Root responses to neighbouring plants in common bean are mediated by nutrient concentration rather than self/non-self recognition

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Abstract. Plants are reported to over-proliferate roots in response to belowground competition, thereby reducing reproductive biomass. This has been cited as an instance of the 'tragedy of the commons'. Many of the studies that report this response suggest that plants can sense neighbours and discriminate between 'self' and 'non-self' roots. To test the alternate hypothesis that root responses to a neighbouring plant are mediated by resource depletion, common bean plants were supplied with the same phosphorus (P) fertiliser dose in varying rooting volumes, or with neighbouring plants separated by plastic film, nylon mesh, or no barrier to vary access to a neighbour. Phosphorus concentration, but not the presence of a neighbour or rooting volume, strongly influenced biomass allocation to roots. Root architecture was significantly altered by both neighbours and P availability. When exposed to the roots of a neighbour, plants altered the vertical and horizontal distribution of roots, placing fewer roots in soil domains occupied by roots of a neighbour. These results support the hypothesis that root responses to neighbouring plants are mediated by resource depletion by the neighbour rather than sensing of 'non-self' roots and show that the presence of a neighbour may affect root architecture without affecting biomass allocation to roots.

Additional keywords: nutrient uptake, P, root, root architecture, self/non-self discrimination.

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Introduction

Competition – both above- and belowground – is a major factor shaping plant communities and is thought to be partly responsible for the diversity of vegetation in different ecosystems (Goldberg and Barton 1992; Wilson and Tilman 2002; Moore 2003; Passarge *et al*. 2006). The intensity of above- and belowground competition depends on the availability of nutrients and light in the environment (Wilson and Tilman 1993) and above- and belowground responses to competition may be independent (Murphy and Dudley 2007). However, compared with aboveground competition, root competition is less well understood since it is affected by multiple soil resources (Casper and Jackson 1997) as well as by animals (Endlweber and Scheu 2006) and is difficult to observe directly.

Interactions may occur among roots of an individual plant (intraplant or self competition) or between roots of different plants (interplant or non-self competition). Root architecture may have important effects on the intensity of both intra- and interplant competition. Steeper basal root angles in common bean resulted in more intensive intraplant competition (Ge *et al*. 2000; Rubio *et al*. 2001) and more similar root architecture leads to more intensive interplant competition

according to geometric modelling (Rubio *et al*. 2001, 2003). Interplant root competition leads to over-production of roots and consequently to a reduction in reproductive biomass; this root over-proliferation response (ROR) has been cited by others as an instance of 'the tragedy of the commons' (Gersani *et al*. 2001). The over-proliferation of roots in the presence of a neighbour is reported to be evidence of the ability of plants to differentiate between their own roots (self) and the roots of a competitor (non-self) (Gersani *et al*. 2001; Falik *et al*. 2003). Schenk (2006) demonstrated that the self/non-self studies of Gersani *et al*. (2001) could be simply explained by taking soil volume into account. Hess and de Kroon (2007) reanalysed data from several published studies and concluded that plant responses to the available soil volume could explain some of the results that had been attributed to competition. In several experiments that were not confounded by volume there was an increase in root mass that depended solely on the identity of neighbours (de Kroon *et al*. 2003; Holzapfel and Alpert 2003; Gruntman and Novoplansky 2004). However, these non-confounded studies do not show a subsequent decrease in shoot biomass or seed production, so the importance of ROR in belowground competition has mixed experimental support.

Indeed, it is not clear whether competition has occurred in these non-confounded experiments, since the more restrictive definitions of competition require that the 'growth, survival, or fecundity' of neighbours be reduced (Casper and Jackson 1997). Neither is it fully clear by what mechanism the presence of a neighbour affects the growth of individual plants.

Competition for nutrients may play an important role in root interactions, but its effect is often confounded by differences in nutrient availability and effective rooting volume (McConnaughay and Bazzaz 1991; Matthes-Sears and Larson 1999). Plant responses to soil volume could confound the interpretation of the effects of competition (Schenk 2006; Semchenko *et al.* 2007). O'Brien and Brown (2008), in a mathematical model, consider the separate effects of volume per plant (V) and nutrient concentration per unit volume (N) on root proliferation and the outcome of competition and conclude that when N is held constant, increasing V should produce an almost linear increase in root proliferation and net nutrients available for reproduction; When $V \times N$ is held constant, roots per plant should at first increase and then decline with increasing volume. Nutrient concentration and soil volume used in experiments should, therefore, be explicitly considered in order to clarify the outcome of competition. We hypothesised that nutrient availability rather than the presence of a neighbour itself affects biomass allocation.

Phosphorus is nearly immobile in soil (Barber 1995) and its absorption by roots results in localised depletion zones, thereby influencing local P availability at a millimetre scale (Lynch 1995). The P depletion volume is positively correlated with root length, as well as other root traits, such as basal root gravitropism (Ge *et al.* 2000; Lynch and Brown 2001; Rubio *et al.* 2001; Walk *et al.* 2004). Resource availability at this scale should affect belowground resource competition. It is possible that many of the responses to the presence of a neighbour attributed to plants sensing the presence of a neighbour's roots may actually be mediated by root responses to local nutrient availability, especially the immobile nutrients such as P, and plants may not be able to distinguish between self competition and non-self competition. Phosphorus availability rather than competition may determine root biomass allocation.

Most studies addressing the effects of belowground competition focus on root biomass but overlook root architecture. Because P mobility in soil is diffusion-limited and because P is generally concentrated in the epipedon, P acquisition is very sensitive to the location of root foraging. For this reason root architecture may play a more important role in P acquisition than root biomass. Several factors affect P acquisition by plants, including rhizosphere modification, root morphology and root architecture (Hinsinger *et al.* 2005; Lynch and Brown 2006). There is much physiological evidence that root architecture responds to P availability (Bonser *et al.* 1996; Williamson *et al.* 2001; Linkohr *et al.* 2002; Chevalier *et al.* 2003; Miller *et al.* 2003; Jiang *et al.* 2007). Various root architectural traits increase P acquisition by enhancing topsoil foraging (Lynch and Brown 2001) and patch exploitation (Borch *et al.* 1999; Farley and Fitter 1999); these include basal root growth angle (Bonser *et al.* 1996; Liao *et al.* 2001; Ho *et al.* 2005), production of shoot-borne roots (Miller *et al.* 2003; Walk *et al.* 2006), increased axial elongation (Ma *et al.* 2003) and reduced

lateral branching in low P domains (Borch *et al.* 1999). In contrast, competition for P may elicit changes in root architecture, thereby facilitating resource partitioning (Callaway *et al.* 2003). Lynch (2005) points out that the changes in root architecture induced by low P availability can be interpreted as precision foraging. Such precision foraging by roots could lead to root avoidance as roots preferentially avoid regions where soil nutrients have been depleted by other roots. This would tend to reduce resource competition among neighbouring roots. Since root architectural changes do not necessarily imply altered allocation of photosynthate to the root system, altering root architecture should be 'cheaper' than increasing biomass allocation to roots and root architecture may be expected to exhibit greater plasticity in response to the presence of a neighbour than will root biomass. It is hypothesised that root architecture should respond to the presence of a neighbour much earlier than root biomass.

In summary, we propose two hypotheses: (1) soil P concentration rather than the presence of a neighbour will determine biomass allocation to roots; and (2) root architecture will exhibit greater plasticity to the presence of a neighbour than will root biomass. In order to test these hypotheses, we conducted two experiments with common bean (*Phaseolus vulgaris* L.), one to test the importance of nutrient concentration and the presence of a neighbour and another to characterise root architectural responses to the presence of a neighbour.

Materials and methods

Experiment 1: Effects of P dose, P concentration and neighbour

Experimental design

In this experiment, two P dosages (low P = 84 mg pot^{-1} and high P = 164 mg pot^{-1} of P) were added to each of three soil volumes (4, 8 and 12 L), resulting in five concentrations of P (7.3, 11, 14.7, 22 and 44 mg P kg^{-1} soil, see Table 1). There were two neighbour treatments (one plant or two plants per pot); pots with one plant (no neighbour) received both the low P and high P dosages, whereas pots with two plants (with neighbour) received the high P dosage. In this way, each of two plants with high P

Table 1. Phosphorus, volume and neighbour treatments in Experiment 1

We regarded 4 L of soil volume as 1 unit volume and P equivalent to 22 P mg kg^{-1} of media as 1 unit P in this calculation; P dose = volume × P added/22 mg kg^{-1} soil, HP = 0.42 and LP = 0.84 g pot^{-1} of triple super phosphate

Treatment	Description	Volume	P added P (mg kg^{-1} soil)	P dose	P dose per plant
4·LP·1	4 L, LP, one plant	1	22	1	1
4·HP·1	4 L, HP, one plant	1	44	2	2
4·HP·2	4 L, HP, two plants	1	44	2	1
8·LP·1	8 L, LP, one plant	2	11	1	1
8·HP·1	8 L, HP, one plant	2	22	2	2
8·HP·2	8 L, HP, two plants	2	22	2	1
12·LP·1	12 L, LP, one plant	3	7.3	1	1
12·HP·1	12 L, HP, one plant	3	14.7	2	2
12·HP·2	12 L, HP, two plants	3	14.7	2	1

would have the same P available per plant as the single plant in low P (Table 1). Each treatment was replicated four times.

Plant growth

The growth medium consisted of 50% sand, 35% coarse vermiculite and 15% red soil (C horizon from a limestone-derived silt loam; fine, mixed, semiactive, mesic Typic Hapludalf). The red soil was oven dried and finely powdered before mixing with the other media components. The characteristics of this solid media were: pH 6.9 (1 : 1 = soil : water), Olsen-extracted P 4.0 mg kg^{-1} soil, CEC 4.5 mEqn 100 g^{-1}. Three sizes of plastic (polyethylene) sleeves were used to achieve volumes of 4, 8 or 12 L. The height of all plastic sleeves was constant so that soil column height was the same for all pot sizes, but the diameter was varied to achieve the desired volume. Each plastic sleeve was placed inside a 12 L pot and then was filled with medium into which either one or two doses of P as finely ground triple super phosphate (0–46–0) had been thoroughly mixed according to the experimental design (Table 1). The space between the pot and plastic sleeve was filled with sand and gravel to support the sleeve and to assure that all pot sizes had equivalent thermal mass (Fig. 1*a*).

Uniform seeds of common bean (*Phaseolus vulgaris* L., RIL 30 from the L88 population developed by J. Kelly at Michigan State University), were surface sterilised, scarified and germinated in rolls of brown germination paper (Anchor Paper Co., St Paul, MN, USA) saturated with 0.5 mM CaSO$_4$ for 2 days at 25°C before planting. All pots were irrigated daily using an automatic drip system which delivered 100 mL day^{-1} of a modified nutrient solution (Epstein 1972) that lacked P and consisted of 3.1 mM NO$_3$, 1.8 mM K, 1.2 mM Ca, 1.4 mM SO$_4$, 1.0 mM NH$_4$, 0.825 mM Mg, 0.05 mM Cl, 5 μM Fe-EDTA, 2 μM B, 1.5 μM Mn, 1.5 μM Zn, 0.143 μM Mo and 0.5 μM Cu. Nutrient solution pH was adjusted to 5.5–5.8. Plants were grown between April and June 2005 in a climate controlled greenhouse at University Park, Pennsylvania, USA (40°49′N, 77°49′W). The average temperature was 24°C ranging from a maximum of 28°C (day) to a minimum of 22°C (night), the photoperiod was 14/10 h (day/night) and the maximum midday photosynthetic flux densities reached 1000 μmol photons m^{-2} s^{-1}.

Biomass, P and root measurements

Plants were harvested 28 days after planting (DAP). A representative sample of each root system was selected by removing the roots from approximately one-quarter of the media. These subsamples were scanned and the resulting images were analysed to calculate root length using image analysis software (WinRhizo Pro 2002, Regent Instruments, Quebec, Canada). The remainder of the roots were washed from the soil, dried and weighed. After scanning, root subsamples were dried at 60°C for 48 h and weighed. Specific root length (root length per gram root biomass) was calculated from the subsample root biomass and root length and total root length calculated from total root system dry mass and specific root length. Shoot and root tissue were dried at 60°C for 2 days and weighed. Tissue P concentration was determined spectrophotometrically (Murphy and Riley 1962). The allometric partitioning coefficient *k*, was calculated as the
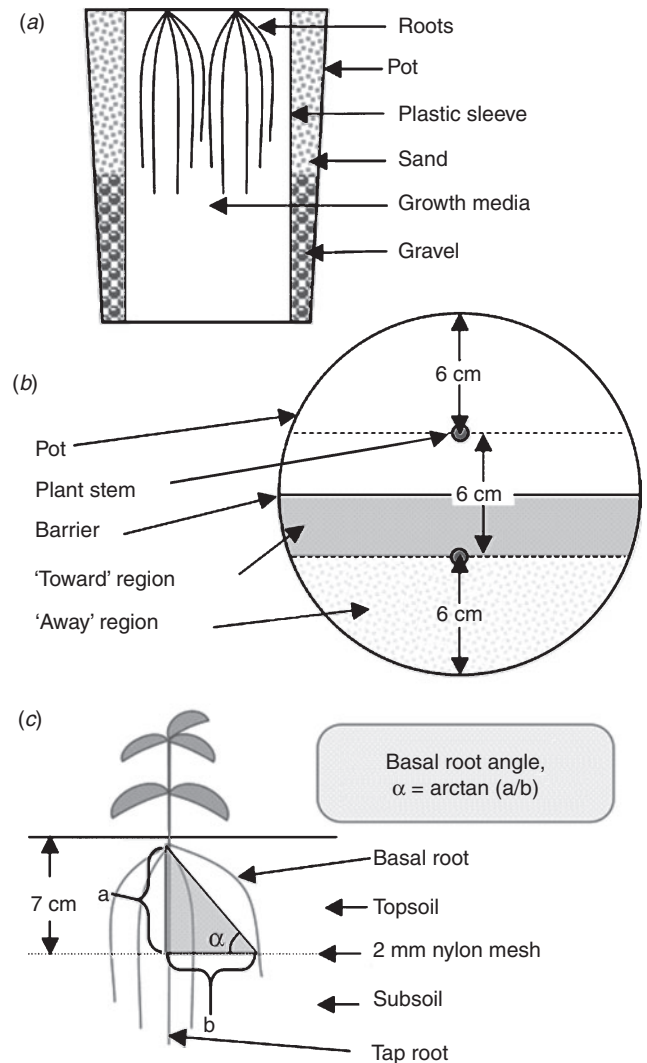

Fig. 1. Schematic views of pots used in (*a*) Experiment 1, (*b*) Experiment 2 and (*c*) of the evaluation of basal root angle of common bean plants. The 2 mm nylon mesh was embedded at 7 cm depth. The barrier between plants was nylon mesh, plastic film or no barrier. The roots in the 'away' region would grow away from the neighbour and those in the 'towards' region towards the neighbour.

slope of the regression of the logarithm of root biomass on the logarithm of shoot biomass (Niklas and Enquist 2002).

Depletion zone fraction was estimated from the depletion zone radius and root length. The radius of the P depletion zone; which typically extends about 1 mm from the root surface (Gahoonia and Nielsen 1992) was estimated from the P diffusion rate and age of the root (Ge *et al*. 2000), as follows for each possible age of roots:

$$r_{dz} = r_r + 2(De \times t)^{1/2}, \tag{1}$$

where r_{dz} is the depletion zone radius (cm); r_r is the root radius (cm); *De* is the effective diffusion coefficient for P (here $De = 10^{-8}$ cm^2 s^{-1}); and *t* is the age of the root(s). We partitioned the total root length according to root age based on the assumption of exponential growth in root length.

Phosphorus depletion zone volume was then calculated as follows for each age of root:

$$P \text{ depletion volume} = \pi \times r_{dz}^2 \times L, \qquad (2)$$

where L is root length; and V is the total volume of pot used in the experiment. These were summed over all root ages and divided by the total soil volume to determine the P depletion volume fraction.

Statistical analysis

All treatments were replicated four times. Data were analysed in R ver. 2.10 (R development core team 2010), with mixed effects models using the package 'nlme' (Pinheiro *et al.* 2009), with replicate modelled as a random effect. When two plants were grown together, the average biomass and root length were reported for comparison with single plants. All interactions that could be tested were included, but because this experiment was not fully factorial (two plants in low P were not included), not all interactions could be tested. To ensure that potentially important interactions were not overlooked, models were fit by beginning with a full model and removing non-significant effects, following procedures outlined by Crawley (2005) and Zuur *et al.* (2009). The effect of P dose and the presence of a neighbour were tested by comparing specific pairs of treatments using mixed models, rather than testing all treatments (Table 2).

Experiment 2: Effects of P stress and neighbour

Experimental design

Two plants were grown in each pot with one of three neighbour treatments: (i) no neighbour ('Isolated') – pot divided into two root zones with a barrier of polyethylene allowing no root interaction; and (ii) partial access to neighbour ('Partial') – pot divided with a 40 µm nylon mesh barrier (Anping Hengxing Bolting Cloth Co. Ltd, Hebei, China) allowing soil solution to move freely but confining roots; and (iii) full access to neighbour ('Full') – no barrier, roots free to intermingle.

Since two plants were grown in every pot, all plants experienced similar levels of aboveground competition. Because the mesh used in the 'Partial' treatment did not allow root penetration, the availability of diffusion limited nutrients (like P) should be similar in the 'Partial' and 'Isolated' treatments; but the soil solution could pass through the mesh so that the roots might be able to respond to their neighbours if there was transmission of soluble signalling compounds between neighbouring plants. The mesh in the PC treatment could also allow root hairs or mycorrhizal hyphae to pass.

Two P treatments were applied: a control treatment (HP) received P at the rate of 42 mg kg^{-1} P as triple super phosphate fertiliser (0–46–0) and a low P treatment (LP) received only half this concentration of P (21 mg kg^{-1}). Nitrogen and potassium were supplied as ammonium nitrate and potassium sulfate at 115 and 104 mg kg^{-1} soil N and K$_2$O respectively. All nutrients were thoroughly mixed into the media.

Table 2.   Results of linear models comparing specific treatments of the effects of rooting volume, P concentration and presence of a neighbour on the biomass production, biomass partitioning and root length of common bean (*Phaseolus vulgaris*)

Significance indicated by: *p from 0.05 to 0.01; **p from 0.01 to 0.001; ***p from 0.001 to 0.0001; ****$p < 0.0001$

Effect	Shoot biomass		Root biomass		Root length	
	d.f.	*F*-ratio	d.f.	*F*-ratio	d.f.	*F*-ratio
Absolute quantity of P (one plant LP vs one plant HP, all volumes)						
Pot volume	2/17	2.853	2/17	1.268	2/15	1.674
P dose	1/17	17.40***	1/17	12.94**	1/15	11.79**
Pot volume × P dose	–	–	–	–	2/15	4.356*
R^2		0.642		0.558		0.789
P concentration (P dose/volume, all treatments)						
Pot volume	2/28	3.750*	2/28	2.946	2/29	4.220*
P concentration	1/28	39.86****	1/28	24.71****	1/29	34.59****
R^2		0.745		0.642		0.596
Neighbour, with same P concentration (one plant HP vs two plants HP, all volumes)						
Pot volume	2/16	12.94***	2/16	5.504*	2/15	2.887
Neighbour	1/16	0.4140	1/16	0.3550	1/15	4.563*
Pot volume × competition	–	–	–	–	2/15	4.557*
R^2		0.671		0.575		0.582
Neighbour, with same P dose/plant (one plant LP vs two plants HP, all volumes)						
Pot volume	2/14	0.7781	2/14	1.271	2/15	1.625
Neighbour	1/14	152.3****	1/14	50.06****	1/15	36.69****
Pot volume × competition	2/14	33.56****	2/14	8.176**	2/15	4.757*
R^2		0.948		0.871		0.806
Neighbour, as in 'tragedy of the commons' experiments (one plant LP 4 L vs two plants HP 8 L)						
Neighbour	1/3	3.666	1/3	1.241	1/3	9.668
R^2		0.736		0.531		0.617

Plant growth

The growth medium consisted of 55% sand, 40% coarse vermiculite and 5% red soil (as described above). In each pot, a 24 cm diameter piece of 2 mm nylon mesh was placed at a depth of 7 cm to maintain the basic architecture of the root system when the media was washed away at harvest (Fig. 1). When filling pots for the 'Partial' and 'Isolated' treatments, a rigid plastic sheet was used to support the barriers while the pot was filled to ensure that volume on both sides remained equal. Germination of seeds was as described above, but the genotype of common bean was a different member of the same RIL population (RIL 13 from the L88 population). Two germinated seeds were planted in each pot at a depth of 4 cm. Plants were irrigated twice daily with 100 mL of nutrient solution and allowed to drain freely. The nutrient solution contained (in addition to the N,P and K concentrations listed above): 2 mM Ca, 2 mM SO_4, 0.5 mM Mg, 0.05 mM Cl, 2.5 µM Fe-EDTA, 1.25 µM B, 1 µM Mn, 1 µM Zn, 0.25 µM Mo and 0.25 µM Cu. Nutrient solution pH was adjusted to 5.5–5.8. Plants were grown between June and July 2007 in the greenhouse described above, with an average temperature of 26°C, ranging from 30°C at day to 23°C at night. The photoperiod was 14/10 h (day/night) and the maximum midday photosynthetic flux density was 1400 µmol photons $m^{-2} s^{-1}$.

Biomass, P and root measurement

Plants were harvested at 15 and 30 DAP and the DW of the shoots were measured after drying at 60°C for 48 h. The basal root angle for each basal root was measured 15 DAP. The roots above the nylon mesh were cut and washed from the media carefully and the locations where the taproot and basal roots (for each plant) passed through the nylon mesh were individually marked. The distances between basal root and taproot interception of the screen and the height of the basal root origin above the nylon mesh were measured and the basal root angle for each basal root was estimated using from the ratio of these distances – the tangent (Fig. 1c). Root length and root biomass were determined as described in the first experiment at 15 and 30 DAP, but were determined separately for the surface horizon (above the nylon screen) and the deeper horizon. At second harvest (30 DAP) the plants had already developed adventitious roots which were included in root tallies for each region.

The soil in each pot was divided between the two plants along the plane where the barriers were installed. Each half was divided into two regions: the 'towards' region, which was nearest the neighbour and the 'away' region, which was furthest from the neighbour (Fig. 1b). The horizontal distribution of basal roots in two regions of each pot, the 'towards' and 'away' regions, was characterised by counting the number of basal roots in each region. Representative subsamples of root tissue were scanned and the resulting images analysed as detailed above and dried at 60°C for two days. Tissue P content was analysed as described above.

Statistical analysis

All treatments had four replicates. The data were analysed in SAS version 6.12 (SAS Institute, Cary, NC, USA). Under a given main factor, such as P application level, the mean for each neighbour treatment was compared and the main factors

subsequently were tested for significant difference using Tukey's HSD. All interactions were included and non-significant interactions were dropped. Pairwise comparisons were conducted to analysed root architectural parameters, but some pairs of treatments were not tested because the parameters were not measured, for instance basal root angle at 30 DAP, shallow root fraction at 15 DAP.

Results

Experiment 1: Effects of P dose, P concentration and neighbour

Plant biomass was affected by P availability rather than the presence of a neighbour. Plants receiving the double dosage of
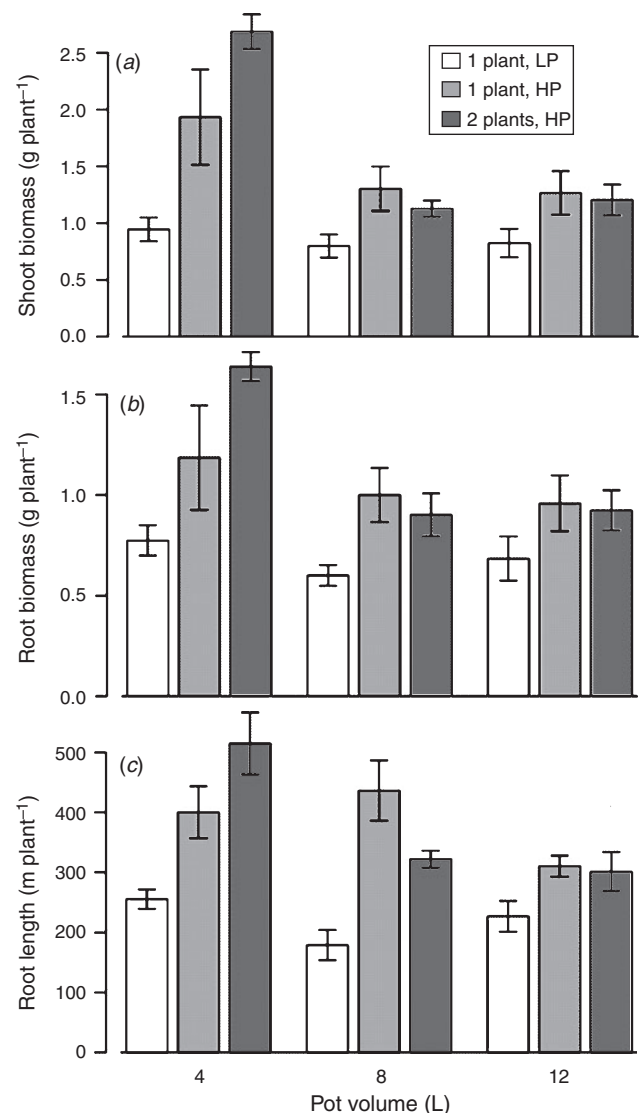

Fig. 2. (*a*) Shoot biomass, (*b*) root biomass and (*c*) root length per plant of common bean (*Phaseolus vulgaris*) grown in varying volumes of media with varying P levels with and without a neighbour. Values are per plant. Low P (LP) was equivalent to 22, 14.7, 7.3 mg kg^{-1} P in 4, 8 and 12 L of media. High P (HP) was equivalent to 44, 22 and 14.7 mg kg^{-1} P in the same volumes. Bars represent mean of four replicates ± s.e.

P produced more root and shoot biomass ($P=0.0004$ root, $P=0.0002$ shoot) across all treatment combinations. The greatest biomass was produced by plants receiving the largest amount of P in the smallest volume of soil (i.e. the greatest P concentration; Fig. 2*a, b*). Even when the double dose of P was diluted in a triple volume of soil, there was sufficient P available to allow plants with neighbours to reach root and shoot biomass comparable to plants with no neighbour (rightmost two bars in Fig. 2).

Comparing the one plant with a single dose of P (LP) treatment with the one plant with a double dose of P (HP) treatment shows that the amount of P added was strongly and positively correlated with shoot and root biomass (Fig. 2; Table 2). Furthermore, root length was affected by doubling the quantity of P only in the smaller (4 and 8 L) volumes. Shoot and root biomass and root length in all treatments increased with and was well correlated with the soil P concentration (Table 2).

The effect of a neighbour can be tested in two different ways: comparing one plant with two plants with the same quantity of soil P (one plant HP vs two plants HP) and by comparing one plant with two plants with the same quantity of P per plant

Table 3. Estimation of P depletion volume as percent of total volume (%)

Data shown in each column are the means of four replicates ± s.e. and the values for each volume are the means of 12 replicates ± s.e. Means followed by different lowercase letters were significantly different for a given volume at $p < 0.05$ and means followed by different uppercase letters were significantly different among three volumes at $p < 0.05$. These values assume $De = 1.0 \times 10^{-9}\,cm^2\,s^{-1}$ and root radius of 0.0362 cm

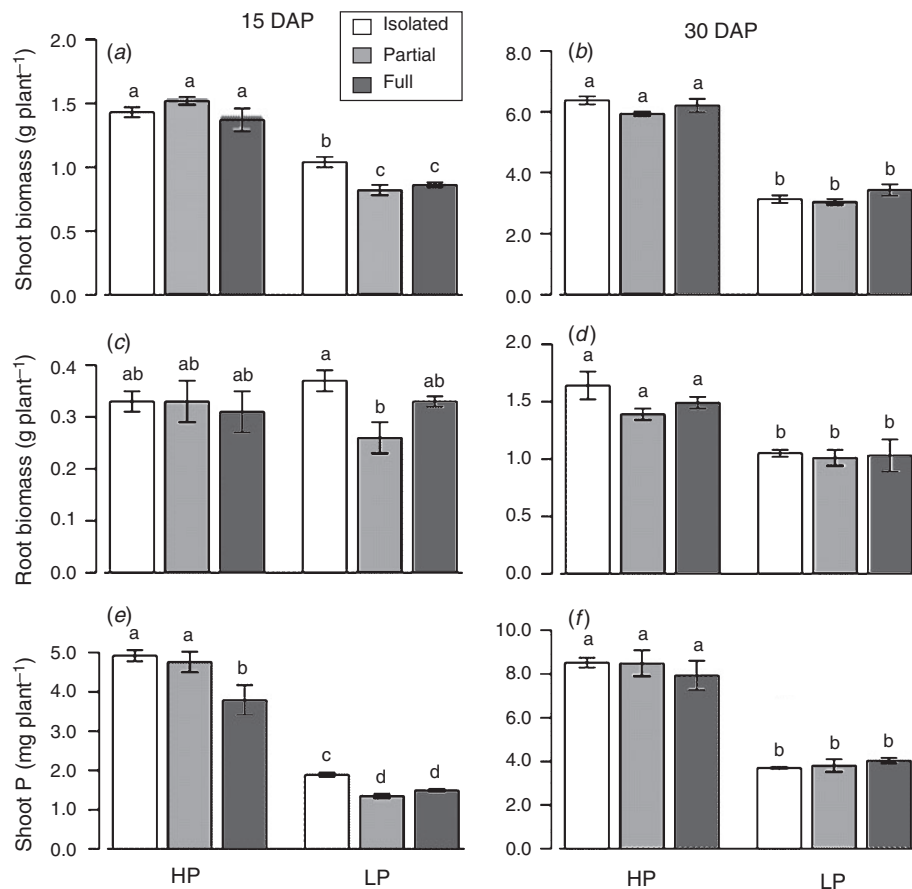| Treatments	Phosphorus depletion volume fraction	
4·LP·1	$9.32 \pm 0.57a$	–
4·HP·1	$14.64 \pm 0.58b$	–
4·HP·2	$35.3 \pm 2.31c$	–
Mean for $V = 4$ L	–	$19.76 \pm 3.46B$
8·LP·1	$3.31 \pm 0.45a$	–
8·HP·1	$7.64 \pm 0.78b$	–
8·HP·2	$11.67 \pm 0.49c$	–
Mean for $V = 8$ L	–	$7.54 \pm 1.07A$
12·LP·1	$2.68 \pm 0.26a$	–
12·HP·1	$3.75 \pm 0.20a$	–
12·HP·2	$7.00 \pm 0.57b$	–
Mean for $V = 12$ L	–	$4.48 \pm 0.59A$

Fig. 3. The shoot and root biomass and shoot phosphorus content of common bean (*Phaseolus vulgaris*) grown in control (HP) and low P (LP) treatments and with Full, Partial (Partial) or No access to the roots of a neighbour at (*a, c, e*) 15 and (*b, d, f*) 30 days after planting (DAP); bars represent mean of four replicates ± s.e. The ANOVA for this data is shown in the Appendix. Note that the *y*-axis scales differ for each panel.

(one plant LP vs two plants HP). For the first test, the neighbour did not alter shoot or root biomass and affected root length differently depending on pot volume (Fig. 2, light grey vs dark grey bars; Table 2). For the second test, where both number of plants and quantity of P were varied, the effect of a neighbour was significant and was greatest in the smallest volumes for shoot and root biomasses as well as for root length (Fig. 2, white vs dark grey bars; Table 2). However, in this test the presence of a neighbour is confounded with the quantity of P, which has strong effects on plant growth (Table 2). The presence of a neighbour was not a significant predictor of root or shoot biomass or of root length when comparing single plants with double plants with double volume and the same P concentration (Table 2).

Biomass allocation between shoots and roots, as reflected by the allometric partitioning coefficient (the slope of the relationship between the logarithms of shoot and root biomass), was not affected by either quantity of soil P ($P = 0.770$) or presence of a neighbour ($P = 0.378$) (Fig. 5a).

Roots depleted a greater fraction of soil P (estimated from depletion zone volumes) in smaller soil volumes (Table 3). High P increased the depletion zone fraction in the 4 and 8 L soil volumes, but not in the 12 L volume.

Experiment 2: Effects of P stress and neighbour

Low P concentration reduced shoot biomass at both harvests and root biomass at the second harvest. However, the allometric partitioning coefficient was constant, indicating that P availability altered the overall growth rate, but not the allocation pattern (Fig. 5b). The neighbour treatments did not affect either root or shoot biomass (Fig. 3; Table A1). Both neighbour and P addition affected shoot P content at 15 DAP but only P addition significantly altered shoot P content at 30 DAP (Table A1). Phosphorus content of plants with full access to the neighbour was less than that of plants with no neighbour at 15 DAP (Fig. 3e).

Phosphorus availability significantly altered root length and root architecture. Under Low P, full access to the neighbour increased root length compared with no access to the neighbour at 15 DAP but this difference was not evident at 30 DAP. Low P significantly reduced the shallow fraction of both root mass and length, whereas full access to the neighbour reduced the shallow root length fraction only (Table 4).

Overall, neighbour treatments did not alter the angles of the basal roots, although the angle of the basal roots differed slightly between 'towards' and 'away' regions (Table 4). At 15 DAP, the basal roots in the 'towards' region had steeper (deeper) angles than roots in 'away' region across all neighbour and P level combinations. Low P tended to increase (steepen) the basal root angles in the 'towards' region in full- and no neighbour, but this trend was not significant. The radial distribution of basal roots at 15 DAP was also affected by the neighbour treatments. Plants having full access to the neighbour positioned fewer roots in the 'towards' region (adjacent to the neighbouring plant), compared with plants with no access to the neighbour (Fig. 4). By 30 DAP radial root deployment was equivalent in 'towards' and 'away' regions.

Table 4. The effects of P and neighbour treatments on root architecture traits of common bean (_Phaseolus vulgaris_)

Data shown are the means of four replicates ± s.e. The fraction of shallow root mass or shallow root length refers the percentage of total mass or length of the roots found in the top 7 cm soil. The basal root angle is measured from horizontal, so that greater angle indicates deeper roots. Significant differences are indicated by: *p from 0.05 to 0.01; **p from 0.01 to 0.001; NS, not significant ($p > 0.005$). Means followed by different letters (a–c) were significantly different among neighbour treatments at $p < 0.05$. LP, low P; HP, high P

Root architecture trait	Root length (m plant^{-1})		Shallow root mass fraction (%)		Shallow root length fraction (%)		Basal root angle by region (degrees)			
							'Towards'		'Away'	
Means										
Neighbour treatment	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP
15 days after planting										
Full	49.1a	66.4a	–	–	–	–	31.0a	40.1a	30.0a	27.2a
Partial	50.1a	44.4c	–	–	–	–	35.5a	26.0b	26.6a	25.5a
Isolated	51.6a	59.6b	–	–	–	–	31.9a	39.5a	27.9a	29.5a
30 days after planting										
Full	328.0a	223.2a	28.5a	21.7a	9.4b	3.3a	–	–	–	–
Partial	249.4b	144.1b	31.5a	19.0a	18.8a	4.1a	–	–	–	–
Isolated	313.0a	183.6ab	31.1a	19.8a	13.4b	5.5a	–	–	–	–
ANOVA results										
Replicate	NS		NS		NS		NS			
Harvest (15 or 30 DAP)	**		–		–		–			
Phosphorus	**		**		**		NS			
Neighbour	**		NS		**		NS			
Region('Towards' or 'Away')	–		–		–		**			
Harvest × P	**		–		–		–			
Harvest × neighbour	**		–		–		–			
Phosphorus × neighbour	*		NS		*		NS			
Adjusted R^2	0.956		0.839		0.850		0.473			

Discussion

Phosphorus concentration rather than self/non-self recognition influences root response to the presence of a neighbour

This study was designed to critically test the ROR and the alternative hypothesis that roots respond to resource availability rather than the presence of a competitor. We found no evidence in support of the ROR. The presence of a neighbour did not alter biomass allocation to roots (Fig. 2; Table 2; Table A1) and the comparison of one plant in 4 L of LP media with two plants in 8 L of HP media, which most closely mimics the conditions of the Gersani *et al.* (2001) experiment, yields no significant effects on shoot or root biomasses and only marginal effects on root length (Table 2). Notably, allometric partitioning between roots and shoots was unaltered by the presence of a neighbour (Fig. 5). However, reduced P availability increased relative biomass allocation to roots (Fig. 3a–d), in agreement with many previous reports (reviewed by Lynch and Ho 2005). These results are consistent with the hypothesis that any root proliferation in response to the presence of a neighbour occurs as a response to resource depletion, rather than as a response to 'non-self' roots.

Increasing P concentration significantly affected shoot and root biomasses and had a stronger effect than that of rooting
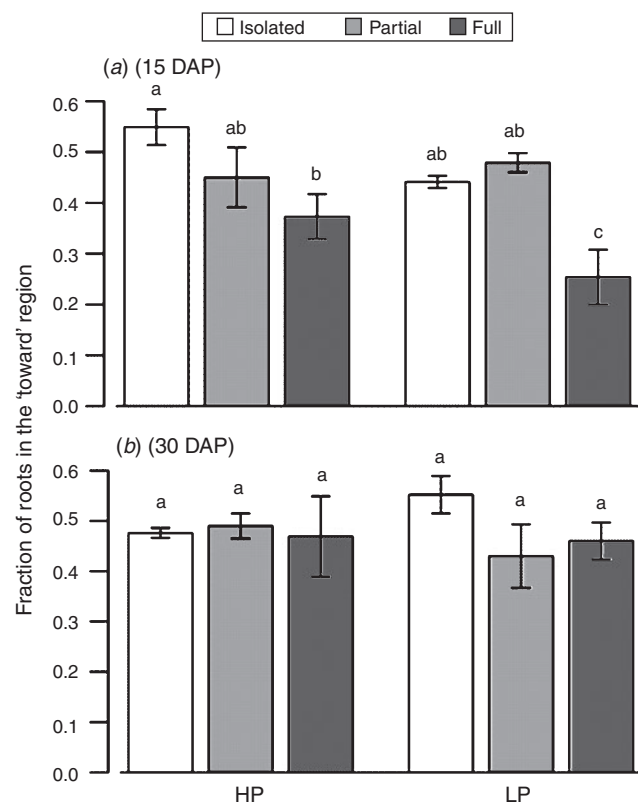
volume or the presence of a neighbour (Table 2). This is consistent with the idea that nutrient availability is one of the driving forces in root competition (Wilson and Tilman 1993). Further, in the smallest soil volume (4 L), where competition for soil resources should be most intense because of greater root length density and overlap of depletion zones (Table 3), we observed no change in either root or shoot biomass when a neighbour was present, as would be predicted by the ROR (Fig. 2).

The degree of resource depletion (of P) should be positively related to root length. When one plant grows in a pot only intraplant competition exists. Enlarging the soil volume should lead to a decrease of both root length density (root length/soil volume) and P depletion zone fraction (Fig. 2c; Table 2). This should allow a decline in the overlap of depletion zones and therefore a reduction in intraplant competition. Whether such a
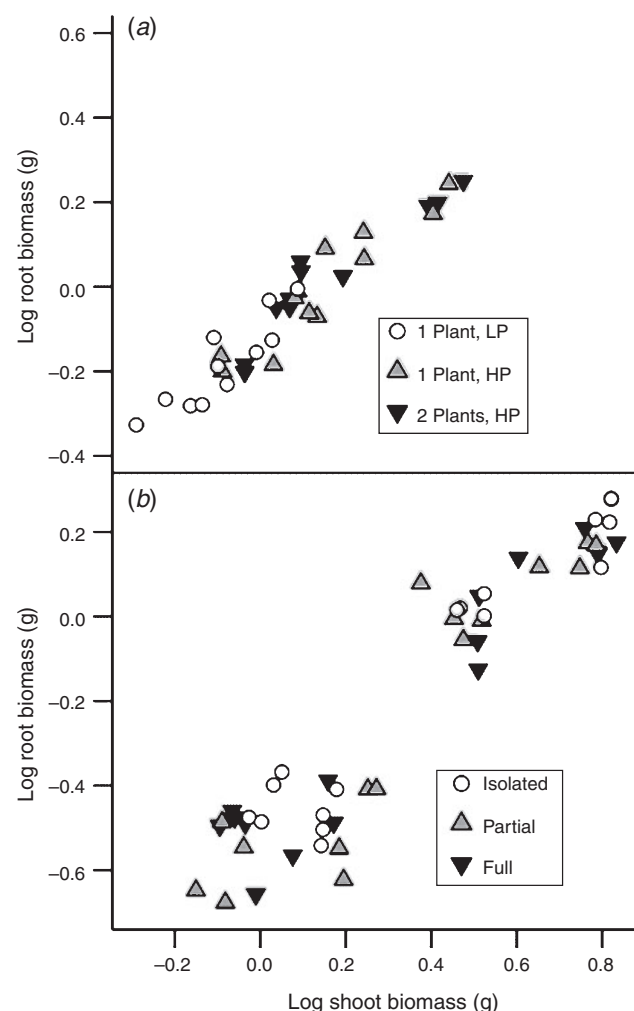

Fig. 4. The fraction of basal roots in the 'towards' region with two levels of P availability and three levels of access to the roots of a neighbour at (*a*) 15 and (*b*) 30 days after planting (DAP). Bars represent mean of four replicates ± s.e., values indicated by the same letters (a–c) were not significantly different at $p \leq 0.05$ among all treatments in each harvest date.

Fig. 5. Root : shoot allometry of common bean (*Phaseolus vulgaris*) grown in varying volumes of media with (*a*) varying P levels with and without a neighbour and (*b*) with different levels of access to neighbouring roots and P treatments. Low P (LP) was equivalent to 22, 14.7 and 7.3 mg kg^{-1} P in 4, 8 and 12 L of media. High P (HP) was equivalent to 44, 22 and 14.7 mg kg^{-1} P in the same volumes. Full, Partial and Isolated refer to levels of access to neighbour roots.

decline in self-competition occurs will depend on the overall root length density and the architecture of the root system. A decline in self-competition would reduce the cost of resource acquisition, potentially leading to an increase in shoot biomass, though such an increase may be small. While we observed no such increase in shoot biomass with increasing rooting volume (Fig. 2), our results are otherwise consistent with the idea that root competition for P (both inter- and intraplant) should increase in smaller rooting volumes, as root length density (root length/volume) and the overlap of P depletion zones, increases (Fig. 2; Table 3).

We note however, that even in the smallest soil volume with two plants, the total estimated depletion zone volume was less than 40% (Table 3) and if we assume a similar range for the ratio of interplant to intraplant competition as reported by Rubio *et al*. (2001) for plants separated by 6 cm (9.4–35.3%), then the actual volume in which interplant competition would occur is likely less than 5% of the total volume. If the maximum likely volume of interplant competition is this small, interplant competition may be relatively unimportant and the lack of response to a neighbour in the 8 and 12 L pots is expected. It is possible that interplant competition for a more mobile nutrient, like nitrate, would result in different responses to the presence of a neighbour.

Root architecture will exhibit greater plasticity in response to the presence of a neighbour than will root biomass

Most studies that have addressed root competition have investigated the alteration of root biomass and have rarely considered the role root architecture may play in belowground competition. Root architecture has been shown to be an important determinant of P acquisition by increasing foraging efficiency (Lynch 2005). This study analysed root architectural modification in order to critically test the hypothesis that root architecture will exhibit greater plasticity in response to the presence of a neighbour than will root biomass.

Root architectural traits, such as root length and vertical and horizontal distribution, were altered by the presence of a neighbour (Fig. 4; Table 4) even though root biomass was similar across the three neighbour treatments (Fig. 3*c*, *d*). Thus, root architecture was modified without alteration of root biomass. Root architectural plasticity may reduce competition for soil resources without altering biomass partitioning between shoot and roots. The presence of a neighbour caused the plants to increase root length (Table 3), place fewer roots in the 'toward' region than in the 'away' region (Fig. 4) and place fewer roots in the topsoil than in the subsoil (Table 4). These architectural responses may reduce the overlap of P depletion zones thus reducing the intensity of both intraplant and interplant competition. Structural–functional plant models (Ge *et al*. 2000; Rubio *et al*. 2001; Postma and Lynch 2011) could be used to estimate the effect of such architectural responses on P acquisition. Thus, root architecture exhibits greater plasticity in response to the presence of a neighbour than root biomass, providing support for the second hypothesis. Changes in root biomass alone may not adequately characterise root responses to a neighbour and root architecture should also be considered.

Conclusion

Root architecture was found to be more sensitive to the presence of a neighbour than root biomass. In these studies, the presence of a neighbour did not alter root biomass, but did alter root architecture and in some cases root length, in a manner that could reduce interplant competition in both vertical and horizontal dimensions. An analogous architectural response occurs in shoot responses to aboveground competition, termed the stress-induced morphogenic response (Potters *et al*. 2007). Root architecture is regulated by P concentration (Lynch and Brown 2008), including root gravitropic setpoint angle (Bonser *et al*. 1996), production of shoot-borne roots (Miller *et al*. 2003; Kim *et al*. 2008), axial elongation (Ma *et al*. 2003), lateral branching (Borch *et al*. 1999; Desnos 2008) and root hair formation (Bates and Lynch. 1996; Ma *et al*. 2001). Increased axial elongation in response to lower P availability (Ma *et al*. 2003) could account for some of the increased root length attributed to competition. Results from these studies are consistent with the hypothesis that architectural responses to local P availability are more important than self/non-self recognition or soil volume in root responses to neighbouring plants.

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Appendix

Table A1. The ANOVA summary for biomass and P content of shoot and root biomass for the Experiment 2

Significance indicated by: *p from 0.05 to 0.01; **p from 0.01 to 0.001; ***p from 0.001 to 0.0001; NS, not significant ($p > 0.05$)

Effects	Shoot biomass	Root biomass	Phosphorus content
15 days after planting			
Replicate	NS	NS	NS
Phosphorus	***	NS	***
Neighbour	NS	NS	**
Phosphorus × neighbour	**	NS	*
Adjusted R^2	0.970	0.308	0.950
30 days after planting			
Replicate	NS	NS	NS
Phosphorus	***	***	***
Neighbour	NS	NS	NS
Phosphorus × neighbour	NS	NS	NS
Adjusted R^2	0.972	0.736	0.913