

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

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13 **With 4925-4969 words, 7 figures** (2 tables in the supporting material)

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26 **Summary**

27 ● Root growth is influenced by soil nutrients and neighbouring plants, but how
28 these two drivers affect root interactions and regulate plant growth dynamics is
29 poorly understood. Here, interactions between the roots of maize (*Zea mays*) and
30 faba bean (*Vicia faba*) are characterized.

31 ● Maize was grown alone (maize) or with maize (maize/maize) or faba bean
32 (maize/faba bean) as competitors under five levels ~~–variable rates~~ of phosphorus
33 (P) supply, and with homogeneous or heterogeneous P distribution.

34 ● Maize had longer root length and greater shoot biomass and P content when
35 grown with faba bean than with maize. At each P supply rate, faba bean had a
36 smaller root system than maize but greater exudation of citrate and acid
37 phosphatase, suggesting greater capacity to mobilize P in the rhizosphere.
38 Heterogeneous P availability enhanced root-length density of maize but not faba
39 bean. Maize root proliferation in the P-rich patches was associated with increased
40 shoot P uptake.

41 ● Increased P availability by localized P application or by the presence of faba bean
42 exudation stimulated root morphological plasticity and increased shoot growth in
43 maize in the maize/faba bean mixture, suggesting root interactions of
44 neighbouring plants can be modified by increased P availability.

45

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

48 **Introduction**

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

64 Root morphological and physiological plasticity is determined by various
65 environmental factors, such as availability of soil resources and their distribution
66 (Jackson *et al.*, 1990; Hutchings & de Kroon, 1994; Hodge, 2004; Cahill *et al.*, 2010;
67 Andersen *et al.*, 2014). Soil nutrient availability can profoundly affect root
68 morphology and physiology. For example, cluster-root formation and citrate
69 exudation in *Lupinus albus* can be induced by low phosphorus (P) conditions, and
70 inhibited by increased P supply (Shen *et al.*, 2005; Lambers *et al.*, 2006; Li *et al.*,
71 2008). Faba bean roots have no significant growth response to localized nutrient
72 supply (Li *et al.*, 2014a), suggesting relatively poor morphological plasticity to
73 variable nutrient availability, and potentially a low capacity to take full advantage of
74 nutrient-rich patches. However, in many species, root growth and distribution are
75 affected by soil nutrient heterogeneity (Drew, 1975; Li *et al.*, 2008; Jing *et al.*, 2010).
76 In response to nutrient-rich patches, plants tend to stimulate root growth and alter root

77 distribution, with increased root proliferation in the local nutrient-rich zone (Drew,
78 1975; Hodge *et al.*, 1999; Hodge, 2004). In addition, some species show root
79 physiological responses to localized nutrient enrichment (eg. an increase in the
80 nutrient uptake capacity per unit root length) (Jackson *et al.*, 1990). Hence, foraging
81 for nutrients is determined by root morphological and physiological dynamics in
82 response to soil environmental conditions, and may result in differences in
83 competitive ability, which, to a large extent, affects plant productivity and nutrient
84 uptake in a mixed system.

85 Growing mixtures of maize and faba bean is used widely to improve grain yield
86 and P-use efficiency in cropping ecosystems in many parts of the world (intercropping)
87 (Li *et al.*, 2007; Zhang *et al.*, 2010; Shen *et al.*, 2011, 2013). Root interactions have a
88 profound impact on P uptake and yield in the maize/faba bean system (Li *et al.*, 2003,
89 2014b), with rhizosphere acidification caused by faba bean increasing mobilization of
90 organic/inorganic P sources and facilitating P uptake by target maize (Li *et al.*, 2007).
91 The roots of maize and faba bean intermingle and appear to grow together; hence,
92 complementarity of the spatial root distribution of intercropped species contributes to
93 interspecies facilitation in maize/faba bean intercropping compared to the
94 maize/maize monocropping system (Li *et al.*, 2006). However, the detailed
95 mechanisms underlying root interactions in the maize/faba bean system are still
96 unclear.

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

104 To investigate the effects of heterogeneous nutrient availability on root growth,
105 distribution and thus competition intensity in the maize/faba bean mixture system, we
106 conducted experiments with varying P supplies and homogeneous or heterogeneous P

107 distribution to test the hypothesis that neighbouring faba bean would not compete
108 strongly with target maize because faba bean roots have low morphological plasticity
109 that is compensated for by significant physiological plasticity in variable soil P supply.
110 We then demonstrate that increased maize growth in the maize/faba bean mixtures is
111 consistent with the hypothesis that spatial heterogeneity in P availability induced by
112 faba bean exudation ~~would~~ stimulates root morphological plasticity in maize,
113 resulting in improved maize shoot growth and nutrient uptake.

114 **Materials and Methods**

115 *Experimental set-up*

116 *Experiment 1*

117 To investigate how maize roots respond to faba bean in soil with variable P supply,
118 and test how soil P availability regulates root interactions between maize and faba
119 bean, a pot experiment was conducted in a glasshouse with five soil P supply levels
120 and three cropping treatments. Maize was grown alone as a single species (single
121 maize treatment), mixed with other maize plants (maize/maize treatment) or
122 intercropped with faba bean (maize/faba bean treatment) in soil supplied with 0, 50,
123 100, 200 or 500 mg P kg⁻¹ soil, corresponding to soil Olsen-P values of 3.5, 36, 51,
124 123 and 270 mg kg⁻¹, respectively. We choose the soil P supply levels based on a
125 preliminary experiment with the same soil: (1) deficient: 0 and 50 mg P kg⁻¹ soil; (2)
126 moderate: 100 mg P kg⁻¹ soil; (3) adequate: 200 mg P kg⁻¹ soil, and (4) high: 500 mg P
127 kg⁻¹ soil (to mimic high-input maize cropping system in intensive agriculture of
128 China). There were 15 treatment combinations in the study, with four replicates per
129 treatment.

130 The soil was collected from Shangzhuang experimental station in Beijing, China,
131 air-dried and passed through a 2-mm sieve. Soil properties were as follows: Olsen-P
132 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 (NO₃⁻
133 and NH₄⁺), available K 32.3 mg kg⁻¹, and pH 8.2 (the ratio of soil to CaCl₂ solution
134 was 1:2.5). The pot was filled with 1.5 kg of air-dried soil. To ensure the nutrient

135 | supply was adequate for plant growth, soil was also fertilized with basal nutrients at
136 the following rates (mg pot⁻¹): Ca(NO₃)₂·4H₂O, 1687; K₂SO₄, 200; MgSO₄·7H₂O, 65;
137 Fe-EDTA, 8.78; MnSO₄·H₂O, 10; ZnSO₄·7H₂O, 15; CuSO₄·5H₂O, 3; H₃BO₃, 1; and
138 Na₂MoO₄·5H₂O, 0.25. Phosphorus was applied as Ca(H₂PO₄)₂·H₂O.

139 The genotype of maize (*Zea mays* L.) was cv. ZD958, and the genotype of faba
140 bean (*Vicia faba* L.) was cv. Lincan5. Maize and faba bean seeds were
141 surface-sterilized in 30% v/v H₂O₂ for 20 min, washed with deionized water, soaked
142 in CaSO₄ saturated solution for 12 hours and then germinated in Petri dishes covered
143 with wet filter papers for 1-2 days at 22°C. All the pots were arranged in a completely
144 randomized design, and were re-randomized weekly during the experiment. The
145 plants were watered every day to maintain field capacity (18%, w/w).

146 Plants were harvested 38 days after sowing (DAS) and separated into shoots and
147 roots. Following root excavation, the soil adhering to roots was defined as rhizosphere
148 soil and was sub-sampled for carboxylate and acid phosphatase measurements. Roots
149 were transferred to a tube containing 50 mL of 0.2 μM CaCl₂ and gently shaken to
150 dislodge the rhizosphere soil, followed by shaking for 5-10s to create homogeneous
151 suspension. A suspension volume of 10 mL was taken by pipette to a 10-mL
152 centrifuge tube for carboxylate analysis by HPLC (see below), and a 0.5 μL aliquot of
153 suspension was placed in a 2-mL centrifuge tube for acid phosphatase measurement
154 (see below).

155 *Experiment 2*

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

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

165 Phosphorus was supplied as $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ in a homogeneous or heterogeneous
166 pattern. For the heterogeneous P treatment, a 3.5-cm P-rich layer (227.5 g soil)
167 containing 46.2 mg P (200 mg P kg^{-1} soil) was manually mixed and placed at the
168 center of the rhizo-box (Fig. 1), referred to as the P-rich patch, and the remaining soil
169 without P additions was the background soil (1072.5 g soil). For the homogeneous P
170 treatment, the same total P (46.2 mg P) was spread evenly throughout the soil (35 mg
171 P kg^{-1} soil).

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

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

183 Both experiments were conducted in a glasshouse at China Agricultural University,
184 Beijing (latitude: $40^\circ 01' \text{ N}$, longitude: $116^\circ 16' \text{ E}$). In experiment 1, temperature in the
185 glasshouse was maintained at $21\text{--}25^\circ \text{ C}$ during the day and $15\text{--}18^\circ \text{ C}$ at night, with
186 12-14 h daytime throughout the growth period. In experiment 2, temperature was
187 maintained at $24\text{--}28^\circ \text{ C}$ during the day and $18\text{--}20^\circ \text{ C}$ at night, with 14-19 h daytime.

188 *Measurements*

189 *Plant biomass and phosphorus uptake*

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

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, $3.5 \text{ cm} \times 1.5 \text{ cm} \times 35 \text{ cm}$), 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.

193 8 mL of 30% v/v H₂O₂. Shoot P was analyzed by the molybdovanadophosphate
194 method at 440 nm by spectrophotometry (Varian Vista-Pro CCD) (Johnson & Ulrich,
195 1959).

196 *Root parameter measurement*

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

201 *Carboxylate and acid phosphatase exudation*

202 Carboxylates in the rhizosphere soil were analyzed using a reversed phase
203 high-performance liquid chromatography (HPLC) system according to a previous
204 report (modified from Shen *et al.*, 2003 and Wang *et al.*, 2010). The chromatographic
205 separation was conducted on a 250 × 4.6 mm reversed-phase column (Alltima C18, 5
206 Micrometers; Alltech Associates, Inc., Deerfield, IL, USA). The mobile phase was 25
207 mmol L⁻¹ KH₂PO₄ (pH 2.25) with a flow rate of 1 mL min⁻¹ at 31°C. Detection of
208 carboxylates was carried out at 214 nm.

209 To determine the activity of acid phosphatase in the rhizosphere soil, 0.5 mL
210 aliquots of soil suspensions were transferred into a 2-mL Eppendorf vial with 0.4 mL
211 sodium acetate buffer and 0.1 mL *p*-nitrophenyl phosphate (NPP) substrate added.
212 Vials were incubated at 30°C for 60 min, and the reaction was terminated by adding
213 0.5 mL of 0.5 M NaOH. Absorption was measured at 405 nm (Alvey *et al.*, 2000).

214 *Statistical analyses*

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

$$RII = \frac{\sum \left(\frac{x_i - x_{i'}}{X} \right)}{n}$$

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

230 The i and i' are two randomly selected individuals (in the present study, $i = 1, \dots, 4$,
 231 because each treatment had 4 replicates) of the same species belonging to two
 232 different cropping treatments. The n is the number of $(x_i - x_{i'})$ values. In the
 233 present study, n is equal to 16 because 4 replicates (4 random individuals) were set in
 234 each pair of cropping treatments (single maize and maize/maize, or single maize and
 235 maize/faba bean).

236 Analysis of variance (ANOVA) was conducted using the SPSS statistical software
 237 (SPSS19.0). Significant differences among means were separated by LSD at the $P \leq$
 238 0.05 probability level. Plant growth and root length were subjected to two-way
 239 ANOVA to assess the effects of plant species, soil P supply, and their interaction in
 240 experiment 1 as well as the effects of heterogeneous/homogeneous P supply and
 241 competition in experiment 2.

242 **Results**

243 *Root interactions between maize and faba bean grown with variable soil P supply* 244 *(exp 1)*

245 Shoot biomass and P content in target maize increased with soil P supply (Fig. 2).
 246 Compared with the single maize treatment at five levels of P supply, shoot biomass of
 247 target maize in the mixtures was not affected by the presence of faba bean, but was
 248 significantly reduced when maize was grown with maize (Fig. 2a). Shoot P content of

249 target maize showed a similar response to shoot biomass when grown either as single
250 or with neighbours (Fig. 2b). In the maize/maize mixture, a decrease in maize shoot P
251 content was affected by soil P availability and the presence of neighbouring maize,
252 however, there was no significant interaction (Fig. 2b; Table S1). In the maize/faba
253 bean mixture, shoot P content in target maize increased with soil P supply, but not
254 with the neighbour presence; the interactive effect was not significant (Fig. 2b; Table
255 S1). Phosphorus content in shoots of target maize was higher in the maize/faba bean
256 than maize/maize mixtures at 36 and 270 mg kg⁻¹ (soil Olsen-P levels). When
257 comparing shoot P content in target maize between the maize/maize and maize/faba
258 bean treatments, we found ~~the a main effects~~ of P supply and neighbour, as well as ~~the~~
259 ~~their~~ interaction (Fig. 2b; Table S1) ~~had a significant influence~~. The interaction was
260 significant because the effect of faba bean on increasing maize shoot P content
261 (compared to competing maize plants) became more pronounced as soil P supply
262 increased (Fig. 3).

263 Relative interaction intensity (RII) of maize grown with maize decreased with soil
264 P supply, whereas in the maize/faba bean mixture, RII of target maize increased from
265 3.5 to 36 mg Olsen P kg⁻¹ and remained unchanged with further increases in soil P
266 (Fig. 3). RII of maize in maize/maize was significantly lower than that of maize
267 grown with faba bean, except in the lowest P treatment (3.5 mg Olsen P kg⁻¹) (Fig. 3).
268 Compared with the maize/maize mixture, RII in maize/faba bean was significantly
269 greater in treatments with Olsen P at 36, 51, 123 and 270 mg kg⁻¹.

270 Root length of target maize in the maize/faba bean or maize/maize mixture
271 significantly increased with P supply (Fig. 4; Table S1). In the maize/faba bean
272 mixture, root length reached the maximum at 51 mg Olsen P kg⁻¹ and remained
273 similar at higher P supplies (Fig. 4), with non-significant effects of neighbour and
274 interaction (Fig. 4; Table S1). Root length of maize when competing with maize
275 attained the highest value at 123 mg Olsen P kg⁻¹, and was significantly influenced by
276 soil P supply and neighbour, but there was no interactive effect (Fig. 4; Table S1).
277 Compared with the maize/maize mixture, root length of maize in maize/faba bean was
278 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

279 and maize/maize mixtures depended on soil P supply, competing plant species and the
280 interaction (Fig. 4; Table S1), with the effect of faba bean on increasing maize root
281 length (relative to competing maize plants) being largest at intermediate P supply
282 (36-51 mg Olsen P kg⁻¹) (Fig. 4).

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

287 Citrate concentration and the acid phosphatase activity were significantly greater in
288 the rhizosphere of faba bean than maize (Fig. 5b, 5c). Increased P supply depressed
289 citrate exudation by faba bean. In contrast, the activity of acid phosphatase in the faba
290 bean rhizosphere was increased from the lowest P treatment (3.5 mg Olsen P kg⁻¹) to
291 123 mg Olsen P kg⁻¹, and then decreased with a further increase in P supply. There
292 were no evident effects of P treatments on citrate exudation and secretion of acid
293 phosphatase in maize, which were at relatively low values compared with faba bean
294 (Fig. 5b, 5c).

295 *The effect of spatial variability in P supply on root interactions between maize and* 296 *faba bean (exp. 2)*

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

303 Soil P heterogeneity and neighbouring species both influenced shoot P content in
304 target maize (Fig. 6b; Table S2). Compared to homogeneous P supply, the P content in
305 shoots of target maize in ~~the~~ heterogeneous environment increased by 139%, 144%
306 and 75% in single maize, maize/maize and maize/faba bean, respectively (Fig. 6b). In
307 the maize/maize mixture, shoot P content of target maize was influenced by P
308 heterogeneity only (Fig. 6b; Table S2). However, in mixture with faba bean, it was

309 significantly affected by both P heterogeneity and neighbouring species (faba bean),
310 but there was no interactive effect (Fig. 6b; Table S2). When the two mixtures were
311 compared, shoot P content in target maize was higher in the maize/faba bean than
312 maize/maize mixtures, which was influenced by soil P supply pattern and
313 neighbouring species, but the interaction was not significant (Fig. 6b; Table S2). Thus,
314 shoot P content of maize was significantly increased by heterogeneous P supply, as
315 well as the presence of faba bean, compared with the maize/maize system.

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

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

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

339 **Discussion**

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

357 In the maize/faba bean mixture, the competition intensity remained unimodal and
358 was lower in the maize/faba bean than maize/maize system at the same soil P supply
359 (Fig. 3). The effect of soil P availability on shoot P content and root length of target
360 maize was significantly dependent on neighbouring species, and was greater in the
361 maize/faba bean than maize/maize mixtures (Table S1). Target maize shoot P content
362 was significantly ~~influeneed-decreased~~ by neighbouring maize ($P < 0.001$) but not faba
363 bean ($P = 0.071$) (Table S1), suggesting that the presence of faba bean resulted in lower
364 competition intensity than the presence of maize. Previous studies indicated that plant
365 species win competition for a shared resource by using ~~of~~ the resources available in
366 hotspots more rapidly as a result of greater root growth plasticity (Grime, 1994;
367 Craine *et al.*, 2005), or by depleting the resource to lower concentrations than their

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.

368 competitors (Tilman, 1982). In the present study, the difference between root
369 competition in the maize/faba bean and maize/maize mixtures could be explained in
370 two ways. Firstly, lower root length of faba bean compared to neighbouring maize
371 could provide a greater available soil volume for target maize roots to exploit,
372 indicating the competitive importance of morphological root responses to the abiotic
373 environment (similarly, plasticity in root morphology of *Pseudoroegneria spicata*
374 improved its capacity to withstand competition from the more vigorous, but less
375 plastic, *Agropyron desertorum*; Huber-Sannwald *et al.*, 1996). Secondly, a larger
376 amount of citrate and acid phosphatase in the faba bean rhizosphere soil would have
377 improved mobilization of sparingly-soluble soil P, thus effectively increasing the
378 amount of accessible P for target maize (see: Hinsinger, 2001; Jones *et al.*, 2003).
379 Hence, nutrient mobilization by root exudation could lead to increased acquisition of
380 limiting resources by decreasing intensity of interspecies competition through niche
381 complementarity (i.e. maize and faba bean accessing different P fractions; it was
382 found that faba bean can access to sparingly soluble P (or unavailable for maize)
383 through root exudation, but maize can mainly use soluble or available soil P,
384 indicating a niche complementarity) (Hinsinger *et al.*, 2011; Shen *et al.*, 2011, 2013;
385 Brooker *et al.*, 2014; Li *et al.*, 2014b). This result supports hypothesis 1 that
386 neighbouring faba bean provides low competition intensity to target maize by its
387 relatively low root morphological plasticity, which was compensated for by high
388 physiological plasticity (strong exudation) to alter soil P availability in the maize/faba
389 bean system.

390 In response to heterogeneous P supply in the present study, maize showed
391 significant root proliferation in the nutrient-rich soil volume (Fig. 7a), which
392 conferred competitive advantage to maize in heterogeneous compared to
393 homogeneous nutrient supply (see also Robinson *et al.*, 1999); hence, shoot P content
394 in maize was greater in the heterogeneous than homogeneous P treatments in the
395 maize/faba bean mixture (Fig. 6b). The reasons why faba bean contributed to
396 increased shoot P content in maize in heterogeneous P supply could be explained as
397 follows. Firstly, heterogeneous P supply increased maize root proliferation (but not

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

See my comments

398 that of faba bean, data not shown) in P-enriched soil (Fig. 7), as in the earlier study
399 (Li *et al.*, 2014a) in which heterogeneous nutrient supply did not influence faba bean
400 root growth in the single faba bean treatments. Increased maize root proliferation in
401 the localized fertilizer zone could cause higher competition intensity in maize/maize
402 compared with maize/faba bean. Secondly, faba bean roots were located mostly
403 outside P-enriched zones (i.e. in soil not fertilized with P), and shoot biomass and P
404 content in faba bean were similar in heterogeneous and homogeneous P supply
405 treatments (data not shown), which is consistent with the previous findings that shoot
406 growth and nutrient uptake of single faba bean was not influenced by heterogeneous P
407 availability (Li *et al.*, 2014a).

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

425 Even though the present study showed that root interactions could be significantly
426 influenced by heterogeneous P supply and root traits of neighbouring species in the
427 maize/faba bean and maize/maize mixtures, we do not know yet how common such

428 interactions might be in other agroecological systems. In maize/faba bean
429 intercropping, a more asymmetric root distribution between maize and faba bean
430 would occur in heterogeneous than homogeneous nutrient supply (cf. Schenk, 2006),
431 probably resulting in an increasing potential for spatial niche complementarity as
432 mentioned above. Interspecies facilitation based on nutrient-mobilizing mechanisms
433 (Shen *et al.*, 2011, 2013; Li *et al.*, 2014b) may be used to help explore potential P
434 resources in soils through selecting neighbouring species or optimizing plant
435 interactions based on root proliferation and the rhizosphere processes to alter
436 competition. Alternatively, a plant species can achieve competitive superiority by
437 depleting nutrient resources to lower concentrations than its competitors (Tilman,
438 1982). In the present study, maize had competitive superiority for nutrient acquisition
439 not only by stimulating root growth in the presence of faba bean, but also by
440 exploiting additional nutrient resources made available in the rhizosphere soil of faba
441 | bean. Nutrient ~~spatial heterogeneity availability induced~~ increased by neighbouring
442 faba bean plants could modify maize root growth and thus change root interactions in
443 the maize/faba bean mixture. This study provided novel support for the
444 nutrient-driven root interactions regulating growth dynamics of plant species in the
445 mixed stand. Furthermore, heterogeneous nutrient supply could be considered a useful
446 strategy for modifying root/rhizosphere interactions to optimize plant combinations
447 and underpin improved crop productivity and nutrient uptake in the agroecological
448 systems.

449 **Conclusions**

450 Both nutrient supply and neighbouring species significantly modified root interactions
451 between maize and faba bean, influencing P uptake and biomass production. At
452 | different soil P ~~supplies~~ supply, faba bean exhibited relatively poor morphological, but
453 strong physiological root plasticity. This contributed to relatively low competition
454 intensity in the maize/faba bean compared with maize/maize mixture. Localized P
455 supply enhanced root proliferation of target maize (but not faba bean) in the

456 maize/faba bean mixture. Enhanced root exudation of citrate and acid phosphatase by
457 faba bean could facilitate soil P availability to benefit maize growth in the maize/faba
458 | bean mixture. The study provided ~~the new insights~~ into root/rhizosphere interactions
459 | in the maize/faba bean intercropping in the systems with variable P supply, which is
460 | important in developing strategies for rhizosphere management through optimizing
461 | plant combinations and soil nutrient supply to increase crop productivity and
462 | nutrient-use efficiency. The challenge of enhancing food supply without using extra
463 | nutrients could be ~~appropriate~~accomplished by maximizing the efficacy of
464 | intercropping as a means of delivering more crop production per ~~drop-unit~~
465 | rate.

Comment [z8]: too conversational.

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603 **Figure legends**

604 **Fig. 1** A schematic diagram of rhizo-box.

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

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

613 **Fig. 4** Experiment 1: effects of neighbour on root length of target maize grown at five
614 levels of P supply. The error bars indicate \pm standard errors. Within a P supply, stars
615 denote significant differences between the maize/maize and maize/faba bean mixtures,
616 $*P \leq 0.05$, $**P < 0.01$, ns = not significant.

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

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

629 between homogeneous and heterogeneous treatments; stars indicate significant
630 differences, * $P \leq 0.05$.

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