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REGULAR ARTICLE

Maize responds to low shoot P concentration by altering root morphology rather than increasing root exudation

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Abstract
Background and aims Alterations in root growth and rhizosphere processes in maize (*Zea mays* L.) occur under phosphorus (P) deficiency, but the dynamics of root morphological and physiological modifications with increasing shoot P concentration remain unclear. This study investigated root responses to a wide gradient in shoot P status.
Methods A range of maize shoot P concentrations (1.0–4.0 mg g^{-1}) was established using controlled pot experiment with eleven rates of P supply from 0 to 1200 mg P kg^{-1} soil. Root morphology and rhizosphere processes were characterized 28 days after planting.
Results Maize reached maximum biomass at shoot P concentration of 2.7 mg g^{-1}. Root morphological responses (i.e. total root length, specific root length and proportion of fine roots) showed a strong increasing trend with decreasing shoot P concentration (1.1–1.3 mg g^{-1}), but they decreased when shoot P concentration was extremely low (below 1.1 mg g^{-1}). In contrast, with increasing shoot P concentration, root morphological responses decreased, but root physiological responses (rhizosphere acidification, acid phosphatase activity and carboxylate exudation in the rhizosphere) were enhanced, and no decrease was noted even at high shoot P concentration (4.0 mg g^{-1}) corresponding to excess P supply.
Conclusions Increasing maize shoot P concentration induced a decrease in root morphological responses and an enhancement in root exudation, with maize response to P deficiency being dependent on root morphological rather than physiological traits.

Keywords Phosphorus deficiency · Root growth · Root exudation · Rhizosphere processes

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Introduction

Phosphorus (P), as one of the most growth-limiting macronutrients, needs to be taken up from soil by plant roots (Raghothama 1999; Vance et al. 2003; Vitousek et al. 2010). However, only 10–25% of applied fertilizer P is taken up by crops in the first growing season (Johnston et al. 2014), due to phosphate adsorption and fixation in soil (Schachtman et al. 1998; Hinsinger 2001; Shen et al. 2011). Root systems display high plasticity to the low concentration and heterogeneous distribution of P in soil to enhance the capacity to

acquire P, including root morphological and physiological strategies (Vance et al. 2003; Lambers et al. 2006; Shen et al. 2011). A greater proportion of photosynthates allocated to root growth, higher root branching, and increased production of fine roots are typical morphological responses that result in enhanced exploration of the soil volume (Hermans et al. 2006; Postma et al. 2014; Lynch 2015). Plants may also increase proton release, carboxylate exudation and phosphatase secretion into the rhizosphere to change soil properties and mobilize sparingly soluble inorganic and organic P (Jones 1998; Neumann and Römheld 1999; Richardson et al. 2011; Lopez-Arredondo et al. 2014).

It is generally accepted that these adaptive responses are controlled by (i) plant internal P status and (ii) P availability in the rooting medium (Shane et al. 2003a; Shen et al. 2003, 2005; George et al. 2011; Giehl et al. 2014). Shane et al. (2003a) reported that shoot P status regulated cluster root growth and citrate exudation in white lupin (*Lupinus albus* L.) grown with a divided root system in which one root half was supplied with P and the other half was not. Our previous studies also suggested that cluster root formation and citrate exudation in white lupin were regulated by the shoot P concentration, but these processes were also affected profoundly by localized external P supply (Shen et al. 2005). Hence, cluster root growth and citrate exudation in white lupin were triggered by shoot P starvation, and suppressed by increasing shoot P concentration associated with external P supply (Shane et al. 2003a, b; Li et al. 2008; Wang et al. 2013). Similar responses have been shown in the species from the Proteaceae and Pasture legume families (Denton et al. 2007; Lambers et al. 2011; Pang et al. 2009; Suriyagoda et al. 2012). However, some studies on root adaptive responses to different P availability in the rooting medium reported contradictory results. Faba bean (*Vicia faba* L.) had a poor root morphological response to varying P supply (Lyu et al. 2016). In chickpea (*Cicer arietinum* L.), carboxylate concentrations in the rhizosphere steadily increased during the plant development, suggesting there was no simple correlation with shoot or soil P status (Wouterlood et al. 2004a, 2005). In soybean (*Glycine max* L.), P deficiency did not enhance proton release into the rhizosphere (Tang et al. 2009; Zhou et al. 2009). Moreover, root exudation of phosphatase by canola (*Brassica napus* L.) increased with increasing P supply (Marschner et al. 2007; Solaiman et al. 2007; Zhang et al. 2009). Hence, it appears that different plant species have developed different strategies to regulate root morphological and physiological responses to variable P supply.

Maize is one of the most widely cultivated crop plants in the world and is used as human and animal food, forage and a source of bioethanol. Under P deficiency, maize roots exhibit extensive morphological alterations (Zhu et al. 2005, 2010; Zhang et al. 2012; Postma et al. 2014; Miguel et al. 2015) that underpin efficiency of P acquisition. P-efficient maize genotypes have greater root/shoot ratio, higher root hair density, more lateral roots and longer total root length than P-inefficient ones (Hajabbasi and Schumacher 1994; Bates and Lynch 2001; Gaume et al. 2001; Liu et al. 2004; Zhu and Lynch 2004; Zhu et al. 2005; Corrales et al. 2007; Lynch 2011). However, adaptive physiological responses of maize to variable P supply are unclear. P-deficient maize does not acidify the rhizosphere soil significantly (George et al. 2002a; Liu et al. 2016), or may even alkalinize the culture solution in the initial stages of P deficiency (Liu et al. 2004; Li et al. 2007; Zhou et al. 2009). Moreover, P-deficient maize often showed decreased (Liu et al. 2004, 2016; Corrales et al. 2007; Li et al. 2010a; Lyu et al. 2016), but rarely increased (Gaume et al. 2001) concentration of carboxylates in the rhizosphere. Regarding acid phosphatase, maize might increase its exudation into the rhizosphere under P deficiency (Sachay et al. 1991; Yun and Kaeppler 2001), but this effect was frequently not observed (George et al. 2002b; Corrales et al. 2007; Carvalhais et al. 2011; Liu et al. 2016; Lyu et al. 2016). Hence, maize root physiological responses to variable P supply as well as their interactions with root morphological responses have yet to be clarified. In addition, it should be borne in mind that a majority of studies mentioned above were done in hydroponic or sand culture (eg. Sachay et al. 1991; Gaume et al. 2001; Yun and Kaeppler 2001; Liu et al. 2004; Corrales et al. 2007; Carvalhais et al. 2011) that might not reflect natural growth conditions (Oburger and Schmidt 2016). Therefore, the aims of this study were to (1) quantify the dynamics of root growth traits and rhizosphere processes in maize grown in soil with variable P supply; (2) characterize the relationship between root morphological or physiological responses and shoot P concentration varying from deficient to optimal to excess.

Materials and methods

Experimental set-up

The experiment was conducted in a naturally-lit greenhouse at the China Agricultural University. Maize (*Zea mays* L. cv. ZD958) was grown in a calcareous silt loam soil with low P availability, which was collected from a long-term fertilizer experiment in Beijing (Latitude: 40°01′N, Longitude: 116°16′E). Soil was air-dried, then sieved to 2-mm and thoroughly mixed. Soil properties were as follows: pH 8.40 (1: 2.5, soil:water), Olsen-P 1.7 mg kg^{-1}, organic carbon 12 g kg^{-1}, total N 0.7 g kg^{-1}, and exchangeable K 82 mg kg^{-1}. There were 11 P application rates (0, 2.5, 5, 10, 25, 50, 75, 150, 300, 600 and 1200 mg P kg^{-1} soil, supplied as KH_2PO_4). Each pot was filled with 1 kg of air-dried soil. To ensure that the supply of other nutrients was adequate for plant growth, soil was also supplemented with basal nutrients at the following rates (mg pot^{-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.

Maize seeds were surface sterilized (30 min in 10% *v*/v H_2O_2 solution), rinsed, imbibed (8 h in saturated $CaSO_4$ solution), and germinated in a dark and humid environment for 2 days at 22 °C. Four uniformly germinated seeds were planted per pot, and the seedlings were thinned to two plants per pot at the 3-leaf stage. Five replicates were grown for each P treatment, and pots were arranged in a completely randomized design. All pots were watered every day to weight to maintain 75% field capacity. Greenhouse temperatures were maintained at 20–25 °C during the day and 15–18 °C at night, with 12–14 h daytime throughout the growth period.

Harvest and measurements

Plants were harvested at the 5-leaf stage (28 days after planting, DAP), when visual growth differences among the P rate treatments were obvious (Fig. S1). At harvest, shoots were cut at the soil surface, oven-dried at 105 °C for 30 min and then at 70 °C for 3 days, and weighed. After sampling the rhizosphere exudates (see the next section), all visible roots in each pot were picked by hand and placed in individual, marked plastic bags. These roots were washed free of soil and then frozen at -20 °C before measurement of root morphology. The bulk soil was also sampled. After air-drying, soil samples were ground to pass through a 2-mm sieve for analysis of soil Olsen-P.

Cleaned root samples were dispersed in water in a transparent tray (30 × 20 × 3 cm) and then scanned with an EPSON scanner at 400 dpi (Epson Expression 1600 pro, Model EU-35, Japan). Root images were analyzed with software Win-RHIZO (Regent Instruments Inc., Quebec, Canada) to obtain total root length and the root length in different root diameter classes. Afterwards, root dry weight was determined by weighing the oven-dried samples. Specific root length was calculated from root length and root dry weight, and the proportion of fine roots (diameter 0–0.2 mm) to total root length was calculated (Jing et al. 2010).

Plant materials were digested with a mixture of 5 mL of concentrated sulfuric acid and 8 mL of 30% *v*/v H_2O_2, and then shoot P was analyzed by the vanado-molybdate method by spectrophotometry at 440 nm (Johnson and Ulrich 1959). Soil P availability was determined according to the Olsen method (Olsen et al. 1954), the air-dried soil being extracted with 0.5 M $NaHCO_3$ at pH 8.5 (180 rpm, 25 °C).

Root exudate collection from the rhizosphere soil

The method for rhizosphere exudate collection was modified from Veneklaas et al. (2003) and Pearse et al. (2007). Roots were carefully lifted out of the soil and shaken to remove the loosely adhering soil around the roots (considered to be bulk soil), and the tightly adhering soil around the root was defined as rhizosphere soil (Veneklaas et al. 2003). After that roots were transferred into a 200-mL vials containing a measured amount of 0.2 mmol L^{-1} $CaCl_2$ solution depending on root volume (Veneklaas et al. 2003; Pearse et al. 2007). Roots were repeatedly dunked (about 60 s in this study) into solution until as much rhizosphere soil as possible was removed. Care was taken to minimize root damage. Two 0.5-mL aliquots of soil suspension were transferred into 2-mL centrifuge tubes for measurement of acid phosphatase activity (Alvey et al. 2001; Neumann 2006). Two 8-mL sub-samples were stored in an ice box. One was for measurement of rhizosphere pH. To the other, we added microbial inhibitor Micropur (Sicheres Trinkwasser, Germany) at 0.01 g L^{-1} and also three drops of concentrated phosphoric acid before storing at -20 °C until analysis of carboxylates by HPLC; the HPLC analysis was done after having passed the soil

suspension through a 0.22-μm filter (Shen et al. 2003; Wang et al. 2007).

Determination of rhizosphere soil pH, acid phosphatase activity and carboxylate content in the rhizosphere soil

The amount of rhizosphere soil collected differed among the treatments. In order to eliminate effect of different soil:water ratio on pH determination of rhizosphere extract solution, a modified pH (soil:water ratio was adjusted to 1:2.5) was calculated from the measured pH by an equation according to Li et al. (2010a). The bulk soil pH was measured using the conventional method at soil:water ratio of 1:2.5. In order to analyse rhizosphere acidification in different P treatments, we define Δ soil pH as a difference between rhizosphere pH and bulk soil pH (rhizosphere pH - bulk soil pH). Measurement of rhizosphere soil pH was completed on the harvest day, and the bulk soil pH was measured after soil samples were air-dried.

For determining acid phosphatase activity in the rhizosphere soil, 0.5-mL aliquots of soil suspensions were transferred into a 2-mL centrifuge tube with 0.4 mL sodium acetate buffer (pH 5.2) and 0.1-mL of 0.15 mol L^{-1} *p*-nitrophenyl phosphate (PNP) substrate. After incubation for 30 min at 25–30 °C, adding 0.5 mL of 0.5 mol L^{-1} NaOH terminated the reaction. The absorption of supernatants was measured at 405 nm (Alvey et al. 2001; Neumann 2006).

Carboxylates were analyzed using a reversed-phase high-performance liquid chromatography (HPLC) system based on the method described by Lyu et al. (2016). The chromatographic separation was conducted on a 250 × 4.6 mm reversed-phase column (Alltima C18, 5 μm; Alltech Associates, Inc., Deerfield, IL, USA). The mobile phase was 25 mmol L^{-1} KH_2PO_4 (pH 2.3) with a flow rate of 1 mL min^{-1} at 31 °C; detection of carboxylates was carried out at 214 nm.

Statistical analyses

One-way analysis of variance was performed using the SAS statistical software (SAS 8.1, USA), and significant differences among means were assessed using Duncan's multiple range analysis test ($P \leq 0.05$).

The linear-plateau model was used to evaluate the critical shoot P concentration for the optimal shoot growth of maize (Teng et al. 2013), and the exponential equation was used to establish the relationships between root/shoot ratio, Δ soil pH and shoot P concentration or soil Olsen-P (Li et al. 2008). Empirical polynomial (inverse third order) equations were used in SigmaPlot (SigmaPlot 10.0, USA) to analyse the relationship between root morphological traits (i.e. total root length, specific root length or proportion of fine roots) and shoot P concentration or soil Olsen-P based on the published method (Deng et al. 2014).

Results

Plant growth and P uptake

At harvest, with P application rates increasing from 0 (P0) to 1200 mg P kg^{-1} soil (P1200), soil Olsen P increased from 1.5 to 594 mg kg^{-1}, and maize increased shoot biomass by 370% and root biomass by 110% (Table 1). Shoot P concentration ranged from 1.0 to 4.0 mg g^{-1}, and P uptake increased by 18-fold from P0 to P1200 (Table 1).

There was a significant relationship between shoot biomass and soil Olsen-P (Fig. 1a), with shoot biomass increasing together with soil Olsen-P at first, and when soil Olsen-P surpassed approx. 200 mg kg^{-1}, shoot growth levelled off. Compared to the treatment without P added, the root/shoot ratio decreased by 110% in the P1200 treatment. The root/shoot ratio declined as soil Olsen-P increased to about 100 mg kg^{-1}, and then levelled off with a further increase in soil Olsen-P (Fig. 1c).

To estimate the critical shoot P concentration for maximum shoot biomass production, the regression analysis revealed that the response of shoot biomass to shoot P concentration fitted a linear-plateau model ($R^2 = 0.87$, $P < 0.01$) (Fig. 1b). The regression equations showed that the critical shoot P concentration was 2.7 mg g^{-1}, corresponding to about 90% of the relative shoot dry weight (Fig. 1b). Also, the root/shoot ratio declined substantially with increasing shoot P concentration, reaching a plateau at approx. 2 mg P g^{-1} shoot dw (Fig. 1d).

Root morphology

The total root length ranged from 10 to 19 m $plant^{-1}$, and it decreased by 47% in the treatment with P1000 in comparison to the P75 treatment (Table S1). Total root length initially increased with an increase in shoot P

Table 1 Shoot biomass, root biomass, shoot P concentration and P uptake under different P fertilization rates. Plants were sampled at the 5-leaf stage (28 days after planting)

P rate (mg P kg^{-1} soil)	Olsen-P (mg kg^{-1})	Shoot biomass (g $plant^{-1}$)	Root biomass (g $plant^{-1}$)	Shoot P concentration (mg g^{-1})	P uptake (mg $plant^{-1}$)
0	1.5 (0.10) e	0.44 (0.03) e	0.13 (0.01) e	1.01 (0.04) f	0.44 (0.02) f
2.5	1.9 (0.09) e	0.38 (0.02) e	0.13 (0.01) e	0.96 (0.02) f	0.37 (0.02) f
5	2.4 (0.08) e	0.42 (0.02) e	0.12 (0.01) e	1.00 (0.01) f	0.42 (0.02) f
10	3.3 (0.10) e	0.45 (0.03) e	0.13 (0.01) e	1.05 (0.03) f	0.47 (0.03) f
25	7.6 (0.18) e	0.56 (0.03) de	0.15 (0.01) de	1.11 (0.02) ef	0.62 (0.04) ef
50	15 (0.31) e	0.75 (0.05) d	0.18 (0.00) cd	1.16 (0.02) ef	0.87 (0.06) ef
75	23 (0.31) e	1.03 (0.08) c	0.19 (0.01) bc	1.33 (0.07) e	1.37 (0.13) de
150	54 (2.3) d	1.27 (0.11) b	0.22 (0.02) b	1.67 (0.06) d	2.10 (0.13) d
300	102 (2.4) c	1.44 (0.14) b	0.22 (0.02) b	2.10 (0.06) c	3.01 (0.28) c
600	178 (11) b	1.86 (0.13) a	0.27 (0.03) a	2.81 (0.14) b	5.27 (0.56) b
1200	594 (33) a	2.08 (0.10) a	0.28 (0.01) a	3.96 (0.22) a	8.32 (0.78) a

Values are means of five biological replicates (SE). Statistical differences ($P \leq 0.05$) between P fertilization rates are indicated by different letters in each column

concentration, peaking when shoot P concentration was about 1.1–1.3 mg g^{-1} (Fig. 2a) at the P fertilization rate near 75 mg P kg^{-1} soil (Table S1). Even though shoot P concentration continued to improve with further increases in P supply, total root length gradually declined to a plateau (around 12 m $plant^{-1}$) at shoot P concentration > 2 mg g^{-1}.

The relationships of shoot P concentration with specific root length or with the proportion of fine roots to total root length were similar to the total root length,

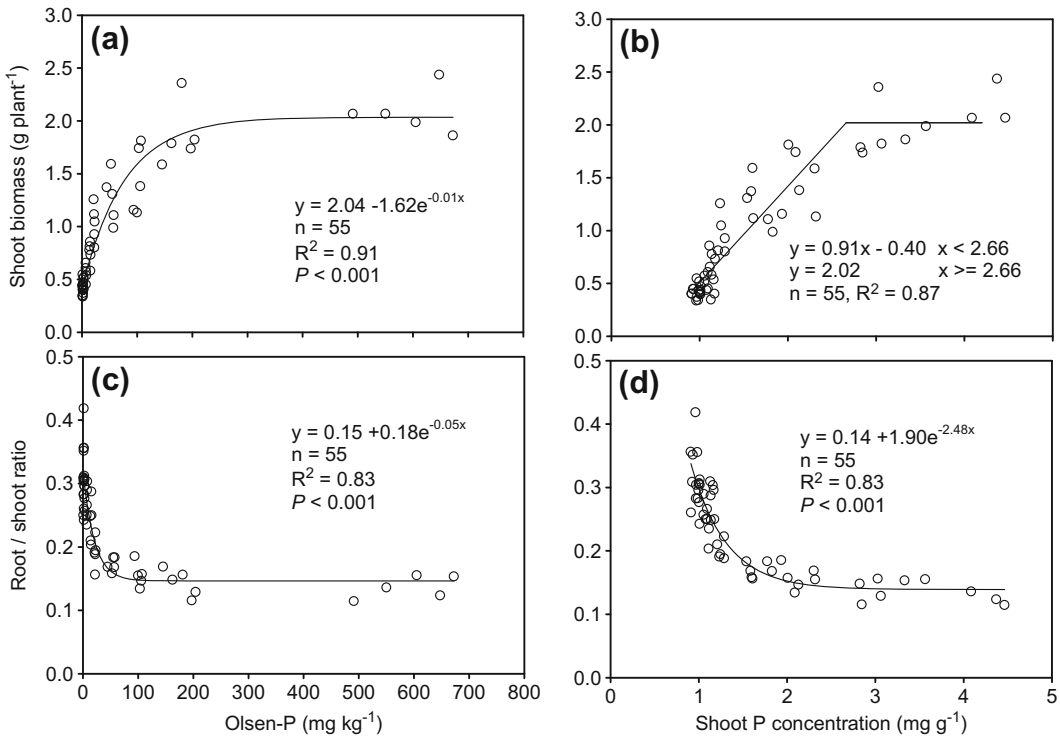

Fig. 1 Shoot biomass and root/shoot ratio as a function of increasing soil Olsen-P (**a**, **c**) and shoot P concentration (**b**, **d**). Data points represent individual replicates

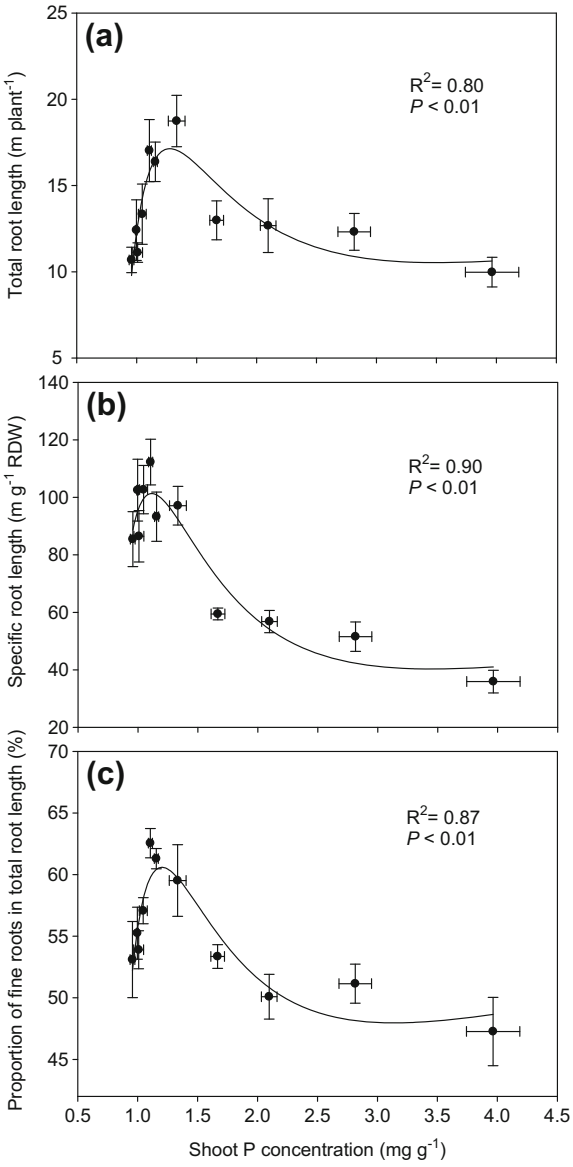

Fig. 2 Maize root morphological traits in response to increasing shoot P concentration: (**a**) total root length, (**b**) specific root length and (**c**) the proportion of fine roots (diameter 0–0.2 mm) in total root length. Each symbol represents the mean (±SE) of five replicates. RDW, root dry weight

with peaks at the critical shoot P concentration around 1.1–1.3 mg g^{-1}, followed by declining to a plateau with an increase in shoot P concentration (Fig. 2b, c). With the shoot P concentration decreasing from around 4 to between approx. 2 and 1.1–1.3 mg g^{-1}, the specific root length and the proportion of fine roots increased. The specific root length peaked at 112 m g^{-1} root dry weight in the P50 treatment, whereas the proportion of fine roots reached the maximum (63%) in the P25 treatment (Table S1). With shoot P concentration decreasing to below 1.1–1.3 mg g^{-1}, the specific root length decreased to 85 m g^{-1} root dry weight and the proportion of fine roots decreased to 54% (Table S1).

Rhizosphere soil pH

The bulk soil pH at harvest was about 8.2 in the treatments ranging from P0 to P600, but a decline occurred in the treatment with P1200 (Fig. S3a) corresponding to high soil Olsen-P (Fig. 3a). The rhizosphere soil pH declined from 8.1 to 6.7 with increasing P fertilization (Fig. S3b) and increasing soil Olsen-P (Fig. 3b). Acidification of the rhizosphere soil decreased as soil Olsen-P increased, reaching a plateau (approx. -1.2 pH units) at around 100 mg Olsen-P kg^{-1} (Fig. 3b).

The relationship between Δ soil pH (due to rhizosphere soil acidification) and shoot P concentration was separated into two phases, with a plateau at the critical level of approx. 2 mg P g^{-1} shoot dw (Fig. 3c). When shoot P concentration was <2 mg g^{-1}, Δsoil pH had a negative linear relationship with shoot P concentration.

Acid phosphatase activity in the rhizosphere soil

At P application rates ≤50 mg P kg^{-1} soil, acid phosphatase activity in the rhizosphere soil (RS-APase) was similarly low, and then increased significantly with increasing soil P supply (Fig. S4a). Compared with the P0 treatment, the RS-APase activity in the P1200 treatment increased by 150% (Fig. S4a). The positive linear relationship between RS-APase activity and shoot P concentration explained 44% of variation (r = 0.66, P < 0.001) (Fig. 4). Based on the regression equation, RS-APase activity increased from 380 to 958 μg PNP h^{-1} g^{-1} soil when shoot P concentration increased from 1.0 to 4.0 mg g^{-1}.

Rhizosphere soil carboxylates

The predominant carboxylates in the rhizosphere were citrate, trans-aconitate, malate and succinate at variable P fertilization treatments. Trace amounts of tartarate and fumarate were also detected (data not shown). Concentration of carboxylates in the rhizosphere regardless of shoot P concentration decreased in the order: succinate > trans-aconitate > malate > citrate (Figs. 5, S5). The concentration of these four carboxylates tended to increase in the rhizosphere soil when P rate increased

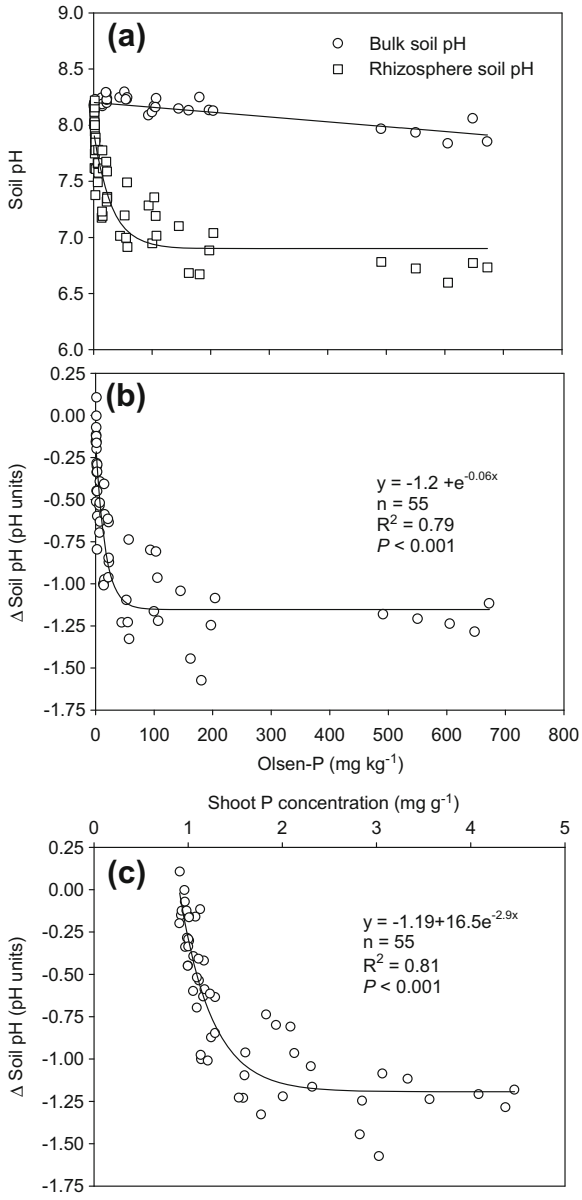

Fig. 3 Relationships of soil Olsen-P with the rhizosphere or bulk soil pH (**a**), and the relationship of Δ soil pH (rhizosphere soil pH minus bulk soil pH) with soil Olsen-P (**b**) or shoot P concentration (**c**). Data points represent individual replicates

above 50 mg P kg^{-1} soil, with the maximum concentration reached in the P1200 treatment (Fig. S5). The citrate and trans-aconitate concentrations were, respectively, 8.70-fold and 13-fold greater in the P1200 than P0 treatments. Similarly, positive linear relationships were observed between carboxylate concentration in the rhizosphere and shoot P concentration (Fig. 5a–d). The slopes (μmol carboxylate g^{-1} soil for each mg P g^{-1}

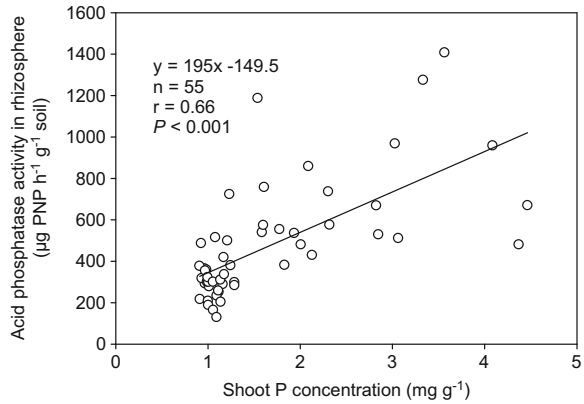

Fig. 4 Correlation between acid phosphatase activity in the rhizosphere soil and shoot P concentration. Data points represent individual replicates

shoot dw) were 0.26 for citrate, 0.70 for trans-aconitate, 0.45 for malate and 1.42 for succinate.

Discussion

Shoot P concentration in response to a wide range of soil P supply

Shoot or leaf P concentration is not only a useful indicator of P limitation, but is also used as a reliable tool in the diagnosis of crop yield depressions (Barry and Miller 1989; Bollons and Barraclough 1999). Shoot P concentration in maize significantly increased with increasing P additions (Table 1), and the critical level of shoot P concentration for shoot biomass production was 2.7 mg g^{-1} at the 5-leaf stage (Fig. 1b), which was consistent with previous literature (Jones 1983), but higher than 1.4 mg g^{-1} reported by Zia et al. (1988) for maize shoot P at the same stage. The various results may be due to different maize genotypes, soil properties or culture conditions (i.e. temperature, rooting volume). Shoot P concentration for maize in present study ranged from 1.0 to 4.0 mg g^{-1} (Table 1), suggesting that the experiment was suitable for estimating the root morphological and physiological responses to shoot P concentration covering deficiency, optimum and excess.

The relationship between root morphological responses and shoot P concentration

Root morphology plays an important role in P uptake. The main morphological responses of maize to low-P

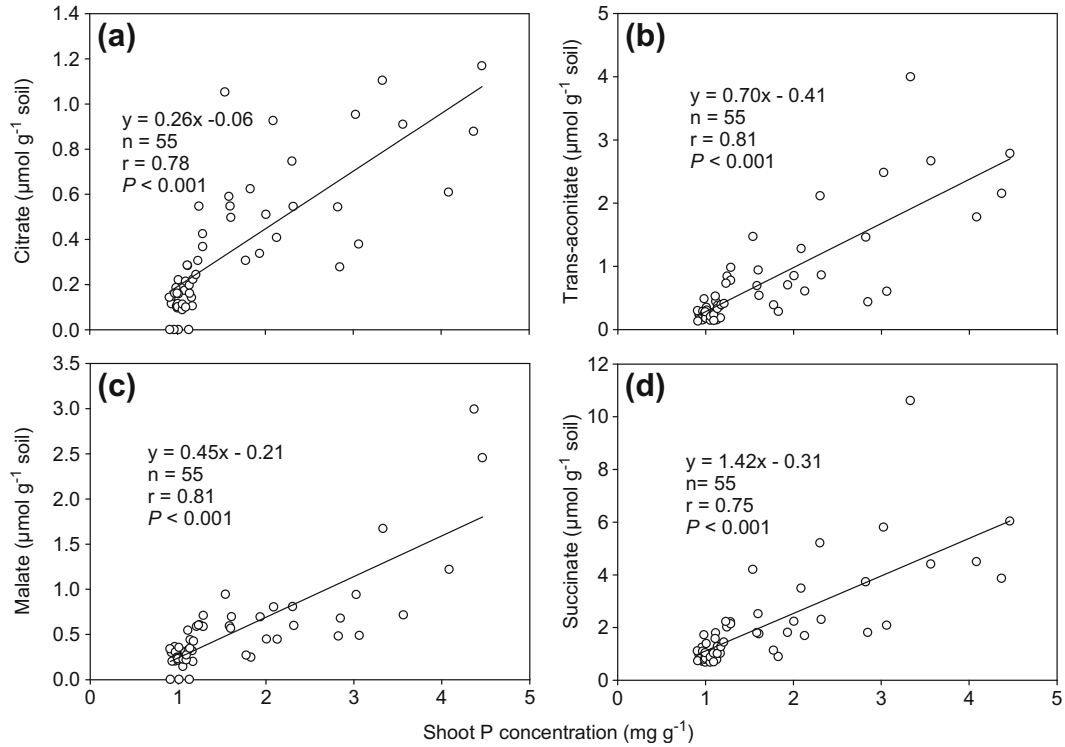

Fig. 5 Correlation between shoot P concentration and concentration of various carboxylates in the rhizosphere soil (**a**–**d**). Data points represent individual replicates

stress include a higher root/shoot ratio, finer roots, a more highly branched root system, and increased specific root length (Zhu and Lynch 2004; Zhang et al. 2012; Deng et al. 2014; Fernandez and Rubio 2015). A wide range of root morphologies were observed with increasing shoot P concentration from severe P deficiency to excess P (Figs. 2a–c; Table S1).

Within a range from sufficient to excess P supply (corresponding to the shoot P concentration between 2.7 and up to 4.0 mg g^{-1}), maize root morphological parameters (total root length, specific root length, proportion of fine roots and root/shoot ratio) were relatively low and stable (Figs. 1d, 2a–c), which would result from soil P availability at high P supply no longer being the limiting factor for plant growth. Fine roots are critical for nutrient uptake and are strongly influenced by soil nutrient availability (Yuan and Chen 2012; Zhang et al. 2012; Li et al. 2016); our results demonstrated that the proportion of fine roots can be regulated by the shoot P status, the proportion decreasing significantly and then plateaued (Fig. 2c) in response to high shoot P concentration due to high soil P availability.

At shoot P concentration below 2–3 mg g^{-1}, all the root morphological parameters in maize (total root length, specific root length, and root/shoot ratio) increased significantly (Figs. 1d, 2a, b and Table S1). An increase in the root/shoot ratio and total root length (Figs. 1d, 2a) are universal responses to P deficiency in maize (Mollier and Pellerin 1999; Zhang et al. 2012; Deng et al. 2014), suggesting P-deficient plants invested more carbon to form new roots and increase the P foraging capacity (Fernandez and Rubio 2015). Similarly, the higher specific root length (Fig. 2b) associated with a decrease in root diameter at low-P supply (cf. Fig. S2c) and low shoot P concentration (Fig. 2c) indicated a root system with a decreased metabolic demand per unit of root length (Eissenstat 1992; Zobel et al. 2007; Pang et al. 2009). Overall, these responses would increase the root surface area available for soil exploration and acquisition of soil P at a minimal energy cost (Vance et al. 2003; Lambers et al. 2006; Lynch 2015).

At shoot P concentration ≤ 1.1 mg g^{-1}, shoot and root biomass were maintained (Table 1), but total root length and proportion of fine roots exhibited a decrease (Figs. 2a, c), suggesting that the extremely low soil P

availability resulted in low shoot P concentration and significantly altered maize root morphology, in accordance with the published reports on maize (Deng et al. 2014) and other plant species (Hill et al. 2006; Pang et al. 2009; Teng et al. 2013). Due to ongoing and severe P deficiency, the leaf growth and photosynthesis in P-deficient maize could decrease significantly (Mollier and Pellerin 1999; Plénet et al. 2000), and subsequently the root growth and function would be severely inhibited.

Compared with shoot growth, root growth is less inhibited under P deficiency (Marschner 2012). We found that the critical shoot P concentration for governing root morphological responses (1.1–1.3 mg g^{-1}, Table S1) was lower than the optimal shoot P concentration for shoot biomass production (2.7 mg g^{-1}, Fig. 1b), indicating that maize root growth was more sensitive than shoot growth to reducing soil P supply. Maize was adapted to decreasing P supply firstly by enhancing fine roots production, even though the total root biomass did not exhibit a significant change. Hence, maize showed a large root morphological plasticity to cope with variable P supply, and these morphological responses were governed by shoot P concentration.

The relationship between root physiological responses and shoot P concentration

To effectively increase P uptake, root morphological changes in response to P deficiency are often accompanied by the activation of physiological responses, such as increased root exudation involved in P mobilization (Vance et al. 2003; Lambers et al. 2006; Shen et al. 2011). However, in the present study, maize root physiological responses were not consistent with the previous studies. We found that P deficiency did not enhance rhizosphere acidification, rhizosphere APase activity and the carboxylate exudation in maize; instead, these parameters increased continuously with an increase in shoot P concentration up to a maximum recorded at 4.0 mg g^{-1} (Figs. 3, 4, and 5).

Modification of soil pH by roots is considered one of the strongest responses to P deficiency by many plant species (Hinsinger et al. 2003, 2005; Rengel and Marschner 2005). However, our results showed that rhizosphere acidification was not induced by P deficiency, but increased with shoot P concentration increasing from the deficient level, then reached a steady state without decreasing even when P was supplied at the above-optimal levels and shoot P concentration was in excess (Figs. 3, S3). This finding was in contrast with the previous reports on maize with no significant change in the rhizosphere pH under low P (George et al. 2002a; Liu et al. 2016). One possible explanation may be different duration and severity of P deficiency in different studies, with the rhizosphere acidification by maize in response to P deficiency potentially being maintained for a short time only (cf. Faget et al. 2013, who observed that the roots of maize acidified the rhizosphere for 7 days after transplanting); hence, our results might represent a later response of maize to P deficiency (our sampling time: 28 days after planting). The other reason was increased shoot K^+ accumulation at high P supply (cf. Figs. 3b and S6b). The increased release of protons may compensate for excess uptake of cations over anions (Hinsinger et al. 2003; Braschkat and Randall 2004).

Increased carboxylate exudation by P-deficient plants has been reported in some studies (e.g. white lupin: Li et al. 2008; Wheat: Pearse et al. 2006; maize: Gaume et al. 2001). In the present study, P-deficient maize had decreased carboxylate concentration in the rhizosphere (Figs. 5, S5), which was in accordance with some previous studies (Corrales et al. 2007; Li et al. 2010a; Liu et al. 2004, 2016, Lyu et al. 2016). Several factors may be responsible for this result: (i) different duration and severity of P starvation, and (ii) in particular variable plant age (cf. Wouterlood et al. 2004b). In addition, most of the reported hydroponic experiments were sampled after a short P-starvation period (e.g. 18 days after seedling transfer, Gaume et al. 2001; 13–15 days after germination, Carvalhais et al. 2011), whereas we characterized the later responses to P deficiency in soil-grown maize. It should also be kept in mind that the patterns of carboxylate exudation in response to P supply in maize may be genotype-specific (Gaume et al. 2001; Corrales et al. 2007).

Our study was carried out in non-sterile soil conditions, thus the carboxylates could have originated from either roots or microbial populations (Ryan et al. 2001; Bais et al. 2006; Jones et al. 2009). Root excretion of carboxylates could be due to enhanced N assimilation from nitrate (Neumann and Römheld 2001; Maistry et al. 2014), which was the only form of N supplied in this study. On the other hand, increased microbial biomass and enhanced abundance of microbial communities in the rhizosphere were observed in the P-fertilized

treatments (Tang et al. 2016), with these microbial populations potentially making a contribution to increasing carboxylate exudation.

Increased acid phosphatase secretion by plants is generally thought to be associated with a low plant P status (e.g. white lupin: Gilbert et al. 1999; wheat: Yadav and Tarafdar 2001, Ciereszko et al. 2011; maize: Yun and Kaeppler 2001). In the present study, P deficiency did not enhance the RS-APase activity in maize (Figs. 4, S4). This result is in accordance with the recent studies (Liu et al. 2016; Lyu et al. 2016). Moreover, with the P supply increasing, RS-APase activity had a positive correlation with shoot P concentration (Fig. 4). A plausible hypothesis is that as shoot P concentration increased, there was an increase in the concentration of P cycling between shoots and roots, which might have resulted in some organic P being lost from roots into the rhizosphere, and increased APase secretion could have recaptured some of that exuded P in the rhizosphere (Barrett-Lennard et al. 1993; Jones et al. 2009). However, RS-APase may originate from either roots or microbes (Tarafdar and Claassen 1988; Richardson et al. 2009; Lambers et al. 2006). It is known that rhizodeposited carbon can significantly enhance soil microbial communities (Richardson and Simpson 2011; Spohn and Kuzyakov 2013), and our results show that the RS-APase activity (Fig. 4) increased concomitantly with an increase in carboxylate exudation (Fig. 5). Therefore, it is speculated that microbial phosphatases may make an important contribution to increasing RS-APase activity with increasing shoot P concentration. Such an explanation, however, requires experimental confirmation by simultaneous determination of the microbial community distribution and the RS-activity around the root, e.g. by soil zymography and fluorescence-in situ-hybridization (FISH) (Spohn et al. 2013, 2015).

The relationship between root responses and soil P supply

All the root morphological and physiological traits were correlated with soil P supply (Figs. 1c, 3a, b, S2, S4b, and S5e-h) as well as shoot P concentration (Figs. 1d, 2, 3c, 4, and 5). Hence, it was difficult to distinguish the effects of shoot P status and soil P supply on root responses. Although some previous studies showed local nutrient supply stimulated root proliferation in nutrient-deficient plants (Drew 1975; Hodge 2004; Li et al. 2010b), this effect of nutrient supply to root traits could be suppressed by high shoot nutrient concentration (Zhang and Forde 1998). Thus, shoot P status is likely to be a more important factor regulating root traits than soil P supply, except potentially at very low P availability.

Conclusions

Shoot P status significantly affected maize growth and the root response. At shoot P concentrations below 2.7 mg g^{-1} (the critical shoot P concentration for the optimum growth of maize seedlings in the present study), most root morphological adaptations (total root length, specific root length, the proportion of fine roots (diameter 0–0.2 mm) in the total root length, and root/shoot ratio) involved with P acquisition were triggered, whereas they were suppressed under either high (3–4 mg g^{-1}) or very low shoot P status (below 1.1–1.3 mg g^{-1}). In addition, P deficiency did not enhance root physiological adaptations (rhizosphere acidification, acid phosphatase activity and the carboxylate exudation), and no decrease was noted even at high shoot P (4.0 mg g^{-1}) corresponding to excess P supply. The modifications of root morphological and physiological traits in maize were differentially influenced by increasing shoot P concentration, with root morphological rather than physiological adaptations occurring in response to P deficiency.

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