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Neighbouring plants modify maize-root foraging for phosphorus: coupling nutrients and neighbours for improved nutrient-use efficiency

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Summary

- Nutrient-distribution and neighbours can impact plant growth, but how neighbours shape root foraging strategy for nutrients is unclear. Here, we explore new patterns of plant foraging for nutrients as affected by neighbours to improve nutrient-acquisition.
- Maize (*Zea mays*) was grown alone (maize), or with maize (maize/maize) or faba bean (*Vicia faba*) (maize/faba bean) as a neighbour on one side and with or without phosphorus (P)-rich zone on the other in a rhizo-box experiment.
- Maize demonstrated root avoidance in maize/maize, with reduced root-growth in 'shared' soil, and increased growth away from its neighbours. Inversely, maize proliferated roots in the proximity of neighbouring faba bean roots that had greater P availability in the rhizosphere (due to citrate and acid phosphatase exudation) than maize roots. Maize proliferated more roots, but spent less time to reach, and grow out of, the P-patches away from neighbours in maize/maize than maize/faba bean. Maize shoot-biomass and P uptake were greater in the heterogeneous P treatment with maize/faba bean than maize/maize.
- The foraging strategy of maize roots is an integrated function of heterogeneous distribution of nutrients and neighbouring plants, consequently improving nutrient acquisition and maize growth. Understanding the foraging patterns is critical for optimizing nutrient management in crops.

Keywords: root foraging, nutrient heterogeneity, root-root interactions, phosphorus acquisition, nutrient-use efficiency, *Zea mays* (maize).

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Introduction

Plants experience significant challenges in obtaining nutrients as a result of spatial variation in the distribution of soil nutrients and neighbours (Callaway *et al.*, 2003; Cahill *et al.*, 2010; Cahill & McNickle, 2011). Consistent with a general understanding of dynamic foraging behaviour in many animal species (Charnov, 1976; Krebs *et al.*, 1977), plants can detect and respond to variations in nutrient availability and distribution in the soil environment (Kelly, 1990; Karban, 2008; McNickle *et al.*, 2015). For example, when encountering a nutrient-rich patch, some species invest resources into proliferating roots, particularly increasing the proportion of fine roots (Drew, 1975; Hodge, 2004; Jing *et al.*, 2010). In addition to changing root distributions in response to localized nutrient supply, plants can also modify nutrient uptake kinetics (Jackson *et al.*, 1990). As root foraging is an active process involving search for nutrients, plants invest more resources into highly enriched patches than they do in homogeneous environments (McNickle & Cahill, 2009; Karst *et al.*, 2012). Collectively, these root-foraging responses can significantly affect nutrient acquisition as well as plant growth and yield (Hodge, 2004; Cahill & McNickle, 2011).

The nutrient uptake benefits of root growth in a particular soil volume depend not only on nutrient concentration, but also on occupancy patterns of neighbours (Cahill *et al.*, 2010). Studies are increasingly finding that plants have highly varied nutrient acquisition responses to the presence of neighbour roots (Semchenko *et al.*, 2007; Mommer *et al.*, 2012; Abakumova1 *et al.*, 2016; Zhang *et al.*, 2016), with some studied species demonstrating avoidance (i.e. roots growing away from the neighbour) (Gersani *et al.*, 1998; Schenk *et al.*, 1999), and some species tending to grow roots near neighbour roots (aggregation) (Maina *et al.*, 2002; de Kroon, 2007). Despite the fact that nutrient depletion (Gersani *et al.*, 1998; Schenk, 2006) and root exudates (Semchenko *et al.*, 2014) can trigger different responses in root-root interactions, plant growth is mainly determined by soil nutrient availability; hence, adaptations that allow plants to acquire nutrients efficiently in the presence of neighbours can strongly

influence plant performance (Cahill & McNickle, 2011; Padilla *et al.*, 2013). The dynamics of root foraging patterns for heterogeneously-distributed nutrients by individual plants are typically influenced by the co-occurring plants (Cahill *et al.*, 2010; Mommer *et al.*, 2012; Zhang *et al.*, 2016). Nonetheless, it remains unclear how plants integrate the information on nutrient and neighbour distributions in their foraging response that influences nutrient acquisition, plant growth, crop yield and even the outcome of ecological interactions.

Maize production is essential to food security in China and throughout the world. Because phosphorus (P) is poorly available in soils due to slow diffusion and strong fixation (Hinsinger, 2001; Shen *et al.*, 2011), low P-use efficiency is one of the main factors that limit maize yield. Maize/faba bean intercropping is widely adopted in Chinese farming systems, particularly in northwest China, and is a good example of cropping systems that enhance P-use efficiency and deliver yield advantages (Zhang *et al.*, 2004, 2010; Li *et al.*, 2007, 2014a; Shen *et al.*, 2013). In the maize/faba bean, maize roots spread underneath faba bean roots and showed greater compatibility of the spatial root distribution between the intercropped maize and faba bean in comparison to the maize/maize monocropping (Li *et al.*, 2006). Whilst some of the maize crop yield and P uptake benefits from root proliferation in localized (heterogeneously-supplied) nutrients zone are understood (Jing *et al.*, 2010, 2012; Ma *et al.*, 2013; Shen *et al.*, 2013; Li *et al.*, 2014b), there is a considerable potential to improve maize yield and/or nutrient-use efficiency through understanding the integrated effects of patchy nutrient distribution and the neighbour species on maize root foraging behaviours.

In order to test how neighbouring plants modify maize-root foraging for heterogeneously-distributed P, and to understand the resulting impacts on P uptake and maize productivity, we conducted a rhizo-box study with varying neighbour species and soil P supply patterns. Specifically, we aimed to test the hypothesis: (1) root foraging strategy is an integrated function of nutrient distribution and neighbour species. The maize shows the specific root placement pattern in response to neighbouring faba bean compared with neighbouring maize, consequently causing the

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varied root P foraging strategies in P-rich patches, such as root proliferation, and the time maize roots spend on acquiring heterogeneously-supplied P; and (2) the maize P nutrition is governed by modified root foraging behaviours influenced by the neighbour species and heterogeneously-supplied P.

Materials and Methods

Experimental set-up

We constructed plastic rhizo-boxes 40 cm long, 12 cm wide and 30 cm deep (Fig. 1a, also refer to McNickle & Cahill, 2009) and the target maize was planted in the center of the rhizo-box. Such a planting design was based on the previous field experiment in that maize roots were concentrated mainly in the top 30 cm of soil, and nutrient-rich patches were located 4-10 cm (we used 8 cm in the present study) away from the maize plants (Jing *et al.*, 2010; Ma *et al.*, 2013; Zhang *et al.*, 2019). In the rhizo-box experiment, one side (40 cm long and 30 cm deep) of the rhizo-box was removable for ease of soil and root sampling. A single clear acrylic tube (6 cm in diameter) was inserted horizontally 10 cm below the top edge of the rhizo-box (Fig. 1a). A minirhizotron camera (Bartz Technology Corporation, Santa Barbara, CA, USA) was used to take images of roots during the experiment, enabling us to track root growth along the tube.

The soil was collected from Shangzhuang experimental station of China Agricultural University (latitude: 40° 14′ N, longitude: 116°19′ E) in Beijing, China, air-dried and sieved through a 2-mm sieve. The soil contained (per kg) 11.5 g organic carbon, 0.72 g total N, 8.5 mg available N (NO_3^-+NH_4^+), 2.6 mg $NaHCO_3$-extractable P, 32 mg NH_4Ac-extractable K, and had pH of 8.2 (the ratio of soil to 0.01 M $CaCl_2$ solution was 1:2.5). Before the treatments were established, basal nutrients in solution were uniformly added to the soil at the following rates (mg per rhizo-box containing 15 kg soil): $Ca(NO_3)_2{\cdot}4H_2O$, 16,870; K_2SO_4, 2000; $MgSO_4{\cdot}7H_2O$, 650; Fe-EDTA, 87.8; $MnSO_4{\cdot}H_2O$, 100; $ZnSO_4{\cdot}7H_2O$, 150; $CuSO_4{\cdot}5H_2O$, 30; H_3BO_3, 10; and $Na_2MoO_4{\cdot}5H_2O$, 2.5.

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There were six treatment combinations of soil P supply patterns (homogeneous or heterogeneous) and maize planting treatments (alone, with neighbouring maize or with faba bean) (Fig. 1b). Each treatment had six replicates. The amount of P (applied as $Ca(H_2PO_4)_2 \cdot H_2O$) was the same in all treatments, but varied in spatial distribution: (1) 525 mg P mixed evenly throughout the soil, resulting in 35 mg P kg^{-1} soil (homogeneous treatment); (2) 255 mg P concentrated in a single rectangular column (4 cm × 12 cm × 30 cm, 1.5 kg soil, spanned the width and depth of each rhizo-box), and the other 270 mg P spread throughout the background soil (13.5 kg soil), resulting in 170 and 20 mg P kg^{-1} soil in the P-rich zone and the background soil, respectively (heterogeneous treatment).

Based on the size of the rhizo-box and the soil volumes partitioned to neighbour, P-rich patch and the zone beyond the patch (Fig. 1c), total soil (15 kg) was divided into three lots: 10.5 kg soil in volumes 1 and 2, 1.5 kg soil in the P-rich patch (volume 3) and 3 kg in soil volume beyond the P-rich patch (volume 4). Before loading the soil into the rhizo-box, the corresponding nutrients were added and thoroughly mixed with the soil. With the help of several thin plastic separators of different sizes, we loaded the soil into the corresponding rhizo-box zones.

The cultivar of maize (*Zea mays* L.) was ZD958, and the cultivar of faba bean (*Vicia faba* L.) was Lincan5. Maize and faba bean seeds were surface-sterilized in 30% v/v H_2O_2 for 20 min, washed with deionized water, soaked in saturated $CaSO_4$ solution for 12 hours, and germinated on wet filter papers in Petri dishes for 4 days at 22 °C; seedlings were then transplanted into the rhizo-boxes. The target maize was grown in the center of the rhizo-boxes; on one side, 8 cm away from the target maize was a neighbouring plant (maize or faba bean), and 8 cm away from the target maize on the other side was the P-rich zone (in the heterogeneous treatments) (Fig. 1c). This design of the rhizo-box experiment allowed us to measure and monitor how the neighbours affected target maize root foraging for the localized nutrient.

The experiment was conducted in a glasshouse at China Agricultural University, Beijing (latitude: 40° 01′ N, longitude: 116° 16′ E). Temperature in the glasshouse was maintained at 23-27 °C during the day and 13-16 °C at night, with a 13:11 h

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light:dark cycle. The plants were gently watered every day to maintain field capacity (30%, v/v, checked by TDR100), and were allowed to grow for 45 days.

Measurements

Root imaging. Images of roots were taken with a BTC 2 Minirhizotron camera system at 15× magnification. Three transects were used along the tube (see Fig. 1a), allowing the visualization of roots above (top transect) and on either side (90° to the top transect) in a clockwise or anti-clockwise direction. Given that small plants had few roots in the sight of the camera before the 17th day, we took the first images on day 18 after sowing. Digital images of roots were taken in 43 frames (1.35 cm × 2 cm) along each transect at 2-3 d intervals to track root growth across the patch or in the related soil volume. After 44 days of growth, we took a final series of digital images. On day 45, all plants were harvested.

Shoot and root analyses. After 45 days of plant growth, the target maize and neighbouring species were sampled separately and divided into shoots and roots. To trace root foraging patterns of target maize, we regarded the rhizo-box edge close to the neighbour (i.e. opposite direction of the P-rich zone) as the starting point (0 cm) to divide roots into four volumes of soil in the heterogeneous or homogeneous treatments (Fig. 1c); volume 1: between the rhizo-box edge on the left-hand side and the stem of target maize (0-20 cm); volume 2: between the stem of target maize and the P-rich zone (20-28 cm); volume 3: P-rich zone (28-32 cm); and volume 4: between the distal P-rich zone edge and the other rhizo-box edge (32-40 cm).

Shoots were oven-dried at 105 °C for 30 min and then at 65 °C for 3 days for dry mass determination. Phosphorus concentration in shoots was determined after digestion with a mixture of 5 mL of concentrated sulfuric acid and 8 mL of 30 % v/v H_2O_2. Shoot P was analyzed by the molybdovanadophosphate method spectrophotometrically (UVmini-1240, Kyoto, Japan) at 440 nm (Johnson & Ulrich, 1959).

At harvest, the removable side of the rhizo-box was taken off, the soil was brushed away little by little from roots, and the neighbouring maize was removed carefully,

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whereas the target maize roots stayed undisturbed; then, the root system of target maize was cut into four parts corresponding to soil volumes 1, 2, 3 and 4 (Fig. 1c). In the maize/faba bean treatments, maize roots and faba bean roots could be distinguished by color, i.e. maize roots were whitish and faba bean roots brownish; after root removal, the soil was sectioned into four parts and roots were further sampled.

Roots were placed in an icebox for transport to the lab and were then washed in deionized water. The target maize roots were scanned on an EPSON root scanner at 400 dot-per-inch resolution (Epson Expression 1600 pro, Model EU-35, Tokyo, Japan), and were then dried at 65 °C for 3 days for the root mass measurement. The root length was analyzed by Win-RHIZO software (Regent Instruments Inc., Quebec, QC, Canada). The length of fine roots (diameter ≤0.2 mm) was calculated based on the classification of root diameters by Win-RHIZO software.

Statistical analyses

To assess the horizontal root distribution pattern of target maize, root mass production towards either the neighbouring plants or the P-rich zone (i.e. root-placement pattern) was analyzed by comparing root mass in region A (Fig. 1c) to the summed root mass in region B (including soil volumes 2, 3 and 4) (Fig. 1c).

The proportion of root overlap between two neighbouring plants per unit area was calculated from the minirhizotron camera images (1.35 cm × 2 cm) to evaluate difference in the root-placement patterns between the maize/maize and maize/faba bean treatments (see Fig. 1c). Root length overlap was analyzed in the minirhizotron camera images using WinRhizoTron V. 2005 software (Regents Inc., Quebec, QC, Canada), and was divided by the total observable area in region A to assess how long both types of roots grew together per unit soil area.

To estimate how roots proliferate in specific soil zones when responding to neighbouring species, we measured the proportion of the target maize total root length and the fine root (diameter ≤0.2 mm) length in the P-rich zone with respect to the total root length in the whole rhizo-box in the heterogeneous treatment. Similarly, total root

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length and fine root length in the soil volume corresponding to P-rich patch were divided by total root length of the target maize in the whole rhizo-box; both root traits were used to compare foraging capacity in the homogeneous with heterogeneous treatments (Mou *et al.*, 1997; Jing *et al.*, 2010; Li *et al.*, 2014b; Wen *et al.*, 2017).

The days the target maize roots needed to reach, and grow out of, the P-rich zone were also recorded to evaluate foraging pattern. When the roots of the target maize were observed by the camera in soil volume 3 (the P-rich zone) in three transects, the day was recorded as the time roots grew into the P-rich zone. The patch-leaving time (when the roots grew out of the P-rich zone into volume 4) was recorded by the same method.

We used the one-way analysis of variance (ANOVA) to test the effects of neighbour species on shoot biomass, P uptake, root distribution patterns and root foraging strategies of target maize. When appropriate, post-hoc mean comparisons were conducted using the Tukey's test at 5% probability ($P \leq 0.05$) using the SPSS statistical software (SPSS version 23.0, IBM SPSS Inc., Chicago, IL, USA). Student's t-tests were conducted to detect significant differences in the variables between the homogeneous and heterogeneous treatments.

Results

Target maize shoot mass and P content

The target maize shoot mass and P content were greater in maize/faba bean than maize/maize in either the homogeneous or heterogeneous P-supply treatment, even though maize shoot mass and P content were reduced in the mixture relative to single maize (Figs. 2a, 2b). Heterogeneous P supply increased maize shoot mass (Fig. 2a) and P content (Fig. 2b) in the maize/maize and maize/faba bean systems, whereas it had no significant effect on the shoot biomass and P content of individually grown maize (compared to the same dose of soil P that was distributed homogeneously). Additionally, neighbouring faba bean combined with the heterogeneous P supply stimulated maize growth, resulting in increased maize shoot biomass and P uptake in

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maize/faba bean in the heterogeneous P treatment compared with maize in maize/maize in the homogeneous P treatment (Fig. 2).

Root distribution patterns of target maize

The interaction between neighbour species and nutrient distributions influenced maize total root mass (Figs. 3a, 3b; Table S1). In the homogeneous P supply treatment, total root mass of target maize was lowest in maize/faba bean, followed by the maize/maize and then individual maize treatments (Table S1). In the heterogeneous treatment, total root mass of target maize was lowest in the maize/faba bean treatment, and did not differ between the individual maize and the maize/maize treatments. Compared with maize (single maize and target maize in maize/maize and maize/faba bean) in the homogeneous treatments, heterogeneous P supply increased total root mass of target maize in the maize/maize and maize/faba bean systems, and for individual maize the trend was in the same direction, but not significant.

Comparing the root mass investment by target maize in the two soil volumes (region A with neighbour and region B with P-rich zone or equivalent in the homogeneous P environment, Fig. 1c), individually grown maize proliferated the same amount of roots on the sides with and without the nutrient patch (Figs. 3a, 3b). However, root proliferation varied in response to the combination of neighbour species and nutrient patch location. In the homogeneous P treatment, maize grew more roots on the side away from a neighbour, regardless of whether the neighbour was maize or faba bean. In contrast, in the heterogeneous P treatment, maize grown with faba bean produced similar root mass on the neighbour and the P-rich sides, but increased root allocation on the neighbour-free/P-rich side when grown with neighbouring maize.

Even though the maize grown with faba bean did not alter root distribution between the two sides of the rhizo-boxes (with neighbour vs with P-rich patch; Fig. 3), the proportion of root overlap between target maize and neighbouring plants (Fig. 4) and their root images (Figs. 5, S1) taken by the camera both proved an adjustment in maize root placement within the soil zone shared with the faba bean neighbour.

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When soil P was homogeneously distributed (Figs. 4a, 4b), the proportion of root overlap between maize and neighbouring faba bean in region A was greater than the root overlap between maize and neighbouring maize in the maize/maize treatment at day 44 (Fig. 4c), although it was similar after 30 and 37 days (Figs. 4a, 4b). In the heterogeneous treatment, the proportion of root overlap per unit of soil area was greater in the maize/faba bean treatment than maize/maize after 30, 37 and 44 days (Fig. 4). The soil P supply pattern had little impact on this proportion of root overlap between target maize and neighbouring plants, except for the maize/maize mixture at day 37 (Fig. 4b). In addition, root images taken with the camera showed that when maize and faba bean roots co-occurred, maize roots grew alongside the faba bean roots regardless of the P supply pattern (Fig. 5; also see Fig. S1).

Root foraging strategies for heterogeneously-supplied P

Under homogeneous P supply, the presence of neighbours (either maize or faba bean) increased maize root foraging capacity in the soil zone corresponding to the P-rich patch location compared with the single maize treatment (Figs. 6a, 6b). When soil P was heterogeneously distributed, neighbouring faba bean did not affect the proportion of target maize total root length and the fine root (diameter ≤0.2 mm) length in the P-rich zone (Figs. 6a, 6b). In contrast, neighbouring maize stimulated target maize root proliferation (Fig. 6a), but not that of fine roots (Fig. 6b), in the P-rich location. Hence, the target maize showed lower foraging capacity in the P-rich patch in the maize/faba bean than maize/maize treatments (Figs. 6a, 6b). Compared with maize in the homogeneous P treatment, the heterogeneous P had no effect on root foraging capacity of target maize grown with faba bean, but improved maize root foraging capacity in single maize and maize/maize (Figs. 6a, 6b).

The time roots of target maize required to reach and grow beyond P-rich zone (or the corresponding soil volume in the homogeneous treatment) was recorded to examine the capacity of roots to seek nutrients (Fig. 7). In the homogeneous and heterogeneous treatments, neighbouring faba bean had no impact on time maize roots spent reaching and growing out of the P-rich zone in maize/faba bean. However, the

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time maize reached and exited the P-rich zone was shorter in the maize/maize than single maize treatments. The time in which target maize roots entered and exited the P-rich zone was longer in maize/faba bean than maize/maize except for the time maize took to reach P-rich patch in the homogeneous treatment. The difference in time the target maize exited the P-rich patch in maize/faba bean and maize/maize was confirmed by the greater target maize root mass and total root length in the soil zone beyond the P-rich patch in the maize/maize than maize/faba bean treatments (Fig. S2). The P supply treatments did not influence the time roots took to reach or grow beyond the P-rich zone (or corresponding soil volume in the homogeneous treatment) regardless of neighbours (Fig. 7).

Discussion

Our results clearly indicated that plant neighbour presence altered the maize root system spatial symmetry. Root growth of an individual plant is generally symmetrical around the plant axis under homogeneous soil nutrient supply (Brisson & Reynolds, 1997; Schenk, 2006). In the present study, single maize grown in the homogeneous-P treatments produced similar root mass in the two soil volumes adjoining its axis, suggesting a balanced investment in development of individual maize roots (Fig. 3). However, any neighbours to maize represented potential competition, but neighbouring faba bean was less competitive than neighbouring maize (Zhang *et al.*, 2016). Plants appear to minimize neighbour competition by directing assimilates to roots in soil away from the neighbouring plant, thereby enhancing root development in those zones and reducing competitive encounters (Figs. 3, 4) (Maina *et al.*, 2002; Callaway *et al.*, 2003; Jesch *et al.*, 2018). Although the present study did not consider root locations in vertical direction because species tested used different depths in soil profile as part of their foraging strategy (Li *et al.*, 2006), the results clearly indicated that once neighbouring maize or faba bean was present, greater root mass was distributed in the soil region away from the neighbouring plant in a horizontal direction, more so in maize/maize than maize/faba bean.

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In maize/maize, the target maize preferentially allocated the root mass into the soil volume away from the neighbouring maize (Fig. 3), leading to the formation of individual plant root territories as mentioned elsewhere (Caldwell *et al.*, 1991; Schenk *et al.*, 1999), so that the new roots could increase nutrient acquisition by avoiding competition for the same nutrient resources. Compared with maize grown with maize, the spatial distribution of maize roots in maize/faba bean was determined by several factors as follows. Firstly, in contrast to maize, faba bean could access the insoluble soil organic/inorganic P fractions (unavailable to maize) by exuding carboxylates and acid phosphatase, suggesting faba bean and maize had different soil P niches, as mentioned in the published reports (Li *et al.*, 2007, 2014a; Hinsinger *et al.*, 2011). Secondly, the proportion of root overlap was greater in maize/faba bean than maize/maize, indicating a tendency of maize roots to grow along faba bean roots (Figs. 4, 5, S1). Hence, the increased P availability induced by faba bean root exudates could support not just faba bean growth, but also that of maize. Thirdly, in addition to P mobilization by faba bean in the rhizosphere, the smaller and more shallow root system of faba bean compared with neighbouring maize could allow a greater soil volume (see also Li *et al.*, 2006; Zhang *et al.*, 2016) to be explored by roots of the target maize in the maize/faba bean than in the maize/maize mixtures; this idea was also supported by a previous study on maize/bean and maize/bean/squash system (Postma & Lynch, 2012). Hence, belowground competition for nutrients between target maize and neighbouring plants in maize/maize or maize/faba bean is fundamental for determining maize root system distribution through the soil. However, how root size and root exudation separately shape the response pattern of targeted maize roots warrant further investigation. In summary, the results indicated a role of the root/rhizosphere interactions driven by the neighbouring species in regulating the spatial asymmetry of maize roots in the maize/faba bean compared with maize/maize mixture.

The neighbour-induced rooting patterns (Figs. 3, 4, 5, S1) and their roles in modifying target plant root foraging for nutrients, particularly in the nutrient-rich zones (Figs. 6, 7), were characterized in the present study. Before roots touched the

P-rich patch, maize root foraging behaviours were mainly influenced by neighbours. Compared with neighbouring maize, target maize roots tended to stay close to the neighbouring faba bean (region A) because faba bean mobilized soil P by exudation to feed maize in the soil (i.e. in soil with increased Olsen-P concentration) (Figs. 4, 5, S1) rather than extend only into the P-rich patch. Hence, the time required to reach the P-rich zone by target maize was longer in maize/faba bean than maize/maize (Fig. 7a). After reaching the patch, root proliferation in P-rich patch was affected by both neighbouring species and heterogeneously-supplied P. Due to the lower root biomass allocated to P-rich patch by target maize in maize/faba bean than maize/maize (Fig. 3), maize produced shorter total root length and fine roots length in P-rich patch (Fig. 6), inducing a lower nutrient depletion rate in P-rich soil volume (Padilla *et al.*, 2013; Zhang *et al.*, 2019) but had longer time for nutrient acquisition and growing out of the patch in maize/faba bean than maize/maize (Figs. 7b, S2). Consistent with the previous study showing that the capacity of plants to proliferate roots into nutrient-rich patches was linked with the distribution of the root system through the soil (McNickle *et al.*, 2015), the results presented here showed a substantial role of the neighbouring faba bean with low root morphological changes but high physiological activity in regulating the target maize root placement pattern, and thus root foraging strategies in the P-rich patches (hypothesis 1).

One of the most interesting arguments is the optimality of plant performance governed by the foraging strategies (Weiner *et al.*, 2010; Anten & Vermeulen, 2016). Optimal foraging does not mean that an organism must always respond to a resource stimulus (Clergue *et al.*, 2005; Gordon, 2011) as has been assumed and expected in many plant studies. Indeed, the reduced proliferation of target maize roots in the P-rich zones when grown with faba bean possibly minimized the carbon cost and increased efficiency of P capture as reported before (McNickle & Cahill, 2009; Lynch, 2015). This study provides a direct support to the idea that an adaptive response should increase the benefits, while minimizing the costs, of a set of responses, subject to certain constraints (Smith, 1978). In the present study maize roots grew along faba bean roots and could capture the P mobilized by faba bean in

the common rhizosphere, thereby suppressing the need to forage for P in the P-rich patch by proliferating roots, which would be associated with a relatively large carbon cost.

In addition, maize in the maize/faba bean mixture could use the P in the soil volume unoccupied by faba bean and also the P in the P-rich patch, leading to greater maize P uptake and productivity in the maize/faba bean than maize/maize mixtures in the rhizo-box (Fig. 2), which was confirmed by an additional field experiment (see the methods and data in SI; Fig. S4). The present study integrated the multiple environmental cues (neighbour and nutrient) for understanding of biologically-complex interactions between rhizosphere properties and root foraging, indicating that interspecific variation in behavioural types governed nutrient uptake and crop yield (hypothesis 2).

Root foraging traits are the important drivers of many ecosystem processes, such as carbon and nutrient cycling, and the formation and structural stability of soil (Bardgett *et al.*, 2014). It is crucial to understand how different combinations of plant traits (such as different phylogeny, root sizes and various resource-use strategies) affect species coexistence and regulate ecosystem function. Understanding the details of plant foraging behaviour can enhance our knowledge on root-root interactions and nutrient-use efficiency as well as crop productivity, and contribute to revealing how these processes vary in the changing environments (e.g. variable nutrient concentrations and distribution patterns) in natural ecosystems, and how different trait combinations can influence system functions and sustainability in agroecosystems. For example, increasing biodiversity in agriculture (e.g. intercropping) could enhance soil fertility without external inputs and protect crops against pests and diseases while ensuring adequate crop productivity (see e.g. Brooker *et al.*, 2015; Gaba *et al.*, 2014; Duchene *et al.*, 2017; Isbell *et al.*, 2017; Weiner, 2017). However, the success of this agroecological practice depends to a great extent on local field conditions and is still potentially diminished by competition between intercropped species, particularly when influenced by unfavourable local climate, growth conditions, fertilization practices or choice of species (Hauggaard-Nielsen & Jensen, 2005). The present

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research underlines the importance of designing cropping systems based on ecological principles and the delivery of ecosystem services (including crop yield) to enhance agroecosystem sustainability and nutrient-use efficiency, and minimize substrate and energy inputs, which is partially highlighted in the previous studies (Isbell *et al.*, 2017; Weiner, 2017). Hence, managing root foraging behaviours might allow designing more productive and sustainable cropping systems and developing more precise and efficient agronomic recommendations.

Conclusions

The results demonstrated that plant root foraging patterns emerge from the interactions of soil nutrient distribution and neighbours presence. The root/rhizosphere interactions induced by neighbours are the underlying mechanism driving maize root spatial distribution and thus governing root foraging for heterogeneously-supplied P. The target maize showed greater foraging capacity for P in the P-rich soil zone in maize/maize cropping than maize/faba bean intercropping, shown as greater total root length as well as fine root length in the P-rich patch, but shorter time to reach, and grow out of, the P-patch zone. The effect of nutrient supply and neighbours on changing root growth and positioning corresponded to increases in the target maize P uptake and shoot mass (higher with the faba bean than maize neighbour, and higher in heterogeneous than homogeneous P supply). Faba bean intercropped with maize stimulated the target maize to acquire more P than neighbouring maize did in maize/maize, which was consistent with the greater shoot biomass of the target maize; the heterogeneous P supply further improved the target maize P nutrition. The present study showed that intercropping maize with faba bean and localized application of P increased the target maize P uptake and shoot biomass.

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Author contributions

D.Z. and J.S. designed research; D.Z., R.H., Y.L. and J.S. performed the experiments; D.Z., H.L., X.T. R.H. and Y.L. collected and analyzed data; D.Z., J.S., Z.R., F.Z., W.R.W., W.J.D. and J.F.C. wrote the paper.

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630 **Figure captions**

631 Fig. 1 (a) Schematic diagram of the experimental rhizo-boxes with a horizontal tube
632 for a minirhizotron camera. One side (40 cm long and 30 cm deep) of the rhizo-box
633 was removable for ease of soil and root sampling. (b) Representation of experimental
634 design with three planting treatments (left-to-right: single maize, maize/maize and
635 maize/faba bean) in the homogeneous (Hom) and heterogeneous (Het) nutrient
636 environments. (c) Arrangement of target maize (in the middle of the rhizo-box),
637 neighbour (on the left side, represented by a red arrow) and P-rich zone (on the right
638 side, vertical light brown column), the four soil volumes [→(1)↔(2)↔(3)↔(4)←] for
639 separate root harvesting, and the two regions (A and B) for the root-placement pattern
640 analysis.

641 Fig. 2 Shoot mass (a) and shoot P content (b) of target maize in various planting
642 patterns and with soil P supply as homogeneous (Hom) or heterogeneous (Het).
643 Different lowercase letters denote significant differences ($P \leq 0.05$) among the target
644 maize data in the single maize, maize/maize and maize/faba bean systems in the
645 homogeneous treatments, and different capitals denote significant differences (P
646 ≤ 0.05) in the heterogeneous treatments. Means + SE (n=6). For target maize, the
647 t-tests were run to assess the differences in shoot mass or shoot P content between the
648 heterogeneous and homogeneous treatments: *, $P \leq 0.05$; ns = not significant.

649 Fig. 3 Root mass of target maize (position 0 on the X-axis) in region A with a
650 neighbour (a) and region B with the P-rich zone (vertical light brown column) (b) in
651 various planting patterns and with homogeneous (Hom) or heterogeneous (Het) soil P
652 supply. Different lowercase letters denote significant differences ($P \leq 0.05$) among the
653 target maize data in the single maize, maize/maize and maize/faba bean systems in the
654 homogeneous treatment, and different capital letters denote significant differences (P
655 ≤ 0.05) in the heterogeneous treatment. The asterisks on the side of horizontal bars
656 denote the differences in root mass between the homogeneous and heterogeneous
657 treatments. The asterisks within horizontal bars represent the treatment differences in

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658 root mass between region A containing a neighbour and region B containing the
659 P-rich zone. The darkening bars from top to bottom represent planting patterns from
660 single maize, maize/maize to maize/faba bean. Means + SE (n=6). The t-tests were
661 run to detect significant difference between the heterogeneous and homogeneous
662 treatments: *, $P \leq 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns = not significant.

663 Fig. 4 Proportion of root overlap between neighbouring two plants in region A (see
664 Fig. 1c) calculated from the minirhizotron camera images that were taken at 10-cm
665 depth after 30 (a), 37 (b) and 44 days (c) with soil P supply either homogeneous
666 (Hom) or heterogeneous (Het). All images were taken in the soil volume bordered by
667 the lines projected from the faba bean and maize stems in region A at 15×
668 magnification. Different lowercase letters denote significant differences ($P \leq 0.05$)
669 between the target maize data in the maize/maize and maize/faba bean systems in the
670 homogeneous treatments, and different capital letters denote significant differences in
671 the heterogeneous treatments ($P \leq 0.05$). Means + SE (n=6). The t-tests were run to
672 detect significant difference between the heterogeneous and homogeneous treatments:
673 * $P \leq 0.05$ and ns = not significant.

674 Fig. 5 Minirhizotron camera images at 10-13 cm depth taken after 23 days of growth
675 (top row, 1-a and 2-a) and after 6 additional days (29 days of growth; bottom row, 1-b
676 and 2-b), whereby roots of the target maize approached and grew along the faba bean
677 roots. All images were taken in the soil volume bordered by the lines projected from
678 the faba bean and maize stems in region A (see Fig. 1c) at 15× magnification. The
679 numbers in the bottom right corner represent replications and the day when the image
680 was taken. Note the maize roots (whitish roots indicated by blue arrows) growing
681 along faba bean roots (brownish roots indicated by red arrows).

682 Fig. 6 Proportion of root length (a) and fine root (diameter ≤0.2 mm) length (b) of
683 target maize in the P-rich zone with respect to the total (whole rhizo-box) root length
684 as influenced by the P supply pattern (Hom, homogenous; Het, heterogeneous).
685 Different lowercase letters denote significant differences ($P \leq 0.05$) among the target

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686 maize data in the single maize, maize/maize and maize/faba bean systems in the
687 homogeneous treatments, and different capital letters denote significant differences (P
688 $\leq$0.05) in the heterogeneous treatments. Means + SE (n=6). The t-tests were run to
689 assess the differences in the target maize data between the heterogeneous and
690 homogeneous treatments: *, $P \leq 0.05$; **, $P < 0.01$; ns = not significant.

691 Fig. 7 The number of days the target maize roots needed to reach (a) and grow out of
692 (b) the P-rich zone in the single maize, maize/maize and maize/faba bean treatments
693 with soil P supply either homogeneous (Hom) or heterogeneous (Het). When the roots
694 of the target maize were observed by the camera in soil volume 3 (the P-rich zone)
695 (see Fig. 1c) from the three transects, that day was recorded as the time roots grew
696 into the P-rich zone. The patch-leaving time (when the roots grew out of the P-rich
697 zone into volume 4, Fig. 1c) was recorded by the same method. Different lowercase
698 letters denote significant differences ($P \leq 0.05$) among the target maize data in the
699 single maize, maize/maize and maize/faba bean systems in the homogeneous
700 treatments, and different capital letters denote significant differences ($P \leq 0.05$) in the
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702 differences in the patch-reaching (a) and -leaving time (b) by target maize between
703 the heterogeneous and homogeneous treatments: ns = not significant.

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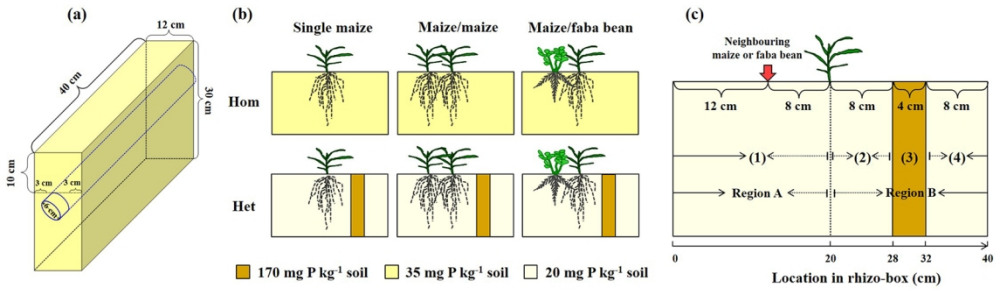

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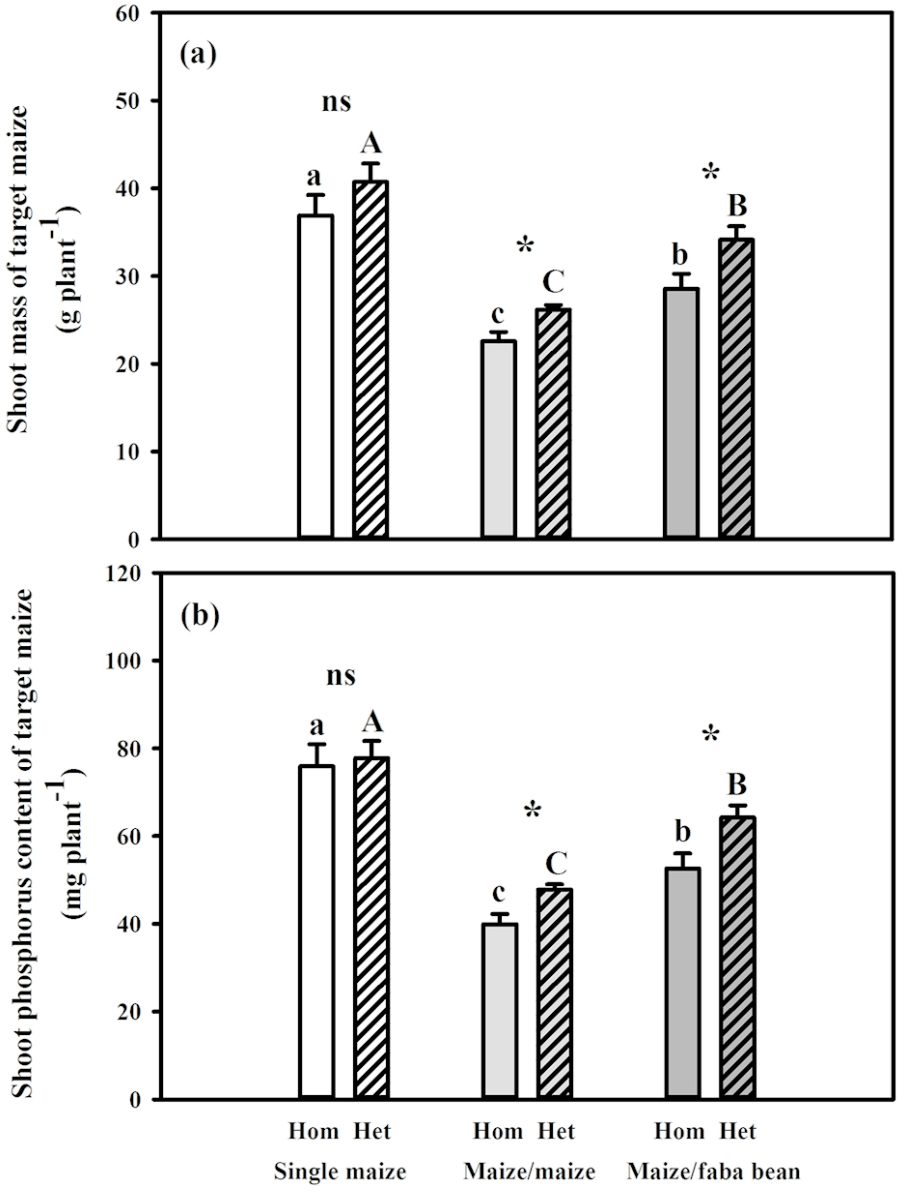

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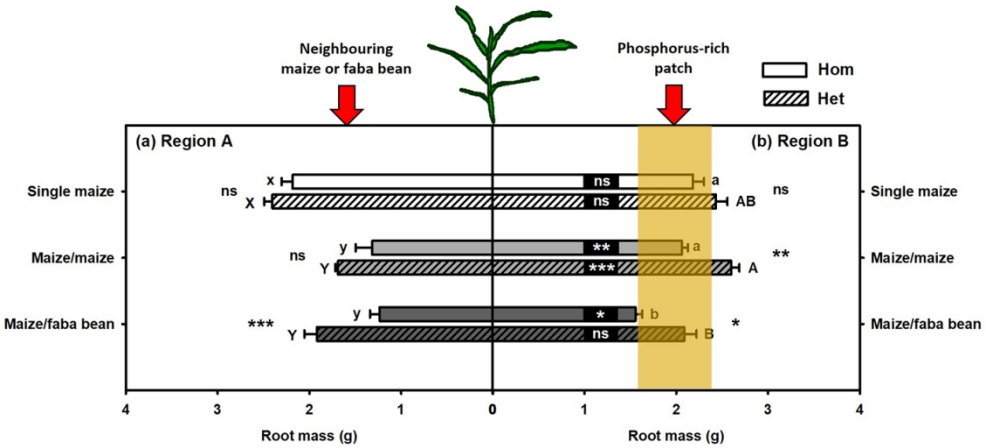

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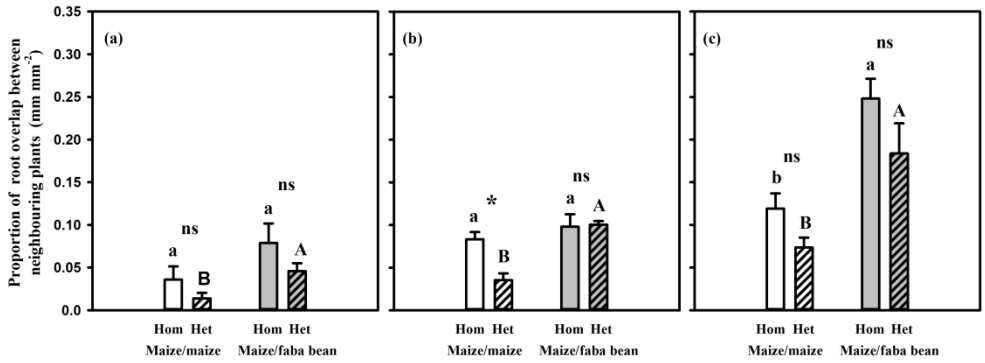

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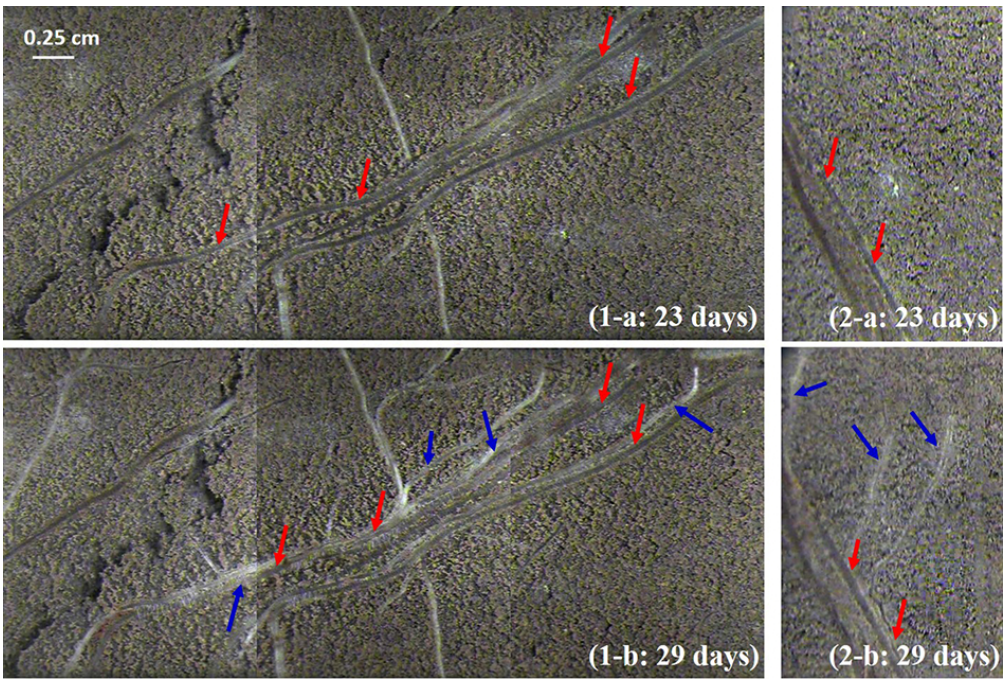
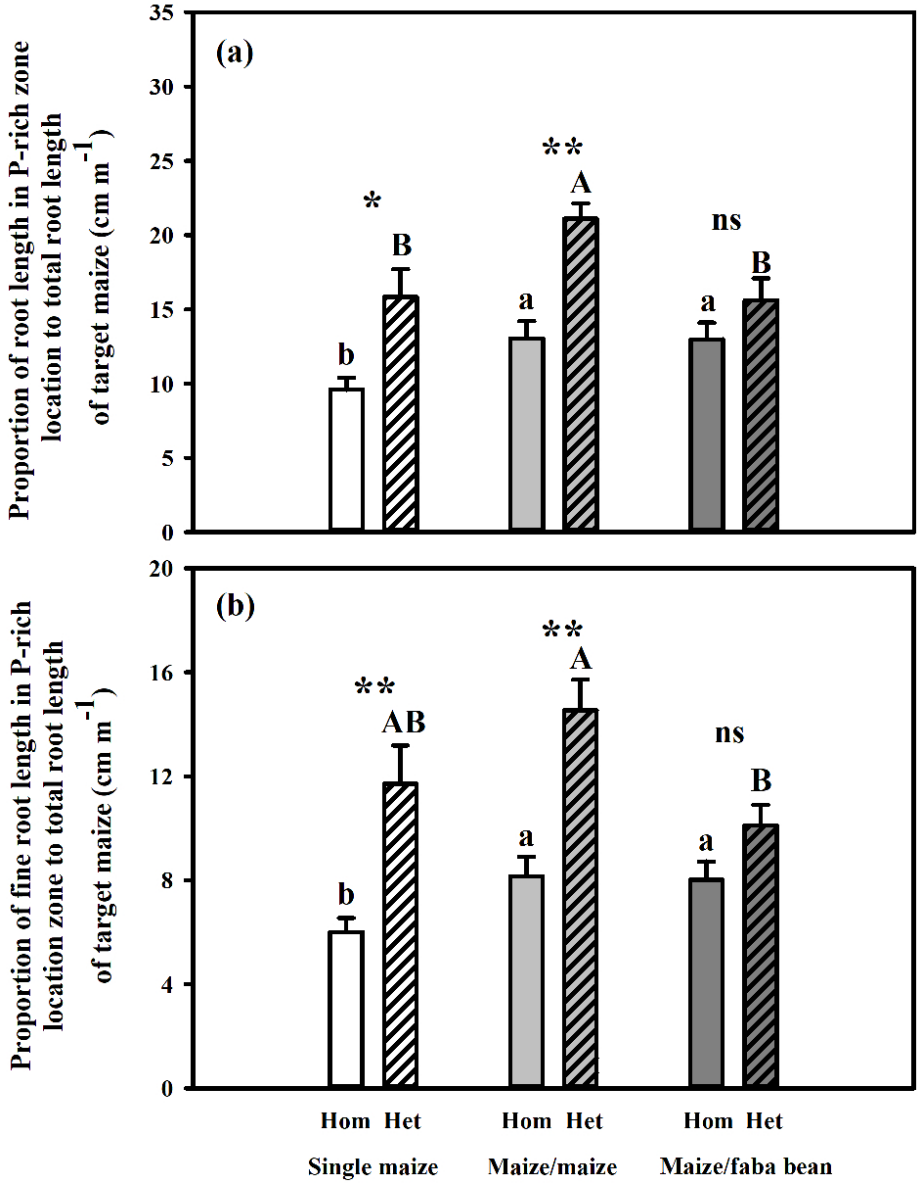


Fig. 5 Minirhizotron camera images at 10-13 cm depth taken after 23 days of growth (top row, 1-a and 2-a) and after 6 additional days (29 days of growth; bottom row, 1-b and 2-b), whereby roots of the target maize approached and grew along the faba bean roots. All images were taken in the soil volume bordered by the lines projected from the faba bean and maize stems in region A (see Fig. 1c) at 15× magnification. The numbers in the bottom right corner represent replications and the day when the image was taken. Note the maize roots (whitish roots indicated by blue arrows) growing along faba bean roots (brownish roots indicated by red arrows).



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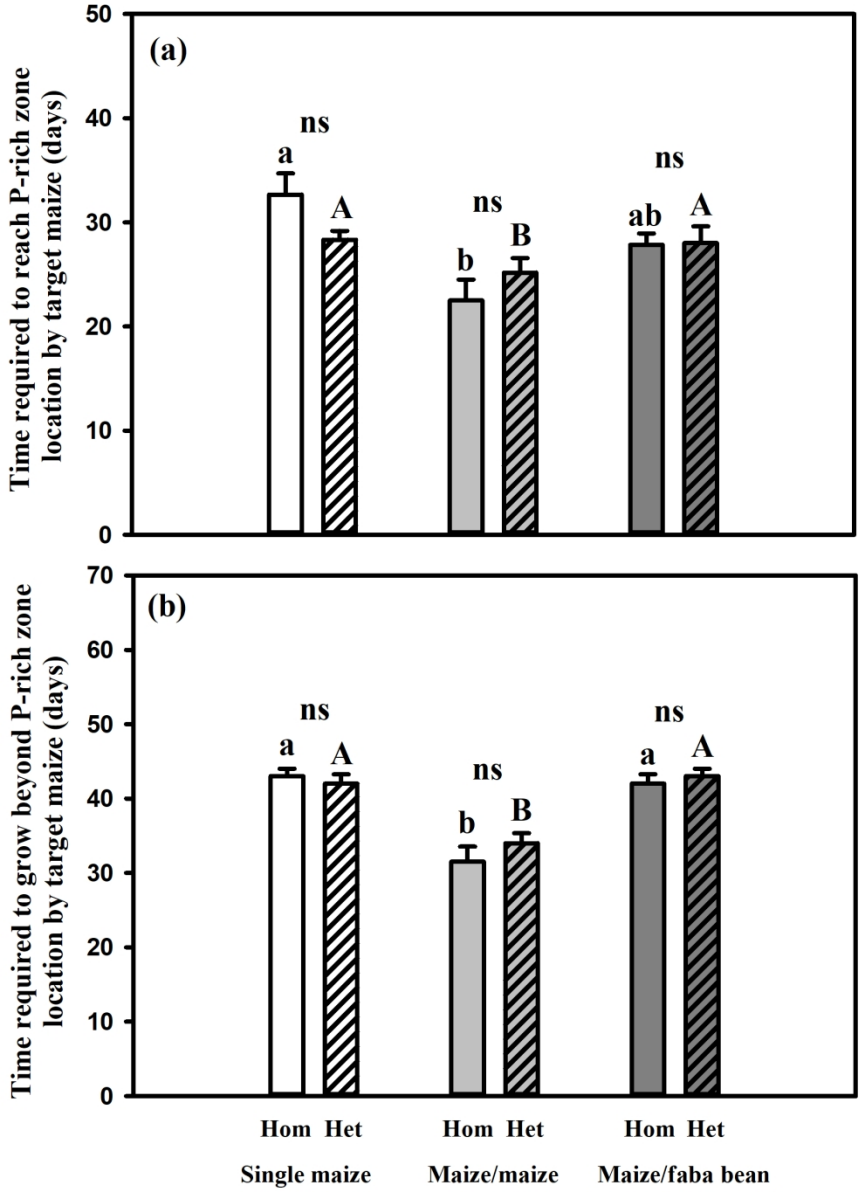

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150x207mm (300 x 300 DPI)