

Phosphorus-efficient pastures: legume root traits for improved nutrient foraging

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Abstract

The critical soil phosphorus (P) requirement (P supply for 90% maximum yield) of many temperate pastures is determined by the high P requirements of key pasture legumes (commonly *Trifolium* or *Medicago* spp.). Legumes that yield well with a lower critical P requirement could reduce the fertiliser requirement of these pastures.

Pot experiments were used to: (i) identify legumes with root traits likely to confer P acquisition efficiency, and (ii) test the impact of these traits on critical P requirement. An initial screen of the root hair length (RHL) and specific root length (SRL) of 13 legumes and two grasses was undertaken. Growth and root morphology of five legumes (*Trifolium subterraneum*, *T. hirtum*, *Ornithopus sativus*, *O. compressus*, *Biserrula pelecinus*) and one grass (*Dactylis glomerata*) were subsequently compared in detail using a sandy loam soil (8.3 mg/kg Colwell P) that was amended by applying P to the top 5 cm of the soil profile (0, 15, 30, 70, 135, 250 mg P/kg). Shoot and root growth were assessed after six weeks.

Significant variation in RHL (0.12–0.75 mm) and SRL (98–603 m/g) was found among the legumes, with most being substantially shorter (RHL) or lower (SRL) than the grasses. In the P-response experiment, *Ornithopus* species (the only legumes with RHL and SRL approaching that of the grasses) had critical P requirements that were less than half that of *T. subterraneum*.

Selecting legumes that maximise root foraging via long, thin roots with long root hairs may reduce the critical P requirement of pastures.

Introduction

The critical external phosphorus (P) requirement (P required for 90% maximum yield) of many temperate pastures is determined by the high P requirements of key pasture legumes (commonly *Trifolium* or *Medicago* spp.; Ozanne et al. 1969). Selecting legumes with lower external requirements for P could reduce the amounts of P fertiliser that need to be applied to pastures because soil managed at lower soil test P concentrations are likely to sorb less P (Simpson et al. 2014). The features of plants that allow them to acquire P more effectively are longer, finer roots with longer root hairs and improved ability to forage for P (Richardson et al. 2011).

This work aimed to: (i) identify potentially useful variation in root morphology associated with nutrient foraging among a range of novel legume species being developed in Australia (Nichols et al. 2007), and (ii) test the impact of these traits on critical external P requirement.

Method

Variation in root hair length and specific root length

The root hair length (RHL) and specific root length (SRL) of 14 legumes and two grasses (Table 1) were assessed in separate experiments. In the first experiment for RHL, a sandy loam soil was steam pasteurised, sieved (<5 mm) and mixed with lime to raise pH (CaCl₂) to 5.5. A complete nutrient solution was applied to the soil (extractable P 33 mg/kg; Colwell 1963). Pots (90 mm diam.; 200 mm height) were filled with 1.33 kg of the soil. Two plants were established per pot and inoculated with an appropriate strain of *Rhizobium*. Soil was maintained at approximately 75% field capacity. Plants were grown in a controlled environment cabinet (15–20°C; photon flux density 600 μmol/m²/s¹; 12 h light/ dark). Four replicates were grown per species. Roots were washed from the soil four weeks after seedling emergence. Root hairs were imaged using a fluorescence microscope fitted with a camera. RHL of ten root hairs was measured on ten images per replicate using Image J (Rasband 1997–2014).

In the second experiment, SRL was assessed on three- and six-week old plants. SRL of the legumes was correlated between harvests ($R^2=0.75$) and results are only presented for the six-week old plants.

Pots (90 mm diam.; 400 mm height) were filled with 2.746 kg of recycled potting mix containing added superphosphate (extractable P 55 mg/kg; Colwell 1963). One plant was established per pot and inoculated with an appropriate strain of *Rhizobium*. Soil was maintained at approximately 75% field capacity by watering with a P-free nutrient solution. The plants were grown in a glasshouse (15–20°C) under natural lighting in May to July 2013 in Canberra, Australia. Five replicates were grown per species. Plants were harvested six weeks after sowing. Roots were washed from the soil, scanned to determine root length using WinRHIZO (Regent Instruments Inc.) and dry mass determined after drying at 70°C. SRL was calculated as length per unit root mass. Data from both experiments were analysed using ANOVA in R (R Core Team).

Growth in response to phosphorus application

In the third experiment, a subset of the species (Figure 1) was selected to determine growth in response to P application and critical external P requirement. Soil was collected, pasteurised, sieved and limed as per the screen for RHL, and amended with a P-free nutrient solution. Pots (90 mm diam.; 200 mm height) were filled with a bottom layer of 1.0 kg of low P soil followed by a top layer of 0.333 kg of the same soil (11% moisture) that had been amended by mixing with KH₂PO₄ at rates of 0, 15, 30, 70, 135 and 250 mg P/kg (oven dry soil) to establish six P application treatments (n=5 replicates). This topsoil-subsoil arrangement was used to mimic the stratification of P that occurs in soil under pastures. Plants were grown in a controlled environment cabinet (15–20°C; photon flux density 900 µmol/m²/s; 12 h light/dark) as microwards with reflective sheets fitted around each pot and raised to equal plant height to reproduce the light conditions in a pasture. Soil was maintained at approximately 75% field capacity. Shoots and roots from the fertilised topsoil and from the subsoil layer were harvested six weeks after sowing. Roots from the topsoil were scanned to determine root length using WinRHIZO (Regent Instruments Inc.). Dry mass of roots and shoots was determined after drying at 70°C. Total P uptake by the plants was determined by ashing root and shoot dry matter at 550°C, dissolving the ash in HCl and determining the P concentration of the solution using malachite green. A Mitscherlich response curve [$y = a - b \cdot (e^{-cx})$] was fitted to the shoot dry mass data in R (R Core Team). Critical external P requirement was determined as the P application rate corresponding with 90% of maximum shoot yield. Root mass fraction (root mass in each soil layer as a proportion of total plant mass) was determined. Root mass fraction and total P uptake per unit root length were analysed using ANOVA in GenStat 16th Ed (VSN International).

Results

RHL of the legumes ranged 6-fold from 0.12 to 0.75 mm (Table 1). SRL of the legumes ranged 3-fold from 98 to 320 m/g (Table 1). The key legume species used in temperate pastures in Australia, *T. subterraneum*, had relatively short root hairs and low SRL. The *Ornithopus* spp. and *B. pelecinus* had RHLs and SRLs that approached that of the grasses.

Table 1. Root hair length and specific root length of 12 legume and two grass species.

Scientific name	Common name	Root hair length (mm)	Specific root length (m/g)
<i>Bituminaria bituminosa</i>	tedera	0.12	98
<i>Trifolium tumens</i>	talish clover	0.21	nd
<i>Trifolium incarnatum</i>	crimson clover	0.23	259
<i>Trifolium subterraneum</i>	subterranean clover	0.23	159
<i>Trifolium spumosum</i>	bladder clover	0.25	239
<i>Trifolium ambiguum</i>	Caucasian clover	0.28	187
<i>Trifolium purpureum</i>	purple clover	0.29	177
<i>Trifolium hirtum</i>	rose clover	0.30	290
<i>Medicago sativa</i>	lucerne	0.37	209
<i>Lotus corniculatus</i>	birdsfoot trefoil	0.44	205
<i>Biserrula pelecinus</i>	biserrula	0.56	299
<i>Ornithopus sativus</i>	French serradella	0.73	320
<i>Ornithopus compressus</i>	yellow serradella	0.75	307
<i>Phalaris aquatica</i>	phalaris	0.86	371
<i>Dactylis glomerata</i>	cocksfoot	1.02	603
LSD ($P=0.05$)		0.06	45

Trifolium hirtum was also notable in that it had the longest root hairs and highest SRL amongst the *Trifolium* species. It was surmised that the root traits of these species might confer an advantage for P acquisition efficiency relative to *T. subterraneum*. The growth of these five species in response to P was compared using *D. glomerata* as a benchmark species with a low critical P requirement.

Shoot growth of all species increased in response to addition of P. *Dactylis glomerata* had the highest maximum yield and lowest critical external P requirement (Table 2). The *Ornithopus* spp. yielded as well as *T. subterraneum* but achieved this with less than half the amount of applied P (Table 2). *Biserrula pelecinus* and *T. hirtum* had critical external P requirements that were intermediate to that of *T. subterraneum* but also had significantly lower yields.

Table 2. Critical external P requirement and parameters for a Mitscherlich curve [$y = A - B \cdot (e^{-Cx})$] in response to addition of P for five legumes and one grass species. Different lowercase letters denote significant differences ($P < 0.05$; $n = 5$).

Species	Critical external P (mg P/pot)	Intercept Yield at P0 (g/pot)	A Max. yield (g/pot)	B	C
<i>Dactylis glomerata</i>	$6.6 \pm 0.6a$	$2.22 \pm 0.07a$	$3.69 \pm 0.03a$	-1.47 ± 0.06	0.811 ± 0.017
<i>Ornithopus compressus</i>	$7.6 \pm 0.5a$	$1.12 \pm 0.05b$	$2.87 \pm 0.03b$	-1.76 ± 0.06	0.788 ± 0.016
<i>Ornithopus sativus</i>	$11.3 \pm 0.5b$	$0.83 \pm 0.04c$	$2.70 \pm 0.03c$	-1.87 ± 0.06	0.841 ± 0.011
<i>Trifolium subterraneum</i>	$26.7 \pm 1.3c$	$0.41 \pm 0.05d$	$2.68 \pm 0.05c$	-2.27 ± 0.06	0.923 ± 0.005
<i>Biserrula pelecinus</i>	$17.3 \pm 1.0d$	$0.36 \pm 0.05d$	$2.04 \pm 0.04e$	-1.69 ± 0.06	0.885 ± 0.010
<i>Trifolium hirtum</i>	$21.1 \pm 1.5e$	$0.36 \pm 0.06d$	$2.03 \pm 0.04e$	-1.67 ± 0.06	0.905 ± 0.008

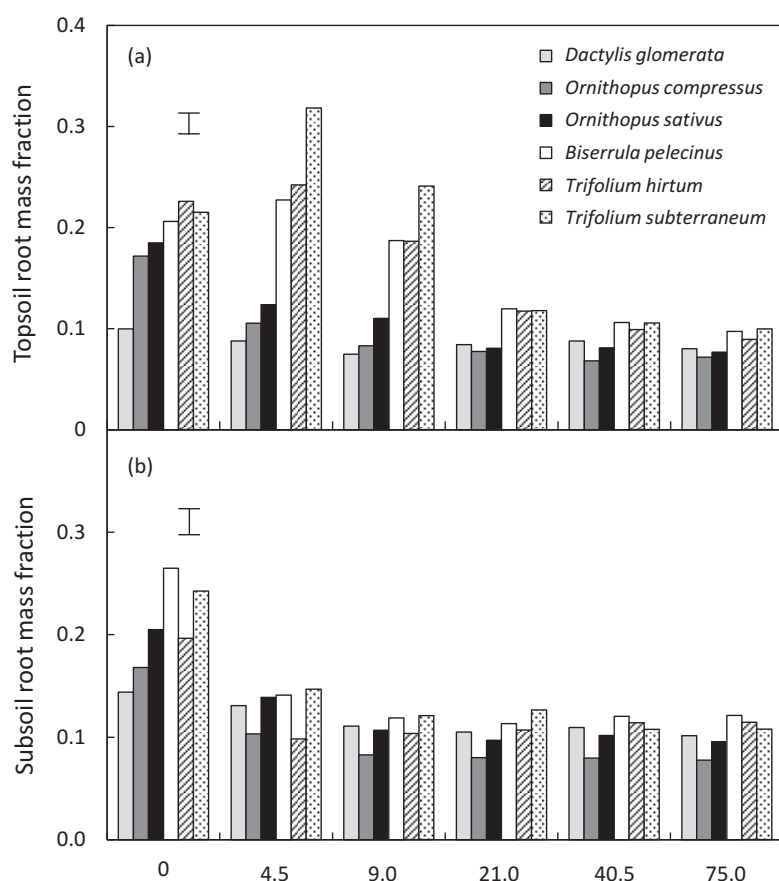
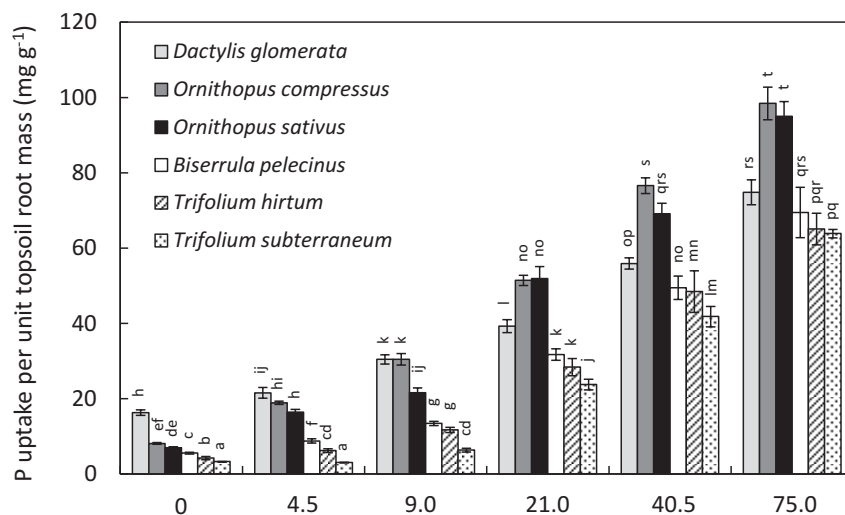


Figure 1. Root mass fraction for (a) topsoil and (b) subsoil roots for five legumes and one grass grown at six rates of P applied to the topsoil. Bar shows LSD for two-way interaction ($P < 0.05$; $n = 5$).

All species increased their proportional allocation of dry matter to roots (i.e. root mass fraction [RMF]) in response to decreasing supply of P; the effect was most pronounced in the P-amended topsoil (Figure 1). Species with higher critical external requirements for P tended to allocate proportionately more biomass to roots and demonstrated the largest increases in RMF in response to low P supply. Increases in RMF were most pronounced at levels of P supply at, or just below the critical external P requirement of each species.



P uptake per unit root mass in the fertilised topsoil increased in response to addition of P (Figure 2). The *Ornithopus* spp. generally had higher P uptake per unit root mass than the other legumes.

Figure 2. P uptake per unit mass of roots in the P-fertilised topsoil for five legumes and one grass grown at six rates of P applied to the topsoil. Different letters denote differences for two-way interaction ($P < 0.05$; $n = 5$).

Discussion

Trifolium subterraneum and *M. sativa* have short root hairs and low SRL relative to some of the grasses with which they are commonly grown. This limits the ability of these species to forage for P and contributes to their high external P requirement. However, significant variation in root traits associated with nutrient foraging was found among a range of alternative legumes that are being developed for temperate pastures in Australia. Most notably, *O. compressus*, *O. sativus* and *B. pelecinus* had RHLs and SRLs approaching that of *D. glomerata* and *P. aquatica*.

Ornithopus compressus and *O. sativus* had a critical external requirement more similar to that of *D. glomerata* but yielded as well as *T. subterraneum*. These species achieved this despite allocating proportionately less biomass to roots in response to low P supply. Relatively high rates of P uptake per unit root mass in the *Ornithopus* spp. supported the hypothesis that long root hairs and high SRL allows these species to efficiently explore soil with concomitant advantages for P acquisition that contribute to their low critical external requirement for P.

Conclusion

Selecting legumes that can maximise nutrient foraging via long, thin roots with long root hairs may reduce the critical P requirement of pastures.

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