Increased soil P availability induced by faba bean root exudation stimulates root growth and P uptake in neighbouring maize

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Summary

- Root growth is influenced by soil nutrients and neighbouring plants, but how these two drivers affect root interactions and regulate plant growth dynamics is poorly understood. Here, interactions between the roots of maize (*Zea mays*) and faba bean (*Vicia faba*) are characterized.
- Maize was grown alone (maize) or with maize (maize/maize) or faba bean (maize/faba bean) as competitors under five levels ~~variable rates~~ of phosphorus (P) supply, and with homogeneous or heterogeneous P distribution.
- Maize had longer root length and greater shoot biomass and P content when grown with faba bean than with maize. At each P supply rate, faba bean had a smaller root system than maize but greater exudation of citrate and acid phosphatase, suggesting greater capacity to mobilize P in the rhizosphere. Heterogeneous P availability enhanced root-length density of maize but not faba bean. Maize root proliferation in the P-rich patches was associated with increased shoot P uptake.
- Increased P availability by localized P application or by the presence of faba bean exudation stimulated root morphological plasticity and increased shoot growth in maize in the maize/faba bean mixture, suggesting root interactions of neighbouring plants can be modified by increased P availability.

***Key words*:** Phosphorus uptake, *Zea mays* (maize), *Vicia faba* (faba bean), root interactions, heterogeneous phosphorus supply, rhizosphere processes

Introduction

Root interactions among plants play an important role in determining the performance of individuals in natural communities as well as crop productivity in agroecological systems. The foraging capacity of roots is a key trait in belowground competition and is dependent on morphological (root architecture) and physiological plasticity (eg. exudation of organic and inorganic compounds, nutrient uptake) in response to the soil environment. To compete for soil resources (water and nutrients), plants invest in root growth to maximize root length density and can out-compete neighbouring plants (Cahill *et al.*, 2010; Cahill & McNickle, 2011). For example, *Rumex palustris* increased root growth in nutrient-rich areas and as a result became the superior species in competition with *Agrostis stolonifera* (Mommer *et al.*, 2012). In addition to root growth, root physiological plasticity (ie. activity) can be critical in obtaining a long-term competitive advantage (Fransen *et al.*, 2001). Modelling studies have suggested that physiological responses may be more important for the capture of patchy nitrogen (N) than morphological responses (Jackson & Caldwell, 1996; Robinson, 1996; Dunbabin *et al.*, 2003).

Root morphological and physiological plasticity is determined by various environmental factors, such as availability of soil resources and their distribution (Jackson *et al.*, 1990; Hutchings & de Kroon, 1994; Hodge, 2004; Cahill *et al.*, 2010; Andersen *et al.*, 2014). Soil nutrient availability can profoundly affect root morphology and physiology. For example, cluster-root formation and citrate exudation in *Lupinus albus* can be induced by low phosphorus (P) conditions, and inhibited by increased P supply (Shen *et al.*, 2005; Lambers *et al.*, 2006; Li *et al.*, 2008). Faba bean roots have no significant growth response to localized nutrient supply (Li *et al.*, 2014a), suggesting relatively poor morphological plasticity to variable nutrient availability, and potentially a low capacity to take full advantage of nutrient-rich patches. However, in many species, root growth and distribution are affected by soil nutrient heterogeneity (Drew, 1975; Li *et al.*, 2008; Jing *et al.*, 2010). In response to nutrient-rich patches, plants tend to stimulate root growth and alter root distribution, with increased root proliferation in the local nutrient-rich zone (Drew, 1975; Hodge *et al.*, 1999; Hodge, 2004). In addition, some species show root physiological responses to localized nutrient enrichment (eg. an increase in the nutrient uptake capacity per unit root length) (Jackson *et al.*, 1990). Hence, foraging for nutrients is determined by root morphological and physiological dynamics in response to soil environmental conditions, and may result in differences in competitive ability, which, to a large extent, affects plant productivity and nutrient uptake in a mixed system.

Growing mixtures of maize and faba bean is used widely to improve grain yield and P-use efficiency in cropping ecosystems in many parts of the world (intercropping) (Li *et al.*, 2007; Zhang *et al.*, 2010; Shen *et al.*, 2011, 2013). Root interactions have a profound impact on P uptake and yield in the maize/faba bean system (Li *et al.*, 2003, 2014b), with rhizosphere acidification caused by faba bean increasing mobilization of organic/inorganic P sources and facilitating P uptake by target maize (Li *et al*., 2007). The roots of maize and faba bean intermingle and appear to grow together; hence, complementarity of the spatial root distribution of intercropped species contributes to interspecies facilitation in maize/faba bean intercropping compared to the maize/maize monocropping system (Li *et al.*, 2006). However, the detailed mechanisms underlying root interactions in the maize/faba bean system are still unclear.

Much work on root traits in response to P supply has been conducted in maize or faba bean separately (Li *et al.*, 2014a), but how altered nutrient gradients and spatial distribution affect root-root interactions in the mixture, and thus regulate plant growth dynamics, is largely unknown. Maize and faba bean roots grow well together, but the root interactions based on complementary and niche differentiation in space might be enhanced with a better understanding of how spatial changes in soil nutrient availability affect root growth, distribution and hence competition.

To investigate the effects of heterogeneous nutrient availability on root growth, distribution and thus competition intensity in the maize/faba bean mixture system, we conducted experiments with varying P supplies and homogeneous or heterogeneous P distribution to test the hypothesis that neighbouring faba bean would not compete strongly with target maize because faba bean roots have low morphological plasticity that is compensated for by significant physiological plasticity in variable soil P supply. We then demonstrate that increased maize growth in the maize/faba bean mixtures is consistent with the hypothesis that spatial heterogeneity in P availability induced by faba bean exudation ~~would~~ stimulates root morphological plasticity in maize, resulting in improved maize shoot growth and nutrient uptake.

Materials and Methods

Experimental set-up

Experiment 1

To investigate how maize roots respond to faba bean in soil with variable P supply, and test how soil P availability regulates root interactions between maize and faba bean, a pot experiment was conducted in a glasshouse with five soil P supply levels and three cropping treatments. Maize was grown alone as a single species (single maize treatment), mixed with other maize plants (maize/maize treatment) or intercropped with faba bean (maize/faba bean treatment) in soil supplied with 0, 50, 100, 200 or 500 mg P kg^{-1} soil, corresponding to soil Olsen-P values of 3.5, 36, 51, 123 and 270 mg kg^{-1}, respectively. We choose the soil P supply levels based on a preliminary experiment with the same soil: (1) deficient: 0 and 50 mg P kg^{-1} soil; (2) moderate: 100 mg P kg^{-1} soil; (3) adequate: 200 mg P kg^{-1} soil, and (4) high: 500 mg P kg^{-1} soil (to mimic high-input maize cropping system in intensive agriculture of China). There were 15 treatment combinations in the study, with four replicates per treatment.

The soil was collected from Shangzhuang experimental station in Beijing, China, air-dried and passed through a 2-mm sieve. Soil properties were as follows: Olsen-P 3.5 mg kg^{-1}, organic C 11.5 g kg^{-1}, total N 0.72 g kg^{-1}, available N 8.5 mg kg^{-1} (NO_3^- and NH_4^+), available K 32.3 mg kg^{-1}, and pH 8.2 (the ratio of soil to $CaCl_2$ solution was 1:2.5). The pot was filled with 1.5 kg of air-dried soil. To ensure the nutrient supply was adequate for plant growth, soil was also fertilized with basal nutrients at the following rates (mg pot^{-1}): $Ca(NO_3)_2·4H_2O$, 1687; K_2SO_4, 200; $MgSO_4·7H_2O$, 65; Fe-EDTA, 8.78; $MnSO_4·H_2O$, 10; $ZnSO_4·7H_2O$, 15; $CuSO_4·5H_2O$, 3; H_3BO_3, 1; and $Na_2MoO_4·5H_2O$, 0.25. Phosphorus was applied as $Ca(H_2PO_4)_2·H_2O$.

The genotype of maize (*Zea mays* L.) was cv. ZD958, and the genotype of faba bean (*Vicia faba* L.) was cv. 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 $CaSO_4$ saturated solution for 12 hours and then germinated in Petri dishes covered with wet filter papers for 1-2 days at 22°C. All the pots were arranged in a completely randomized design, and were re-randomized weekly during the experiment. The plants were watered every day to maintain field capacity (18%, w/w).

Plants were harvested 38 days after sowing (DAS) and separated into shoots and roots. Following root excavation, the soil adhering to roots was defined as rhizosphere soil and was sub-sampled for carboxylate and acid phosphatase measurements. Roots were transferred to a tube containing 50 mL of 0.2 μM $CaCl_2$ and gently shaken to dislodge the rhizosphere soil, followed by shaking for 5-10s to create homogeneous suspension. A suspension volume of 10 mL was taken by pipette to a 10-mL centrifuge tube for carboxylate analysis by HPLC (see below), and a 0.5 μL aliquot of suspension was placed in a 2-mL centrifuge tube for acid phosphatase measurement (see below).

Experiment 2

To address the question of how soil P heterogeneity affects root interactions between maize and faba bean, we set up a rhizo-box experiment comprising two P supply treatments and three cropping treatments (maize, maize/maize and maize/faba bean) with four replicates of each treatment. We chose the same soil, genotypes of maize and faba bean, and crop treatments as in experiment 1.

To record root growth and distribution, we constructed microcosm PVC-rhizo-boxes (20 × 1.5 × 35 cm, Fig. 1), containing irrigation holes, a viscose fleece for moisture distribution, transparent plastic foil for soil-covering and a Perspex front lid with screws. All rhizo-boxes were filled with 1.3 kg air-dried soil.

Phosphorus was supplied as $Ca(H_2PO_4)_2·H_2O$ in a homogeneous or heterogeneous pattern. For the heterogeneous P treatment, a 3.5-cm P-rich layer (227.5 g soil) containing 46.2 mg P (200 mg P kg^{-1} soil) was manually mixed and placed at the center of the rhizo-box (Fig. 1), referred to as the P-rich patch, and the remaining soil without P additions was the background soil (1072.5 g soil). For the homogeneous P treatment, the same total P (46.2 mg P) was spread evenly throughout the soil (35 mg P kg^{-1} soil).

Maize and faba bean seeds were handled as in experiment 1. To ensure nutrient supply for plant growth, the same amounts of basal nutrients as in experiment 1 were added to soil, followed by thorough mixing.

Before planting, all rhizo-boxes were irrigated through the bottom irrigation holes. After 15 days of growth, the rhizo-boxes were irrigated every 2 days until the final harvest. After 30 days of growth, the target species (maize) and neighbouring species were separated, and shoots and roots of each species were collected for biomass measurements. Shoot P content was analysed in maize. In the heterogeneous treatments, roots grown in vs. out of the P-rich patch were sampled separately for length measurement, and the corresponding roots at the same location were sampled in the homogeneous treatments.

Both experiments were conducted in a glasshouse at China Agricultural University, Beijing (latitude: 40° 01′ N, longitude: 116°16′ E). In experiment 1, temperature in the glasshouse was maintained at 21-25 °C during the day and 15-18 °C at night, with 12-14 h daytime throughout the growth period. In experiment 2, temperature was maintained at 24-28 °C during the day and 18-20 °C at night, with 14-19 h daytime.

Comment [z1]: How were the P additions done?

Phosphorus-rich soil (227.5 g soil) with 46.2 mg P concentrated in a single rectangular column (3.5 cm length, spanned the width and the deep of the rhizo-boxes, 3.5 cm × 1.5 cm × 35 cm), and the background soil (1072.5 g soil) without P added, were separately placed in the rhizo-boxes, refer to Fig. 1.

Comment [z2]: Why were there different environmental conditions for the two experiments?

We did these two experiments separately.

Measurements

Plant biomass and phosphorus uptake

Shoots were oven-dried at 105°C for 30 min and then at 65°C for 3 days before weighing for dry biomass determination. Phosphorus concentration in shoots was determined after digestion with a mixture of 5 mL of concentrated sulphuric acid and

8 mL of 30% v/v H_2O_2. Shoot P was analyzed by the molybdovanadophosphate method at 440 nm by spectrophotometry (Varian Vista–Pro CCD) (Johnson & Ulrich, 1959).

Root parameter measurement

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

Carboxylate and acid phosphatase exudation

Carboxylates in the rhizosphere soil were analyzed using a reversed phase high-performance liquid chromatography (HPLC) system according to a previous report (modified from Shen *et al*., 2003 and Wang *et al*., 2010). The chromatographic separation was conducted on a 250 × 4.6 mm reversed-phase column (Alltima C18, 5 Micrometers; Alltech Associates, Inc., Deerfield, IL, USA). The mobile phase was 25 mmol L^{-1} KH_2PO_4 (pH 2.25) with a flow rate of 1 mL min^{-1} at 31°C. Detection of carboxylates was carried out at 214 nm.

To determine the activity of acid phosphatase in the rhizosphere soil, 0.5 mL aliquots of soil suspensions were transferred into a 2-mL Eppendorf vial with 0.4 mL sodium acetate buffer and 0.1 mL *p*-nitrophenyl phosphate (NPP) substrate added. Vials were incubated at 30ºC for 60 min, and the reaction was terminated by adding 0.5 mL of 0.5 M NaOH. Absorption was measured at 405 nm (Alvey *et al.*, 2000).

Statistical analyses

To investigate the effect of faba bean on target maize, the Relative Interaction Intensity (RII) was calculated in experiment 1. RII can be used to compare the performance of plants growing in mixtures or alone in order to estimate the magnitude of competitive responses of the target plant species (Markham & Chanway, 1996; Howard & Goldberg, 2001; Weigelt & Jolliffe, 2003). Calculation of RII was based on the method proposed by Wilson & Keddy (1986) and modified by Markham & Chanway (1996). A formula of Markham & Chanway (1996) was adopted to calculate RII (modified from Valladares *et al*., 2006).

$$RII = \frac{\sum\left(\frac{xi - xi'}{X}\right)}{\mathrm{n}}$$

where xi is shoot biomass of target maize in the presence of neighbours and xi' is biomass of target maize in the absence of neighbours. X is either xi or xi', whichever was larger. RII is symmetrical around zero and constrained between +1 and -1 (Markham & Chanway, 1996), so that magnitudes of competition or facilitation can be compared. If maize is unaffected by its neighbour, RII will be zero. Negative RII values represent the relative competition between neighbouring plants; positive values indicate that target maize can benefit from neighbour presence.

The *i* and *i*' are two randomly selected individuals (in the present study, i =1, …, 4, because each treatment had 4 replicates) of the same species belonging to two different cropping treatments. The n is the number of $(xi - xi')$ values. In the present study, n is equal to 16 because 4 replicates (4 random individuals) were set in each pair of cropping treatments (single maize and maize/maize, or single maize and maize/faba bean).

Analysis of variance (ANOVA) was conducted using the SPSS statistical software (SPSS19.0). Significant differences among means were separated by LSD at the $P \leq 0.05$ probability level. Plant growth and root length were subjected to two-way ANOVA to assess the effects of plant species, soil P supply, and their interaction in experiment 1 as well as the effects of heterogeneous/homogeneous P supply and competition in experiment 2.

Results

Root interactions between maize and faba bean grown with variable soil P supply (exp 1)

Shoot biomass and P content in target maize increased with soil P supply (Fig. 2). Compared with the single maize treatment at five levels of P supply, shoot biomass of target maize in the mixtures was not affected by the presence of faba bean, but was significantly reduced when maize was grown with maize (Fig. 2a). Shoot P content of target maize showed a similar response to shoot biomass when grown either as single or with neighbours (Fig. 2b). In the maize/maize mixture, a decrease in maize shoot P content was affected by soil P availability and the presence of neighbouring maize, however, there was no significant interaction (Fig. 2b; Table S1). In the maize/faba bean mixture, shoot P content in target maize increased with soil P supply, but not with the neighbour presence; the interactive effect was not significant (Fig. 2b; Table S1). Phosphorus content in shoots of target maize was higher in the maize/faba bean than maize/maize mixtures at 36 and 270 mg kg^{-1} (soil Olsen-P levels). When comparing shoot P content in target maize between the maize/maize and maize/faba bean treatments, we found ~~the~~ a main effect~~s~~ of P supply and neighbour, as well as ~~the~~ their interaction (Fig. 2b; Table S1)~~ had a significant influence~~. The interaction was significant because the effect of faba bean on increasing maize shoot P content (compared to competing maize plants) became more pronounced as soil P supply increased (Fig. 3).

Relative interaction intensity (RII) of maize grown with maize decreased with soil P supply, whereas in the maize/faba bean mixture, RII of target maize increased from 3.5 to 36 mg Olsen P kg^{-1} and remained unchanged with further increases in soil P (Fig. 3). RII of maize in maize/maize was significantly lower than that of maize grown with faba bean, except in the lowest P treatment (3.5 mg Olsen P kg^{-1}) (Fig. 3). Compared with the maize/maize mixture, RII in maize/faba bean was significantly greater in treatments with Olsen P at 36, 51, 123 and 270 mg kg^{-1}.

Root length of target maize in the maize/faba bean or maize/maize mixture significantly increased with P supply (Fig. 4; Table S1). In the maize/faba bean mixture, root length reached the maximum at 51 mg Olsen P kg^{-1} and remained similar at higher P supplies (Fig. 4), with non-significant effects of neighbour and interaction (Fig. 4; Table S1). Root length of maize when competing with maize attained the highest value at 123 mg Olsen P kg^{-1}, and was significantly influenced by soil P supply and neighbour, but there was no interactive effect (Fig. 4; Table S1). Compared with the maize/maize mixture, root length of maize in maize/faba bean was higher (Fig. 4). The difference in root length of target maize between maize/faba bean

Comment [z3]: Would it be more useful to talk about increased root growth in terms of relative biomass allocation? I suspect that while root growth increases with increasing P, it's a relatively smaller fraction of total biomass.

See my comments

and maize/maize mixtures depended on soil P supply, competing plant species and the interaction (Fig. 4; Table S1), with the effect of faba bean on increasing maize root length (relative to competing maize plants) being largest at intermediate P supply (36-51 mg Olsen P kg^{-1}) (Fig. 4).

For the neighbouring species (faba bean or maize), root length was significantly lower in faba bean than maize, except at 3.5 mg Olsen P kg^{-1} (Fig. 5a). There was no effect of P supply level on root length of neighbouring faba bean, but root length of neighbouring maize significantly increased with increasing P supply (Fig. 5a).

Citrate concentration and the acid phosphatase activity were significantly greater in the rhizosphere of faba bean than maize (Fig. 5b, 5c). Increased P supply depressed citrate exudation by faba bean. In contrast, the activity of acid phosphatase in the faba bean rhizosphere was increased from the lowest P treatment (3.5 mg Olsen P kg^{-1}) to 123 mg Olsen P kg^{-1}, and then decreased with a further increase in P supply. There were no evident effects of P treatments on citrate exudation and secretion of acid phosphatase in maize, which were at relatively low values compared with faba bean (Fig. 5b, 5c).

The effect of spatial variability in P supply on root interactions between maize and faba bean (exp. 2)

Shoot biomass of target maize in the maize/maize and maize/faba bean mixtures was significantly stimulated by heterogeneous P supply, although the total amount of P applied was the same ~~between~~ for the homogeneous and heterogeneous treatments. A similar trend was found in the single maize treatment, but the differences were not significant (Fig. 6a). When soil P was supplied heterogeneously, the biomass of target maize was 100% higher when grown with faba bean than with maize.

Soil P heterogeneity and neighbouring species both influenced shoot P content in target maize (Fig. 6b; Table S2). Compared to homogeneous P supply, the P content in shoots of target maize in the heterogeneous environment increased by 139%, 144% and 75% in single maize, maize/maize and maize/faba bean, respectively (Fig. 6b). In the maize/maize mixture, shoot P content of target maize was influenced by P heterogeneity only (Fig. 6b; Table S2). However, in mixture with faba bean, it was significantly affected by both P heterogeneity and neighbouring species (faba bean), but there was no interactive effect (Fig. 6b; Table S2). When the two mixtures were compared, shoot P content in target maize was higher in the maize/faba bean than maize/maize mixtures, which was influenced by soil P supply pattern and neighbouring species, but the interaction was not significant (Fig. 6b; Table S2). Thus, shoot P content of maize was significantly increased by heterogeneous P supply, as well as the presence of faba bean, compared with the maize/maize system.

Root length density (RLD) of target maize was greater in the P-rich soil volume in the heterogeneous than that in the same place in homogeneous treatments (~~3.9, 2.9 and 2.4 times greater in the single maize, maize/maize and maize/faba bean systems, respectively,~~ Fig. 7a). In heterogeneous environment, RLD of target maize did not differ among the single maize, maize/maize and maize/faba bean treatments, whereas RLD of maize grown in homogeneous environment was higher in the maize/faba bean than single maize treatment. Root length density (RLD) of target maize in the P-rich soil volume was significantly influenced by heterogeneous P supply and not by neighbour competition in either maize/maize or maize/faba bean systems (Fig. 7a; Table S2).

Comment [z4]: Does not make sense-please revise

Outside the P-rich soil volume, RLD of target maize was increased significantly with heterogeneous compared with homogeneous P supply in the maize/maize treatment, but the effect was not significant in the single maize and maize/faba bean treatments (Fig. 7b). The increased RLD outside the P-rich soil volume of maize in maize/maize was caused by heterogeneity, but there was no effect of neighbour presence (Fig. 7b; Table S2). The heterogeneous P supply increased maize RLD outside the P-rich soil volume in maize/faba bean in comparison with single maize, but there was no significant difference between the maize/faba bean and maize/maize mixtures (Fig. 7b). In the maize/faba bean system, maize RLD outside the P-rich soil volume was enhanced by neighbour presence, but not by heterogeneity (Fig. 7b; Table S2). Compared with the maize/maize and maize/faba bean treatments, RLD of target maize outside the P-rich soil volume was influenced by soil P heterogeneity and neighbouring species (Fig. 7b; Table S2).

Discussion

In the present study, the maize/faba bean treatment was compared to maize alone or maize/maize treatment. Any neighbours with maize would potentially represent competition, but our findings indicated neighbouring faba bean was less competitive than neighbouring maize (Fig. 3). With increasing P supply level, relative interaction intensity (RII) of target maize in maize/maize mixtures significantly decreased (Fig. 3), suggesting an increase in competition intensity with soil P supply from 3.5 to 270 mg Olsen-P kg^{-1}. According to Hess & de Kroon (2007), plants can sense and coordinate root growth based on available soil volume and a nutrient supply pattern. In the present study, ~~in the maize/maize mixtures at low P supply,~~ maize shoot growth was P-limited at low P supply (Fig. 2a), which ~~would have lowered the translocation of photosynthetic products to belowground parts for production of new roots~~was also relatively consistent with low root length (Fig. 4), resulting in low competition for the same soil P in the maize/maize mixtures. However, as root growth significantly increased with an increase in soil P supply level, the intensity of maize competition in the maize/maize mixtures for the same available soil volume was strengthened, resulting in a ~~lower~~ more negative RII and thus high competition at higher soil P supply.

Comment [z5]: Specify increased soil P supply.

Comment [z6]: This is speculative—you have actual root length numbers, why not refer to them?

Revised here.

In the maize/faba bean mixture, the competition intensity remained unimodal and was lower in the maize/faba bean than maize/maize system at the same soil P supply (Fig. 3). The effect of soil P availability on shoot P content and root length of target maize was significantly dependent on neighbouring species, and was greater in the maize/faba bean than maize/maize mixtures (Table S1). Target maize shoot P content was significantly ~~influenced~~ decreased by neighbouring maize (P<0.001) but not faba bean (P=0.071) (Table S1), suggesting that the presence of faba bean resulted in lower competition intensity than the presence of maize. Previous studies indicated that plant species win competition for a shared resource by using ~~of~~ the resources available in hotspots more rapidly as a result of greater root growth plasticity (Grime, 1994; Craine *et al.*, 2005), or by depleting the resource to lower concentrations than their competitors (Tilman, 1982). In the present study, the difference between root competition in the maize/faba bean and maize/maize mixtures could be explained in two ways. Firstly, lower root length of faba bean compared to neighbouring maize could provide a greater available soil volume for target maize roots to exploit, indicating the competitive importance of morphological root responses to the abiotic environment (similarly, plasticity in root morphology of *Pseudoroegneria spicata* improved its capacity to withstand competition from the more vigorous, but less plastic, *Agropyron desertorum*; Huber-Sannwald *et al.*, 1996). Secondly, a larger amount of citrate and acid phosphatase in the faba bean rhizosphere soil would have improved mobilization of sparingly-soluble soil P, thus effectively increasing the amount of accessible P for target maize (see: Hinsinger, 2001; Jones *et al.*, 2003). Hence, nutrient mobilization by root exudation could lead to increased acquisition of limiting resources by decreasing intensity of interspecies competition through niche complementarity (i.e. maize and faba bean accessing different P fractions: it was found that faba bean can access to sparingly soluble P (or unavailable for maize) through root exudation, but maize can mainly use soluble or available soil P, indicating a niche complementarity) (Hinsinger *et al.*, 2011; Shen *et al.*, 2011, 2013; Brooker *et al.*, 2014; Li *et al.*, 2014b). This result supports hypothesis 1 that neighbouring faba bean provides low competition intensity to target maize by its relatively low root morphological plasticity, which was compensated for by high physiological plasticity (strong exudation) to alter soil P availability in the maize/faba bean system.

Comment [z7]: Aren't the roots accessing the same P fractions, once the faba exudates have made the P available?

See my comments

In response to heterogeneous P supply in the present study, maize showed significant root proliferation in the nutrient-rich soil volume (Fig. 7a), which conferred competitive advantage to maize in heterogeneous compared to homogeneous nutrient supply (see also Robinson *et al.*, 1999); hence, shoot P content in maize was greater in the heterogeneous than homogeneous P treatments in the maize/faba bean mixture (Fig. 6b). The reasons why faba bean contributed to increased shoot P content in maize in heterogeneous P supply could be explained as follows. Firstly, heterogeneous P supply increased maize root proliferation (but not that of faba bean, data not shown) in P-enriched soil (Fig. 7), as in the earlier study (Li *et al*., 2014a) in which heterogeneous nutrient supply did not influence faba bean root growth in the single faba bean treatments. Increased maize root proliferation in the localized fertilizer zone could cause higher competition intensity in maize/maize compared with maize/faba bean. Secondly, faba bean roots were located mostly outside P-enriched zones (i.e. in soil not fertilized with P), and shoot biomass and P content in faba bean were similar in heterogeneous and homogeneous P supply treatments (data not shown), which is consistent with the previous findings that shoot growth and nutrient uptake of single faba bean was not influenced by heterogeneous P availability (Li *et al*., 2014a).

The high physiological plasticity of faba bean in terms of exudation of carboxylates and acid phosphatase could mobilize soil P and increase P availability to provide adequate P supply for its own growth and could even contribute to satisfying demand of neighbouring plants (see experiment 1). A shallow root system of faba bean (Li *et al*., 2006) provided a niche for maize roots to increase root length density (Fig. 7b) and P uptake (Fig. 6b) compared with maize in the maize/maize treatment. Hence, increased P uptake by maize may be attributed to root interactions between maize and faba bean being facilitated in the heterogeneous P supply treatment. The results indicated that ~~spatial heterogeneity in P availability~~the increased soil P availability induced by physiological root plasticity in faba bean stimulated morphological root plasticity in maize, resulting in improved maize growth and P uptake. In contrast, in the maize/maize mixture, high morphological root plasticity caused strong competition, further intensified in the heterogeneous P treatment by greater root proliferation in the P-rich patches, and resulting in lower shoot growth and nutrient uptake compared with maize grown with faba bean. The results provide support for hypothesis 2 that maize would benefit from the root physiological traits of neighbouring faba bean as well as from heterogeneous P supply.

Even though the present study showed that root interactions could be significantly influenced by heterogeneous P supply and root traits of neighbouring species in the maize/faba bean and maize/maize mixtures, we do not know yet how common such interactions might be in other agroecological systems. In maize/faba bean intercropping, a more asymmetric root distribution between maize and faba bean would occur in heterogeneous than homogeneous nutrient supply (cf. Schenk, 2006), probably resulting in an increasing potential for spatial niche complementarity as mentioned above. Interspecies facilitation based on nutrient-mobilizing mechanisms (Shen *et al.*, 2011, 2013; Li *et al.*, 2014b) may be used to help explore potential P resources in soils through selecting neighbouring species or optimizing plant interactions based on root proliferation and the rhizosphere processes to alter competition. Alternatively, a plant species can achieve competitive superiority by depleting nutrient resources to lower concentrations than its competitors (Tilman, 1982). In the present study, maize had competitive superiority for nutrient acquisition not only by stimulating root growth in the presence of faba bean, but also by exploiting additional nutrient resources made available in the rhizosphere soil of faba bean. Nutrient ~~spatial heterogeneity~~ availability ~~induced~~ increased by neighbouring faba bean plants could modify maize root growth and thus change root interactions in the maize/faba bean mixture. This study provided novel support for the nutrient-driven root interactions regulating growth dynamics of plant species in the mixed stand. Furthermore, heterogeneous nutrient supply could be considered a useful strategy for modifying root/rhizosphere interactions to optimize plant combinations and underpin improved crop productivity and nutrient uptake in the agroecological systems.

Conclusions

Both nutrient supply and neighbouring species significantly modified root interactions between maize and faba bean, influencing P uptake and biomass production. At different soil P ~~supplies~~supply, faba bean exhibited relatively poor morphological, but strong physiological root plasticity. This contributed to relatively low competition intensity in the maize/faba bean compared with maize/maize mixture. Localized P supply enhanced root proliferation of target maize (but not faba bean) in the maize/faba bean mixture. Enhanced root exudation of citrate and acid phosphatase by faba bean could facilitate soil P availability to benefit maize growth in the maize/faba bean mixture. The study provided ~~the~~ new ~~in~~sights into root/rhizosphere interactions in the maize/faba bean intercropping in the systems with variable P supply, which is important in developing strategies for rhizosphere management through optimizing plant combinations and soil nutrient supply to increase crop productivity and nutrient-use efficiency. The challenge of enhancing food supply without using extra nutrients could be ~~appropriate~~ accomplished by maximizing the efficacy of intercropping as a means of delivering more crop production per ~~drop~~ unit of fertilizer rate.

Comment [z8]: too conversational.

Acknowledgements

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Figure legends

Fig. 1 A schematic diagram of rhizo-box.

Fig. 2 Experiment 1: effects of neighbour on shoot biomass (a) and shoot P content (b) of target maize grown at five levels of P supply. The error bars indicate ± standard errors. Within a P supply, different letters denote significant differences among target maize data in the single maize, maize/maize and maize/faba bean treatments ($P \leq 0.05$).

Fig. 3 Experiment 1: relative interaction intensity (RII) of target maize with its neighbours in the maize/maize and maize/faba bean mixtures. The error bars indicate ± standard errors. Within a P supply, stars denote significant differences between the maize/maize and maize/faba bean mixtures, $^{**}P<0.01$, ns = not significant.

Fig. 4 Experiment 1: effects of neighbour on root length of target maize grown at five levels of P supply. The error bars indicate ± standard errors. Within a P supply, stars denote significant differences between the maize/maize and maize/faba bean mixtures, $^{*}P \leq 0.05$, $^{**}P<0.01$, ns = not significant.

Fig. 5 Experiment 1: root length (a), and citrate concentration (b) and acid phosphatase activity (c) in the rhizosphere of neighbours (maize and faba bean). The error bars indicate ± standard errors. Within a P supply, stars denote significant differences between neighbouring species (maize *vs* faba bean), $^{*}P \leq 0.05$, $^{**}P<0.01$, ns = not significant.

Fig. 6 Experiment 2: effects of homogeneous vs heterogeneous P supply on shoot biomass (a) and shoot P content (b) of target maize. Hom: homogeneous P supply; Het: heterogeneous P supply. The error bars indicate standard errors. Different lower case letters denote significant differences among target maize ($P \leq 0.05$) data in the single maize, maize/maize and maize/faba bean treatments in the homogeneous treatments, and capitals denote significant differences in the heterogeneous treatments. For each parameter and species treatment, t-tests were run separately to detect difference between homogeneous and heterogeneous treatments; stars indicate significant differences, * $P \le 0.05$.

Fig. 7 Experiment 2: effects of homogeneous/heterogeneous P supply on target maize root length density within (a) and outside P-enriched soil volume (b). Hom: homogeneous P supply; Het: heterogeneous P supply. The error bars indicate standard errors. Different lower case letters denote significant differences among target maize ($P \le 0.05$) data in single maize, maize/maize and maize/faba bean mixtures in the homogeneous treatments, and capitals denote significant differences in the heterogeneous treatments. For each soil volume and each species treatment, t-tests were run separately to detect significant difference between homogeneous and heterogeneous treatments; stars indicate significant differences, * $P \le 0.05$.