Growth and root dry matter allocation by pasture legumes and a grass with contrasting external critical phosphorus requirements

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Abstract

Background and Aims: This work aimed to quantify the critical external requirement for phosphorus (P) (i.e. external P concentration required for 90% of maximum yield) for a number of temperate legume species and understand differences in dry matter allocation, P distribution and P acquisition efficiency among these species.

Methods: Shoot and root growth of five legume and one grass species was assessed in response to six rates of P mixed into the top 45 mm of soil in a pot experiment. *Dactylis glomerata* and *Trifolium subterraneum* were used as benchmark species; they are commonly grown together in mixed temperate pastures and have low and high critical external requirements for P, respectively. Growth was compared with four potential alternative legume species: *Ornithopus compressus*, *Ornithopus sativus*, *Biserrula pelecinus* and *Trifolium hirtum*, that have root morphologies better suited to soil exploration and nutrient acquisition than that of *Trifolium subterraneum*.

Results: *Dactylis glomerata*, *Ornithopus compressus* and *Ornithopus sativus* had maximum yields equal to or greater than *Trifolium subterraneum* but achieved this at rates of P less than half that of *Trifolium subterraneum*. *Biserrula pelecinus* and *Trifolium hirtum* had critical P requirements between that of *Trifolium subterraneum* and the *Ornithopus* species, but also had lower yields. Root dry matter of *Dactylis glomerata* and the *Ornithopus* species in the fertilised soil layer was only marginally changed in response to low P supply. In contrast, *Trifolium subterraneum*, *Trifolium hirtum* and to a lesser extent *Biserrula pelecinus* markedly increased root dry matter allocation to this soil layer. Species with lower critical P requirements were able to take up more P per unit root dry mass than those with higher critical P requirements, particularly at lower levels of P addition.

Conclusions: The high P acquisition efficiencies of the *Ornithopus* species and *Dactylis glomerata* were likely to have contributed to their low critical external P requirements. It was surmised that differences in root morphology traits underpin the differences in acclimation to low P stress and P acquisition efficiency among the species.

Key words: root mass fraction; phosphorus deficiency; subterranean clover; serradella; rose clover; cocksfoot

Introduction

It is known that the grasses and the legume component (commonly *Trifolium* or *Medicago spp*.) of temperate mixed pastures have very different critical phosphorus (P) requirements (here defined as the concentration of extractable-P in soil, or the amount of P applied for 90% of maximum growth rate) (Mengel et al. 2001). The grass species achieve near maximum growth at much lower extractable-P concentrations in the soil than the associated legume (Ozanne et al. 1969; 1976; Hill et al. 2006). This is attributed to grasses having roots that can explore soil more effectively (nutrient foraging). In particular, temperate grasses have long, fine roots and long root hairs that enable development of relatively large specific root hair cylinder volumes (i.e. the soil volume defined by the root diameter and root hair length per unit root dry mass; Evans 1977; Horst et al. 1993; Gahoonia and Nielsen 1997; 2004; Hill et al. 2006). The grasses also have lower critical internal P concentrations (i.e. the concentration of P in herbage at near-maximum growth rate) (Pinkerton and Randall 1994).

Grass-legume pastures in Australia rely on legume nitrogen (N) fixation as the sole source of N input. The disparity in the P requirement of the grasses and legumes means that the high P requirement of the legume determines the P fertiliser application rate for high pasture production. However, the P use efficiency of the pasture systems is low because P accumulates in Australian soils as a result of their high propensity for P sorption (Simpson et al. 2014; 2015). Pastures used for sheep and beef production, for example, require 5–9 units of P to be applied as fertiliser for an output of 1 unit of P exported in animal products (Weaver and Wong 2011). However, long-term monitoring of the P balance of grazed pasture systems has shown that lower P inputs will be achieved if pastures can be managed at lower concentrations of extractable P in the soil without reducing production (Simpson et al. 2014; 2015). Legumes that are as productive as current mainstream pasture legume species (e.g. *T. subterraneum; T. repens*) at lower soil test P concentrations are needed to achieve this.

There have been many studies of the P requirement of *Trifolium* spp., most of which have demonstrated only marginal differences amongst genotypes; where differences are reported, interpretation has sometimes been confounded by differences in yield and ontogeny (McKell et al. 1962; Jones et al. 1970). Attempts to select for improved P acquisition efficiency and/

or productivity under low P conditions have been relatively unsuccessful. For example, while significant genotypic variation in root morphology traits, and/ or response to P, has been identified within *T. repens* (Caradus 1981; Crush et al. 2008; Jahufer et al. 2008), the value of this for improving the productivity of *T. repens* under low P conditions in the field has not been fully realised (e.g. Caradus 1994; Caradus and Dunn 2000).

Temperate pastures in southern Australia rely heavily on the use of three main groups of pasture legumes: *T. subterraneum* (acid soils, temperate southern Australia), *T. repens* (high rainfall, temperate zone) or annual *Medicago* spp. (low rainfall, neutral-alkaline soils). However, over several decades Australia has invested in the development of "novel" legumes, through breeding and ecotype selection, to fill soil or environmental niches not already covered by the main pasture legumes and, in some instances, as potential alternatives to the mainstream species (Nichols et al. 2007). The P requirements of the alternative legumes are largely unknown although it is reported that *Ornithopus compressus* can yield as well as *T. subterraneum* in the sandy soils of Western Australia with about half the amount of applied P (Paynter 1990). The features of grass root systems and of other species that are likely to enable plants to achieve high yield at low critical P levels include high specific root length and long root hairs (Lynch 2007; Evans 1977). Yang et al. (2015) have reported that some of the novel pasture legumes have high specific root lengths, long root hairs and root hair cylinder volumes approaching that of some grasses. This may confer improved potential for P acquisition in low P soil and lower critical external requirements for P than is achieved by the mainstream legume species.

Here we report the first study of the response to P fertiliser by five alternative pasture legumes and a companion grass species and assess their root dry matter allocation, internal P distribution and P acquisition efficiency in response to P application.

Materials and Methods

Plant material

Five annual pasture legumes and one perennial grass were selected on the basis of differences in their root morphology traits (Yang et al. 2015) to represent a range in potential for nutrient foraging capability. Two of the species are commonly grown as companions in temperate

pastures. *Trifolium subterraneum* L. (subterranean clover cv. Leura), is a cultivar of the most widely-used legume in the pastures of southern Australia. It has relatively low specific root length (171 m g^{-1}) and short root hairs (0.23 mm). *Dactylis glomerata* L. (cocksfoot cv. Porto) is often grown in pastures with *T. subterraneum.* It is reputed to grow well in infertile soils (Lolicato and Rumball 1994) and has a high specific root length (603 m g^{-1}) and long root hairs (1.10 mm). The other legumes used in this experiment are presently considered as potential alternatives to *T. subterraneum* for use in temperate pastures (Nichols et al. 2007). *Biserrula pelecinus* L. (biserrula cv. Mauro) has high specific root length (299 m g^{-1}) and intermediate root hair length (0.56 mm); *Ornithopus sativus* Brot. (French serradella cv. Margurita) has a high specific root length (320 m g^{-1}) and long root hairs (0.73 mm); *Ornithopus compressus* L. (yellow serradella cv. Santorini) has high specific root length (307 m g^{-1}) and long root hairs (0.75 mm) and *Trifolium hirtum* All. (rose clover cv. Hykon) has high specific root length (290 m g^{-1}) and short root hairs (0.37 mm) (Yang et al. 2015).

Soil and nutrient treatments

A sandy loam soil (Yellow Chromosol; Isbell 1996) with a low concentration of extractable P (8.3 mg kg^{-1} P; Colwell, 1963) was collected from Ginninderra Experiment Station, Canberra, ACT, Australia (35°10'30"S, 149°02'33.4"E). The soil was steam pasteurised (60-65°C) to reduce levels of disease inoculum, sieved to < 5 mm and mixed with lime (1.06 g $CaCO_3$ kg^{-1}) to raise pH (1:5 w/v; 0.01M $CaCl_2$) to 5.5 and lower the concentrations of Al^{3+} to negligible levels. Nutrients were then mixed into the soil at rates of 41.1 mg kg^{-1} soil $MgSO_4.7H_2O$, 43.0 mg kg^{-1} $CaSO_4.2H_2O$, 169 mg kg^{-1} KNO_3, 27.5 mg kg^{-1} $(NH_4)_2SO_4$, 16.7 mg kg^{-1} NH_4NO_3, 119 µg kg^{-1} H_3BO_3, 759 µg kg^{-1} $MnCl_2.4H_2O$, 359 µg kg^{-1} $ZnSO_4.7H_2O$, 33.3 µg kg^{-1} $CuSO_4.5H_2O$, 72.1 µg kg^{-1} $(NH_4)_2MoO_4$, 19.8 µg kg^{-1} $CoCl_2 \cdot 6H_2O$ and 1530 µg kg^{-1} Fe-EDTA. These nutrient additions ensured that all nutrients except P were in adequate supply. Six P-fertilised soil treatments were established by mixing KH_2PO_4 with sub-samples of the amended soil at rates of 0, 15, 30, 70, 135 and 250 mg P kg^{-1} soil. This resulted in extractable P concentrations of 8.3, 19.2, 30.9, 58.4, 111 and 203 mg kg^{-1} (Colwell 1963). Pots (cylindrical PVC; 87 mm internal diameter, 190 mm soil height) were filled with a bottom layer of the soil (1.00 kg; 11% moisture) that was not fertilised with P (the subsoil) and then with a topsoil layer of P-fertilised soil (0.333 kg; 11% moisture). The resultant P application rates were 0, 4.5, 9.0, 21.0, 40.5 and 75.0 mg P pot^{-1}. The mixing of phosphate throughout the topsoil layer of the pots mimicked the stratified concentration of P in fields

that results from application of P fertiliser to the soil surface of grazing lands. The boundary of the fertilised topsoil and unfertilised subsoil layers was marked by placing small alkathene beads around the interior edge of the pot. To ensure that the grass had an adequate supply of N for growth, an additional 21 mg N pot^{-1} (supplied as NH_4NO_3) was applied to the *D. glomerata* plants at 32 days after sowing. Further N was not applied to the legumes in order to promote N_2-fixation.

Plant growth conditions and experimental design

Seed (50 mg viable seed pot^{-1}) of each species was spread evenly across the pot surface area and covered with the topsoil to achieve a 5 mm sowing depth. Five replicate pots of each species at each rate of applied P were prepared. Plants were grown in a controlled-environment growth cabinet with 12 hours of light (720 µmol $quanta^{-1}$ m^2 s^{-1}) and 12 hours dark, at 25/15°C, respectively. Pots were arranged in a randomised complete block design and rotated within blocks every 3 to 7 days to minimise the effect of light gradients within the cabinet. When plant growth exceeded the rim of the pot, sleeves with a reflective inner surface were fitted to the outside of the pots and raised daily to equal plant height. This was used to reproduce light conditions in a pasture sward. Soil moisture was maintained at approximately 75 to 80% of field capacity by daily weighing and watering of pots. Seven days after sowing, the legumes were inoculated with rhizobium; Group C for *Trifolium* spp., Group S for *Ornithopus* spp. and Biserrula special for *B. pelecinus*.

Harvest and measurements

Plants were harvested six weeks after sowing when still in the vegetative growth stage (40 days after sowing for *T. hirtum*; 41 days for *B. pelecinus*, *O. sativus* and *T. subterraneum*; 42 days for *D. glomerata* and *O. compressus*). Shoots were cut at the surface of the soil and dried at 70°C for dry mass determination. Soil was removed from the pots as an intact core and cut at the interface of the fertilised topsoil (0-45 mm depth) and the subsoil (45-190 mm depth) as identified by the alkathene beads. Roots from each layer were washed from the soil. A sub-sample of each was weighed fresh and stored in 50% ethanol at 4°C for subsequent measurements of root morphology (not reported here). An additional sub-sample was weighed fresh and immediately dried to facilitate calculation of a correction factor to account for loss of root mass during storage in the ethanol solution (Crush et al. 2010). The total dry mass of roots in each soil layer was determined after drying all samples at 70°C. The mass of the sub-sample of roots stored in 50% ethanol was corrected to allow for loss of mass (Root

DM = $DM_{[after\ alcohol\ storage]}$ * correction factor) using the following correction factors determined for each species and, where necessary, for an effect of P application: (*O. sativus* 1.06; *O. compressus* 1.13; *D. glomerata* 1.13; *T. subterraneum* 1.15; *T. hirtum* 1.32; *B. pelecinus* 1.64 at 0 mg P kg^{-1} and 1.27 for 15–250 mg P kg^{-1}).

Shoots and root samples that had not been stored in ethanol were milled to a fine powder and 25 to 50 mg samples ashed in a muffle furnace for 4 hours at 550°C. The ashed material was dissolved in 2M HCl and P concentration determined colorimetrically using malachite green (Irving and McLaughlin 1990).

Statistical analysis

For each species, the yield of shoot dry matter growth in response to P application was analysed by fitting a Mitscherlich non-linear curve (Equation 1) in R (R Core Team, 2013).

$$y = a - b*(e^{-cx}) \quad [1]$$

where y is the shoot dry matter and x is the P application rate.

The critical external P requirement of each species was defined as the amount of P applied to achieve 90% of maximum yield. Estimates and confidence intervals for critical P and maximum yield (a) were determined by least squares and assume that the model is approximately linear around the estimate. Estimates and confidence intervals for other parameters, including the shoot yield at no P addition to the soil, were obtained by re-parameterising the model to the value of the key parameter using R and GenStat 16th Edition (VSN International, UK). Differences between the critical P concentrations, the maximum yield (or asymptote) and the zero P yields were tested by considering the estimates and approximate standard errors for each measure simultaneously, and testing for significant pairwise differences. Significance was determined by calculating a standardised difference that weighted the two contributing standard errors. Values greater than two standard errors were considered significantly different (P = 0.05). No adjustment was made for multiple comparisons.

For each species, critical internal P concentration was determined as the herbage P concentration corresponding to the critical external P application rate based on curvilinear responses between shoot P concentration and rate of P addition (Hill et al. 2005). Internal P-use efficiency (PUE; g DM/ g shoot P) was calculated using two alternative approaches.

"Physiological PUE" was determined as: (shoot yield at 90% maximum yield - shoot yield at 0 mg P pot^{-1}) divided by (total shoot P at 90% maximum shoot yield - total shoot P at 0 mg P pot^{-1}) (Baligar et al. 2001). Based on the Rose et al. (2015) definition of PUE, the reciprocal of shoot P concentration of each species was also determined at a common shoot P content ("PUE at 6.2 mg shoot P"). Linear relationships between ln(shoot P) and PUE (calculated as above) were fitted in GenStat 16th Edition (VSN International, UK) and PUE was predicted at ln(6.2 mg shoot P per pot). This was the lowest shoot P content that allowed all species to be compared at a common shoot P content in the P application range below the critical P requirement of all species. Propagation of errors for critical Shoot P and PUE were performed using the Delta method (Agresti 2002).

Root mass fractions were calculated separately as the mass of roots in the topsoil, subsoil or total root system divided by the total plant mass. P uptake per unit topsoil root mass was calculated as the total plant P (i.e. P in topsoil roots, subsoil roots and shoots) divided by the mass of roots in the topsoil. Relative shoot yield was calculated as: shoot yield at the given level of P pot^{-1} divided by the maximum potential shoot yield determined from the asymptote of Equation 1.

The effect of P addition on shoot dry matter, root mass fraction and P uptake per unit root mass in the topsoil layer of the six species was analysed using general analysis of variance in GenStat 16th Edition (VSN International, UK). A split-plot analysis of variance with Species and P addition as whole-plots and Soil Depth as split-plots was used to analyse root dry matter. Likewise, a split-plot analysis of variance with Plant Part as the sub-plot was used to analyse the P concentration of the roots in the topsoil and subsoil, and the shoots.

Simple linear regression with groups in GenStat 16th Edition (VSN International, UK) was used to fit regressions for the range over which the response between relative shoot yield (considered an indicator of P sufficiency) and topsoil root mass fraction was linear, and to assess differences in intercept and gradient of the regressions between species.

Results

Shoot dry matter response and critical external P requirement

Shoot dry matter of all species increased in response to addition of P, however, the initial slope of the P response function (c in Equation 1) and the maximum shoot yield (a in Equation 1) varied among species (Fig. 1 and Table 1). *Dactylis glomerata* had the highest maximum shoot yield (3.69 g pot^{-1}), almost double that of the lowest yielding species (*B. pelecinus* and *T. hirtum*; Fig. 1, Table 1). *Ornithopus compressus*, *O. sativus* and *T. subterraneum* had maximum yields that were intermediate to those of *D. glomerata* and *B. pelecinus* or *T. hirtum*.

Dactylis glomerata had the lowest critical external P requirement and *T. subterraneum* had the highest critical external P requirement; the latter was significantly higher than all other species (Table 1). *Ornithopus compressus* and *O. sativus* had relatively low critical external P requirements more comparable to that of the. The critical external P requirement of *O. compressus* did not differ significantly from that of *D. glomerata*. The critical external P requirements of *B. pelecinus* and *T. hirtum* were between that of *T. subterraneum* and the *Ornithopus* spp.

Root dry matter

The legumes allocated a similar mass of roots to the fertilised topsoil layer (0-45 mm depth) and the unfertilised subsoil layer (45-190 mm depth) at the higher rates of P application (Fig. 2). Some of the legumes made adjustments to root dry matter allocation in response to low P supply. Where this occurred (i.e. at or below the critical P application rate), more root dry matter was allocated to the fertilised topsoil than the unfertilised subsoil. In contrast to the legumes, *D. glomerata* allocated more dry matter to the subsoil layer at all P application rates.

Trifolium subterraneum, *B. pelecinus* and *T. hirtum* produced 1.3–1.6 fold more root dry matter in the P-fertilised topsoil when grown at soil P fertility levels immediately below their critical P level (9–21 mg kg^{-1}; Fig. 2). However, these species were not able to achieve this dry mass allocation at P application rates <4.5 mg P pot^{-1} and their root dry mass in the unfertilised soil was significantly less than that at all other rates of P addition.

Ornithopus sativus only marginally adjusted root dry mass in the topsoil in response to P supply below its critical P level; root dry matter of *O. sativus* peaked at P addition of 9 mg pot^{-1} (Fig. 2). *Ornithopus compressus* did not adjust root dry mass at P levels below its

critical P level. In contrast to the legumes, root dry mass of the grass was decreased in response to lower supply of P; root dry mass of *D. glomerata* grown at 0 mg P pot^{-1} was 75% of the maximum root dry mass achieved at ≥ 21 mg P pot^{-1}.

In the subsoil, the root dry mass of all species, except *O. compressus*, was lowest in the unfertilised treatment. *Ornithopus compressus* did not adjust its root mass and the reductions in root mass by *O. sativus* and *D. glomerata* were relatively small. The species with higher critical P requirements, *T. subterraneum*, *B. pelecinus* and *T. hirtum*, achieved subsoil root dry masses that were only about 50% of their maximum root mass in the fertilised topsoil. For these species, lower root dry mass occurred when P supply rates were less than the critical P level for the species.

Root mass fraction

All species increased the proportional allocation of biomass to their roots (i.e. total root mass fraction) in response to lower soil P fertility (Fig. 3a). Adjustments in root mass fraction occurred regardless of whether or not the species had increased root dry matter in the fertilised topsoil layer in response to lower soil P fertility (Fig. 2). For example, *D. glomerata* allocated less dry matter to roots at the lowest level of P fertility (Fig. 2f) but root mass fraction was nevertheless increased at this level of P fertility.

Generally, species that had lower critical external P requirements (Table 1), had lower overall relative allocation of biomass to roots compared to the species with higher critical external P requirements. For example, relative allocation of biomass to roots ranged from 15 to 34% (over the tested range of P addition) for *O. compressus* (low external P requirement), compared to 21 to 46% for *T. subterraneum* (high external P requirement). Despite these differences, all legumes more than doubled their relative allocation of biomass to roots in response to P stress while that of the grass increased by only a third.

The root mass fraction of roots in the topsoil differed considerably from that in the subsoil (Fig. 3b and 3c, respectively). The overall adjustment the species made in total root mass fraction (Fig. 3a) was largely the result of adjustments to biomass allocation in the fertilised topsoil. Adjustment to root mass fraction in the subsoil only occurred when no P fertiliser had been applied to the topsoil resulting in a soil profile with a uniformly low extractable P concentration at all depths. Under this circumstance, adjustment to root mass fraction in the

subsoil was similar to that occurring in the topsoil. Regressing root mass fraction against relative shoot yield (an index of P sufficiency) demonstrated that all of the species had adjusted root mass fraction in response to P stress (Fig. 4). The total root mass allocation response to P stress (i.e. the gradient of the total root mass fraction-relative shoot yield relationship) was similar for most of the species, but significantly lower allocation responses (i.e. the gradients) were observed for *T. hirtum* and *D. glomerata* (Fig. 4a). By contrast, the root mass allocation response to P stress in the fertilised topsoil layer differed substantially among the species with *T. subterraneum* > *T. hirtum* = *B. pelecinus* > *Onithopus species* > *D. glomerata* (Fig. 4b). The root mass fraction allocated to the topsoil layer was expected to represent the roots that were most involved in nutrient foraging.

Tissue P concentration, critical internal P requirement and internal P use efficiency

Shoot P concentration, topsoil root P concentration and subsoil root P concentration of all species increased with higher rates of P application (Fig. 5). The concentration of P in topsoil roots, subsoil roots and shoots was generally comparable for each legume grown in soil at rates of P application below its critical external P requirement. Above the critical external P requirement of each legume species, the P concentration of topsoil roots increased to a greater extent than that of the shoot or subsoil root P concentration. For example, above 9 mg P pot^{-1}, the shoot P concentration of *O. compressus* increased from 4.0 to 7.1 g kg^{-1} of dry matter, compared to 5.0 to 9.0 g kg^{-1} for topsoil roots and 2.6 to 3.8 g kg^{-1} for subsoil roots. *Dactylis glomerata* differed from the legumes in that the P concentration of the shoot was greater than that of topsoil and subsoil roots. In contrast to the legumes, the P concentration of the roots in each layer was similar.

Insets to Fig. 5 and associated regressions (Table 2) demonstrate that a disproportionate increase in the P concentration of topsoil roots of *T. subterraneum* and *T. hirtum* occurred when P application exceeded the critical P requirement of these species. In contrast, the shoot P concentration of all other species increased linearly and was correlated with the increase observed for root P concentration. In contrast to the legumes, the P concentrations of *D. glomerata* roots in each layer were similar, even at rates above the critical external P requirement.

Curvilinear fits (Table 3) between rate of P addition and shoot P concentration (Fig. 5) were used to determine the internal P concentration of shoot dry matter for the six species that corresponded with their critical external P requirement (Table 1). The critical internal shoot P concentration of the species determined by this method ranged from 2.3 g kg^{-1} for *D. glomerata* to 3.5 g kg^{-1} for *B. pelecinus*. Most of the legumes had a similar critical internal P concentration (range 3.0 to 3.5) in shoot dry mass. However, the critical internal P requirement for *O. compressus* was lower and more similar to that of the grass. Physiological PUE calculated at 90% relative yield was similar among all species (250-267 g dry mass g^{-1} P), except for *O. compressus*, which was higher (327 g dry mass g^{-1} P; Table 3). When PUE was compared at a common amount of shoot P content (in this case, 6.2 mg shoot P pot^{-1}), *D. glomerata* had the highest PUE (350 g dry mass g^{-1} P) and *B. pelecinus* and *T. hirtum* the lowest PUE (248 and 254 g dry mass g^{-1} P, respectively).

P uptake per unit mass of the roots in the topsoil

P uptake per unit mass of roots in the topsoil increased in response to increasing supply of P (Fig. 6). Species that had lower critical external P requirements had larger P uptake per unit mass of root, with differences most pronounced at the lower levels of P addition (i.e. below the critical external P requirement for each of the species). For example, at 4.5 mg P pot^{-1}, P uptake per unit root mass of *O. compressus* (18.9 mg P g^{-1} root) was 6-fold larger than that of *T. subterraneum* (3.0 P g^{-1} root). At 75 mg P pot^{-1}, P uptake per unit root mass of both species was larger again but the difference between the two species was less pronounced (1.5-fold).

Relationship between topsoil root dry mass allocation, topsoil P uptake per unit root dry mass and nutrient foraging potential

Topsoil root mass allocation responses (i.e. the gradients in Fig. 4b) were negatively correlated with previously reported estimates (Yang et al. 2015) of the specific root hair cylinder volumes of the species (Fig. 7a). Topsoil P uptake per unit root dry mass was positively correlated with these estimates of specific root hair cylinder volumes (Fig. 7b).

Discussion

Dry matter partitioning and root morphology acclimation by plants growing in P deficient soil

Increased proportional allocation of plant dry matter to the root system is a general response to nutrient deficiency (Brouwer 1962) and an acclimation that particularly assists continued acquisition of a relatively immobile nutrient such as P (Lynch and Brown 2001; Nielsen et al. 2001). This response is typically characterised by proliferation of roots in relatively concentrated zones of P in an otherwise P deficient soil and is often observed in surface soils due to P-enrichment from cycling of organic material or application of fertilisers (Drew et al. 1975; Manske et al. 2000; Lynch and Brown 2001). In the present experiment, acclimation of roots to P-deficiency essentially only occurred in the P-enriched layer of the soil profile. For this reason, assessments of the allocation of root biomass in response to P stress, and P uptake per unit root dry mass could be considered using root growth in the P-enriched layer alone.

When relative shoot yield was used as an index of the level of "P sufficiency" experienced by each species it was apparent that increased allocation of biomass to roots in the fertilised soil was triggered when P supply began to reduce yield (i.e. at or near the critical P level of all of the species) and as a response to P stress. Large differences in the acclimation "effort" made by each species were apparent. The largest adjustments in root mass fraction were made by the species with relatively high critical P requirements and less by the species with low critical P requirements. This indicated that a high rate of root mass fraction adjustment alone was not a particularly effective way to achieve a low critical P requirement and may have been more a consequence of P starvation, than it being a strategic acclimation to low P soil.

Internal P distribution, critical internal P concentration and internal P use efficiency

The ability of plants to more efficiently distribute and utilise P internally could also contribute to a lower external P requirement. With the exception of *O. compressus*, the legumes had very similar critical internal P concentrations and physiological PUE based on

the method of Baligar et al. (2001). However, these rankings were not consistent with those calculated using the method of Rose et al. (2015 i.e. PUE at 6.2 mg shoot P). Nevertheless, PUE did not appear to be an overriding influence on the critical P requirement of the legumes. *Trifolium hirtum* has been reported to have a low critical shoot P concentration (Pinkerton et al. 1997) but, it was not different to *T. subterraneum* in the present study.

All legumes developed higher P concentrations in roots directly exposed to the fertilised soil. In contrast, the P concentration of roots of *D. glomerata* were similar in the fertilised topsoil and unfertilised subsoil suggesting a greater capacity for translocation of P to roots exploring low P soil. *Dactylis glomerata* also had a lower critical internal shoot P concentration than most of the legumes.

Root P concentration of most species was linearly correlated with shoot P concentration. However, both *Trifolium* spp. were exceptions in that the P concentration of roots exposed to the fertilised topsoil accumulated P at an increasing rate relative to shoot P concentration suggesting less capacity of their roots to regulate P uptake when exposed to luxury P supply. It may be relevant that *T. subterraneum* is susceptible to P toxicity (Rossiter 1952; Greenwood and Hallsworth 1960; Kim et al. 1985; Culvenor et al. 1989).

Efficiency of P acquisition

In the present study, significant differences in the critical external P requirement were measured amongst the species examined. Species that had a lower external critical P requirement were found to allocate less dry matter to the fertilised soil but absorbed more P per unit root mass than those with a higher critical P requirement. This was particularly evident at low levels of P addition below the critical external P requirement.

Large differences in the root morphology traits associated with nutrient foraging have been reported previously for these species (Yang et al. 2015). The *Ornithopus* spp. and *Dactylis glomerata* had long root hairs and high specific root length, which conferred a high specific root hair cylinder volume (746 and 2314 cm^3 g^{-1}, respectively) i.e. root hair cylinder volume per unit root mass, and high potential for nutrient foraging. In contrast, *T. subterraneum* had short root hairs, low specific root length and a low specific root hair cylinder volume (78 cm^3 g^{-1}). Specific root hair cylinder volumes of *T. hirtum* and *B. pelecinus* were intermediate (177 and 444 cm^3 g^{-1}, respectively). Essentially all of the acclimation to P stress by the species

occurred in the P-enriched topsoil layer. Differences in the rate of adjustment in topsoil root mass fraction to P stress were negatively correlated with the potential specific root hair cylinder volumes (Yang et al. 2015) of the species (Fig. 7a). The results suggest that root morphology differences among the species influenced how much root dry mass was allocated to nutrient foraging. In contrast, P acquisition efficiency (P uptake per unit root dry mass when plants are below their critical P requirement) was positively correlated with the potential specific root hair cylinder volumes. Thus, root morphology differences appear to also be a major factor determining the effectiveness (for P uptake) of the root acclimation response to P stress. Consequently, we surmise that differences in root morphology must also be a significant factor in achieving a low critical external P requirement. Direct assessment of root morphology under simular P stress conditions is needed to confirm this hypothesis and to examine whether other nutrient acquisition traits (e.g. release of P-solubilising exudates; mycorrhiza) have also contributed to the differences among these species in their capacity for P acquisition.

Implications for improving P efficiency of grass-legume pasture systems

O. compressus and *O. sativus* showed the greatest potential to improve P efficiency in pasture systems. Their low critical external P requirements suggest that use of these species, as alternatives to *T. subterraneum* or other similar legumes (e.g. *Trifolium repens*, annual *Medicago* spp.), could reduce P fertiliser application rates to levels that are closer to the requirements of temperate grasses. The critical external P requirements and P uptake per unit dry mass of roots of *B. pelecinus* and *T. hirtum* were also more favourable than that of *T. subterraneum*, but interpretation of their apparent P efficiency was confounded by the fact that they had substantially lower shoot yields.

Conclusions

Species that had a lower external critical P requirement allocated less dry matter to the fertilised soil but absorbed more P per unit root mass than those with a higher critical P requirement. This suggested that differences in their root morphology traits were likely to be a major factor contributing to P acquisition efficiency and, consequently, differences in the critical P requirement among these species.

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Table 1 Critical external phosphorus (P) requirement (mg P pot^{-1}) for five legume and one grass species determined as the rate of P required to achieve 90% of maximum shoot yield from a fitted Mitscherlich response ($y = a - b*(e^{-cx})$). Values ± standard error. Different letters denote significant differences within each column. Intercept reflects yield with no applied P i.e. a-b.

	Critical external P	Parameter			
Species	**(mg P pot^{-1})**	**Intercept**	**a**	**b**	**c**
Dactylis glomerata	6.6 ± 0.6a	2.22 ± 0.07a	3.69 ± 0.03a	1.47 ± 0.06	0.811 ± 0.017
Ornithopus compressus	7.6 ± 0.5a	1.12 ± 0.05b	2.87 ± 0.03b	1.76 ± 0.06	0.788 ± 0.016
Ornithopus sativus	11.3 ± 0.5b	0.83 ± 0.04c	2.70 ± 0.03c	1.87 ± 0.06	0.841 ± 0.011
Trifolium subterraneum	26.7 ± 1.3c	0.41 ± 0.05d	2.68 ± 0.05c	2.27 ± 0.06	0.923 ± 0.005
Biserrula pelecinus	17.3 ± 1.0d	0.36 ± 0.05d	2.04 ± 0.04e	1.69 ± 0.06	0.885 ± 0.010
Trifolium hirtum	21.1 ± 1.5e	0.36 ± 0.06d	2.03 ± 0.04e	1.67 ± 0.06	0.905 ± 0.008

Table 2 Relationship between shoot phosphorus (P) concentration (x) and root P concentration (y) for five legumes and one grass species grown at six levels of P supply (n=5).

Species	Topsoil fit	R^2	Subsoil fit	R^2
Dactylis glomerata	y = 0.66x - 0.02	0.97	y = 0.36x + 0.08	0.96
Ornithopus compressus	y = 1.5x - 0.10	0.95	y = 0.45x + 0.08	0.92
Ornithopus sativus	y = 1.40x - 0.10	0.96	y = 0.50x – 0.0010	0.92
Trifolium subterraneum	$y = 4.25x^2 - 0.71x + 0.14$	0.97	y = 0.70x + 0.04	0.94
Biserrula pelecinus	y = 1.28x - 0.11	0.93	y = 0.63x + 0.04	0.93
Trifolium hirtum	$y = 2.44x^2 + 0.32x + 0.06$	0.96	y = 0.58x + 0.06	0.96

Table 3 Critical internal phosphorus (P) concentration and internal P-use efficiency assessed by alternative approaches for five legume and one grass species based on a polynomial fit between P applied per pot (x) and shoot P concentration (y). Values ± standard error. Different letters denote significant differences.

Species	Critical internal P ($g\ kg^{-1}$)	Physiological PUE[1] ($g\ DW\ g^{-1}\ P$)	PUE at 6.2 mg shoot P[2] ($g\ DW\ g^{-1}\ P$)	Polynomial fit	R^2
Dactylis glomerata	2.3 ± 0.1	263 ± 27	350 ± 14a	$y = -7E\text{-}04x^2 + 0.117x + 1.554$	0.993
Ornithopus compressus	2.4 ± 0.1	327 ±19	313 ± 11bc	$y= -6E\text{-}04x^2 + 0.118x + 1.535$	0.995
Ornithopus sativus	3.0 ± 0.2	267 ± 19	296 ± 10b	$y = -8E\text{-}04x^2 + 0.140x + 1.520$	0.995
Trifolium subterraneum	3.3 ± 0.2	258 ± 17	327 ± 9ac	$y = -7E\text{-}04x^2 + 0.126x + 0.440$	0.996
Biserrula pelecinus	3.5 ± 0.2	250 ± 18	248 ± 8d	$y = -8E\text{-}04x^2 + 0.133x + 1.464$	0.996
Trifolium hirtum	3.2 ± 0.2	267 ± 20	254 ± 8d	$y = -9E\text{-}04x^2 + 0.125x + 0.942$	0.994

[1] After Baligar et al. (2001)

[2] After Rose et al. (2015)

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Fig. 1 Shoot dry weight of five legume and one grass species in response to phosphorus (P) applied in the topsoil of a pot (n=5). Lines show fitted Mitscherlich curves for each species. Bar shows LSD for the Species x P applied interaction (P<0.05).

Fig. 2 Root dry mass in topsoil and subsoil in response to phosphorus (P) applied in the topsoil of a pot for (a) *Trifolium subterraneum* (b) *Trifolium hirtum* (c) *Ornithopus compressus* (d) *Ornithopus sativus* (e) *Biserrula pelecinus* and (f) *Dactylis glomerata* (n=5). Bar shows LSD for the Species x P applied x Soil interaction (P<0.05). Dashed line shows critical external P requirement for each species.

Fig. 3 Root mass fractions for (a) total root mass (b) topsoil root mass and (c) subsoil root mass of five legume and one grass species in response to phosphorus (P) applied in the topsoil of a pot (n=5). Bars show LSD for the Species x P applied interaction (P<0.05).

Fig. 4 (a) Regressions fitted for relationship between relative shoot yield [an indicator of phosphorus (P) sufficiency] and total root mass fraction for five legumes and one grass species. Gradient *Dactylis glomerata* > *Trifolium hirtum* > *Ornithopus sativus* = *Biserrula pelecinus* = *O. compressus* ≥ *T. subterraneum*. Intercept *T. subterraneum* > *B. pelecinus* > *O. sativus* = *T. hirtum* = *O. compressus* > *D. glomerata* (P<0.05). (b) Regressions fitted for linear range between relative shoot yield and topsoil root mass fraction. Gradient *D. glomerata* > *O. sativus* = *O. compressus* > *B. pelecinus* = *T. hirtum* > *T. subterraneum*. Intercept *T. subterraneum* > *T. hirtum* = *B. pelecinus* > *O. compressus* = *O. sativus* > *D. glomerata*) (P<0.05). Dashed vertical line shows critical external P requirement of 90% of maximum shoot yield.

Fig. 5 Tissue phosphorus (P) concentrationof topsoil roots (closed symbols), subsoil roots (open symbols) and shoots (×) in response to P applied to the topsoil of a pot for (a) *Trifolium subterraneum* (b) *Trifolium hirtum* (c) *Ornithopus compressus* (d) *Ornithopus sativus* (e) *Biserrula pelecinus* and (f) *Dactylis glomerata* (n=5). Bar shows LSD for the Species x P applied x Plant part (P<0.05). Dashed line shows critical external P requirement for each species. Inset shows the relationship between shoot P concentration and P concentration of topsoil roots (closed symbols) and subsoil roots (open symbols).

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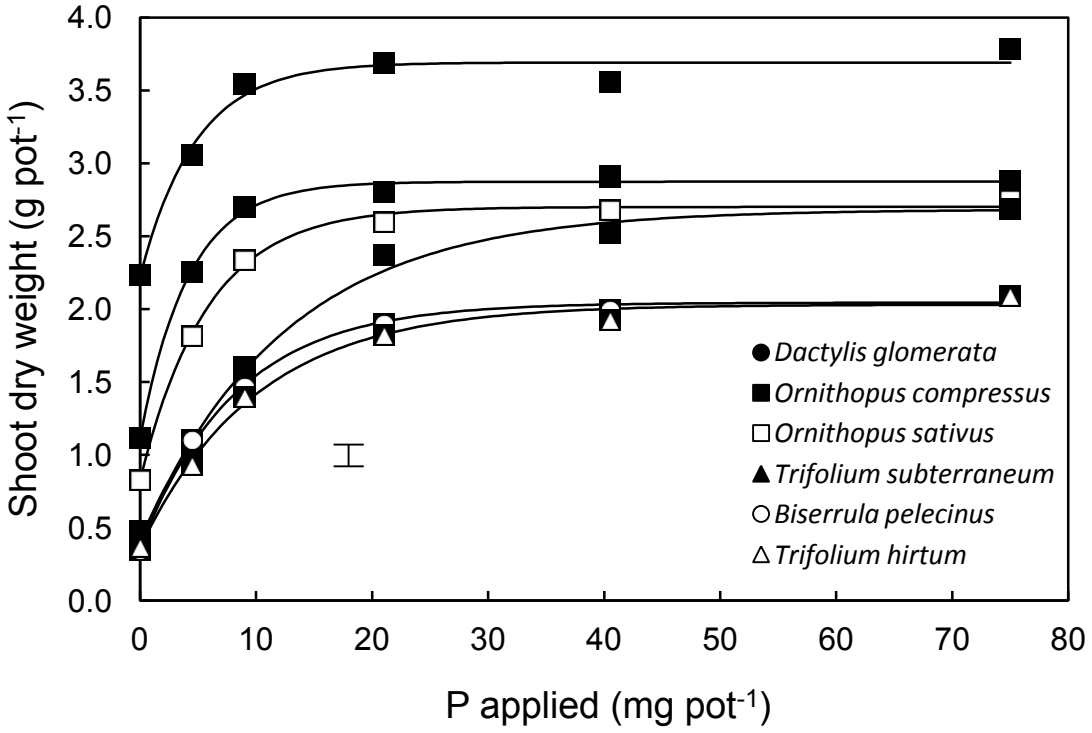

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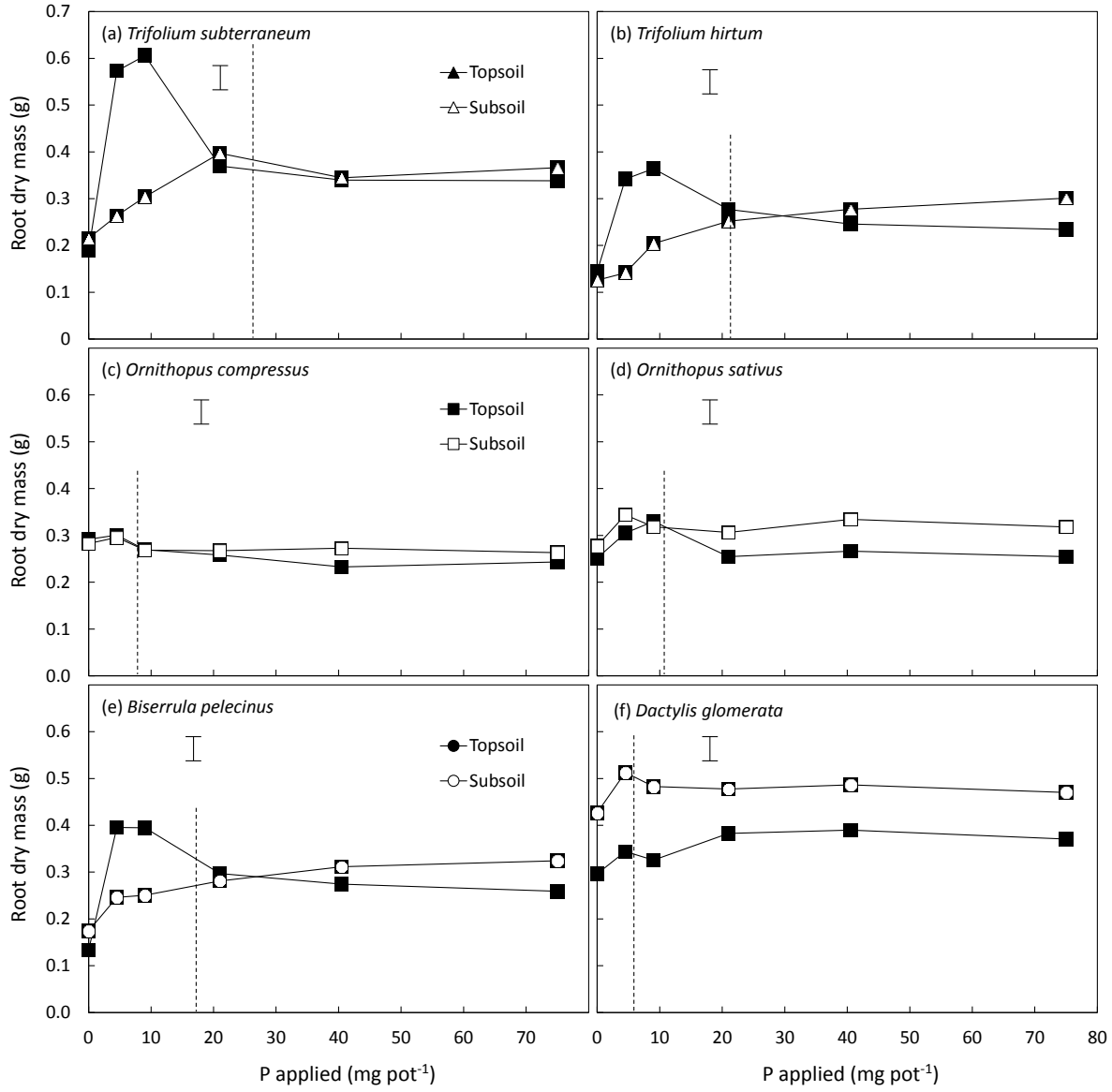

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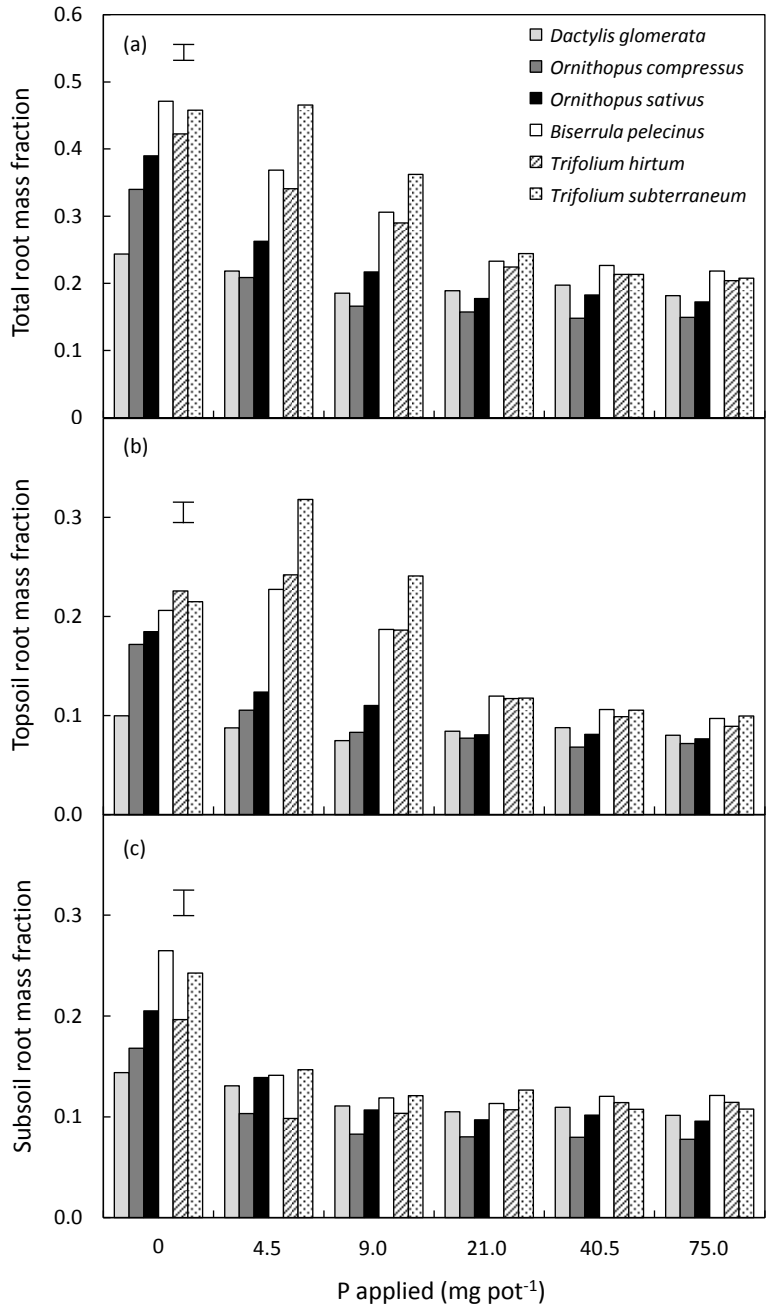

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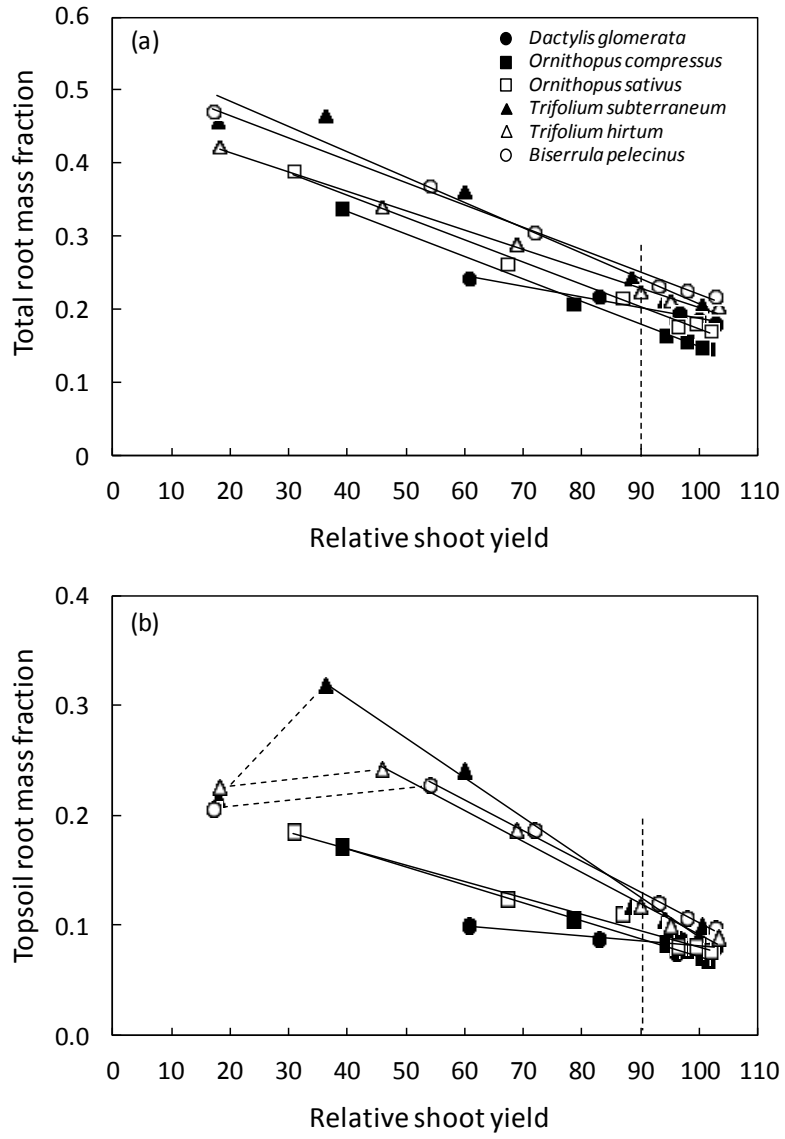

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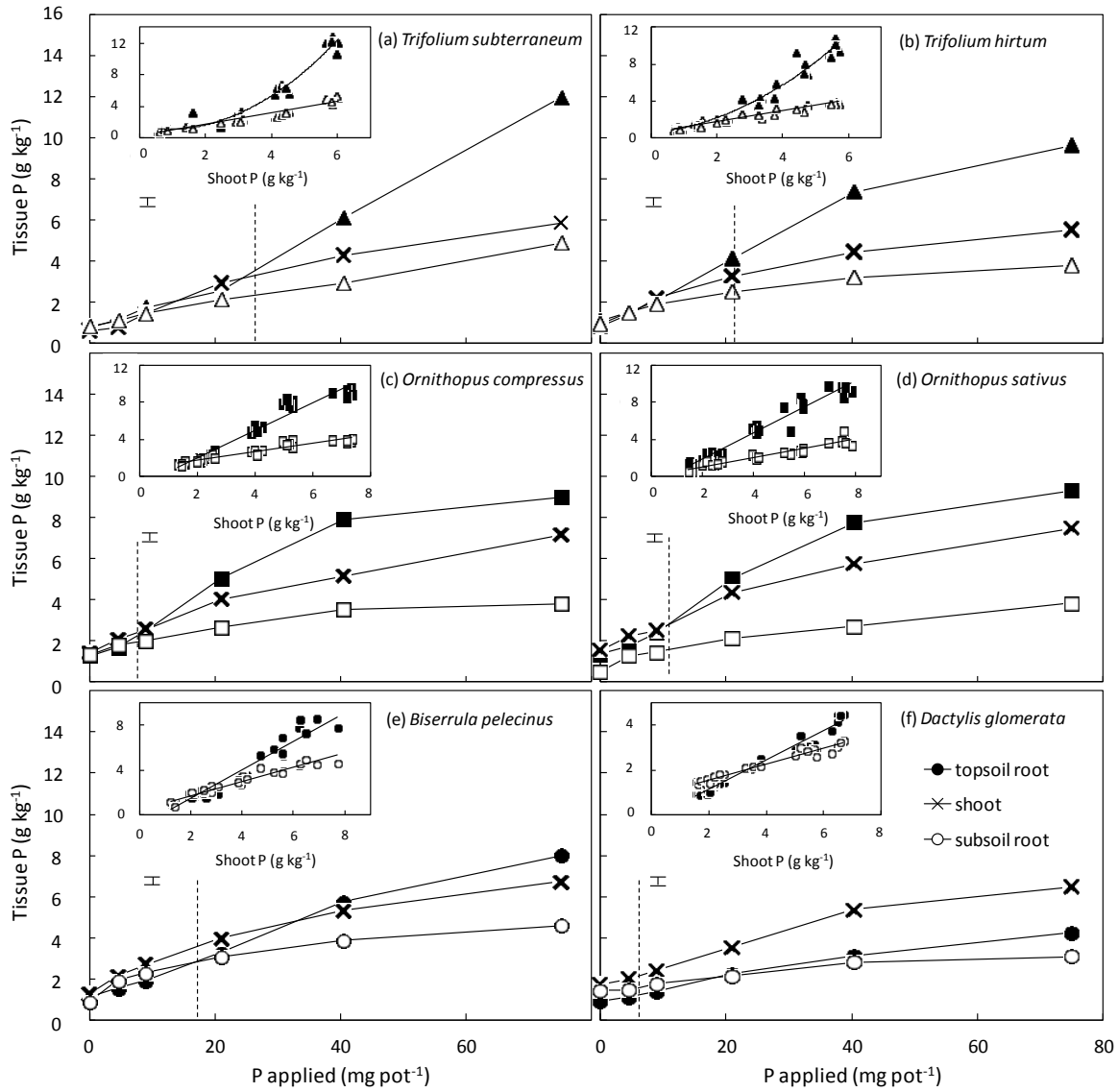

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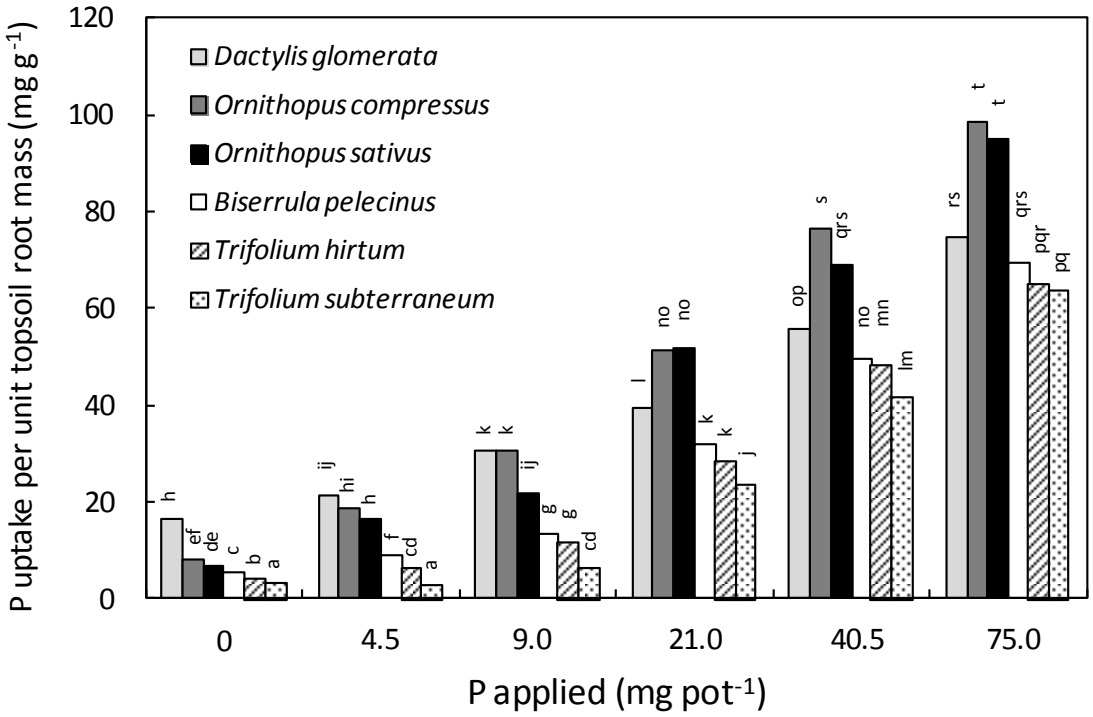

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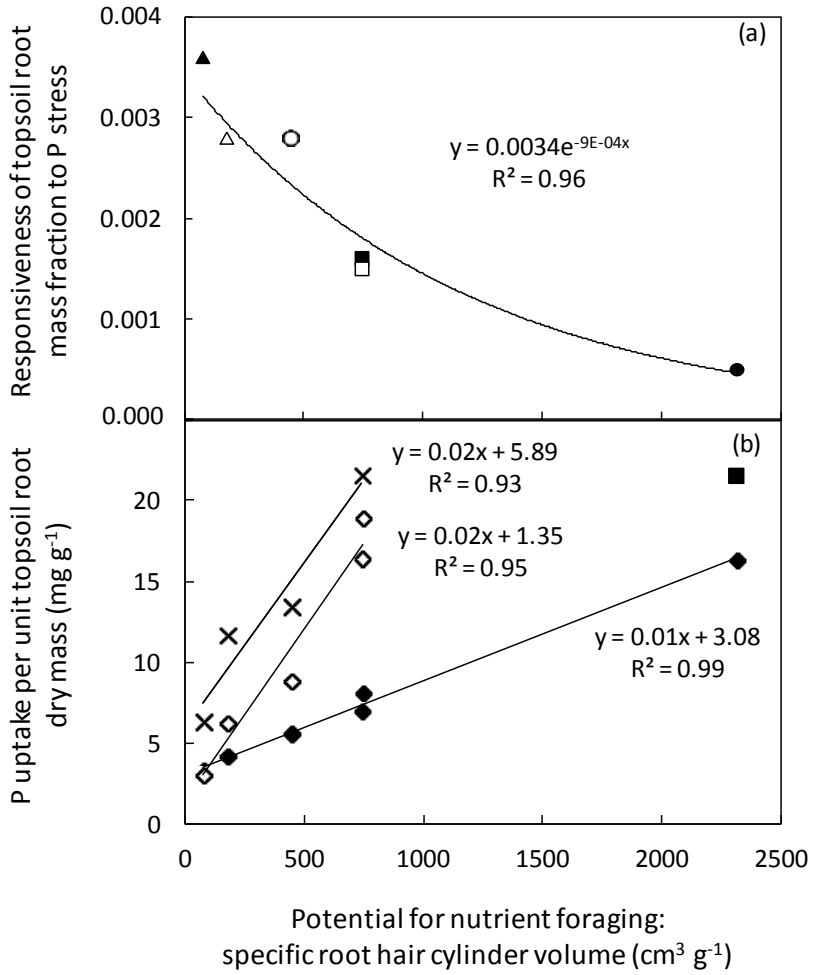

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