur (S) or enhancing microbial activity by adding carbon (C) can increase P bioavailability. However, understanding of the impact of the combined application of P, S and C on P availability in calcareous soils is lacking. In this study, we investigated the effects of heterogeneous (co-localized) supply of different combinations of P, S and C on the maize (*Zea mays* L.) shoot and root growth and soil P availability in calcareous soil with low available P.

Methods A rhizo-box experiment with heterogeneous supply of P, S and C was conducted to assess the responses of maize growth, root morphology and physiology, as well as soil P availability.

Results Heterogeneous low P (P1) with S supply significantly increased shoot and root biomass, shoot P content and P-use efficiency (PUE) compared to homogenous or heterogeneous P1 supply alone. Application of S decreased the rhizosphere soil pH and stimulated the proliferation of maize roots. By contrast, heterogeneous P1 and C supply did not change shoot biomass, P uptake or PUE but decreased root biomass. The addition of C, however, enhanced acid phosphatase activity and carboxylates concentration in the rhizosphere, and increased $NaHCO_3$-extractable P, implying enhanced P availability.

Conclusion Adding S combined with P is an effective approach to engineer the rhizosphere by increasing maize growth and PUE in calcareous soil, whereas adding C increased $NaHCO_3$-extractable soil P but did not influence maize shoot growth.

Keywords P-use efficiency · Rhizosphere processes · Nutrient patches · Root length · Root exudation

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Introduction

Phosphorus (P) is one of the essential nutrients that influence plant growth, crop production and quality. Over the past decades, excessive and inappropriate use of P fertilizers has resulted in the waste of P mineral resources, environmental pollution, and large soil accumulation of residual P in many countries (Zou et al. 2022). Taking China as an example, the amount of fertilizer P applied annually has increased from 1.0 to 4.5 Tg between 1978 and 2019 (FAO), but only about 20% is used by the crop in the first growing season (Zhang et al. 2008).

Calcareous soils are widely distributed in North China Plain, which is one of the most intensively farmed agricultural regions in China (Chen et al. 2006). The availability of P in calcareous soils is low because P can precipitate with Ca (e.g., as dicalcium phosphate, DCP). Over time, DCP is converted into more stable and less plant-available forms, such as octacalcium phosphate and hydroxyapatite (Shen et al. 2011). As a consequence, increasing the P fertilizer-use efficiency and reducing P fixation in calcareous soils remains a great challenge in achieving sustainable P management in China.

As the diffusion rate of P is low (10^{-12} to 10^{-15} m^2 s^{-1}), uptake of P by plants depends heavily on the morphological and physiological characteristics of roots (Schachtman et al. 1998; Shen et al. 2011). During growth, plant lateral roots showed greater proliferation to heterogeneous nutrient supply (whereby nutrients are applied to a soil patch or a band) than to a homogeneous supply as evidenced by an increase in length, biomass, and branching of roots (Drew 1975; Jing et al. 2010). Robinson (1994, 2001) reported that lateral roots proliferated extensively in N- and P-rich soil patches. Through a field experiment conducted on calcareous soil, Jing et al. (2010) found that the heterogeneous application of P and NH_4^+-N (ammonium nitrogen) significantly stimulated the proliferation of maize lateral roots, thereby promoting maize growth and P uptake. In addition to providing the source for N, NH_4^+-N also plays a role in acidifying the rhizosphere environment, which increases P availability in calcareous soils (Jing et al. 2010). Similarly, Havlin et al. (2005) reported increased proliferation of barley root in soil with heterogeneous P supply due to banding P fertilizer. In addition to the morphological response of the root system, the regulation of root physiological traits is also an important plant strategy to enhance soil P acquisition. Currently, most research on root physiological traits is focused on the response and function of root exudates such as carboxylates (e.g., citrate, malate and malonate) that mobilize P from the sorbed mineral or organic fractions (Lambers et al. 2006; Richardson et al. 2011) and phosphatases that enhance the mineralization of organic P to increase plant P availability (Wen et al. 2019).

Amendments that acidify soil have been used to improve the availability of P in calcareous soils (Brownrigg et al. 2022). Elemental sulfur (S) application has been reported to reduce soil pH and therefore increase P availability to plants in calcareous soils (Deluca et al. 1989; Besharati 2017). The biochemical oxidation of S produces sulfuric acid that decreases soil pH and reacts with calcium-bound P minerals, increasing the concentration of soluble P (Deluca et al. 1989; Lindemann et al. 1991). The combined application of reactive phosphate rock with elemental S enhances the dissolution of phosphate rock by the protons produced by S oxidation, thereby increasing the soil Olsen-P content as well as plant biomass (Evans et al. 2006).

Elemental sulfur has to be oxidized to sulfates for utilization by plant root (Malik et al. 2021). The oxidation of elemental S is a microbial process and is thus influenced by the activity of microorganisms in soil (Zhao et al. 2015). Organic carbon (C) addition increases the abundance and activity of microorganisms (Butterly et al. 2009), thereby promoting the oxidation of S. In addition, soil microorganisms also play an important role in the P transformation by mobilizing organic and inorganic P in the soil via secretion of protons, carboxylates and phosphatases (Richardson et al. 2009). The addition of organic C increases microbial activity (Butterly et al. 2009), which may increase the secretion of carboxylates and phosphatases, thereby increasing the availability of soil P, with associated changes in soil P fractions (Stutter et al. 2015). However, microorganisms may also compete with plants for P and rapidly immobilize P in their biomass. The P contained in the microbial biomass in soil typically accounts for 1–10% of the total soil P (Richardson 2001). In low C:P conditions, microorganisms have a net mobilization effect on soil P, whereas at high C:P they have a net immobilization effect (Stevenson and Cole 1999; Xu et al.

2020). It has also been found that organic carbon may limit irreversible P fixation in soil by blocking P sorption sites on aluminum (Al) and iron (Fe) oxides, thus enhancing the readily available P fraction, but this positive effect of organic matter on soil P availability is found only at low soil P saturation (Vermeiren et al. 2022).

Manipulation of plant rhizosphere processes through heterogeneous supply of P, elemental S and organic C may be an effective approach to reducing the application rate of P fertilizer and improving P-use efficiency. Information on the responses of maize in terms of shoot and root growth and P uptake, as well as soil P availability, to the combined application of P, S and C is limited. Hence, the objectives of the study were to test (1) whether the heterogeneous supply of P combined with S and/or organic C would stimulate plant growth and P uptake through altering root morphological and physiological characteristics in maize and (2) how the heterogeneous supply of P combined with S and/or organic C influences soil P fractionation and availability. It is hypothesized that the heterogeneous supply of P combined with S and/or organic C would alter the root morphological and physiological traits in maize grown in calcareous soils, stimulating plant growth and enhancing P availability, thereby reducing the need for high application rates of P fertilizers.

Material and methods

Experimental set up

To investigate the effects of the heterogeneous (co-localized) supply of P, S and/or C on shoot and root growth, P uptake and soil P availability, a rhizo-box experiment was conducted in a naturally lit glasshouse at China Agricultural University, Beijing (40.01°N, 116.16°E). Temperature ranged from a minimum of 18 °C at night to a maximum of 25 °C during the day, with 10–11 h day length throughout the 30-day growing period. Seven treatments were set up: (1) control (no P, S and C addition); (2) homogeneous P1 supply (75 mg P kg^{-1}; 90 mg P per rhizo-box; Hom P1); (3) homogeneous P2 supply (225 mg P kg^{-1}; 270 mg P per rhizo-box; Hom P2); (4) heterogeneous P1 supply (225 mg P kg^{-1} in the middle layer; 90 mg P per rhizo-box; Het P1); (5) heterogeneous (co-localized) P1 and S supply (150 mg S kg^{-1}; 60 mg S per rhizo-box; Het P1 + S); (6) heterogeneous (co-localized) P1 and C supply (480 mg C kg^{-1}; 192 mg C per rhizo-box; Het P1 + C) and (7) heterogeneous (co-localized) P1, S and C supply (Het P1 + S + C) (Fig. 1A). After harvest, measurements were made of: (i) shoot and root biomass, shoot P content and P-use efficiency, i.e., by measuring the shoot biomass and shoot P concentration, (ii) one root morphological trait: root length, (iii) two root physiological traits: the acid phosphatase activity and concentration of carboxylates in the rhizosphere, and (iv) soil P fractions: Olsen-P, oxalate-extractable P and microbial biomass P.

Each treatment was established with four replicates. The treatments were arranged in a randomized complete block design and were re-randomized weekly during the experiment. The rhizo-boxes (20 × 1.5 × 35 cm) were filled with 1.2 kg air-dried soil. The soil was collected from Shangzhuang experimental station in Beijing, China (40.14°N, 116.19°E), air-dried and passed through a 2 mm sieve. Soil properties were: Olsen-P 3.1 mg kg^{-1}, organic C 0.4 g kg^{-1}, available N 8.5 mg kg^{-1} (NO_3^- and NH_4^+), available K 32 mg kg^{-1}, $CaCO_3$ 27 g kg^{-1} and pH 8.2 (the ratio of soil to $CaCl_2$ solution was 1:2.5). Olsen-P was determined after extraction with 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5). Soil available N was determined by a continuous flow analyser (TRACS 2000 system, Branand Luebbe, Norderstedt, Germany) after extracting with 0.01 mol L^{-1} $CaCl_2$. Soil available K was determined by a flame photometer (Model 410, Sherwood Scientific Ltd., UK) after extraction with 1 mol L^{-1} NH_4OAc (pH = 7). Soil organic C was determined using a total organic carbon analyser (TOC-5000A, Shimadzu, Japan). The amount of $CaCO_3$ was measured by the acid neutralization method (Rowell 1994). Soil pH was determined by a pH meter (Mettler Toledo, FE20, Switzerland) in 0.01 mol L^{-1} $CaCl_2$ with a ratio of 1:2.5 (soil: solution).

To ensure that the nutrient supply was adequate for plant growth, all essential nutrients other than P were added to each pot as solutions, providing (in mg kg^{-1} on a dry soil basis): 200 N as $Ca(NO_3)_2·4H_2O$, 150 K as K_2SO_4, 45 Ca as $CaCl_2$, 4.27 Mg as $MgSO_4·7H_2O$, 1.65 Mn as $MnSO_4·4H_2O$, 0.12 B as H_3BO_3, 0.51 Cu as $CuSO_4·5H_2O$, 2.26 Zn as $ZnSO_4·7H_2O$, 0.14 Mo as $(NH_4)_6Mo_7O_{24}·4H_2O$, and 0.77 Fe as EDTA-FeNa.

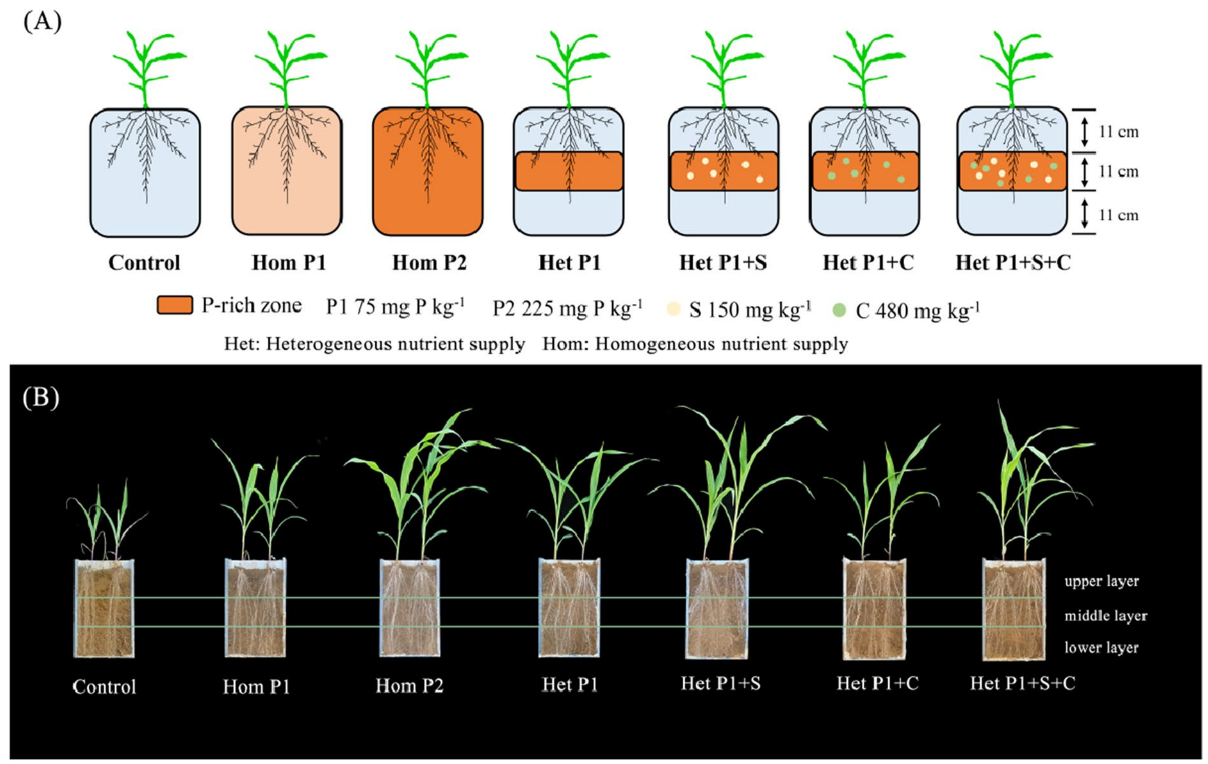

Fig. 1 Diagram of the experimental set-up (**A**). Shoot and root growth under different treatments before harvest (**B**; 30-day-old plants). Control: no P addition, Hom P1: homogeneous P1 (75 mg P kg^{-1}) supply, Hom P2: homogeneous P2 supply (225 mg P kg^{-1}); Het P1: heterogeneous P1 supply; Het P1 + S: heterogeneous (co-localized) P1 and S (150 mg S kg^{-1}) supply; Het P1 + C: heterogeneous (co-localized) P1 and C (480 mg C kg^{-1}) supply; Het P1 + S + C: heterogeneous (co-localized) P1, S and C supply. The colour coding of P supply corresponds to the concentration of P applied to the corresponding zone of rhizo-box

Phosphorus was supplied as KH_2PO_4 in a homogeneous or heterogeneous pattern. Sulfur was supplied as elemental S, and C was supplied as glucose ($C_6H_{12}O_6$) in the relevant treatments. In the homogeneous treatments, P was uniformly applied throughout the rhizo-box. For the heterogeneous treatments, a 11 cm layer fertilized with P, S and/or C (400 g soil) was placed at the center of the rhizo-box; the upper and lower layers soil received the same nutrients as the control treatment. (see Fig. 1 for details of the treatments).

Seeds of maize (*Zea mays* L. cv. Zhengdan 958) were surface-sterilized in 30% v/v H_2O_2 for 30 min, rinsed with deionized water, soaked in saturated $CaSO_4$ solution for 12 h, and then germinated in Petri dishes covered with wet filter papers at 22 °C for 1–2 d. Four seeds were planted per rhizo-box, and were thinned to two per rhizo-box 7 days after planting. The plants were watered with deionized water by weighing every 2 days to maintain soil moisture at 70% of actual field water capacity (200 g kg^{-1}).

Harvest and measurements

All plants were harvested 30 days after planting and separated into shoots and roots. The soil in rhizo-box was cut into three layers (0–11, 11–22, 22–33 cm), and roots were carefully lifted out of the soil and gently shaken to remove the bulk soil (the soil tightly adhering to the roots was considered as rhizosphere soil) (Wen et al. 2019). The roots with rhizosphere soil attached were then transferred to a beaker containing 50 mL of 0.2 mmol L^{-1} $CaCl_2$, gently dunked for 60 s until as much of the rhizosphere soil was dislodged as possible. The beaker was left to stand for 2 min, and the soil suspension from the top layer was sampled for various analyses:

(1) For measurement of rhizosphere pH, 8 mL of soil suspension was transferred into a 15 mL centrifuge tube. Then a pH meter (Mettler Toledo, FE20, Switzerland) was used to assay the rhizosphere soil pH. The amount of collected rhizosphere soil differed among the treatments. To eliminates effect of different soil: $CaCl_2$ ratios on pH determination, a modified pH (soil: $CaCl_2$ ratio adjusted to 1:2.5) was calculated from the measured original pH according to the experimental method of Li et al. (2010). The following equation was derived from the experiment: $y = -0.024\ln(x) + 1.07$, $R^2 = 0.90$, $P < 0.001$, where y is the ratio of modified pH to original pH and x is the dry weight of the rhizosphere soil.

(2) For carboxylate analysis, 8 mL of rhizosphere soil suspension was placed in a 15 mL centrifuge tube, and two drops of microbial inhibitor Micropur (Sicheres Trinkwasser, Munich, Germany) (0.01 g L^{-1}) and two drops of concentrated phosphoric acid were added, followed by storing it at −20 °C. The concentrations of carboxylates in the rhizosphere soil were analyzed using a reversed phase high performance liquid chromatography (HPLC) system following the method of Zhang et al. (2016).

(3) For measurement of acid phosphatase activity, two 0.5 mL aliquots of soil suspension were transferred into separate 2 mL centrifuge tubes containing 0.4 mL of sodium acetate buffer (pH 5.2) and 0.1 mL of 0.15 mol L^{-1} *p*-nitrophenyl phosphate (PNP) substrate. After incubation at 30 °C for 30 min, addition of 0.5 mL of 0.5 mol L^{-1} NaOH terminated the reaction. The absorption of supernatants was measured at 405 nm (Synergy H1 MD, USA) (Neumann 2006).

Shoots were oven-dried at 105 °C for 30 min and then at 75 °C for 3 days, and were weighed for dry biomass determination. Then, dry shoots were ground into powder. To determine shoot P concentrations, the powdered shoot sample was digested in a mixture of 5 mL of concentrated sulfuric acid and 8 mL of 30% v/v H_2O_2. Shoot P concentration was assayed by the molybdovanadophosphate method at 440 nm by spectrophotometry (Synergy H1 MD) (Johnson and Ulrich 1959).

Roots were washed in deionized water and then scanned with an EPSON root scanner at 157 dots-per-cm resolution (400 dpi; Epson Expression 1600 pro, Model EU-35, Tokyo, Japan). The total root length was analysed with software Win-RHIZO (Regent Instruments Inc., Quebec, QC, Canada).

$NaHCO_3$-extractable P (available P) in bulk soil was extracted in 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) and determined colorimetrically. The oxalate-extractable P (moderately labile P) in bulk soil was determined after the extraction with solution containing oxalic acid (0.1 mol L^{-1}) and ammonium oxalate (0.175 mol L^{-1}) at pH 3.0 (McKeague and Day 1966). The extracts were analysed for oxalate-extractable P by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Norris and Titshall 2012). Microbial biomass P (MBP) was calculated as the difference between the amounts of inorganic P (Pi) extracted by 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) from fresh soil fumigated with $CHCl_3$ for 24 h and from unfumigated soil (Brookes et al. 1982).

Statistics

All statistical analyses were performed in R version 4.0.3 (R Development Core Team 2020, Vienna, Austria). The effects of different treatments (reducing P fertilizer input and adding S and C) on shoot and root biomass, shoot P content, P-use efficiency, root length, acid phosphatase activity, and concentrations of carboxylates, Olsen-P, oxalate-extractable P and microbial biomass P were subjected to one-way analysis of variance (ANOVA). Significant difference among means was based on Tukey's test ($P \leq 0.05$).

The P-use efficiency was calculated as follows:

$$\mathrm{P-use\ efficiency}\ (\%) = \left(\mathrm{U_p} - \mathrm{U_c}\right)/\mathrm{P_{added}} \times 100,$$

where Up is the amount of shoot P uptake by maize in P addition treatment (mg), U_c is the amount of shoot P uptake by maize in control treatment without P addition (mg), and P_{added} is the amount of P applied in soil in P addition treatment (mg).

Results

Plant growth and P uptake

The homogeneous P2 (Hom P2) and the heterogeneous P1 and S (Het P1 + S) supplies resulted in significantly higher shoot biomass compared to the other treatments (Fig. 2A). Similarly, the Hom P2 treatment resulted in higher shoot P concentration and P content compared with all the other treatments (Fig. 2B, C). However, there was no difference in shoot P concentration between the homogeneous P1 treatment and heterogeneous P1, P1 + C and P1 + S + C supply (Fig. 2A, B). However, the shoot P content was higher in the Het P1 + S treatment than in the other treatments with heterogeneous P1 supply, except the Het P1 + S + C treatment (Fig. 2C). Compared to Het P1 treatment, the shoot biomass and shoot P content in the Het P1 + S treatment increased by 26% and 49%, respectively (Fig. 2A, C). Similarly, the addition of S increased the use efficiency of P fertilizer,

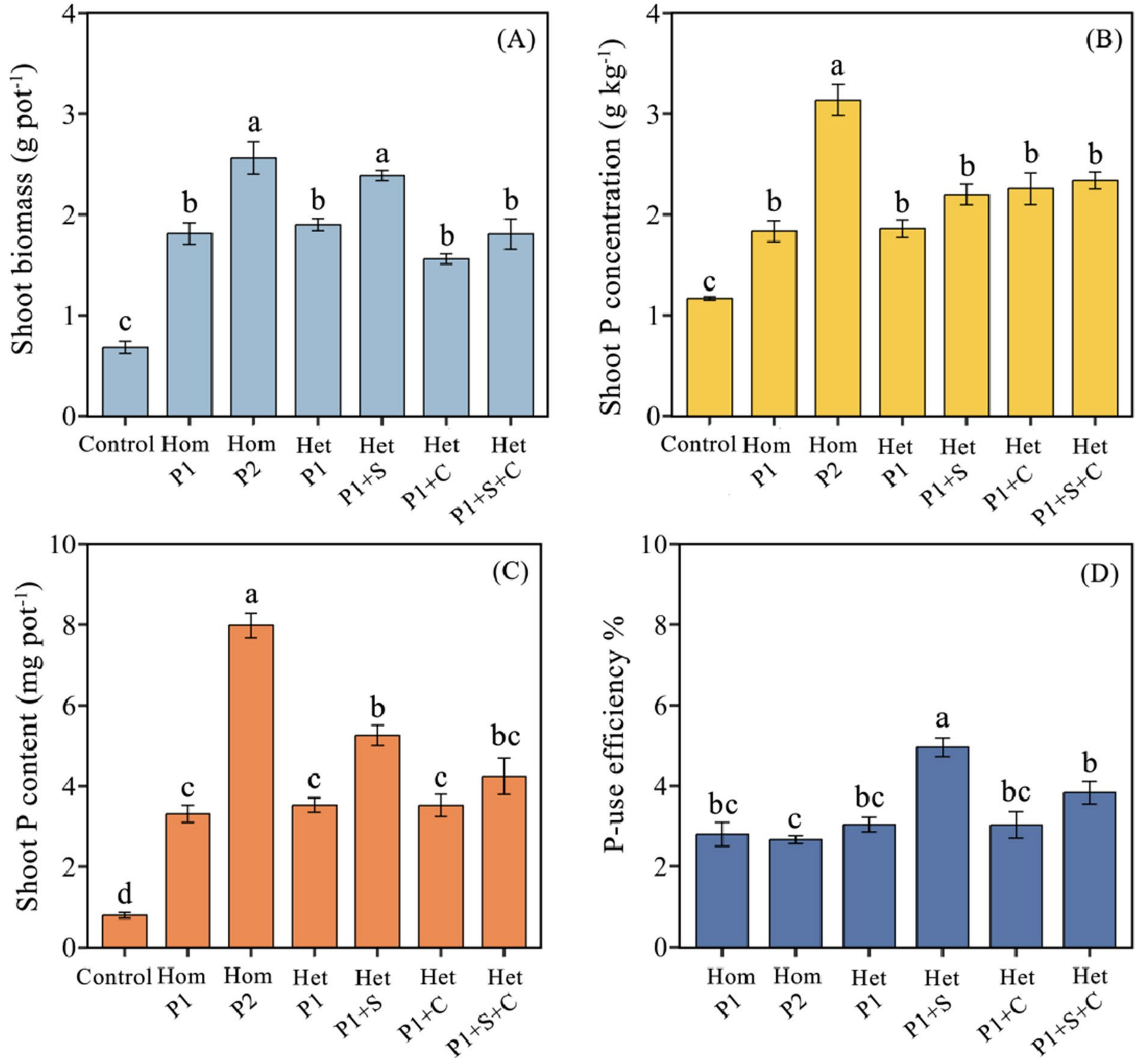

Fig. 2 Maize shoot biomass (**A**), shoot P concentration (**B**), shoot P content (**C**) and P-use efficiency (**D**) after 30 days of growth under different treatments. Data are means ± standard errors ($n = 4$). Different lowercase letters denote significant difference ($P \leq 0.05$). Control: no P addition, Hom P1: homogeneous P1 (75 mg P kg^{-1}) supply, Hom P2: homogeneous P2 supply (225 mg P kg^{-1}); Het P1: heterogeneous P1 supply; Het P1 + S: heterogeneous (co-localized) P1 and S (150 mg S kg^{-1}) supply; Het P1 + C: heterogeneous (co-localized) P1 and C (480 mg C kg^{-1}) supply; Het P1 + S + C: heterogeneous (co-localized) P1, S and C supply

making it significantly higher than in any other treatment (Fig. 2D).

Root growth and distribution

Compared to homogeneous P1 (Hom P1) supply, the root biomass and total root length were significantly stimulated by heterogeneous P1 (Het P1) supply, increasing by 22% and 21%, respectively. Under conditions of heterogeneous P1 (Het P1) supply, both the root biomass and total root length reached the same level as in the Hom P2 treatment. Compared to Het P1 treatment, the addition of S to the heterogeneous P environment increased the root biomass and total root length, whereas the addition of C had the opposite effect. In comparison with the Het P1 treatment, root biomass and total root length in the Het P1 + S treatment increased by, respectively, 11% and 13%, but decreased by 18% and 25%, respectively, in the Het P1 + C treatment (Fig. 3A, B).

In the upper soil layer, the homogeneous P2 (Hom P2) supply resulted in significantly higher root length compared to the other treatments (Fig. 4A). The heterogeneous supply of nutrients increased the root length in the middle soil layer, except in the Het P1 + C treatment. The heterogeneous P1 and S (Het P1 + S) supply resulted in higher root length compared with all the other treatments (Fig. 4B). Similarly, the highest proportion of the total root length located in the middle layer appeared in the Het P1, Het P1 + S and Het P1 + S + C treatments (Fig. 4D). In comparison to Hom P2 treatment, the root lengths in the middle layer in the Het P1, Het P1 + S and Het P1 + S + C treatments increased by 59%, 80% and 51%, respectively (Fig. 4B).

Acid phosphatase activity and carboxylates concentration in different soil layers

Compared to the Hom P2 treatment, the Hom P1 treatment showed a decrease in acid phosphatase activity in every soil layer (Fig. 5A, B, C). In comparison with the Het P1 treatment, the heterogeneous P1, S and C supply (Het P1 + S + C) increased the acid phosphatase activity in the upper, middle and lower soil layers (Fig. 5A, B, C). In the middle soil layer, there was no significant difference in acid phosphatase activity between the Hom P2, Het P1

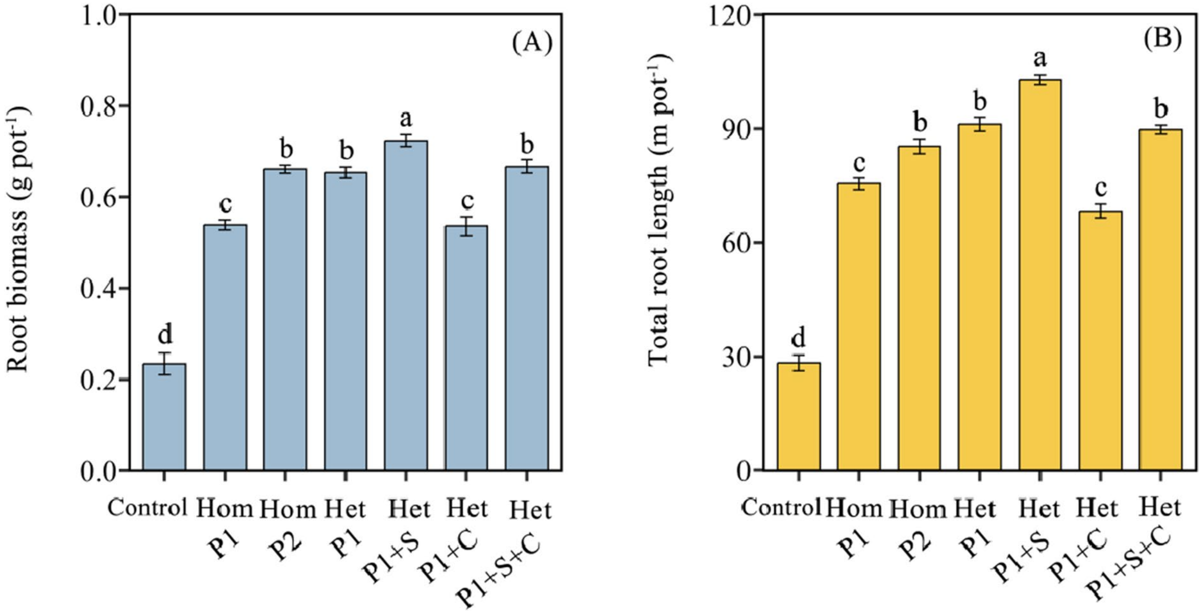

Fig. 3 Maize root biomass (**A**) and total root length (**B**) after 30 days of growth under different treatments. Data are means ± standard errors ($n = 4$). Different lowercase letters denote significant difference ($P \leq 0.05$). Control: no P addition, Hom P1: homogeneous P1 supply (75 mg P kg^{-1}), Hom P2: homogeneous P2 supply (225 mg P kg^{-1}); Het P1: heterogeneous P1 supply; Het P1 + S: heterogeneous (co-localized) P1 and S (150 mg S kg^{-1}) supply; Het P1 + C: heterogeneous (co-localized) P1 and C (480 mg C kg^{-1}) supply; Het P1 + S + C: heterogeneous (co-localized) P1, S and C supply

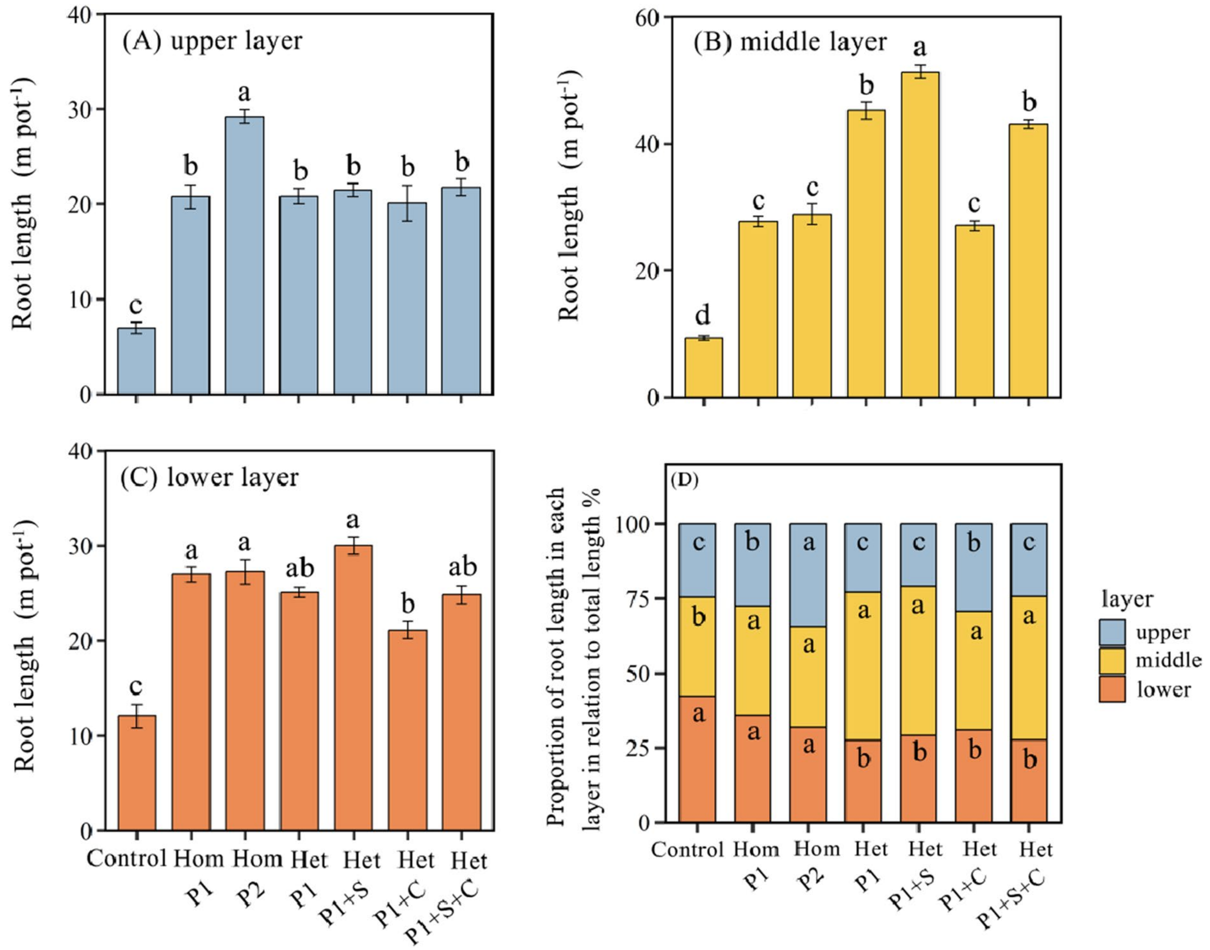

Fig. 4 Maize root length in upper (**A**), middle (**B**) and lower (**C**) soil layers after 30 days of growth under different treatments. Root length in each soil layer as a proportion of total root length under different treatments (**D**). In A-C, data are means ± standard errors ($n=4$). Different lowercase letters denote significant difference among treatments ($P \leq 0.05$). Control: no P addition, Hom P1: homogeneous P1 supply (75 mg P kg^{-1}), Hom P2: homogeneous P2 supply (225 mg P kg^{-1}); Het P1: heterogeneous P1 supply; Het P1 + S: heterogeneous (co-localized) P1 and S (150 mg S kg^{-1}) supply; Het P1 + C: heterogeneous (co-localized) P1 and C (480 mg C kg^{-1}) supply; Het P1 + S + C: heterogeneous (co-localized) P1, S and C supply

and Het P1 + S treatments (Fig. 5B). The Het P1 + C and Het P1 + S + C treatments resulted in higher acid phosphatase activity in middle layer soil compared to other treatments (Fig. 5B).

Compared to the Hom P2 treatment, rhizosphere carboxylate concentrations decreased significantly in the Hom P1 treatment in every layer (Fig. 5D, E, F) and in the Het P1 and Het P1 + S treatments in the upper and lower soil layers (Fig. 5D, F). The rhizosphere carboxylate concentrations in upper and lower soil layers were higher in the Het P1 + C and Het P1 + S + C treatments than in other treatments (Fig. 5D, F). Compared to the Het P1 treatment, the addition of S and C (Het P1 + S, Het P1 + C, Het P1 + S + C) significantly increased the rhizosphere concentration of carboxylates in the middle soil layer (Fig. 5E).

Soil P fractions and rhizosphere soil pH in different soil layers

In the upper and lower soil layers, the homogeneous P2 (Hom P2) supply resulted in significantly higher P availability compared to the other treatments (Fig. 6A, C). In

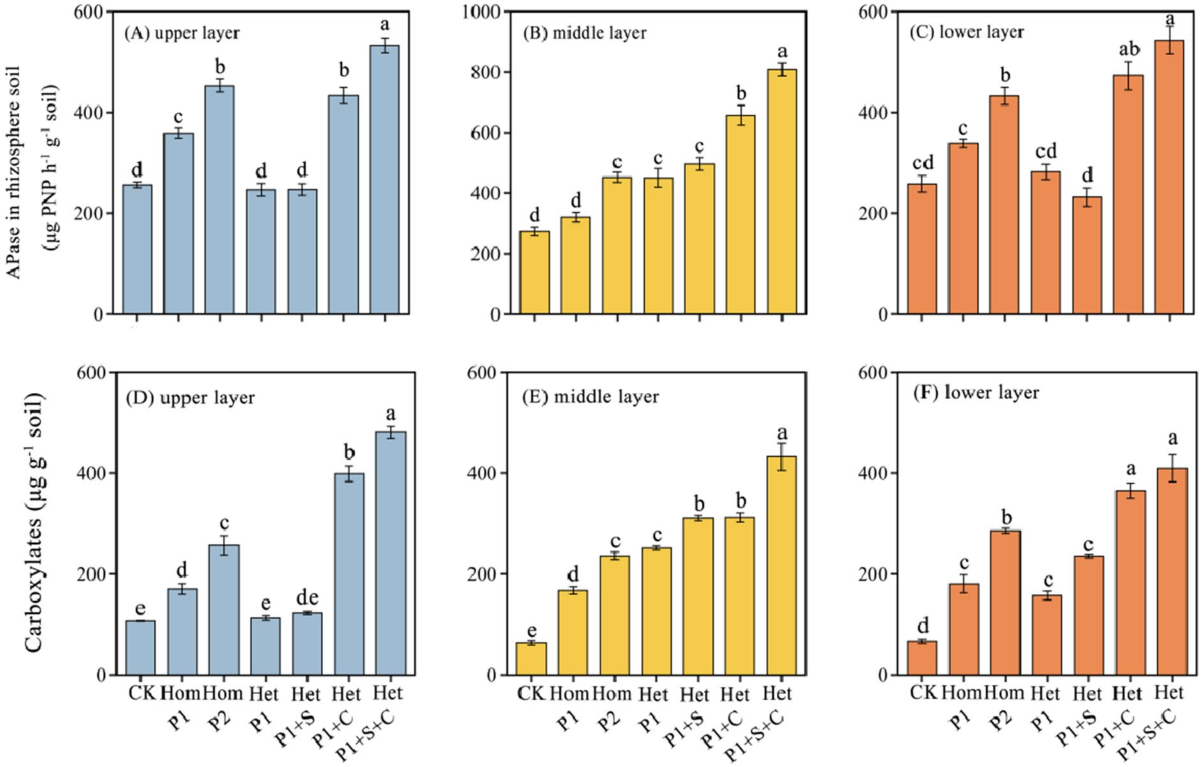

Fig. 5 Acid phosphatase activity (APase; **A**-**C**) and the concentration of carboxylates (D-F) in the rhizosphere soil of upper, middle and lower soil layers after 30 days of maize growth under different treatments. Data are means ± standard errors ($n=4$). Different lowercase letters denote significant difference ($P \leq 0.05$). Control: no P addition, Hom P1: homogeneous P1 supply (75 mg P kg^{-1}), Hom P2: homogeneous P2 supply (225 mg P kg^{-1}); Het P1: heterogeneous P1 supply; Het P1 + S: heterogeneous (co-localized) P1 and S (150 mg S kg^{-1}) supply; Het P1 + C: heterogeneous (co-localized) P1 and C (480 mg C kg^{-1}) supply; Het P1 + S + C: heterogeneous (co-localized) P1, S and C supply

the middle soil layer, the available P was significantly lower in the Het P1 and Het P1+S treatments than in the Hom P2, Het P1+C and Het P1+S+C treatments (Fig. 6B). Similarly, the Hom P2 treatment resulted in higher concentration of moderately labile P in the upper and lower soil layers compared with all the other treatments (Fig. 6D, F). Compared to Het P1, the moderately labile P in the Het P1+S+C treatment decreased by 31%, 32% and 27% in the upper, middle and lower soil layers, respectively (Fig. 6D, E, F). The heterogeneous P1+C and P1+S+C supply resulted in significantly higher microbial biomass P compared to the other treatments (Fig. 6G, H, I).

There was no difference in the rhizosphere soil pH of the upper and lower layers among the treatments (Fig. 7A, C). Application of S (Het P1 + S) significantly (by one pH unit) decreased the rhizosphere soil pH of the middle layer compared to the treatment without S application (Het P1) (Fig. 7B).

Discussion

Maize growth and P availability responses to the heterogeneous supply of P combined with S and/or C

To adapt to their environment, plant roots exhibit high developmental plasticity (Karlova et al. 2021). Such plasticity is crucial for plant P acquisition in soils with low P availability (Kumar et al. 2019). The response of roots to P-rich patches, manifested as an increase in lateral root number and lateral root length, has improved plant growth and P use efficiency (Drew 1975; Jing et al. 2010). In the present study, shoot biomass and P uptake did not differ among homogeneous (Hom P1) and heterogeneous P (Het P1) supplies (Fig. 2), whereas root growth exhibited differences among the two contrasting P supplies (Fig. 3). Two factors could explain this observation. Firstly, the availability of P is

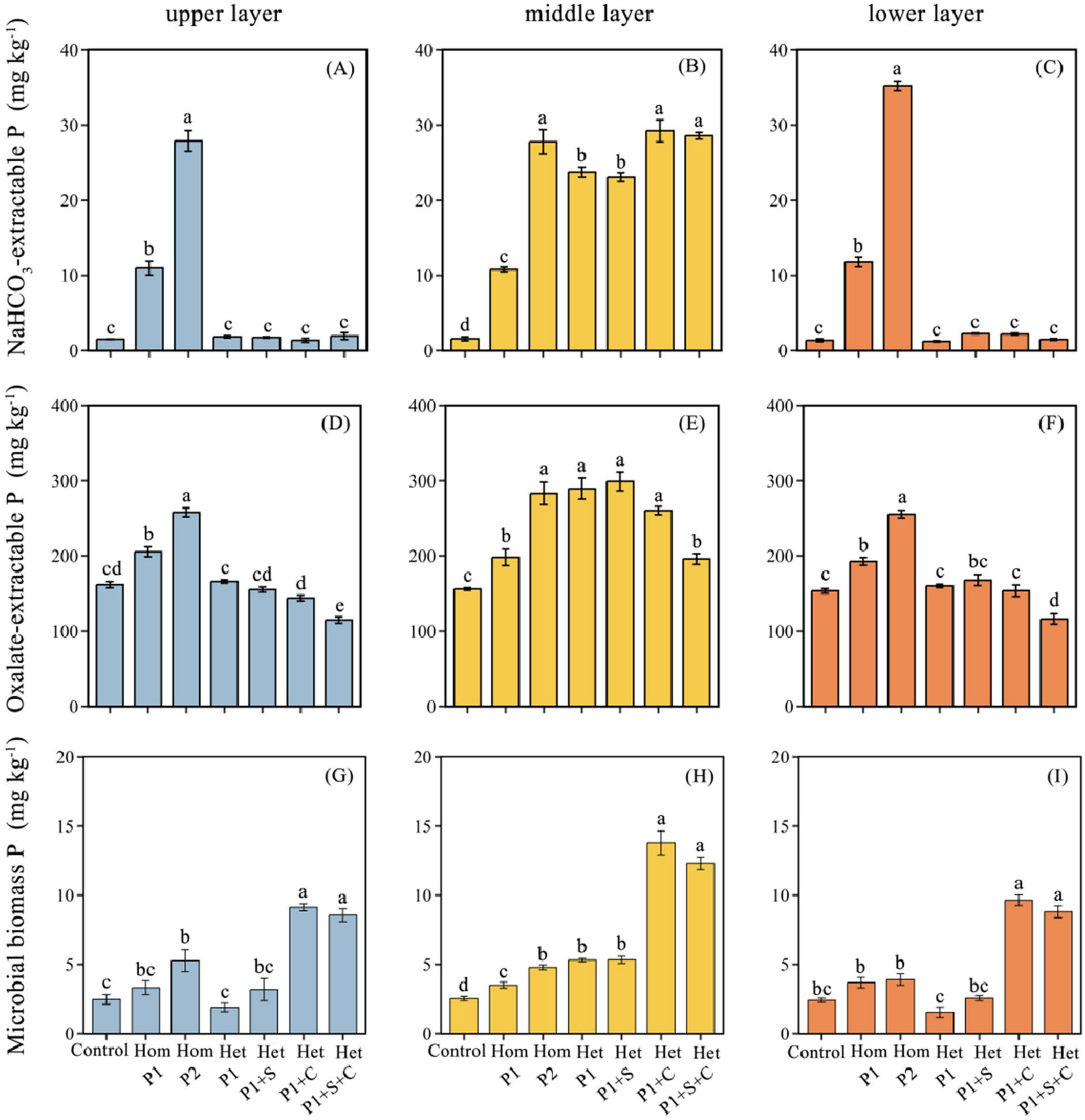

Fig. 6 $NaHCO_3$-extractable P (available P) (**A**-**C**), oxalate-extractable P (moderately labile P) (D-F) and microbial biomass P (**G**-**I**) of upper, middle and lower layers of bulk soil after maize grew for 30 days under different treatments. Data are means ± standard errors ($n = 4$). Different lowercase letters denote significant difference among treatments ($P \leq 0.05$). Control: no P addition, Hom P1: homogeneous P1 supply (75 mg P kg^{-1}), Hom P2: homogeneous P2 supply (225 mg P kg^{-1}); Het P1: heterogeneous P1 supply; Het P1 + S: heterogeneous (co-localized) P1 and S (150 mg S kg^{-1}) supply; Het P1 + C: heterogeneous (co-localized) P1 and C (480 mg C kg^{-1}) supply; Het P1 + S + C: heterogeneous (co-localized) P1, S and C supply

low in calcareous soils (Shen et al. 2011), and heterogeneous P supply may result in excessive root growth of some plant species, which may be less favorable for plant growth compared to homogeneous supply (Li et al. 2014). Therefore, previous studies in calcareous soils have employed heterogeneous placement of both NH_4-N

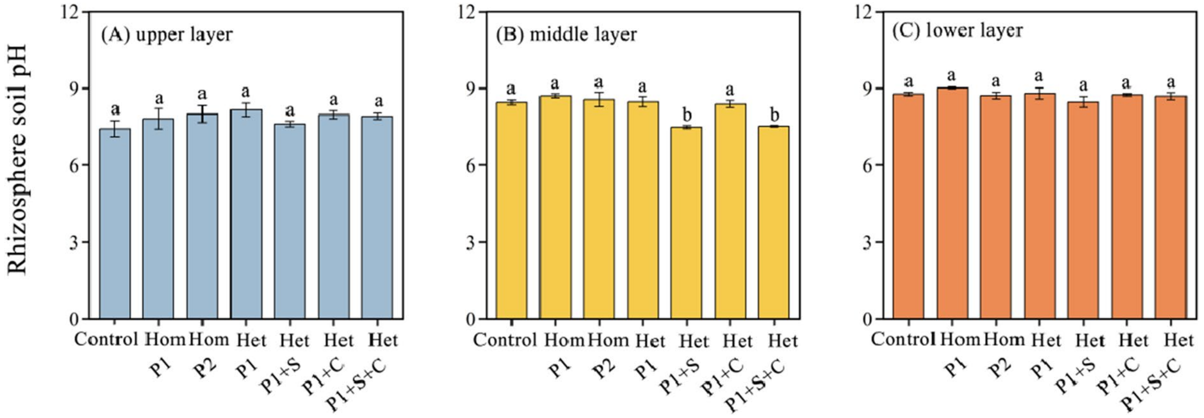

Fig. 7 Rhizosphere soil pH of upper (**A**), middle (**B**) and lower (**C**) layers after 30 days of maize growth under different treatments. Data are means ± standard errors ($n = 4$). Different lowercase letters denote significant difference ($P \leq 0.05$). Control: no P addition, Hom P1: homogeneous P1 supply (75 mg P kg^{-1}), Hom P2: homogeneous P2 supply (225 mg P kg^{-1}); Het P1: heterogeneous P1 supply; Het P1 + S: heterogeneous (co-localized) P1 and S (150 mg S kg^{-1}) supply; Het P1 + C: heterogeneous (co-localized) P1 and C (480 mg C kg^{-1}) supply; Het P1 + S + C: heterogeneous (co-localized) P1, S and C supply

and P, whereby NH_4-N had the effect of acidifying the soil and thus improving soil P availability, significantly promoting maize growth as well as P uptake (Jing et al. 2010; Wang et al. 2021). Secondly, it cannot be excluded that roots responded to heterogeneous P supply in preference to (or faster than) the aboveground plant parts within the time allocated.

Sulfur is commonly used as an amendment for soil to decrease pH and increase P availability to crops (Deluca et al. 1989; Scherer 2001). Jaggi et al. (2005) reported that applying S to neutral and alkaline soils can significantly decrease soil pH (by 0.6–0.7 units) due to biochemical oxidation of S that produces H_2SO_4. In calcareous soils, the high concentration of $CaCO_3$ exacerbates P adsorption as well as precipitation reactions forming stable calcium phosphates, so that P concentrations in soil solution are generally low (<1% of total soil P) (McLaughlin et al. 2011; Shen et al. 2011). Soil pH can regulate nutrient availability. Hopkins and Ellsworth (2005) showed that the available P is highest in soil with pH 5.0–7.0; hence, lowering the pH of calcareous soil is a strategy for increasing the soil P availability. Lower rhizosphere pH in calcareous soil may stimulate root growth by regulating root cell proliferation and loosening the cell wall matrix (Edelmann and Fry 1992; Bloom et al. 2002). Moreover, a decrease in the pH of calcareous soil can dissolve $CaCO_3$ and release P bound to Ca minerals into soil solution, thereby increasing P availability (Jaggi et al. 2005; Ye et al. 2010). As shown in the present study, heterogeneous supply of P1 and S significantly decreased rhizosphere soil pH (Fig. 7B), stimulated the growth of maize shoots and roots as well as P uptake, and improved the P-use efficiency (Figs. 1 and 2). This resulted in lower amounts of available P in the soil after harvesting in the Het P1 + S treatment compared to the treatments with equivalent amount of P addition in the middle soil layer (Hom P2, Het P1 + C, Het P1 + S + C; Fig. 6B).

Soil microorganisms play vital roles in soil P transformation as well as the oxidation of S to produce H_2SO_4 (Richardson 2001; Jaggi et al. 2005). Phosphate-solubilizing microorganisms (PSM) have been reported to solubilize soil inorganic P that is not available to plants. Hence, they may promote P uptake by plants (Jakobsen et al. 2005). The addition of C increases soil microbial activity (Xu et al. 2020), and we hypothesized that it would enhance S oxidation, acidification of calcareous soils, and soil P availability. However, we did not find a positive effect of C addition on plant growth (Fig. 2), but instead found a significant decrease in root biomass and root length (Fig. 3). This is possibly because of the competition for nutrients between microorganisms and plants (Kuzyakov and Xu 2013). The competitiveness of microorganisms is affected by carbon availability, which restricts microbial growth and activity (Demoling et al. 2007; Marschner et al. 2011). However, from a long-term perspective, microbial biomass P can become available and conducive to plant growth upon microbial biomass turnover.

We found an increase in microbial biomass P in the C-addition treatment (Fig. 6B). Previous studies have reported that an increase in soil C can increase transfer of available P into microbial biomass P (Wu et al. 2007). Marschner (2007) reported that adding glucose to the soil stimulated the bacteria to transform available P into microbial biomass P, suggesting that bacteria competed with plants for available P. However, in the present study, contrary to expectations, in the carbon addition treatment we found an increase in soil available P and a decrease in moderately labile P (Fig. 6B; E). It could be due to the conversion of soil moderately labile P into available form via soil microorganisms (Wang et al. 2016; Soltangheisi et al. 2021). The other possible interpretation is that with C depletion, some P got released from the microbial biomass pool into the soil available P pool (Zhang et al. 2014).

Response of root functional traits to the heterogeneous P supply combined with S and/or C

By comparing the length of roots, the concentration of carboxylates and the activity of acid phosphatase responses in each treatment relative to the Hom P2 treatment, we found that heterogeneous P1 and heterogeneous P1 plus S application induced changes in root growth (Figs. 4 and 5). On the other hand, the addition of C altered the concentration of carboxylates and activity of phosphatase in the rhizosphere (Fig. 5). Root exudation of acid phosphatase and carboxylates is important for P mobilization and acquisition (Vance et al. 2003; Lambers et al. 2006).

It is generally accepted that carboxylate secretion and phosphatase activity in the rhizosphere increase under low-soil P conditions (Pearse et al. 2007; Zhang et al. 2016; Zhou et al. 2016). However, in this study, root length was increased significantly in the middle soil layer (P-rich patch) of the Het P1 treatment compared to the Hom P2 treatment (Fig. 4B), but the rhizosphere acid phosphatase activity and carboxylate concentrations were significantly decreased in the upper and lower layers (Fig. 5). The plasticity of root growth in response to heterogeneous nutrients is species-specific, and is greater in graminaceous species, such as maize, than in leguminous species, such as chickpea (Li et al. 2014; Yang et al. 2022). This might have been associated with maize being dependent on the changes in root morphological traits to enhance phosphorus acquisition rather than on root exudation (Lyu et al. 2016).

Some studies reported a decrease in the rhizosphere concentration of carboxylates and activity of acid phosphatase under low soil P availability (Corrales et al. 2007; Liu et al. 2016; Lyu et al. 2016), but others reported the opposite results (Gaume et al. 2001; Yun and Kaeppler 2001). Such variable results might have been due to different durations and conditions of plant growth. We found that in the studies that obtained positive results (i.e., the concentrations of carboxylates and activity of acid phosphatase increased under low P supply), maize was grown hydroponically (Gaume et al. 2001; Yun and Kaeppler 2001), which may not accurately reflect the responses that would occur in the rhizosphere soil (Oburger and Schmidt 2016). In addition, the growth stage and the genotype of maize can also influence the exudation of carboxylates into the rhizosphere (Liu et al. 2004; Santangeli et al. 2024).

There was a significant increase in acid phosphatase activity and the concentration of carboxylates in the maize rhizosphere in the Het P1 + C treatment (Fig. 5). These increases could be attributed to the increased microbial abundance resulting from the addition of C. Greater abundance of microorganisms would mean a greater potential that microorganisms would exude phosphatase and carboxylates, complementing similar plant exudations (Richardson et al. 2009; Zhang et al. 2016). In addition, carboxylates are also produced during the degradation of glucose (Rukshana et al. 2011) that we added as a C source.

The 2-D rhizobox system was adopted in this study to facilitate in situ observation of root growth responses to localized supply of nutrients. However, root growth could be constrained to some extent, by the size of the rhizobox (Neumann et al. 2009). Therefore, in this experiment, maize was harvested immediately after it was observed that the roots touched the sides of the rhizobox, resulting in a growth period of only 30 days for maize. In addition, this experiment had well-defined control treatments as well as four replicates to ensure appropriate statistical analysis for the certainty of research results as mentioned in previous studies (Wang et al. 2021; Jing et al. 2022). Under field conditions, localized application of phosphorus and ammonium can further improve the growth of maize seedlings by stimulating root proliferation and rhizosphere acidification (Jing et al. 2010, 2022). To develop practical measures based on this study, further field experiments should be considered to validate the results of this study, due to variations in light intensity, soil moisture, soil nutrient availability and soil biological activity in farmland soil.

Conclusions

In contrast to homogeneous low P (P1) supply, heterogeneous P1 supply led to proliferation of maize roots, but no significant response was found in the maize shoots. Application of S significantly decreased the rhizosphere soil pH, thereby enhancing soil P availability. It stimulated proliferation of maize roots and promoted maize shoot growth and P content and P-use efficiency. Addition of C caused divergent impacts on P availability. On the one hand, there was a significant increase in acid phosphatase activity and the concentration of carboxylates in the maize rhizosphere and in soil available P. On the other hand, this study did not find a growth-promoting effect of C addition on maize shoots, potentially due to the intensified competition between microorganisms and plants. This was indicated by increased microbial biomass P and the significantly inhibited maize root growth. Long-term field research is needed to gain more insight into the coupling of P and C cycling processes to maximize the synergies between microorganisms and plants.

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Author contributions Jianbo Shen and Dongfang Zheng designed the study. Dongfang Zheng carried out the glasshouse and laboratory work, analysed the data and wrote the manuscript. Yang Lyu, Gerard H. Ros, Wim de Vries, Zed Rengel and Jianbo Shen contributed to the review and editing of the manuscript, data analysis, and language editing.

Data availability The data in this study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

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