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RESEARCH ARTICLE

Maize shows intraspecific facilitation under phosphorus deficiency but competition under nitrogen deficiency when grown under increased plant densities in alkaline soil

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Abstract

Background and aims Neighbouring plants compete for resources in intensive cropping systems when the plant density is high. Most studies on plant density have focused on yield responses, whereas only few studies have paid attention to belowground root-soil-interactions. Knowledge about belowground responses to different plant densities under nitrogen (N) or phosphorus (P) limitation remains scant.

Methods Two pot experiments were conducted in a glasshouse using a calcareous soil (pH 8.4). Five treatments were applied with different amounts of N or P and planted with different plant densities. Shoot and root biomass, and root morphological traits including total root length and proportions of root length in different diameter classes were examined in both the N and P experiment. Root physiological traits including rhizosheath pH, phosphatase activity and carboxylate concentration were measured in the P experiment.

Results Plant biomass, P content and total root length increased with increasing plant density in the P experiment, while plant biomass, N content and

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total root length decreased with increasing plant density in the N experiment. Maize with high plant density released carboxylates and phosphatases under P deficiency.

Conclusion Growing in calcareous soil, maize showed a competition effect at increasing plant density under N limitation, but an intraspecific facilitation effect at increasing plant density under P limitation. This study shows that maize (*Zea mays* L. cv. ZD958) released carboxylates and phosphatases in response to high soil pH under P-limiting conditions. The findings of this work are important towards the sustainability of intensive cropping systems.

Keywords Calcareous soil · Competition · Facilitation · Maize · Nitrogen · Phosphorus · Plant density

Introduction

Crop growth and productivity are often limited by resources including light, water, and nutrients (Freschet et al. 2018). One of the most important ways to increase crop productivity is to increase plant density (Adams et al. 2019; Duvick 2005). Plant density is often high in intensive cropping systems; however, there may be competition for resources among neighbouring individual plants. Yield responds differently to plant density in intercropping systems and sole cropping systems (Mao et al. 2018; Willey and Osiru 1972). Many studies about plant density focus on intercropping systems but pay little attention to belowground interactions in intraspecific competition (Echarte et al. 2011; Zhang et al. 2020b). Under high plant density, incident light capture per plant is decreased, and this drives an increase in specific leaf area and leaf mass fraction but a decrease in photosynthesis (Evans and Poorter 2001; Freschet et al. 2018; Poorter et al. 2012). With slower photosynthetic rates, less biomass will accumulate in plants, and less carbon (C) is allocated to roots. Carbon is important for belowground interactions as both root production and exudation require a large amount of C (de Vries et al. 2019; Ding et al. 2021; Raven et al. 2018). Without sufficient C from photosynthesis, roots might not be able to acquire sufficient nutrients as they require C for nutrient acquisition (Phillips et al. 2011; Raven et al. 2018); thus, they may exhibit nitrogen (N) and phosphorus (P) deficiency. Both N and P are important macronutrients that may limit crop growth and productivity in agroecosystems. Farmers supplement nutrients to avoid deficiency caused by high density through fertiliser application, but often too much fertiliser is applied giving rise to many offsite environmental problems such as water pollution (Cameron et al. 2013; Vitousek et al. 1997; Withers et al. 2014). Many studies have been done to underpin effective fertilisation (Cai et al. 2015; Liu et al. 2022; Scavo et al. 2022), but most of the work on plant density focuses on yield responses (Cong et al. 2023; Ren et al. 2017; York et al. 2015). In contrast, few studies have paid attention to the activity belowground, and our knowledge about rhizosphere responses to different plant densities under low N or P supplies remains scant.

In low-nutrient habitats, numerous nutrient-acquisition and -utilisation strategies evolved in plants, and these mechanisms have been widely studied in different ecosystems (Coskun et al. 2017; Lambers et al. 2022; Luo et al. 2022). Nitrogen is mobile in soil and relatively easy for plants to acquire. However, farmers prefer to apply N fertilisers only once before sowing instead of several times during the growing period (Gao et al. 2015), and thus, there is often N leaching that pollutes groundwater. For this reason, it is important to know plant responses to less N fertiliser supplies that contribute to greater N-use efficiency. Plant root structures change under N deficiency (Maizlish et al. 1980; Wang et al. 2005). Nitrogen deficiency suppresses the shoot growth of maize (*Zea mays* L.) while the root to shoot ratio increases concomitantly with changes in root architecture (Chen et al. 2014; Chun et al. 2005). Maize plants show greater total and lateral root lengths that contribute to N acquisition, and the elongation of individual axial roots increases whereas their number decreases (Chun et al. 2005). However, for crops growing in calcareous soils in northeast China, we do not know how root morphology of maize changes under different plant densities with low N supply, and whether the root to shoot ratio changes with increasing plant density.

In contrast to the mobility of nitrate, phosphate (P_i) is relatively immobile, and does not readily move to the root surface for subsequent active uptake. Mass flow of N in the soil is 150 kg ha^{-1} while that of P is only 2 kg ha^{-1} (Barber 1985), and thus, less P arrives at the root surface. The diffusion coefficient

of phosphate is also very low compared with that of nitrate, and this limits its supply to the roots (Lambers and Oliveira 2019). Growing in low-P soils, roots show morphological changes that increase the root surface areas and absorb more P_i from the soil solution, as well as physiological acclimations that provide greater access of phosphate sorbed onto soil particles (Lyu et al. 2016; Reichert et al. 2022).

At the morphology level, many plants tend to produce more lateral roots, especially fine roots and show high fine root proportions which increase the volume of soil that roots can explore (Shen et al. 2018; Wen et al. 2019). More and longer root hairs and a higher root mass ratio contribute to uptake of all ions but especially P_i in response to the low mass flow and slow diffusion of phosphate in the soil solution (Lambers and Oliveira 2019). Some species growing in extremely P-impoverished habitats show special root structures such as cluster roots and dauciform roots that comprise hundreds to thousands of rootlets or numerous root hairs (Lamont 1982; Shane et al. 2006; Shane and Lambers 2005). At the physiological level, some P-mobilising species release root exudates that mine soil P as well as some micronutrients such as manganese (Mn) and iron (Fe) at the same time (Lambers and Oliveira 2019), and thus make more P available in the soil solutions. These P-mobilising species can release low-molecular-weight exudates including carboxylates that mobilise soil-bound P into soluble P_i and organic P (P_o) (Lambers and Oliveira 2019; Li et al. 2014). Exuded phosphatases can then hydrolyse P_o in the soil solution into P_i for plants to take up (Lambers and Oliveira 2019). At the same time, some non-P-mobilising species cannot mobilise P themselves but can be facilitated by their neighbouring plants to acquire P (Lambers et al. 2022; Zhong et al. 2021). These facilitated plants can take up the P_i in the soil solution mobilised by their neighbouring plants. For example, in south-western Australia, many native Proteaceae plants such as *Hakea* species produce cluster roots, which release large amounts of carboxylates that contribute to P acquisition (Shane et al. 2004). The non-P-mobilising species *Bossiaea eriocarpa* (Fabaceae) can take up the P_i mobilised by their P-mobilising Proteaceae neighbours (Abrahão et al. 2018). Steppe species in Inner Mongolia also exhibit facilitation of P acquisition strategies (Yu et al. 2020). Many crop species form more fine roots and release exudates under P deficiency (Lyu et al. 2016). As for maize, it tends not to release more exudates in low-P soil but alters its root morphology (Wen et al. 2017), while wheat releases phosphatases under P deficiency in addition to root morphological changes (Shen et al. 2018).

Soil pH is also important for nutrient acquisition, and plant are less effective at acquiring P when growing in alkaline soils than in neutral soils (Lambers and Oliveira 2019). The P sorbed on oxides and hydroxides of iron and aluminium in the soil has to be mobilised by carboxylates or protons to readily-exchangeable P in the soil solution for roots to take it up. In acid soils, only chelation is needed to solubilise P_i sorbed onto soil particles. The concentration of hydroxide ions (OH^-) is higher in alkaline soils, and both acidification and chelation are needed to enhance the P_i concentration ($[P_i]$) gradient between the soil and root surface (Lambers and Oliveira 2019). In this study, calcium ions are the main cations present in the calcareous soil, and sorbed phosphate with a negative charge can form bonds with positive calcium ions. The calcium phosphate compounds, which are less soluble in soil must be dissociated first before roots can take up P, and protons can replace calcium ions (Lambers and Oliveira 2019). The maximum activity for acid phosphatases is around pH 5.0–6.5 (Eivazi and Tabatabai 1977). Phytase, which is important for hydrolysing phytate, exhibits optimal activity in acidic conditions ($pH < 4.0$) (George et al. 2005; Turner and Balckwell, 2013). The $[P_i]$ in calcareous soil increases markedly as soil pH declines (Turner and Blackwell 2013). Calcareous soils are common in north China, where P availability is limiting for plant growth (Ding et al. 2019), and, thus, large amounts of P fertilisers are applied by farmers. Growing in calcareous soil with low P availability, it is important to know how plant density might affect plant P-acquisition strategies, and whether crops acidify the soil to make P more available.

How plants respond to different plant densities in intensive cropping systems with low N- and P-fertiliser supplies, especially in terms of rhizosphere processes remains unknown. In this study, we investigated maize growing in calcareous soils under different plant densities with the same low total N and P additions per pot, and then the nutrient supplies were supplemented with the same N and P additions per plant. For the N experiment, we hypothesised that the biomasses of maize plants would be less as a result of

competition in response to high plant density. After supplementing plants with the same amount of N for each plant, the competition at high plant density treatments is expected to be relieved. For the P experiment, we hypothesised that the maize plants acidify the soil and form more fine roots at high plant density because of resource competition. We hypothesised that maize releases more protons, thus significantly decreasing the rhizosheath soil pH at high plant density with the effect to make P more available. Maize shows morphological changes without releasing more carboxylates that mobilise P and phosphatases that hydrolyse P_o in response to high plant density under P deficiency.

Materials and methods

Experimental set-up

Two pot experiments were conducted in a natural-lit greenhouse at China Agricultural University with a day/night average temperature of 28/16 °C, and 45–55% relative air humidity. Maize (*Zea mays* L. cv. ZD958) was selected as the study species and a low-P calcareous silt loam soil with bulk density 1.45 g cm^{-3} in North China Plain was collected from Shangzhuang, Beijing (40°05′40″N, 116°12′32″E). The soil type is a fluvo-aquic soil defined by the Chinese soil classification system, and classified as a calcareous Cambisol according to FAO's classification. The soil was air-dried and sieved through a 2-mm sieve and then mixed thoroughly. The soil pH was 8.4 (1: 2.5, soil: water), and contained: organic C 11.5 g kg^{-1}; total N 0.51 g kg^{-1}; mineral N, NO_3^- and NH_4^+ 8.5 mg kg^{-1}; total P 0.69 g kg^{-1}; Olsen-P 1.68 mg kg^{-1}; total K 14.6 g kg^{-1}; NH_4Ac exchangeable K 82.4 mg kg^{-1}. Each pot (outside diameter, 15 cm; inside diameter, 12 cm; height, 12 cm) was filled with 0.14 kg of air-dried soil.

The first experiment (named the N experiment in the following text) was conducted from August to September 2016. Three treatments were all supplied with the same 60 mg N kg^{-1} soil ($Ca(NO_3)_2{\cdot}4H_2O$) per pot which is low but can support plant growth (Wang et al. 2005), with different plant densities (1–3 plants per pot): 60 (1), 60 (2) and 60 (3). Another two treatments were supplied with the same 60 mg N kg^{-1} soil per plant with different plant densities: 120 (2), and 180 (3). The soil was also supplied with the following nutrients (mg kg^{-1}): KH_2PO_4 1317, K_2SO_4 335, $CaCl_2$ 126, $MgSO_4{\cdot}7H_2O$ 43, EDTA-FeNa 5.8, $MnSO_4{\cdot}4H_2O$ 6.7, $ZnSO_4{\cdot}7H_2O$ 10, $CuSO_4{\cdot}5H_2O$ 2.0, H_3BO_3 0.67, and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.26.

The second experiment (named the P experiment in the following text) was conducted from May to June, 2017. Five similar treatments were applied with different amounts of P (KH_2PO_4) and plant densities. There were three treatments with the same 60 mg P kg^{-1} soil per pot which is low but can support plant growth (Wen et al. 2017), with different plant densities (1–3 plants per pot): 60 (1), 60 (2) and 60 (3). Two further treatments supplied the same 60 mg P kg^{-1} soil per plant with different plant densities: 120 (2), 180 (3). The pots were also supplied with the following nutrients (mg kg^{-1}): $Ca(NO_3)_2{\cdot}4H_2O$ 1687, K_2SO_4 335, $CaCl_2$ 126, $MgSO_4{\cdot}7H_2O$ 43, EDTA-FeNa 5.8, $MnSO_4{\cdot}4H_2O$ 6.7, $ZnSO_4{\cdot}7H_2O$ 10, $CuSO_4{\cdot}5H_2O$ 2.0, H_3BO_3 0.67, and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.26.

The maize seeds were sterilised for 30 min in 10% (v/v) H_2O_2 and then germinated at 23 ℃ for 48 h in a dark and humid environment. Uniformly germinated seeds were planted in the pots when the main roots were around 1 cm. Then two, four and six germinated seeds were selected at the three-leaf stage and planted in the soil in pots. After one week, the seedlings were thinned to one, two, and three plants per pot according to the different treatments. This was a completely randomized design. All pots were arranged randomly on one bench and rearranged and watered every day by weighing to keep the soil moisture at ~ 70% of pot capacity.

Harvests and analyses

The maize plants were harvested at the five-leaf stage (30 days) for both the N and P experiment (Fig. S1). Shoots were severed at the soil surface and kept in paper bags. The shoots were dried at 105 ℃ for 30 min and then at 70 ℃ for 48 h, after that, the shoot weight was measured. All the visible roots were collected and kept in a zip-lock bag, then the fresh roots were kept at −20 ℃ for future measurement after being washed free of soil with deionized (DI) water. The soil in the pots was thoroughly mixed and collected as bulk soil. For the P

experiment, rhizosheath soil was collected. Roots were gently shaken, and the soil still adhered to the roots was defined as rhizosheath soil. The roots with rhizosheaths were rinsed with 0.2 mM $CaCl_2$ for 1 min to collect the rhizosheath soil. The suspension was stored at −20 ℃ and then used to determine the rhizosheath soil pH, carboxylates and acid phosphatase activity. For the carboxylate measurement, two drops of 0.01 g L^{-1} microbial inhibitor Micropur (Sicheres Trinkwasser, Munich, Germany) and two drops of concentrated phosphoric acid were added to the suspension before being stored at −20 ℃ for high-performance liquid chromatography (HPLC) analysis. Rhizosheath soil pH was measured on the harvest day and a modified pH (adjusted to 1: 2.5 soil: water ratio) was calculated from the measured pH (Li et al. 2010).

Root morphological traits were analysed by scanning the whole root systems. Roots were well arranged in the water without overlap in a transparent tray (30 × 20 × 3 cm), and then scanned at 400 dpi with Epson Expression 1600 (Seiko Epson, Nagano, Japan). The Win-RHIZO (Regent Instruments Inc., Quebec, Canada) was used to analyse the total root length and the root length in different root diameter classes. Then the clean roots were oven-dried at 70 ℃ for 48 h and the root dry weight was determined. The proportion of fine roots (diameter 0–0.2 mm), medium-size roots (diameter 0.2–0.4 mm) and thick roots (> 0.4 mm) to total root length was calculated.

Plant material was ground into a fine powder with a stainless-steel multifunctional grinder (DM-200 g, Nanjingdongmai, Nanjing, Jiangsu, China) before being weighed for nutrient analyses. Around 8-mg samples were weighed for leaf N measurement by an elemental analyser (Model NA2500, CE Instruments, Milan, Italy). Additional 0.3000 g leaf samples were weighed and digested with 6 ml concentrated HNO_3 and 2 ml of 30% (v/v) H_2O_2 in a microwave-accelerated reaction system (CEM, Matthews, NC, USA) (Niemelä et al. 2005), and the concentrations of P, K, Ca, Mg, Mn, Zn, Cu, and Fe in the digested solutions were determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies, Santa Clara, California USA). Root nutrient uptake was calculated by dividing the plant nutrient content by total root length.

Root exudate analysis

The stored solution for carboxylate measurement was filtered through sterile Millex GS Millipore 0.22-μm filters and directly analysed for carboxylates using Waters HPLC (Shen et al. 2003). The chromatographic separation was conducted on a 250 × 4.6 mm reversed-phase column (Alltima C18, 5 μm, length 250 mm × 4.6 mm; Alltech Associates, Inc., Deerfield, IL, USA). Detection of carboxylates was carried out at 214 nm. A 25 mM KH_2PO_4 (pH 2.3) mobile phase was used for isocratic elution with a flow rate of 1 mL min^{-1} at 31 ℃.

Acid phosphatase activity was measured using a spectrophotometric method (Neumann 2006). The 0.1 mL substrate was added as 0.15 M *p*-nitrophenyl phosphate (PNP) with 0.4 mL sodium acetate buffer (pH 5.2). Samples were incubated for 30 min at 27 ℃, and then 0.5 mL 0.5 M NaOH was added to stop the reaction. After that, the absorbance of the supernatants was measured spectrophotometrically at 405 nm (Alvey et al. 2001).

Statistics

All statistical analyses were made using the platform R v4.3.2 (R Development Core Team, 2023). Heteroscedasticity and normality of the residuals were assessed for each figure, and the variance was log-transformed if the data failed to pass the heteroscedasticity or normality check. Differences among five treatments with different plant density and nutrient addition in P and N experiments were assessed using one-way ANOVA tests with the package *stats* followed by *least significant difference* post hoc tests (package *multcomp*) in R. Sample size was 4 and full ANOVA tables for each figure have been provided in the Supporting Information (Tables S3-S9). Linear regression analysis was conducted to test the correlations between acid phosphatase activity and shoot P concentration ([P]), and between plant density × 60 (1), 60 (2) and 60 (3)/ 60 (1), 120 (2) and 180 (3) in R.

Results

Plant growth and nutrient uptake

Plant growth and biomass of maize showed different trends in the P and N experiments (Figs. 1, S2).

Shoot biomass increased with increasing plant density in the P experiment (Figs. 1a, b, S2a), while it decreased with increasing plant density among 60 (1)-(3) in the N experiment (Figs. 1a, S2b). With the same low P supply per pot (60 mg P kg^{-1} soil), shoot biomass of 60 (3) (three plants per pot) was around 70% greater than that of 60 (1) (one plant per pot). With the same amount of P per plant, shoot biomass of 180 (3) (180 mg P kg^{-1} soil with three plants per pot) was around 180% greater than that of 60 (1). With the same low N supply per pot, shoot biomass of 60 (3) was about 70% lower than that of 60 (1). Shoot biomass of 120 (2) (120 mg P kg^{-1} soil with two plants per pot) was intermediate between those of 60 (1) and 180 (3). Similar to shoot biomass, root biomass also increased with increasing plant density under P deficiency (Figs. 1c, d, S2c), whereas it decreased with increasing plant density among 60 (1)-(3) under N deficiency (Figs. 1c, d, S2d). In the P experiment, root biomass of 60 (3) was around 60%

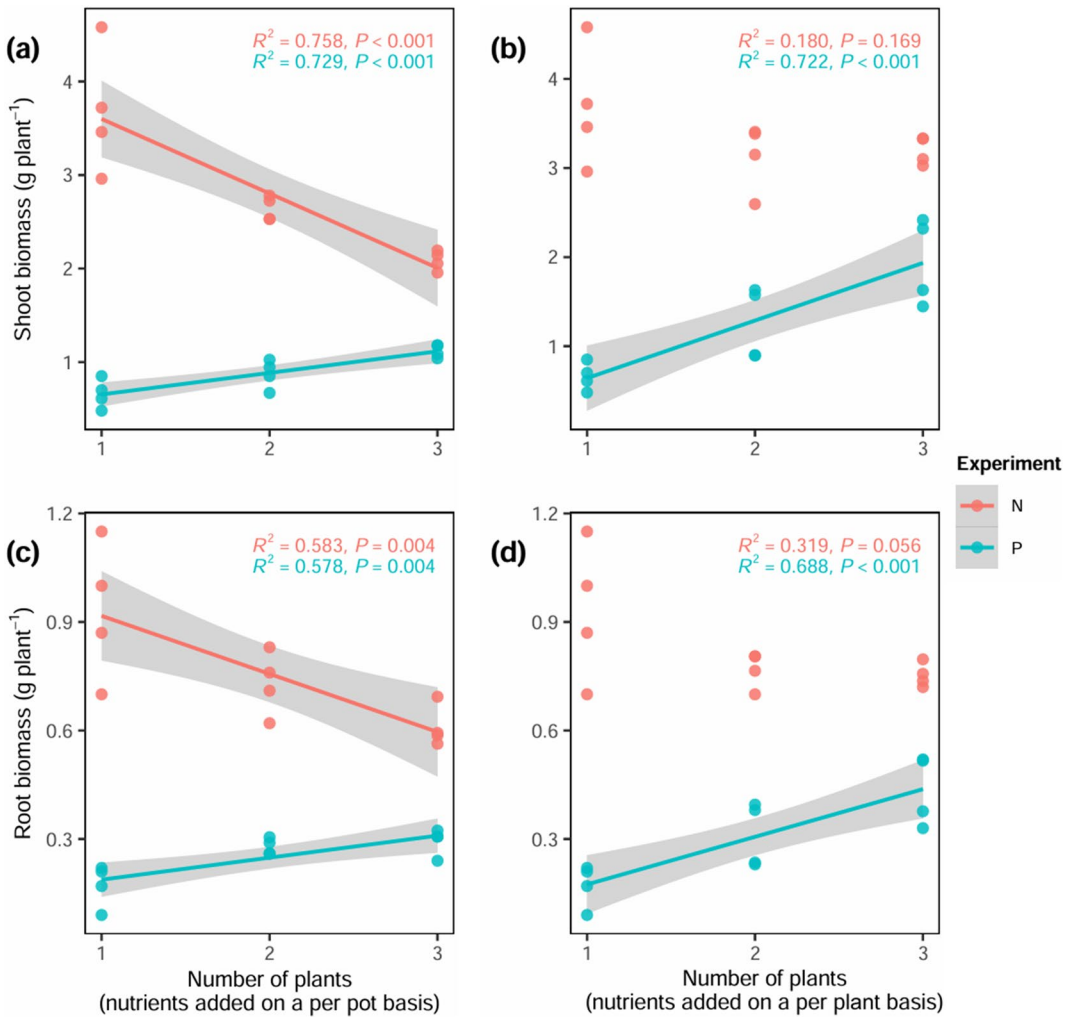


Fig. 1 Correlations between plant biomass and plant density in the phosphorus (P) experiment, and nitrogen (N) experiment. Correlations between shoot biomass and different plant density among (**a**) 60 (1), 60 (2) and 60 (3), and (**b**) 60 (1), 120 (2) and 180 (3); correlations between root biomass and different plant density among (**c**) 60 (1)−60 (3), and (**d**) 60 (1)−180 (3). The shaded area indicates the 95% confidence range, derived from the models. The regression lines and their 95% confidence range are not drawn when $P > 0.05$. Treatment 60 (1), 60 (2) and 60 (3): three treatments supplied with the same 60 mg P kg^{-1} soil per pot with different plant densities (1–3 plants per pot). Treatment 120 (2) and 180 (3): another two treatments supplied with the same 60 mg P kg^{-1} soil per plant with different plant densities (2–3 plants per pot)

greater than that of 60 (1). After being supplemented with the same amount of P per plant, root biomass of 180 (3) was about 140% greater than that of 60 (1). In the N experiment, root biomass of 60 (3) was around 50% less than that of 60 (1). After being supplemented with the same amount of N per plant, the root biomass of 180 (3) was the same with that of 60 (1). However, root/shoot ratios were indistinguishable among all treatments in both the N and P experiments (Figs. S2e, S2f).

When supplied with the same amounts of P per pot, both shoot and root [P] were all indistinguishable in the five treatments with increasing plant density (Figs. 2a-d, S3a, S3c). In the N experiment, when supplied with the same amounts of N per pot, shoot and root N concentrations ([N]) decreased with increasing plant density (Figs. 2a-d, S3b, S3d). With the same low P supply per pot, shoot [P] was intermediate with increasing plant density. When supplemented with the same amount of P per plant, shoot [P] showed an increasing trend with increasing plant density (Fig. 2b). Shoot [P] of 180 (3) was about 20% greater than that of 60 (1). With the same low N supply per pot, shoot [N] of 60 (3) was about 50% lower than that of 60 (1). Shoot [N] of 180 (3) was about 70% lower than that of 60 (1), while shoot [N] of 60 (3) was indistinguishable from that of 180 (3). Shoot N: P ratio decreased with increasing plant densities in both P and N experiments (Figs. 2e, f, S4e, S4f).

In the P experiment, plant P content showed a completely different trend from [P], as it increased with increasing plant density (Figs. 3, S4a, S4c, S4e). Under low P supply, shoot P content of 60 (3) was about 60% greater than that of 60 (1). Shoot P content of 180 (3) was four-fold that of 60 (1). Root P content of 60 (3) was intermediate between that of 60 (1) and 180 (3), while root P content of 180 (3) was significantly greater than that of 60 (1). Total P content showed the same changing trend as shoot P content. As for the root P uptake, with low P supply, that of the treatment with higher plant density was not less than the root P uptake of the treatment with low plant density (Table S1). For the treatment with the same amount of P per plant, root P uptake of 180 (3) was about 53% greater than that of 60 (1).

Plant N content decreased with increasing plant density in the N experiment (Figs. 3, S4b, S4d, S4f). Shoot N content of 60 (1) was about three-fold that of 60 (3), and about two-fold the shoot N content of 180 (3). Root N content of 60 (1) was about 87% greater than that of 60 (3), and about 34% greater than the root N content of 180 (3). As root N content was low compared with shoot N content, total N content followed the same trend as that of shoot N content (Table S2). Root N uptake of 60 (3) was significantly less than that of 60 (1); however, root N uptake of 180 (3) was intermediate between 60 (1) and 60 (3).

Root morphological changes

Maize plants exhibited greater total root length in the N experiment than in the P experiment (Figs. 4a, b). In the P experiment, total root length increased with increasing plant density (Figs. 4a, b, S5a). With the same low P supply per pot, total root length of 60 (3) was around 80% greater than that of 60 (1), and total root length of 180 (3) was about 180% greater than that of 60 (1). The proportion of fine roots also showed a similar trend to total root length. Fine root proportion of 60 (3) was 20% greater than that of 60 (1), and fine root proportion of 180 (3) was 60% greater than that of 60 (1) (Figs. 4c, d). For the proportion of medium-diameter roots, that of 60 (3) was about 20% greater than that of 60 (1), and proportion of medium-diameter roots of 180 (3) was around 55% greater than that of 60 (1) (Figs. 4e, f). As for the proportion of thick root, there was an opposite trend: that of 60 (3) was around 20% lower than that of 60 (1), and the proportion of thick root of 180 (3) was about 25% lower than that of 60 (1) (Figs. 4g, h).

In contrast, in the N experiment, total root length decreased with increasing plant density (Figs. 4, S5). When supplied with the same low amount of N, the total root length of 60 (3) was about 50% of that of 60 (1), while that of 180 (3) was about 65% of the total root length of 60 (1) (Fig. S5b). Fine root and medium-size root proportions were intermediate among five treatments (Figs. S5e, S5f).

Root physiological changes

Acid phosphatase activity increased with increasing plant density (Figs. 5, S6a). At low P supply, the phosphatase activity of 60 (3) was about two-fold that of 60 (1). Phosphatase activity of 180 (3) was around three-fold that of 60 (1). However, phosphatase activity was not correlated with shoot [P] (Fig. S6b).

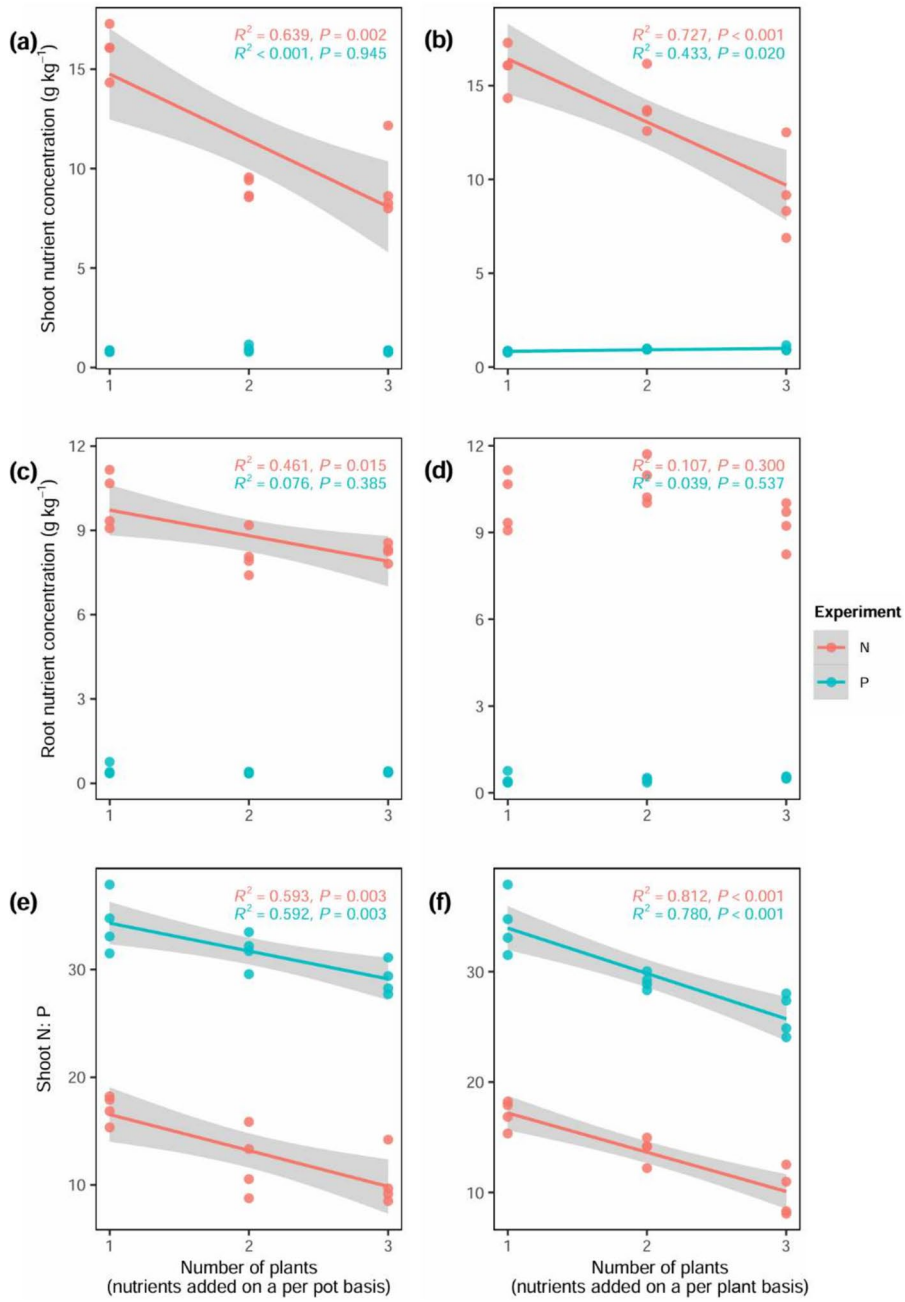


Fig. 2 Correlations between plant nutrient concentrations and plant density in the phosphorus (P) experiment, and nitrogen (N) experiment. Correlations between shoot nutrient concentration and different plant density among (**a**) 60 (1), 60 (2) and 60 (3), and (**b**) 60 (1), 120 (2) and 180 (3); correlations between root nutrient concentration and different plant density among (**c**) 60 (1)−60 (3), and (**d**) 60 (1)−180 (3); correlations between shoot N: P and different plant density among (**e**) 60 (1)−60 (3), and (**f**) 60 (1)−180 (3). The shaded area indicates the 95% confidence range, derived from the models. The regression lines and their 95% confidence range are not drawn when $P > 0.05$. Treatment 60 (1), 60 (2) and 60 (3): three treatments supplied with the same 60 mg P kg^{-1} soil per pot with different plant densities (1–3 plants per pot). Treatment 120 (2) and 180 (3): another two treatments supplied with the same 60 mg P kg^{-1} soil per plant with different plant densities (2–3 plants per pot)

Maize released carboxylates in the P experiment (Fig. 6). Carboxylate concentrations of maize plants were indistinguishable among 60 (1)−60 (3) but showed an increasing trend, and increased when supplied with the same amount of P per plant (Figs. 6a, b, S7 a). The 180 (3) showed a significantly greater carboxylate concentration than that in any other treatment. As for the malate concentration, there was no difference among different plant densities for plants growing at low P supply, while after addition of P, the malate release of 180 (3) was more than four-fold that of 60 (1) (Fig. S7b).

The pH of the rhizosheaths soil was indistinguishable among 60 (1)- 60 (3), while the pH of 60 (1) was significantly higher than that of 180 (3). However, we found minor changes in each treatment and no

correlations between pH and plant density (Figs. 7, S8).

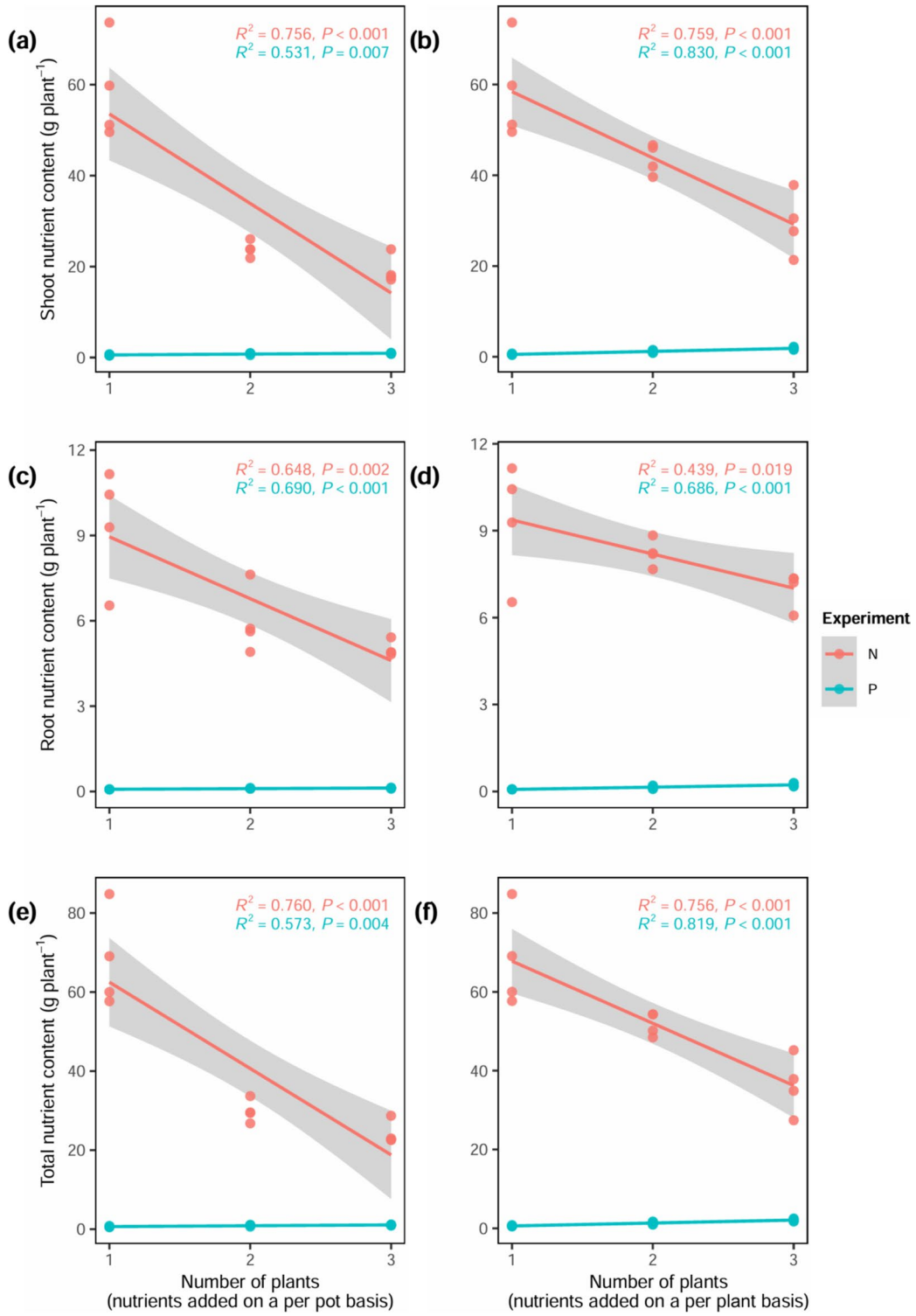
Discussion

Growing in calcareous soils with different plant densities, surprisingly, maize showed an intraspecific facilitation effect under P-limiting conditions, while, as expected, we found a competition effect under N-limiting conditions. Our study showed that the studied variety of maize released both carboxylates and phosphatases, rather than acidifying the rhizosheath soil in response to the high pH soil under high plant density. Increasing plant density had a negative effect on maize productivity when N fertiliser was not sufficient to support plant growth, and this negative effect was relieved but still existed when N fertiliser was supplemented to the same amount of N per plant. In contrast, increasing plant density had a positive effect on maize productivity when P was limiting for plant growth, and this positive effect was even found when P fertiliser was supplemented to the same amount of P per plant.

Plant growth in response to plant density

Growing in the same pot, competition for resources is expected to increase with increasing plant density. However, the growth of maize plants showed different responses to high plant density under P-limiting conditions from that under N limitation (Fig. 1). Both shoot and root biomass decreased in response to increased plant density under low N supply which indicates significant competition, as anticipated. Although the maize plants grew better after being supplemented with the same amount of N per plant, there was still a competition effect with increasing plant density. However, in the P experiment, the result was rather different. Both shoot and root biomass positively correlated with plant density regardless of the nutrient addition (Fig. 1) which indicates an intraspecific facilitation effect in each pot. After being supplemented with the same amount of P per plant, this facilitation effect was even greater. However, the root: shoot ratio was not affected in these experiments, indicating that the plant density had an equally positive effect on maize shoot and root growth. Similar findings were found for Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.); compared with growing single fir cuttings, multiple fir cuttings under competition (growing next to each other) have significantly greater shoot and root biomass while the root: shoot ratio is the same (Wu et al. 2017). It is interesting that the maize plants exhibited an intraspecific facilitation effect when grown with a growth-limiting P supply at the high plant density in the P experiment. Nitrogen is quite mobile in the soil and it can move to the roots for plants to take up without physiological adjustments; however, P is relatively immobile, especially at the high pH of calcareous soils compared with soils of lower pH (Ding et al. 2019; George et al. 2012; Turner and Blackwell 2013), and roots tend to show morphological and physiological changes that allow plants to acquire more P (Reichert et al. 2022; Wen et al. 2017). This might contribute to the intraspecific facilitation effect we found in this study.

In most studies, maize tends to alter its root morphology in response to a low P or N availability, and root exudates are not considered a P-acquisition strategy in maize under low P availability (Jia et al. 2018; Liu et al. 2009; Maizlish et al. 1980). For example, some maize hybrids change their root size and distribution in low-N soils and accumulate more N, and the root length and the primary root numbers increase with increasing N supply (Chen et al. 2014; Maizlish et al. 1980). Wang et al. (2005) found that under N deficiency, maize plants tend to produce longer primary roots that increase the root-soil surface area, and at high N supply, they tend to produce longer lateral roots that contribute to N accumulation. Under P deficiency, maize produces more fine/lateral roots that increase the root-soil surface area which is effective at acquiring immobile P (Jia et al. 2018; Wen et al. 2017). In this study, the total root length decreased with increasing plant density under N deficiency and showed a significant competition effect regardless of the amount of N in the soil (Fig. 4). However, the root morphology did not change in response to increasing densities, because the proportion of different root diameters was indistinguishable among plant densities. Light interception per plant decreases with increasing plant density, and plant photosynthesis and also biomass accumulation decrease (Freschet et al. 2018; Pettigrew et al. 2013). Thus, less carbon is allocated to roots under high plant density, and total root length decreases. In contrast, this negative effect of light interception was not exhibited under different P


(a)
R^2 = 0.756, P < 0.001
R^2 = 0.531, P = 0.007
(b)
R^2 = 0.759, P < 0.001
R^2 = 0.830, P < 0.001
(c)
R^2 = 0.648, P = 0.002
R^2 = 0.690, P < 0.001
(d)
R^2 = 0.439, P = 0.019
R^2 = 0.686, P < 0.001
(e)
R^2 = 0.760, P < 0.001
R^2 = 0.573, P = 0.004
(f)
R^2 = 0.756, P < 0.001
R^2 = 0.819, P < 0.001
Shoot nutrient content (g plant^{-1})
Root nutrient content (g plant^{-1})
Total nutrient content (g plant^{-1})
Number of plants
(nutrients added on a per pot basis)
Number of plants
(nutrients added on a per plant basis)
Experiment
N
P

◂**Fig. 3** Correlations between plant nutrient concentrations and plant density in the phosphorus (P) experiment, and nitrogen (N) experiment. Correlations between shoot nutrient content and different plant density among (**a**) 60 (1), 60 (2) and 60 (3), and (**b**) 60 (1), 120 (2) and 180 (3); correlations between root nutrient content and different plant density among (**c**) 60 (1)−60 (3), and (**d**) 60 (1)−180 (3); correlations between total nutrient content and different plant density among (**e**) 60 (1)−60 (3), and (**f**) 60 (1)−180 (3). The shaded area indicates the 95% confidence range, derived from the models. The regression lines and their 95% confidence range are not drawn when $P > 0.05$. Treatment 60 (1), 60 (2) and 60 (3): three treatments supplied with the same 60 mg P kg^{-1} soil per pot with different plant densities (1–3 plants per pot). Treatment 120 (2) and 180 (3): another two treatments supplied with the same 60 mg P kg^{-1} soil per plant with different plant densities (2–3 plants per pot)

additions in this study. The total root length increased with increasing plant density in low-P soil, and these plants showed significant intraspecific facilitation for total root length (Fig. 4). Different from the N experiment, the proportion of fine roots of 180 (3) was significantly greater in the P experiment, associated with a greater size of the root system because of the intraspecific facilitation. Root exudates might contribute to this belowground facilitation under P-limiting conditions.

Belowground interactions in the P experiment

To further explore the intraspecific facilitation effect in the P experiment in contrast with the competition effect in the N experiment, belowground interactions by the root exudates were considered. Soil P_i availability is low at high soil pH as explained above that the concentration of OH^- is higher in alkaline soils, and both acidification and chelation are needed to enhance the [P_i] gradient between the soil and root surface (Lambers and Oliveira 2019). Some plants are able to acidify the soils by releasing protons that decrease the rhizosheath pH (Lambers and Oliveira 2019), and thus, soil P availability increases. This study was conducted with a low-P calcareous silt loam soil with a pH of c. 8.4, which restricts plant P uptake (Ding et al. 2019; Turner and Blackwell 2013). Soil pH is a key factor that has a major effect on the P chemistry and biological availability. Phosphorus availability is low at pH 8.0–8.5, and higher at pH 6.5–7.5 (Lambers and Oliveira 2019). Many plant species are sensitive to the high pH of calcareous soil (Condron et al. 2005; George et al. 2012). The main cations in the calcareous soils, positive calcium ions, should be replaced by protons first before plant roots can take up P (Barrow et al. 2021). For example, *Lupinus* species are sensitive to the calcareous soil because of the high pH, and they show less nutrient uptake and reduced growth in alkaline soil (Ding et al. 2019). The alkaline soil should be acidified by the plant to make more P available. However, the variation of the rhizosheath soil was minor in this study (Figs. 7, S8). Maize plants did not show a significant pH decrease under high plant density. The citrate and malate are the anion component of the organic acids that can exchange with bound P ions (Lambers and Oliveira 2019). In alkaline soils, carboxylates may also contribute to greater P acquisition.

In contrast with our expectation that maize does not depend on root exudates in response to low P availability, there was a clear effect on carboxylates rather than pH that contributed to the P-mobilisation by maize in calcareous soil. The studied maize plants released carboxylates, especially malate, which increased plant P availability (Fig. 6), rather than depending on decreasing soil pH in this study. Many P-efficient plants release different carboxylates under P deficiency which mobilise soil-bound P into soluble P_o and P_i (Lambers and Oliveira 2019). In agroecosystems, chickpea (*Cicer arietinum*) releases carboxylates including citrate and malonate that mobilise P (Pang et al. 2018); in natural ecosystems, *Banksia* species (Proteaceae) release carboxylates including citrate and malate that enhance P acquisition (Denton et al. 2007); alfalfa (*Medicago sativa* L.) releases tartrate as a major carboxylate under low P supply and high N supply (He et al. 2020); *Adenanthos* species (Proteaceae) release mainly oxalate in P-impoverished soils (Shen et al. 2024a). However, this is not generally found for maize (Wen et al. 2017). In contrast, in the present experiment, maize released carboxylates and the concentrations of carboxylates was not decreased with increased plant density (Fig. 6), and thus would allow maize to acquire more P from the low-P calcareous soil. Our study shows that maize released carboxylates rather than protons and thus increased the P availability in responses to low P availability in a calcareous soil.

Few studies have explored interspecific facilitation, which is important for intercropping in agroecosystems (Gardner and Boundy 1983; Li et al. 2022, 2007, 2003; Turkington and Jolliffe 1996).

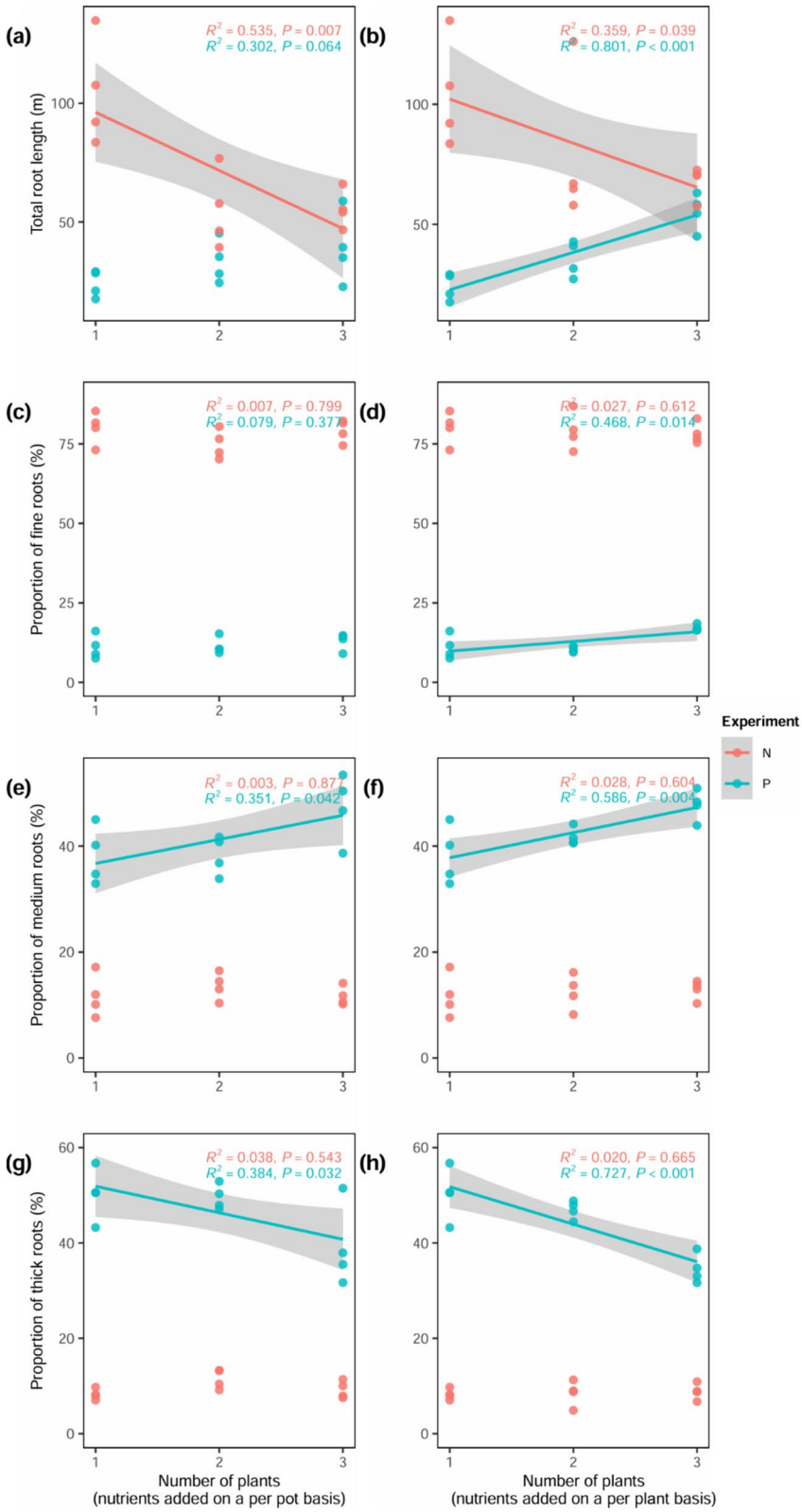


Fig. 4 Total root length and proportion of root length with different diameter classes to total root length of maize plants in the phosphorus (P) experiment, and nitrogen (N) experiment. Correlations between total root length and different plant density among (**a**) 60 (1), 60 (2) and 60 (3), and (**b**) 60 (1), 120 (2) and 180 (3). Correlations between proportion of fine roots and different plant density among (**c**) 60 (1)−60 (3), and (**d**) 60 (1)−180 (3). Correlations between root biomass and proportion of medium roots among (**e**) 60 (1)−60 (3), and (**f**) 60 (1)−180 (3). Correlations between root biomass and proportion of thick roots among (**g**) 60 (1)−60 (3), and (**h**) 60 (1)−180 (3). The shaded area indicates the 95% confidence range, derived from the models. The regression lines and their 95% confidence range are not drawn when $P > 0.05$. Treatment 60 (1), 60 (2) and 60 (3): three treatments supplied with the same 60 mg P kg^{-1} soil per pot with different plant densities (1–3 plants per pot). Treatment 120 (2) and 180 (3): another two treatments supplied with the same 60 mg P kg^{-1} soil per plant with different plant densities (2–3 plants per pot)

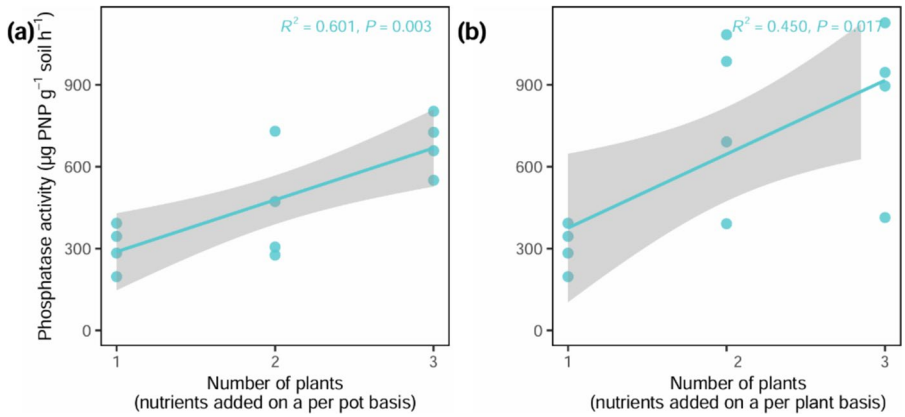


Fig. 5 Correlations between phosphatase activity and different plant density among (**a**) 60 (1), 60 (2) and 60 (3), and (**b**) 60 (1), 120 (2) and 180 (3). The shaded area indicates the 95% confidence range, derived from the models. Treatment 60 (1), 60 (2) and 60 (3): three treatments supplied with the same 60 mg P kg^{-1} soil per pot with different plant densities (1–3 plants per pot). Treatment 120 (2) and 180 (3): another two treatments supplied with the same 60 mg P kg^{-1} soil per plant with different plant densities (2–3 plants per pot)

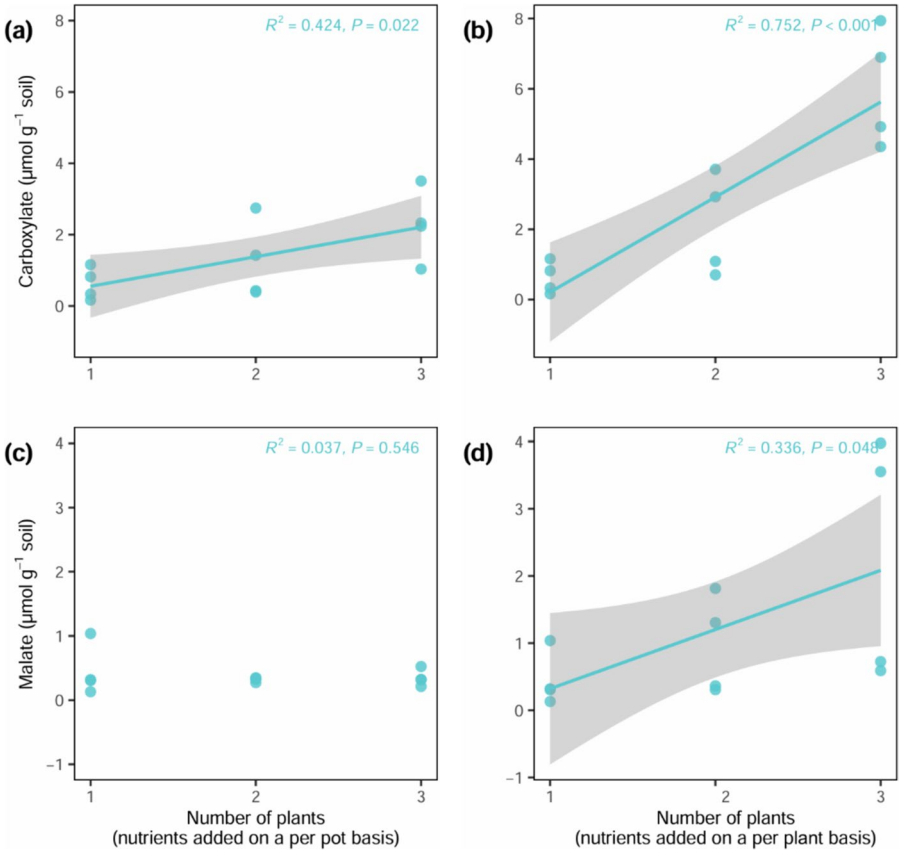


Fig. 6 Correlations between carboxylate concentration and different plant density among (**a**) 60 (1), 60 (2) and 60 (3), and (**b**) 60 (1), 120 (2) and 180 (3) in the phosphorus (P) experiment; correlations between malate concentration and different plant density among (**c**) 60 (1)−60 (3), and (**d**) 60 (1)−180 (3) in the P experiment. The shaded area indicates the 95% confidence range, derived from the models. The regression lines and their 95% confidence range are not drawn when $P > 0.05$. Treatment 60 (1), 60 (2) and 60 (3): three treatments supplied with the same 60 mg P kg^{-1} soil per pot with different plant densities (1–3 plants per pot). Treatment 120 (2) and 180 (3): another two treatments supplied with the same 60 mg P kg^{-1} soil per plant with different plant densities (2–3 plants per pot)

The soluble P_i mobilised by the P-mobilising species can be taken up directly by their neighbouring non-P-mobilising species (Li et al. 2014). For example, P-mobilising faba bean (*Vicia faba*) is intercropped with the non-P-mobilising maize (*Zea mays*) (Li et al. 2007; Zhang et al. 2020a). Phosphorus-mobilising

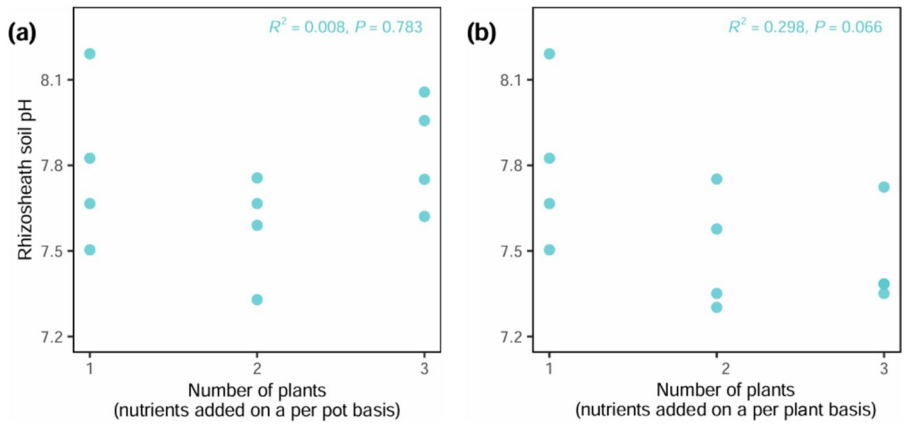


Fig. 7 Correlations between rhizosheath soil pH and different plant density among (a) 60 (1), 60 (2) and 60 (3), and (b) 60 (1), 120 (2) and 180 (3) in the phosphorus (P) experiment. The regression lines and their 95% confidence range are not drawn when $P > 0.05$. Treatment 60 (1), 60 (2) and 60 (3): three treatments supplied with the same 60 mg P kg^{-1} soil per pot with different plant densities (1–3 plants per pot). Treatment 120 (2) and 180 (3): another two treatments supplied with the same 60 mg P kg^{-1} soil per plant with different plant densities (2–3 plants per pot)

peanut (*Arachis hypogaea*) has been grown with maize in pot experiments (El Dessougi et al. 2003; Guo et al. 2014), and P-mobilising chickpea (*Cicer arietinum*) grown with wheat (*Triticum aestivum*) in pot and field experiments (Li et al. 2003; Singh et al. 2019). In this study, maize plants exhibited intraspecific facilitation in response to increasing plant density under P deficiency. The mechanism is similar to that for interspecies facilitation. With increasing plant density, each individual root released more carboxylates, and thus, we found higher carboxylate concentration per plant which contributed to enhanced plant growth. Thus, maize plants showed a facilitation effect under high plant density in the P experiment. However, facilitation depending on carboxylates did not play a role in the N experiment; so, maize plants showed competition effects in response to increasing plant density under N deficiency in this study.

Roots can only take up P_i in the soil solution but not P_o (Lambers 2022). Phosphatases hydrolyse soluble P_o mobilised by carboxylates releasing P_i for plants to take up (Lambers and Oliveira 2019). In addition to carboxylates in this study, phosphatases showed a similar facilitation function in acquiring P for maize growing in calcareous soils with different plant densities (Figs. 5, S6). Some species rely on P-mining strategies based on the release of phosphatases that hydrolyse P_o. The south-western Australian native species *Xylomelum occidentale* and *Adenanthos cygnorum* (Proteaceae) release phosphatases that acquire less tightly bound P_o without carboxylate release (Shen et al. 2024b; Zhong et al. 2021). Native legumes also release large amounts of phosphatases in low-P soils (Olde Venterink 2011; Png et al. 2017). Phosphatase activity is positively correlated with shoot [P] in maize (Wen et al. 2017). However, we found no correlation in this study. Treatments with different plant densities showed intermediate low shoot [P], but they released different amounts of phosphatases, and this explains why no correlation was found between phosphatase activity and shoot [P].

More fine and medium-size roots that have more surface areas accessing P_i in the soil solutions than thick roots (Lambers and Oliveira 2019) were also formed at high plant density in the P experiments. Contrary to what is commonly known that plants produce more fine roots and less thick roots in adjusting to a low P environment (Reichert et al. 2022), maize in this study did not produce more fine roots under P deficiency (60 (3) (Fig. 4), and showed a facilitation effect for root growth under high plant density. In the P experiment, each individual root released more carboxylates and phosphatases that greatly increased root P uptake, and thus, the root growth was facilitated at high density. As for the N experiment, without more exudates to contribute to the facilitation, root growth decreased

with increasing plant density and showed competition effects.

Root nutrient uptake

The nutrient uptake of roots under N or P deficiency was different because of the facilitation. For the N experiment, when the roots were competing for N, they were as effective in taking up N regardless of plant density (Table S2). However, for the P experiment, the root P uptake was enhanced with increasing plant density, when total root length increased, and the size of the root system was larger, especially for the 180 (3), which produced more fine roots (Table S1). At the same time, the facilitated plants, which had larger root systems released more carboxylates making more P available, and this was reflected in a greater amount of P taken up per unit root length.

Despite the same root N uptake (Table S2), total N content (Fig. 3) varied with plant density and showed significant competition due to the high plant density even after being supplied with the same amount of N per plant. The maize plants competed for N under high plant density, and more N per plant was needed to achieve the same total N content per plant. The total P content also confirmed a significant facilitation effect as did plant biomass. When soil P is limiting, plants use all the absorbed P supporting their growth instead of accumulating P in leaves, and thus, show the same low leaf [P] (de Groot et al. 2003; Shane et al. 2003). The shoot [P] (Fig. S3a) were all much lower than the critical shoot [P] value (2.7 g P kg^{-1}) (Wen et al. 2017), and thus plants used all the P supporting growth showing the same shoot [P] (Lambers 2024). In this study, the maize plants showed a greater P content (Fig. 3) and also produced more biomass (Fig. 1) when growing with the same amount of P per pot; so, these plants did not exhibit luxury uptake of P that accumulated and used all the P they acquired to support their growth. The 180 (3) showed significantly higher shoot P content, and thus, had sufficient P to support their growth and showed greater shoot [P]. This explained why, being facilitated, these maize plants absorbed more P and showed a higher shoot P content but not higher shoot [P]. In addition, higher N: P ratios contributed to a higher P-use efficiency under P limitation. However, shoot N: P of 60 (3) was significantly lower than that of 60 (1) in the P experiment which indicates that the P limitation was relieved under higher plant density and that the plant P acquisition was facilitated. Although shoot [P] was not increased, shoot P content increased and shoot N: P ratio decreased which shows strong evidence that plant growth of 60 (3) and 180 (3) were facilitated under high plant density compared to 60 (1). In contrast, shoot N: P ratio showed similar trends with shoot [N] in the N experiment (Fig. 2). In intensive cropping systems, when growing under high plant density in calcareous soil, more N fertiliser (but not P fertiliser) should be applied to the studied variety of maize to support plant growth.

Conclusions

Growing in calcareous soils, maize showed a competition effect at increasing plant density under N-limiting conditions, while it showed intraspecific facilitation at increasing plant density under P-limiting conditions. In the calcareous soil, more N fertiliser should be supplied under higher plant density for the studied maize variety (*Zea mays* L. cv. ZD958) whereas no additional P fertiliser is needed. Maize roots in the present experiment were effective at acquiring P themselves under P-limiting conditions at high plant density but not at taking up N. Interestingly, the studied maize variety depended on carboxylates and phosphatases but not acidification in response to high plant density in the calcareous alkaline soil. Root exudates should not be ignored for maize growing in intensive cropping systems in calcareous soils. The present maize variety might be a good candidate using the organic P in organic production systems with calcareous soils. The results of this study contribute to a better understanding of N and P fertilisation under high plant density. The findings of this work are important for the sustainability of crop ecosystems.

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Author contributions QS and Haigang Li designed the study. QS carried out the experiments and collected the data with the contribution from JX and LW. QS analysed the data with the contributions from JX, YC, JF, Haigang Li and Hans Lambers. QS prepared the manuscript. QS and Hans Lambers

revised the manuscript. All authors read and approved the manuscript.

Data Availability The datasets generated during and/or analysed during the current study are available in the ScienceDB repository, https://www.scidb.cn/en/s/mUzM32.

Declarations

Conflict of interest There is no conflict of interests to influence the work reported in this paper.

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