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Root distributions of Australian herbaceous perennial legumes in response to phosphorus placement

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Abstract. Many Australian plant species have specific root adaptations for growth in phosphorus-impoverished soils, and are often sensitive to high external P concentrations. The growth responses of native Australian legumes in agricultural soils with elevated P availability in the surface horizons are unknown. The aim of these experiments was to test the hypothesis that increased P concentration in surface soil would reduce root proliferation at depth in native legumes. The effect of P placement on root distribution was assessed for two Australian legumes, *Kennedia prorepens* F. Muell. and *Lotus australis* Andrews, and the exotic *Medicago sativa* L. Three treatments were established in a low-P loam soil: amendment of 0.15 g mono-calcium phosphate in either (i) the top 50 mm (120 μg P g^{-1}) or (ii) the top 500 mm (12 μg P g^{-1}) of soil, and an unamended control. In the unamended soil *M. sativa* was shallow rooted, with 58% of the root length of in the top 50 mm. *K. prorepens* and *L. australis* had a more even distribution down the pot length, with only 4 and 22% of their roots in the 0–50 mm pot section, respectively. When exposed to amendment of P in the top 50 mm, root length in the top 50 mm increased 4-fold for *K. prorepens* and 10-fold for *M. sativa*, although the pattern of root distribution did not change for *M. sativa*. *L. australis* was relatively unresponsive to P additions and had an even distribution of roots down the pot. Shoot P concentrations differed according to species but not treatment (*K. prorepens* 2.1 mg g^{-1}, *L. australis* 2.4 mg g^{-1}, *M. sativa* 3.2 mg g^{-1}). Total shoot P content was higher for *K. prorepens* than for the other species in all treatments. In a second experiment, mono-ester phosphatases were analysed from 1-mm slices of soil collected directly adjacent to the rhizosphere. All species exuded phosphatases into the rhizosphere, but addition of P to soil reduced phosphatase activity only for *K. prorepens*. Overall, high P concentration in the surface soil altered root distribution, but did not reduce root proliferation at depth. Furthermore, the Australian herbaceous perennial legumes had root distributions that enhanced P acquisition from low-P soils.

Keywords: alfalfa, *Kennedia prorepens*, *Lotus australis*, lucerne, *Medicago sativa*, phosphatase.

Introduction

Phosphorus (P) is a key substrate in respiration, photosynthesis, and regulation of plant enzymes and an important component of macromolecules (Raghothama and Karthikeyan 2005). While P is a critical macronutrient for plant growth and development, most soil P is unavailable for plant uptake owing to its rapid immobilisation in soil organic and inorganic components (Holford 1997). Since P in soils is relatively immobile and often unavailable, plants have evolved morphological, physiological and biochemical mechanisms to respond to P deficiency (Raghothama 1999; Vance *et al*. 2003; Raghothama and Karthikeyan 2005). Typical morphological responses to low P supply include increased root development, higher root : shoot ratios, finer roots, longer root hairs and the formation of mycorrhizas, all of which facilitate exploration of a greater

Abbreviations used: MCP, mono-calcium phosphate; RMR, root mass ratio; SRL, specific root length.

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soil volume (Smith and Read 1997; Schachtman *et al*. 1998; Raghothama 1999; Gahoonia and Nielsen 2004; Zhu *et al*. 2005). In addition, the supply of P in soils is typically heterogeneous and most plant roots grow preferentially in regions that contain favourably high concentrations (Drew 1975; Fransen *et al*. 1999; Hodge 2004). Plants can also enhance P acquisition by altering their root physiology to increase the rate of nutrient absorption per unit root tissue mass or length (Neumann and Martinoia 2002). Typical root physiological mechanisms that increase P acquisition include the exudation of carboxylates (i.e., malate and citrate) and phosphohydrolases (Gilbert *et al*. 1999; Richardson *et al*. 2000; Ryan *et al*. 2001; Wouterlood *et al*. 2005).

Australian native legumes are considered to have potential for development as pasture species (Robinson *et al*. in press). An understanding of the morphological and physiological traits that affect P acquisition is important to facilitate the development of native Australian legumes for agricultural purposes. Knowledge to assist in the selection of species with enhanced P acquisition will be beneficial in low-input agroecosystems and may improve the productivity and sustainability of high-input agroecosystems.

Very little is known about morphological and physiological root responses of Australian native perennial legumes to P. Many Australian native plants have evolved in P-impoverished environments (Beadle 1966; Handreck 1997) and possess specialised adaptations to low soil P, such as root clusters (Shane and Lambers 2005). Efficient P acquisition mechanisms appear to be poorly regulated at high P availability for some Australian plants (Shane *et al*. 2004) and many native species are sensitive to P toxicity (Handreck 1997). Large quantities of superphosphate have been applied to agricultural soils to improve crop yields in Australia (Bolland *et al*. 1997) and the impact of high P concentrations on the performance of native species is unknown. Of particular concern is the effect that high P concentrations in surface soils could have on root distributions. Enhanced root proliferation in surface soils could lead to a reduction in deep root growth that could affect nutrient and water acquisition deeper in the soil profile and hence successful incorporation of native species into agricultural systems.

The objective of this study was to assess the effect of P concentration and placement on root distribution of two native Australian legumes, *Kennedia prorepens* and *Lotus australis*. Responses were compared with *Medicago sativa*, a key exotic pasture species in Australia, which has undergone significant selection and breeding. We hypothesised that: (i) native legumes would differ from *M. sativa* in their root distribution patterns; (ii) in high-P surface soils native legumes would have increased root proliferation in surface soil and suffer a reduction in deep root growth; and (iii) native legumes possess specialised morphological or physiological adaptations to enhance P acquisition in low-P environments and would, therefore, accumulate more P in their shoots than *M. sativa* when unfertilised.

Materials and methods

Two experiments that tested: (1) the root morphological responses of native legumes and *M. sativa* to P amendment in the surface soil, and (2) the exudation of acid mono-ester phosphatases from native legumes grown with or without P amendment, were performed.

Growth conditions in experiment 1

Root morphological responses of three legumes were assessed by growth in a pot system with differing P supplies. *Kennedia prorepens* F. Muell. [CRC for Plant-based Management of Dryland Salinity (CRC PBMDS), KIMS001, collected from central Western Australia], *Lotus australis* Andrews (CRC PBMDS SA33610, collected from Sellicks Beach, South Australia) and *Medicago sativa* Lindley (cv. Hunterfield) were grown in 1-m tall, 100-mm diameter pots. The experiment consisted of three treatments: a control (C) with no added P, placement of 0.15 g mono-calcium phosphate (MCP) in the top 50 mm of soil (120 $\mu g\ P\ g^{-1}$ soil) ('top', T-treatment), placement of 0.15 g MCP in the T–500 mm of soil (12 $\mu g\ P\ g^{-1}$ soil) ('half', H-treatment). Four replicate pots of each species × treatment combination were established.

Pots were filled with 7.5 kg of non-calcic brown Warranine loam (Dr 2.11, Northcote 1979) collected from the top 150 mm of an unfertilised field site 70 km east of Perth. Soil was sieved, the <4 mm fraction was mixed to ensure homogeneity and was not sterilised. Basic soil characteristics were determined according to Rayment and Higginson (1992) by CSBP Futurefarm analytical laboratories, Bibra Lake, Australia (Table 1). All essential nutrients other than P were provided by amending the field soil with 50 $mL\ kg^{-1}$ of modified Long Ashton's nutrient solution (without P): 2 mM K_2SO_4, 1.5 mM $MgSO_4{\cdot}7H_2O$, 3 mM $CaCl_2{\cdot}2H_2O$, 0.1 mM FeEDTA, 4 mM $(NH_4)_2SO_4$, 8 mM $NaNO_3$, 46 μM H_3BO_3, 9.1 μM $MnCl_2{\cdot}4H_2O$, 0.77 μM $ZnSO_4{\cdot}7H_2O$, 0.32 μM $CuSO_4{\cdot}5H_2O$, 0.1 μM $Na_2MoO_4{\cdot}2H_2O$.

Seeds were imbibed with a 1 : 10 dilution of smoke water to enhance germination (Tieu *et al*. 2001) and sown in seedling trays at staggered times according to their pre-determined germination time. Three seedlings were planted into each pot and thinned to one plant at 3 weeks. All pots were maintained at 60% of field capacity for the duration of the experiment. Weekly additions of 300 mL of 2 mM NH_4NO_3 were applied after week six to ensure an adequate nitrogen supply. Any germinating weeds were removed by hand as necessary. The experiment was set out in a glasshouse at the University of Western Australia, Perth, as a randomised complete block design. The glasshouse was unheated and had an average daytime temperature of 23°C during the experiment, which was conducted from April to August 2005.

Table 1. Characteristics of field soil used in the experiments
Values are means ($n = 2$)

Characteristic	Value
pH ($CaCl_2$)	5.3
pH (H_2O)	6.1
Extractable P (Colwell) ($mg\ kg^{-1}$)	5
Total phosphorus ($mg\ kg^{-1}$)	179
Total nitrogen ($mg\ g^{-1}$)	1.8
Nitrate nitrogen ($mg\ kg^{-1}$)	13
Ammonium nitrogen ($mg\ kg^{-1}$)	2.5
Extractable K (Colwell) ($mg\ kg^{-1}$)	96
Organic carbon (%)	3.0
Reactive iron ($mg\ kg^{-1}$)	1336
% Sand	70
% Silt	9
% Clay	21

Plant and soil analysis for experiment 1

Plants were harvested at 12 weeks and shoots were collected and dried at 80°C. The soil column was separated into six sections: 0–50, 50–100, 100–250, 250–500, 500–750 and 750–1000 mm. The plant roots from each section were collected and stored in 50% ethanol. A 100-g soil sample was collected from each section and dried at room temperature. Root lengths and diameters were analysed with an Epson 1680 scanner and the Winrhizo (Regent Instruments, Quebec, Canada) root scanner program. A subsample of roots from 0 to 50 mm depth was cleared and stained to allow assessment of the percentage of root length colonised by the arbuscular mycorrhizal fungi indigenous to the field soil (Giovannetti and Mosse 1980). The mass of roots used for analysis of mycorrhizal colonisation were estimated from fresh mass. The roots from each section were dried at 80°C for 3 d and weighed.

Bicarbonate-extractable P was analysed from the 0–50, 50–100 and 100–250 mm soil sections by a modification of the method of Colwell (1963). P was extracted from soil with 0.5 M $NaHCO_3$ (pH 8.5) with a soil / solution ratio of 1 : 100, and an extraction time of 16 h at 23°C. A manual colourimetric estimate of P based on the method of Murphy and Riley (1962) was used. Shoot material and seed samples were analysed for P using a nitric acid / perchloric acid digest and the molybdovanadophosphate method (AOAC 1975).

Growth conditions in experiment 2

A two-compartment pot system, made up of two PVC cylinders, each with an internal diameter of 100 and 80 mm deep, was used to study rhizosphere processes (Fig. 1). The two compartments were separated by a polyester mesh with 24-μm pore diameter and connected with strong tape. The upper compartment was packed with 650 g of field soil (Table 1) and the lower compartment with 650 g of fine sand. All essential nutrients other than P were provided by amending the field soil with 100 mL kg^{-1} of minus-P Long Ashton's nutrient solution, as for experiment 1.

The experimental design consisted of two treatments: a high-P treatment [120 μg P (MCP) g^{-1} soil] and a nil-P treatment (C) and the same three plant species as used in experiment 1. The two treatments were replicated five times for each species and pots were arranged in a completely randomised block design in a glasshouse. Seeds were imbibed, germinated and transplanted, as for experiment 1. The pots were pre-moistened, placed in a 50-mm deep tray on a bed of gravel and kept moist with a wicking system (Fig. 1). The water level in the tray was maintained at a constant level by watering every 2 d. The experiment was run from June to August 2005 in the same glasshouse as experiment 1.

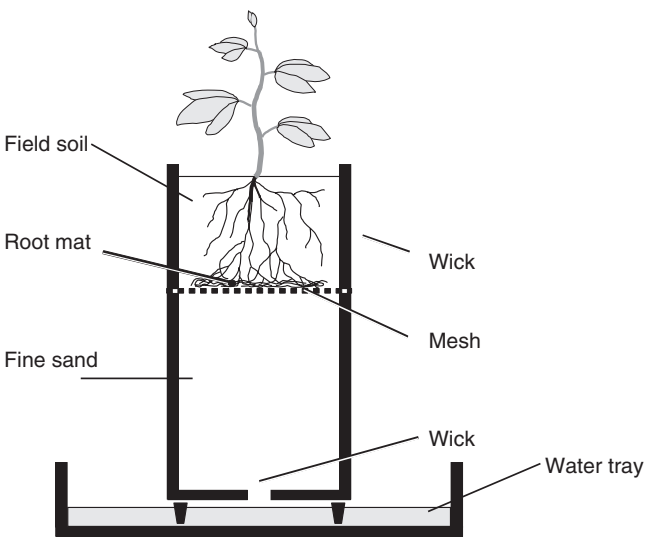

Fig. 1. Schematic representation of the pot system used to study rhizosphere processes in experiment 2, indicating the mesh that was impenetrable to roots and the wicking system used to maintain soil moisture (from Nuruzzaman *et al*. 2006).

Plant and soil analysis in experiment 2

Plant roots in the upper compartment were unable to penetrate the polyester mesh and formed a root mat. The soil below the polyester mesh was considered to represent the rhizosphere.

After 64 d plant shoots were harvested and the soil in the lower compartment was sliced into 1-mm sections directly below the mesh with a custom-made device (Nuruzzaman *et al*. 2006). This device had a round plate with a similar diameter to the pots, which could be accurately moved in 1-mm distances, using a spindle to push soil out of the pot system to collect fractions of soil. The first 5 mm below the mesh was sliced into sections of 1 mm thickness and a final 1-mm slice was taken at 8 mm. Roots and shoots were collected and dried in an oven for 2 d at 80°C. The 1, 2, 3, 4, 5 and 8 mm fractions were analysed for acid phosphomonoesterase (EC 3.1.3.2) activity by the methods of Tabatabai (1994). Sand (1 g) from each fraction was placed into a vial with 0.2 mL toluene, 4 mL modified universal buffer (Skujins *et al*. 1962), pH 6.5 (Tabatabai 1994), and 1 mL of 0.05 M *p*-nitrophenyl phosphate made up in modified universal buffer. Vials were capped, gently shaken and incubated at 37°C for 1 h. Following incubation, 1 mL 0.5 M $CaCl_2$ and 4 mL 0.5 M NaOH were added. A subsample of the soil suspension was taken and filtered. The absorbance of this solution was measured with a spectrophotometer at 420 nm. Controls were prepared as described above, except that the addition of 1 mL of *p*-nitrophenyl solution was made after the addition of 1 mL 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH.

Statistical analyses

One- and two-factor ANOVAs were performed with Genstat edition 7 (VSN International Ltd, Rothamsted, UK). Multiple comparisons were made by Tukey's HSD test. To determine the effects of treatments on root distribution that were independent of plant size, data were normalised by calculating the percentage of root length in each section. To further test for differences in relative root distribution patterns we used non-metric multidimensional scaling (non-metric MDS) with the multivariate statistical package Primer (version 6; Clarke and Warwick 2001). The starting point for this technique is the calculation of a dissimilarity matrix. The dissimilarity among root distributions of individual plants is based on the dissimilarities (i.e. Euclidean distances) between the relative amounts of roots placed in each of the six pot sections. Non-metric MDS calculates the rank order of these dissimilarities and then constructs a two-dimensional map satisfying the conditions imposed by the dissimilarity matrix. A goodness-of-fit value is then calculated by regressing the distances in two-dimensional space with those obtained from the original dissimilarity matrix (six-dimensional space). This goodness-of-fit is expressed as a stress value, with values <0.1 being regarded as 'good ordinations with no real prospect of a misleading interpretation' (Clarke and Warwick 2001). Although other multivariate techniques (e.g. principal components analysis) gave similar results, we choose to use the technique that required the least number of assumptions on our data to be made.

Results

Experiment 1

Root distributions

Root length densities in the top 50 mm were differentially affected by treatments for each species (Table 2; Fig. 2).

Table 2. Significance of experimental treatments for a range of parameters in experiment 1
Significant differences are indicated according to species, treatment, and the interaction between species and treatment (n.s., no significant difference; *$P<0.05$; **$P<0.01$; ***$P<0.001$)

Variable	Species	Treatment	Species × treatment
Root length density (RLD) at 0–50 mm depth	**	***	***
RLD at 0–250 mm depth	**	***	n.s.
Normalised root length (RL) at 0–50 mm depth	***	n.s.	**
Total root mass	*	***	n.s.
Total root length	**	***	n.s.
Specific root length	n.s.	***	n.s.
Mean root diameter	*	n.s.	*
Root mass ratio	***	n.s.	*
% Root length colonised by mycorrhizal fungi	***	n.s.	n.s.
Shoot mass	***	*	n.s.
Shoot [P]	***	n.s.	n.s.

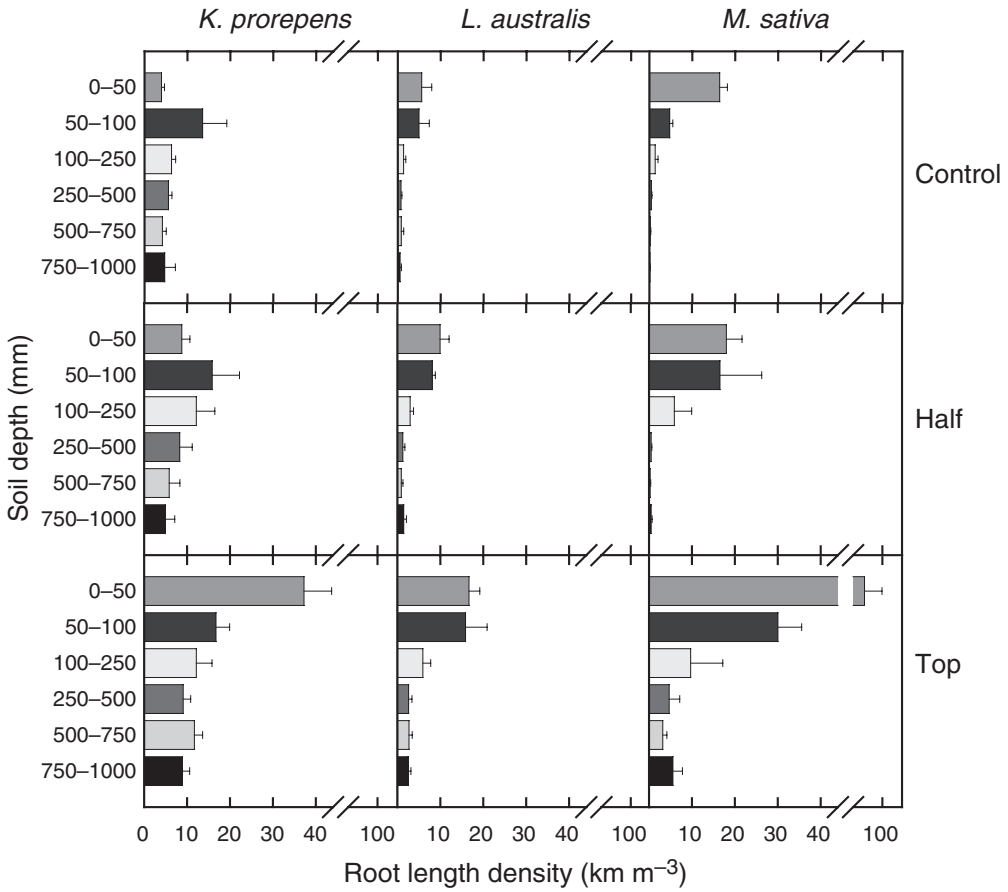

Fig. 2. Effect of P fertiliser placement on root length density in 0–50, 50–100, 100–250, 250–500, 500–750 and 750–1000 mm depths of the pot for *Kennedia prorepens*, *Lotus australis* and *Medicago sativa*. Treatments were C (no applied P); H-, 12 µg P g^{-1} soil applied to the top 500 mm of pots, and T-, 120 µg P g^{-1} soil applied to the top 50 mm of the pot, (experiment 1). Bars show means (+SE, $n = 4$).

In the control treatments *K. prorepens* had the highest root density for over the entire 1-m pot (5.5 km m^{-3}) with the highest root density at 50–100 mm depth (13.5 km m^{-3}). *M. sativa* (1.3 km m^{-3}) and *L. australis* (1.5 km m^{-3}) had lower average root densities, with the highest densities between 0–100 mm (Fig. 2). Placement of P in the top 500 mm of the pot (H-treatment) increased root density of all species, particularly in the top 250 mm (Table 2; Fig. 2).

M. sativa was generally shallow rooted; roots of *K. prorepens* and *L. australis* were more evenly distributed down the pots. Localised placement of P in the top 50 mm T-treatment increased root density more than 5-fold in the top 0–50 mm for *K. prorepens* and 10-fold for *M. sativa*. *L. australis* did not respond as strongly, with only a small increase in root density at 0–50 mm depth when P was supplied in this section of the pot. Root density below 50 mm was increased in the T-compared with the control treatment for each species (Fig. 2). Application of P in the top 500 mm only affected the root densities at 0–50 mm depth for *M. sativa*.

Normalised root length (the percentage of root length present in each section of the pot) increased in the top 50 mm of the pot in response to the (T-treatment) for *K. prorepens*, but not for *M. sativa* and *L. australis* (Table 2; Fig. 3). Over the entire 1-m pot, each of the three species had distinct root distributions; *M. sativa* distributed 39–58% of root length in the top 50 mm of the soil profile (Fig. 3). *K. prorepens* had only 4–16% of root length in the top 50 mm and more than half of its roots were distributed in the bottom half of the pot. *L. australis* had 20–24% of total root length in the top 50 mm and had a more even distribution of roots with depth (Fig. 3).

Analysis of normalised root length using non-metric multi-dimensional scaling provided reliable evidence that *K. prorepens* had root distributions distinct from those of *M. sativa*, and that *L. australis* had root distributions that overlapped with the other two species (Fig. 4). Root distributions of *K. prorepens* showed similar patterns between the C and H- treatments; root distributions of the T- and C treatments were most similar for *M. sativa* (Fig. 4).

Kennedia prorepens had the highest root mass and length across all treatments; root mass and length were similar in *M. sativa* and *L. australis* (Tables 2, 3). The T-treatment had the greatest root mass, followed by the H- and then C treatments. Total root length was highly correlated with shoot P content ($r^2=0.85$, $P<0.001$). Specific root length (SRL, the length per unit root mass) was only affected by treatments and all species had decreased SRL with

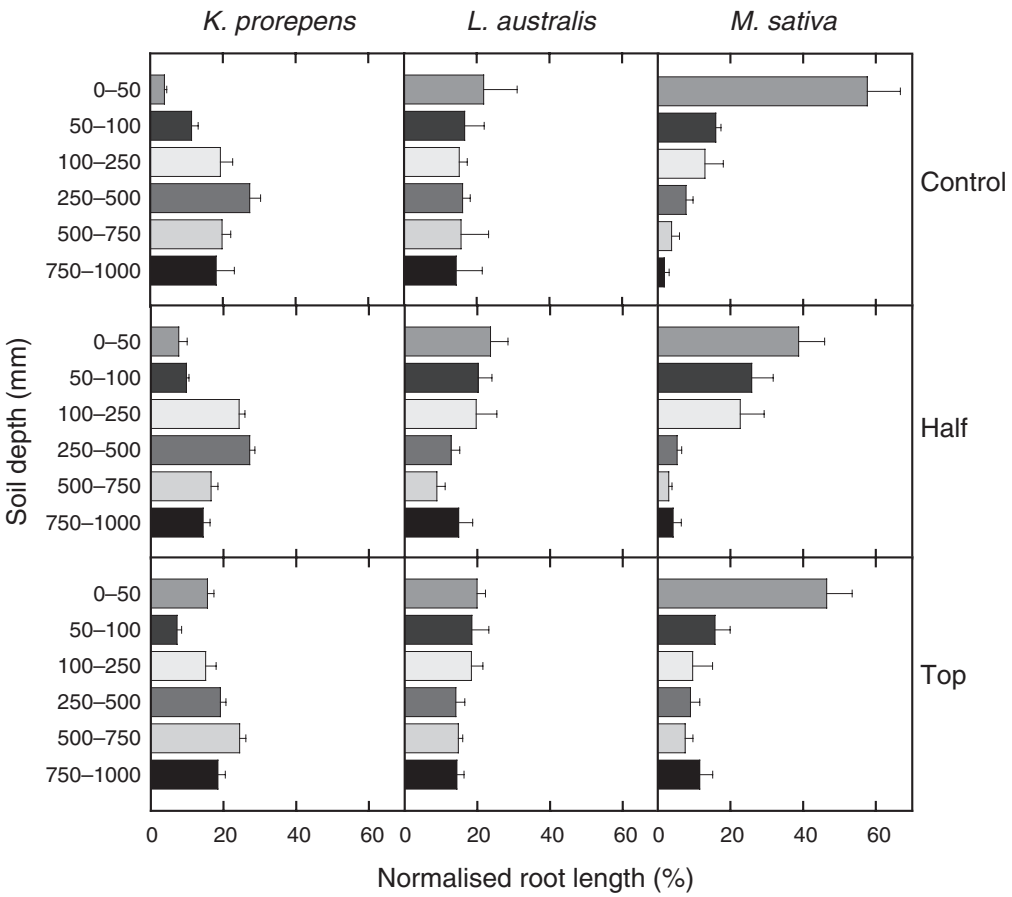

Fig. 3. Effect of P fertiliser placement on normalised root lengths (the percentage of root length present in each portion of the pot) in 0–50, 50–100, 100–250, 250–500, 500–750 and 750–1000 mm depths of the pot for *Kennedia prorepens*, *Lotus australis* and *Medicago sativa*. Treatments were C (no applied P); H-, 12 µg P g^{-1} soil applied to the top 500 mm of pots, and T-, 120 µg P g^{-1} soil applied to the top 50 mm of the pot, (experiment 1). Bars show means (+SE, $n=4$).

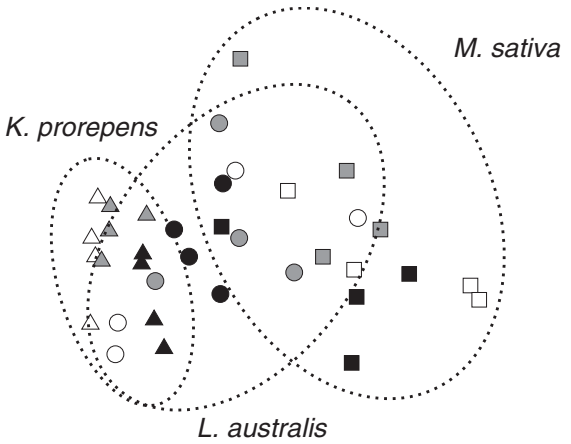

Fig. 4. Non-metric multi-dimensional scaling based on normalised root lengths for *Kennedia prorepens* (triangles), *Lotus australis* (circles) and *Medicago sativa* (squares) for experiment 1. Control (open symbols), H-treatments (grey symbols) and T-treatments (filled symbols). The stress value for this analysis is 0.04. Species are encircled to aid visual assessment.

increased [P] in surface soil (Tables 2, 3). SRL was positively correlated with shoot P content (r^2=0.47, P<0.05). Mean root diameter was differentially affected for each species by treatment (Tables 2, 3) and was greater in the T-treatment for *M. sativa* only.

Root mass ratio (RMR, the proportion of root to total plant mass) increased for *K. prorepens* as surface [P] increased but changed very little for the other species (Tables 2, 3). Approximately half of the total biomass was allocated to roots in *M. sativa*; the native legumes allocated lower proportions of biomass to roots (Table 3).

Mycorrhizal colonisation

All three species had a high percentage of root length colonised by arbuscular mycorrhizal fungi. The degree of colonisation differed between species but was unaffected by treatment (Table 2). *L. australis* (83%) was more highly colonised than *K. prorepens* (45%) or *M. sativa* (55%) (Table 3). In all cases colonisation was generally dense in the root cortex and consisted primarily of arbuscules.

Seed and shoot mass

Kennedia prorepens had larger seeds and a higher seed P concentration than *L. australis* or *M. sativa* (Table 4). The percentage of seed P in shoot material was lower in the H- and T-treatments, compared with the control. Accordingly, the percentage of P in shoots that potentially originated from the seed declined from 14.6% in the C to 6.7% in the T-treatment for *K. prorepens*, from 5.5 to 1.0% in *L. australis* and from 6.7 to 0.8% in *M. sativa* for the same treatments.

Shoot mass differed according to species and P treatment (Table 2). *K. prorepens* had the highest shoot mass across all the treatments followed by *L. australis* and *M. sativa* (Table 4). Compared with the controls, P addition in the T-treatment caused a 2-fold increase in shoot mass of *K. prorepens*, a 10-fold increase for *M. sativa* and a 4-fold increase for *L. australis*. Shoot P concentration differed with species (Tables 2, 4) and the native legumes had a lower shoot [P] than *M. sativa* (Table 4).

Soil phosphorus

Bicarbonate-extractable soil P was measured at the end of the experiment to validate treatment differences throughout the experiment. Controls had the lowest bicarbonate-extractable P in the top 250 mm (1.74 mg P kg^{-1}), the H-treatment was intermediate and the T-treatments had the greatest concentration of extractable P. A higher bicarbonate-extractable P was observed in the 0–50 mm section of the T-treatments for *K. prorepens* (2.98 mg P kg^{-1}) and *M. sativa* (3.14 mg P kg^{-1}) but not for *L. australis* (2.08 mg P kg^{-1}). The results indicate that no leaching of P was observed during the experiment, consistent with the root distribution data.

Table 3. Root parameters of *Kennedia prorepens*, *Lotus australis* and *Medicago sativa* following growth in unamended soil (C), application of P in the top 500 mm of pots (H-), or application of P in the top 50 mm of pots (T-) in experiment 1

Values are the means (SE, n = 4). Results from statistical analyses are provided in Table 2

Species	Treatment	Total root dry mass (mg)	Total root length (m)	Specific root length (m g^{-1})	Root diameter (mm)	Root mass ratio	Mycorrhizal colonization (%)
Kennedia prorepens	C	273 (70)	43 (11)	162 (14)	0.33 (0.01)	0.19 (0.03)	46 (8)
	H-	649 (290)	62 (23)	117 (17)	0.34 (0.01)	0.24 (0.05)	43 (7)
	T-	1352 (346)	94 (16)	75 (8)	0.33 (0.03)	0.31 (0.04)	55 (15)
Lotus australis	C	64 (22)	10 (3)	167 (17)	0.37 (0.01)	0.23 (0.01)	85 (3)
	H-	138 (35)	18 (4)	135 (10)	0.40 (0.01)	0.18 (0.09)	79 (6)
	T-	291 (69)	35 (8)	123 (5)	0.38 (0.02)	0.19 (0.02)	83 (5)
Medicago sativa	C	89 (7)	12 (0.8)	130 (5)	0.35 (0.01)	0.45 (0.05)	39 (26)
	H-	235 (132)	24 (10)	117 (12)	0.33 (0.02)	0.52 (0.02)	46 (12)
	T-	942 (301)	86 (21)	96 (6)	0.42 (0.04)	0.45 (0.03)	81 (5)

Table 4. Initial seed mass, seed P concentration, shoot mass and shoot P concentration of *Kennedia prorepens*, *Lotus australis* and *Medicago sativa* following growth in unamended soil (C), application of P in the top 500 mm of pots (H-), or application of P in the top 50 mm of pots (T-) in experiment 1

Values are the means (SE, $n = 4$). For seeds, values in the same column not followed by the same superscript letter are significantly different at $P < 0.05$. For shoots, results from statistical analyses are contained in Table 2

Species	Treatment	Seed		Shoot	
		Dry mass (mg)	P (mg g^{-1})	Dry mass (g)	P (mg g^{-1})
Kennedia prorepens	C	12.4 (1.9)[a]	4.2 (0.03)[a]	1.4 (0.6)	2.0 (0.1)
	H-			2.0 (0.8)	2.2 (0.1)
	T-			2.9 (0.5)	2.3 (0.3)
Lotus australis	C	2.7 (1.4)[b]	1.9 (0.02)[b]	0.3 (0.04)	2.3 (0.3)
	H-			0.7 (0.2)	2.2 (0.2)
	T-			1.2 (0.2)	2.7 (0.2)
Medicago sativa	C	2.8 (0.5)[b]	1.2 (0.02)[c]	0.1 (0.01)	3.0 (0.1)
	H-			0.2 (0.1)	3.3 (0.8)
	T-			1.2 (0.4)	3.2 (0.2)

Experiment 2

Shoot and root mass of all three species increased with P fertiliser addition (Table 5). *K. prorepens* had the highest shoot and root mass for both treatments, followed by *L. australis* and *M. sativa*. In all species there was greater acid phosphatase activity close to the root mat, which declined as distance from the root mat increased (Fig. 5). For *K. prorepens* phosphatases were influenced by P treatment ($P < 0.001$) and sampling depth ($P < 0.001$). There were no differences in phosphatases with sampling depth and P treatment for *L. australis*, while for *M. sativa* phosphatases decreased with depth ($P < 0.001$) but not treatment (Fig. 5).

Discussion

Root distributions of Australian legumes

Root distribution patterns in our experiments differed markedly among species, which supports our first hypothesis that native legumes would differ from *M. sativa* in their root distribution patterns. In all treatments *M. sativa* had the

Table 5. Shoot and root mass of *Kennedia prorepens*, *Lotus australis* and *Medicago sativa* under two P treatments; nil and 120 μg P (MCP) g^{-1} soil in experiment 2

Values are the means (SE, $n = 5$)

Species	Treatment	Shoot dry mass (mg)	Root dry mass (mg)
Kennedia prorepens	High P	371 (45)	395 (100)
	Nil P	129 (51)	255 (73)
Lotus australis	High P	357 (128)	222 (84)
	Nil P	44 (14)	44 (16)
Medicago sativa	High P	219 (42)	474 (105)
	Nil P	4 (0.89)	20 (5.1)

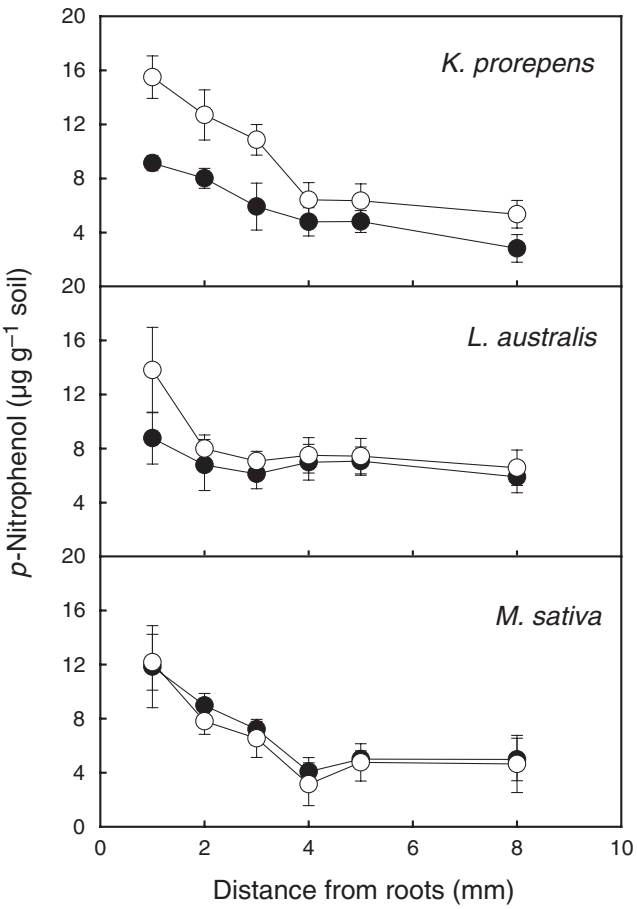

Fig. 5. Effect of applied P (120 μg P g^{-1} soil, ●) and no added P (○) on mono-ester phosphatase activity (measured as *p*-nitrophenol released per gram of soil) measured at 1, 2, 3, 4, 5 and 8 mm from the root mat of *Kennedia prorepens*, *Lotus australis* and *Medicago sativa* roots (experiment 2). Bars show ± standard errors ($n = 5$).

majority of root length concentrated in the top 50–100 mm of soil. In contrast, the native legumes had greater root length below 100 mm. *M. sativa* increased root length in 0–250 mm of soil in the H-treatment, but root distribution was not altered in the T-treatment; instead, more roots were produced with the same root distribution. *K. prorepens* had the greatest plasticity in response to amendment of P in the surface soil and produced significantly more roots at 0–50 mm in the T-treatment.

Clear differences in root distribution for the test species allow us to make predictions about the role of root distributions in P acquisition. Root distributions of *M. sativa* suggest a dimorphic root architecture with the majority of roots close to the surface and few roots at depth, similar to type VI in the classification of Cannon (1949). We hypothesise that this large proliferation of surface roots is required to maximise the acquisition of poorly mobile surface P in environments with reliable rainfall patterns. Topsoil root foraging is considered to be an optimum strategy for acquiring P efficiently from low-P soils, as P is usually greatest in these horizons owing to the contribution of decaying dead leaves, and higher organic matter and microbial activity (Lynch and Brown 2001). In contrast, the few deep roots that were measured in *M. sativa* are thought to function primarily in accessing water, and thus there is less need for extensive root proliferation at depth, since plants require few roots for water uptake (Passioura 1972) and lucerne roots often reach groundwater (Fillery and Poulter 2006; Ward and Micin 2006). Root distribution will undoubtedly change due to ontogeny and environmental conditions (Fitter 1994; Hutchings and de Kroon 1994). *M. sativa*, for example, grown for a similar period in sand had more roots at depth than in the present study, which is possibly a consequence of the soil type or different P nutrition compared with the present work (Bell 2005). However, in mature *M. sativa* stands ~60–70% of total root mass is in the upper 150 mm of soil (Barnes and Sheaffer 1985) and our *M. sativa* seedlings mimicked this root distribution. In contrast to the root distribution of *M. sativa*, *K. prorepens* and *L. australis* had few roots in the surface layers and significant roots at depth, indicating greater soil exploration to acquire P or water from deeper layers in the soil, closer to type VII of Cannon's classification (Cannon 1949). *K. prorepens* originates from very dry environments (mean annual rainfall 280 mm) with variable rainfall and is likely to have a root system genetically programmed to optimise water capture from depth, with a lesser ability for root proliferation in the high-P surface layers (Ho *et al.* 2005). *L. australis* had a uniform root distribution with depth that was intermediate compared with the other species (Fig. 4; similar to Cannon type VII).

We tested only two examples of a large range of native legumes that are being evaluated for agricultural potential. The likelihood is that native legumes will have a wide range of root distributions that are co-optimised for P, water, water-soluble nutrients and anchorage (Lynch and Brown 2001). Diversity in root system morphology and physiology is likely to contribute to marked differences in P acquisition, particularly in environments where moisture and nutrients are temporally and spatially variable. For this reason there is a need to better understand root systems to make the best use of emerging germplasm currently under evaluation. Through selection of species with particular root architectures it may be possible to increase acquisition of poorly soluble P, particularly if these architectures involve enhanced physiological adaptations.

High surface P did not reduce deeper root growth of native legumes

We hypothesised that native legumes would proliferate roots in surface layers of soil that were amended with P and that deep root growth would be reduced. Plants typically respond to patches of available P in soils by increasing root proliferation in those areas (Drew 1975; Fitter 1994; Linkohr *et al.* 2002; Hodge 2004). Nutrients are naturally distributed in a heterogeneous manner in soils (Caldwell 1994) and P in particular normally has a patchy distribution since it is poorly mobile, and is depleted primarily by root interception and diffusion processes rather than mass flow (Barber and Silberbush 1984; Marschner 1995). Surface [P] in agricultural soils is usually high because of fertiliser addition and low mobility of P down the soil profile. Contrary to our hypothesis, proliferation of surface roots did not result in a reduction of deep roots for the native legumes. *K. prorepens* had the greatest plasticity in response to surface amendment of P, increasing root mass in the soil surface. Despite this, *K. prorepens* contained significantly more roots below 50 mm than *M. sativa*; *L. australis* had a greater proportion of roots below 50 mm in the T-treatment compared with other treatments. These results suggest that high surface soil [P] is unlikely to reduce deep root growth of native legumes.

The response of native legumes will, of course, depend on the effects of soil type in mediating P availability. Soils with a low P-buffering capacity (Brennan *et al*. 1994) are likely to provide higher P availability than those of the loam we used. High P concentrations may alter root growth and even lead to P toxicity. Many native Australian plants are sensitive to high leaf P status (Handreck 1997; Shane *et al*. 2004), and the impact of high P availability on shoot and root growth is unknown. Understanding how native legumes respond to a range of soil P concentrations in a soil of low buffering capacity is the subject of a further study (M Denton, M Ryan, M Tibbett, M Wouterlood, R Bennett, unpublished data).

Root morphological and physiological adaptations to increase P acquisition

Native legumes possessed root morphological adaptations that increased P acquisition compared with *M. sativa*, particularly in low-P soils, but did not exude more monoester phosphatases than *M. sativa*. Root systems of native legume differed from *M. sativa* in several fundamental traits associated with efficient P acquisition. Differences in root length and diameter are often considered to be associated with adaptation to particular environments. Most species increase RMR when nutrients are limiting (Christie and Moorby 1975; Hutchings and de Kroon 1994; Hill *et al*. 2006). Slow-growing species from nutrient-poor habitats often have large root systems, whereas roots of fast-growing species from nutrient-rich habitats are typically smaller (Chapin 1980). RMR in our experiments differed from most findings; *M. sativa* is considered to originate in more fertile sites, but allocated more biomass to roots compared with native species. In contrast, *K. prorepens* allocated a low proportion of biomass to roots in the control treatments but RMR of *K. prorepens* increased with increasing surface P supply (H- and T-treatments).

Kennedia prorepens had significantly greater root length than other species in the C- and H-treatments, which is proposed to have enabled greater P acquisition. Although root length is important for acquiring poorly mobile nutrients (Barber and Silberbush 1984), in our experiment the longest root lengths were associated with the T-treatment. Since total root length was highly correlated with shoot P content, P supply appeared to limit root extension.

Specific root length increased in response to P stress, as previously observed (Powell 1974; Christie and Moorby 1975; Fitter 1985) with *K. prorepens* the most responsive, *M. sativa* the least responsive and *L. australis* intermediate. In a recent study, most grasses and dicots increased SRL in response to decreased P supply; in contrast, only half of the species altered SRL in response to decreased N supply (Hill *et al*. 2006). The authors considered that this difference was due to the importance of mass flow in N supply but not P supply. In the present experiments, mean root diameter for the whole root system differed minimally among species and treatments. The increase in root diameter for *M. sativa* in the T-treatment was most probably caused by ontological changes causing thick tap and crown roots in this treatment.

While not a focus of these experiments colonisation by arbuscular mycorrhizal fungi indigenous to the soil was assessed. Soil was not sterilised to eliminate these fungi, as the presence of mycorrhizas represents the normal state of these species under low soil P conditions. Indeed, all species were highly colonised. However, it is impossible to speculate on whether the fungi made a significant contribution to plant P acquisition and whether this differed between species or treatments. While it is likely that the fungi did enhance P-acquisition (Smith and Read 1997), a high level of colonisation does not always confer such benefits, even in low soil P (Ryan and Angus 2003). Notably, colonisation was not affected by P fertilisation.

Efficiency in the acquisition and use of P by legumes

Native legumes accumulated more shoot P than *M. sativa*, particularly in the C treatments. Australian soils often contain a significant amount (80–90%) of soil P in forms that are unavailable for crop use (Holford 1997). However, many native species have adaptations for acquiring this poorly soluble P. The introduction of native legumes into agroecosystems may increase the efficient capture and use of P that is otherwise unavailable to species currently cultivated. Larger seed size and higher [P] in seeds appear to have increased the early vigour of *K. prorepens* in the control treatment. Greater seed P may increase early growth, and enhance the capture of further P, by allowing greater resources for root growth. Thus, as for many Australian species, high seed P in *K. prorepens* is a likely adaptation to low-P environments (Milberg and Lamont 1997).

Greater P availability in the T- *v.* C-treatments caused an enormous increase in shoot mass for all species, indicating that shoot growth was limited by P supply in C treatments. Within a species, shoot [P] was not influenced by treatments — growth diluted any additional P accumulated and there was no evidence of luxury uptake. In all treatments *K. prorepens* had the greatest total shoot P. Although it is unlikely that P was limiting the growth of *M. sativa* in the T-treatment, greater seed resources other than P (i.e., other nutrients, carbon) may have accounted for the increased growth of *K. prorepens*. Shoot [P] was deemed to be adequate for *M. sativa* in all treatments, based on reference materials (Reuter and Robinson 1997). Shoot [P] was lower in the native legumes, indicating that these species had greater P-use efficiency. Nutrient requirements of grassland species frequently differ, which has consequences for competitive abilities under differing soil P status (Hill *et al*. 2004). A perceived reduction in nutrient concentration could also result from the leaves of the native legumes having a higher leaf mass per area (LMA, leaf dry mass per unit area of leaf) compared with those of *M. sativa*, a trait often associated with adaptation to aridity and P-deficient soils (Beadle 1966; Wright *et al*. 2002). LMA was higher for *K. prorepens* ($145\,g\,m^{-2}$), but did not differ significantly between *L. australis* ($91\,g\,m^{-2}$) and *M. sativa* ($86\,g\,m^{-2}$) (M Denton, M Ryan, M Tibbett, M Wouterlood, R Bennett, unpublished data).

Native legumes had greater P acquisition than suggested by the differences in seed mass alone. Acquisition of P by *L. australis* was higher than that of *M. sativa*, while there

was no difference in the seed size between these species. Since total root length was lower in *L. australis* compared with *M. sativa*, the possibility remains that P acquisition by *L. australis* was enhanced through mycorrhizal symbiosis or root physiological processes. Root physiological adaptations to enhance P acquisition typically involve the exudation of carboxylates (i.e. citrate, malate) and phosphatases (Ryan *et al*. 2001; Neumann and Martinoia 2002). Native legumes are adapted to P-impoverished soils and are likely to have mechanisms to make use of poorly soluble P. Since the majority of soil P in ecosystems is in organic forms (Adams 1992), phosphatases are likely to have a pivotal role in mobilising soil P for plant use. Although rhizosphere mono-ester phosphatase activities did not differ between native legumes and *M. sativa*, phosphatases in all treatments were higher in proximity to roots. Although *M. sativa* did not have increased rhizosphere phosphatase activity associated with P-deficiency, many legumes including *Lupinus albus* L., *Trifolium subterraneum* L. and *M. polymorpha* L., had increase root phytase and phosphatase concentrations when subjected to P-deficient conditions (Gilbert *et al*. 1999; Hayes *et al*. 1999; George *et al.* 2004). Other species, such as *Trifolium repens* L., were unresponsive to P-deficiency and did not contain greater phytase or phosphatase concentrations in P-deficient roots (Hayes *et al*. 1999). Further work is required to understand the role of P-solubilising enzymes in the roots of *M. sativa* and Australian native legumes. Phosphatases in the rhizospheres of *K. prorepens* were increased under low P supply and may have resulted from plant- or microbial-derived phosphatases. Carboxylates, di-ester phosphatases or phytases can result in significant P mobilisation (Tadano and Sakai 1991) and these remain to be assessed. Some *Kennedia* species are reported to have root clusters (Brundrett and Abbott 1991; Adams *et al.* 2002) that can concentrate root exudates through morphology and synchronicity of release of exudates (Watt and Evans 1999). If these species can be used in agriculture they may access recalcitrant soil P, which is otherwise unavailable to crop species. The extent to which native legumes rely on root physiological mechanisms in the acquisition of P remain to be elucidated.

In conclusion, the native legume species used in this study had root systems with a significant proportion of roots at depth and these differed markedly from the pattern of root distribution in *M. sativa*, in which the majority of roots were concentrated in the surface soil. While the addition of high concentrations of P increased the root proliferation of Australian legumes in the surface soils, this was not to the detriment of roots produced in the deeper sections of soil. Consequently, high P concentrations in surface soils are unlikely to compromise deep root growth of Australian legumes, and high P soils are unlikely to be a constraint to the introduction of perennial herbaceous Australian legumes into farming systems. The results also indicate that native legumes are able to use more soil P than *M. sativa*.

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