

Field benchmarking of the critical external phosphorus requirements of pasture legumes for southern Australia

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Abstract. In recent decades several pasture legumes have been available in southern Australia as potential alternatives to the most widely used annual pasture legume *Trifolium subterraneum*. Little is known about their soil phosphorus (P) requirements, but controlled environment experiments indicate that at least some may differ in their P fertiliser requirements. In this study, pasture legume varieties, including *T. subterraneum* as the reference species, were grown at up to four sites in any one year over a 3-year period (in total, seven site × year experiments) to measure herbage growth responses in spring to increased soil P availability. A critical soil test P concentration (corresponding to 95% maximum yield) was estimated for 15 legumes and two pasture grasses. The critical soil P requirements of most of the legumes did not differ consistently from that of *T. subterraneum*, indicating their soil fertility management should follow the current soil test P guidelines for temperate Australian pastures. However, the critical P requirement of *Medicago sativa* was higher than that of *T. subterraneum*, but remains ill-defined because extractable soil P concentrations in these experiments were often not high enough to permit a critical P estimate. Three forage crop legumes (*Trifolium incarnatum*, *Trifolium purpureum*, *Trifolium vesiculosum*) and two pasture legumes (*Ornithopus compressus*, *Ornithopus sativus*) had lower critical soil test P concentrations. It may be feasible to manage pastures based on these species to a lower soil test P benchmark without compromising yield.

Additional keywords: critical soil test P, *Ornithopus compressus*, *Ornithopus sativus*, phosphorus fertiliser, serradella, subterranean clover, *Trifolium subterraneum*.

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Introduction

Phosphorus (P) is an essential input for productive grazing enterprises in southern Australia because large areas used for growing dryland grass–legume pastures have soils that are deficient in P for pasture growth (Williams and Andrew 1970), can remain so even after many years of fertiliser use (Scheffe *et al.* 2015) or require continuing applications of fertiliser to maintain optimal P availability for high production (Simpson *et al.* 2015). The soils are also often deficient in other macronutrients (e.g. nitrogen, sulfur and potassium) and micronutrients (e.g. molybdenum) to varying degrees. In these systems, legumes are relied on to supply nitrogen to the pasture system. Provided other nutrient

deficiencies have been resolved, it is recommended that P fertiliser be applied to increase pasture yields until the critical P requirement of the pasture–soil system is achieved (i.e. the extractable soil P concentration required to achieve near-maximum yield). Thereafter, P fertiliser inputs should be moderated to maintain soil P fertility at the critical P concentration (Reuter *et al.* 1995; Simpson *et al.* 2015). Dryland pastures maintained with optimal soil fertility achieve maximum efficiency of rainfall use (e.g. Mills *et al.* 2006) and permit high stocking rates (Lean *et al.* 1997) and, consequently, high land use efficiency. The P balance efficiency of fertiliser use in grazing systems (i.e. P output expressed as a proportion of P inputs) is determined primarily by the rate at

which P accumulates in moderate to high P-sorbing soils (e.g. Simpson *et al.* 2014, 2015), and/or by loss of P via erosion, run-off or leaching (Lewis *et al.* 1981). The P sorption capacity of a soil (i.e. the net capacity for phosphate to sorb to and continue to react with a soil; McLaughlin *et al.* 2011) usually determines whether the rate of P accumulation or rate of P loss dominates the efficiency equation. However, both components of P inefficiency are positively correlated with the extractable P concentration at which a soil is maintained by fertiliser applications (Melland *et al.* 2008; McDowell 2012; Simpson *et al.* 2014). Consequently, from both production and resource use efficiency viewpoints, the most effective use of P fertiliser occurs when a pasture soil is maintained close to the critical P requirement of the pasture–soil system.

The challenge for practical soil fertility management is knowing the critical soil test P concentration at which maximum pasture yield can be achieved. For southern Australia, there are two assessments of historical datasets that have defined the critical P requirements of temperate pastures (Gourley *et al.* 2007, 2019; Moody 2007). Both studies reinterpret several key experiments (e.g. Helyar and Spencer 1977), many of which explicitly examined the response to soil test P by pastures based on *Trifolium subterraneum* L. (subterranean clover) and *Trifolium repens* L. (white clover). These legumes have higher critical P requirements than the grasses with which they are grown (Ozanne *et al.* 1969, 1976; Helyar and Anderson 1970; Jackman and Mouat 1972; Hill *et al.* 2010) and, consequently, grass–legume pastures are fertilised to meet the legume's higher P requirement, because legume N₂ fixation ultimately drives the productivity of the pasture system.

Since the late 1990s, numerous alternative pasture legumes have been developed in Australia, primarily to address areas of the agricultural landscape and farming systems where *T. subterraneum* cannot be used reliably (Loi *et al.* 2005; Nichols *et al.* 2007, 2012). Little is known about the P requirements of these species, but controlled environment experiments indicate that at least some of the species differ in their P fertiliser requirements (Haling *et al.* 2016a; Sandral *et al.* 2018). Herein we report results from field experiments examining the growth during spring of several alternative

legumes and two perennial grasses in response to six levels of soil P availability. The aim of the research was to determine the relative P requirements of the species and to estimate the critical extractable P concentration (i.e. soil test P) for near-maximum yield, under field conditions. *T. subterraneum* is the most commonly grown legume in permanent pastures and mixed crop–pasture systems in southern Australia and was included as a reference species.

Materials and methods

Experiment sites

Four sites were established near Yass (−34.948, 148.930), Burrinjuck (−34.869, 148.672), Belfrayden (−35.116, 146.992) and Beckom (−34.202, 147.033) in southern New South Wales, Australia. The site characteristics are presented in Table 1. The Yass site was sown in 2012 and resown in 2013 and 2014. The Burrinjuck site was sown in 2013 and resown in 2014, whereas the Belfrayden and Beckom sites were only sown in 2014. At each site, 12 pasture cultivars were sown each year and grown at six rates of P supply with three replicates. Plot size was 10 m × 2 m.

Yass and Burrinjuck are in regions of permanent pasture-based livestock systems. Belfrayden and Beckom are in regions where pasture systems are either permanent (non-arable landscapes) or grown in sequence with crops.

Fertiliser application

Lime was applied to a site during its initial preparation if the surface soil pH_{Ca} (0–10 cm) was below 5.2. At Yass and Burrinjuck, 2 Mg lime ha^{−1} was applied and cultivated in to a depth of ~10 cm; at Beckom 1 Mg lime ha^{−1} was applied and incorporated. It was not necessary to apply lime at Belfrayden. The surface soil pH_{Ca} that was achieved is recorded in Table 1. Basal nutrients and P were then applied before sowing at each site: micronutrients (molybdenum trioxide (MoO₃) 0.07 kg ha^{−1}, boric acid (H₃BO₃) 1.75 kg ha^{−1}, copper sulfate (CuSO₄·5H₂O) 1.75 kg ha^{−1} and zinc sulfate (ZnSO₄·7H₂O) 3.5 kg ha^{−1}) were applied using a boom spray with 100 L ha^{−1} water as the carrier; and potassium sulfate (K₂SO₄) and magnesium sulfate (MgSO₄·7H₂O) were applied

Table 1. Mean annual rainfall and rainfall received in each year of the experiments, soil pH_{Ca}, native Colwell P and the phosphorus-buffering index (PBI) for each site in different experimental years (2012–2014)

n.d., not determined in that growing season

	Yass			Burrinjuck		Belfrayden	Beckom
	2012	2013	2014	2013	2014	2014	2014
Rainfall ^A (mm)	781.8	583.5	704.5	625.1	876.0	369.5	415.9
Mean annual rainfall ^A (mm)		681			802	471	498
Soil pH _(Ca) ^B	5.4	5.3	5.1	6.0	5.9	5.8	4.9
Colwell P ^C (mg kg ^{−1})	12	11	8	13	11	14	5
PBI ^D	52	42	n.d.	65	n.d.	82	40

^ALong-term and yearly rainfall data for the sites were sourced from the Australian Bureau of Meteorology (<http://www.bom.gov.au/>, accessed 22 August 2018) and were supplemented for Belfrayden and Beckom using rainfall data collected near these sites during the experiments.

^BpH in CaCl₂ (1 : 5 w/v 0.01 M CaCl₂), Method 4B1 (Rayment and Lyons 2011).

^CColwell (1963), Method 9B2 (Rayment and Lyons 2011).

^DBurkitt *et al.* (2002, 2008); Method 914a (Rayment and Lyons 2011).

at rates of 100 and 60 kg ha⁻¹ respectively. P was applied to the soil surface by hand as triple superphosphate (20.7% P, 1.5% S) to establish six uniformly spread soil P levels. Rates were 0, 15, 30, 45, 60 and 80 kg P ha⁻¹ at Yass (2012) and Burrinjuck (2013), and 0, 15, 30, 50, 65 and 85 kg P ha⁻¹ at Belfrayden and Beckom (2014). Subsequently, maintenance P applications were applied in the same way in autumn at 0, 1, 3, 8, 15, 20 kg P ha⁻¹ at Yass (2013 and 2014) and Burrinjuck (2014). Plots sown to perennial grasses received a total of 80 kg ha⁻¹ year⁻¹ nitrogen in four equal applications during May, July, August and October.

Plant material

Because of the range in annual rainfall (growing season lengths) among the sites, it was not sensible to sow all the pasture varieties at each site. The cultivars of *T. subterraneum* were selected for use at a site using current maturity-type information (Nichols *et al.* 2013). Some of the alternative legumes had not been tested regionally and were without specific recommendations concerning their suitability. Subsequently, several were not resown at some of the sites because they proved to be unsuitable (see Supplementary Materials table S1, available at the journal's website). The annual legume species (common name and cultivar) used in the experiments were as follows: *Trifolium glanduliferum* Boiss (gland clover cv. Prima), *Trifolium hirtum* All. (rose clover cv. Hykon), *Trifolium incarnatum* L. (crimson clover cv. Dixie), *Trifolium michelianum* Savi. (balansa clover cv. Bolta), *Trifolium purpureum* Loisel. (purple clover cv. Electra), *Trifolium spumosum* L. (bladder clover cv. Bartolo), *T. subterraneum* L. (subterranean clover cv. Leura, Narrikup, Izmir), *Trifolium vesiculosum* Savi (arrowleaf clover cv. Zulu II), *Ornithopus compressus* L. (yellow serradella cv. Santorini, Avila), *Ornithopus sativus* Brot. (French serradella cv. Margurita), *Biserrula pelecinus* L. (biserrula cv. Casbah, Mauro), *Medicago truncatula* Gaertn. (barrel medic cv. Sultan-SU) and *Lupinus albus* L. (white lupin cv. Luxor). The perennial legumes were: *Medicago sativa* L. (lucerne cv. SARDI 10), *Trifolium ambiguum* M. Bieb. (Caucasian clover cv. Kuratas), *Trifolium tumens* Steven ex M. Bieb. (talish clover cv. Permatas), *Lotus corniculatus* L. (birdsfoot trefoil line LC07AUYY) and *Bituminaria bituminosa* var. *albomarginata* (L.) C.H. Stirt. (tedera line Tedera 27). The perennial grasses were *Dactylis glomerata* L. (cocksfoot cv. Porto, Uplands) and *Phalaris aquatica* L. (phalaris cv. Advanced AT). All species are primarily used in pastures with the exception of *T. incarnatum* and *T. purpureum* (forage species) and *L. albus* (a crop species).

Seed was sown on 7 May (Yass 2012), 20 May (Yass and Burrinjuck 2013), 25 February (Yass and Burrinjuck 2014) and 2 April (Belfrayden and Beckom 2014). All annual legumes were resown each year at 30 kg viable seed per hectare to avoid possible differences in seed reserves and establishment density. Seeds were sown using DBS direct-drill tines (Ausplow, Cockburn Central, WA, Australia) at a row spacing of 25 cm, aiming typically for seed placement at a depth of 1 cm. Perennial legumes and perennial grasses were sown at 10 kg ha⁻¹ and not resown annually, unless establishment was considered inadequate. The germination of all cultivars was tested and sowing rates were adjusted accordingly. Legumes were

inoculated with appropriate rhizobia before sowing: Group S (WSM471 strain) for the *Ornithopus* spp., WSM1497 for *B. pelecinus*, Group G (WU425) for *L. albus*, Group AM (WSM1115) for *M. truncatula*, Group AL (RRI128) for *M. sativa*, SU343 for *L. corniculatus*, WSM4083 for *B. bituminosa*, Group C (WSM1325) for all the annual *Trifolium* spp., CC283b for *T. ambiguum* and Group B (TA1) for *T. tumens*.

Table S1 provides a full list of all of the cultivars sown at each site in each year and indicates whether the cultivar was harvested or had failed to establish. Yield and critical P results are only reported for cultivars that established and grew successfully.

Establishment and management

Yass 2012 and 2013

The perennial grasses failed to establish adequately at the Yass site in 2012 because of grass weeds (mostly *Vulpia* spp.) that invaded these treatments and could not be controlled. In the legume plots, grass weeds were controlled with 500 mL ha⁻¹ of the grass-selective herbicide Select (Sumitomo Chemical Australia Pty Ltd, Epping, NSW, Australia; 240 g L⁻¹ clethodim). *T. ambiguum* (cv. Kuratas), a perennial legume, established at low densities and plant numbers decreased further over the 2012–13 summer. Consequently, these plants were not harvested and were replaced with other species in autumn 2013. An experimental line of *B. bituminosa* var. *albomarginata* (Tedera 27) was sown in 2012, but winter frosts killed most seedlings. It was subsequently sown in spring (late September) 2012. However, only low numbers of *B. bituminosa* plants survived frosts in the 2013 winter, and peak spring growth could not be assessed adequately. The annual legumes *B. pelecinus* (cv. Mauro) and *T. spumosum* (cv. Bartolo) failed to produce adequate forage over the winter and spring of 2012 and were not assessed. Rutherglen bug (*Nysius vinitor*) was found on *T. spumosum* during spring, and was controlled by spraying with Matador (Nufarm Australia, Laverton North, Vic, Australia; 250 g L⁻¹ lambda cyhalothrin) at 36 mL ha⁻¹.

Yass 2014

At Yass in 2014, the annual legumes *B. pelecinus* (cv. Casbah), *T. spumosum* (cv. Bartolo) and *T. hirtum* (cv. Hykon) failed to produce adequate spring growth. Rutherglen bug (*N. vinitor*) was observed in large numbers on *T. spumosum* in early September and Matador was again applied at 36 mL ha⁻¹. *M. sativa* (cv. SARDI 10) plant populations declined considerably over the 2013–14 summer, and consequently this species was not assessed in spring 2014 (see table S1). A *Sclerotinia* sp. was identified on herbage in small collapsed necrotic patches of *O. sativus* (cv. Margurita) and was controlled with Prosaro 420 SC (Bayer Australia, Pymble, NSW, Australia; 210 g L⁻¹ prothioconazole and 210 g L⁻¹ tebuconazole) at 300 mL ha⁻¹.

Burrinjuck 2013 and 2014

At Burrinjuck in 2013, the sown perennial grasses failed due to preferential grazing by wombats (*Vombatus ursinus*), until they were excluded by erecting a low electrified fence wire, followed by grass weed incursions that could not be selectively

removed with a herbicide. The legume plots received 500 mL ha⁻¹ of the grass-selective herbicide Select (240 g L⁻¹ clethodim) to control grass weeds. The legumes *T. spumosum* (cv. Bartolo), *T. hirtum* (cv. Hykon), *B. pelecinus* (cv. Mauro) and *L. corniculatus* (line LC07AUYF) failed to establish in adequate numbers and were not assessed. The likely reason for this is that these species were not competitive against *Juncus bufonius* L. (toad rush) that had invaded these treatments over winter. In 2014, *T. spumosum* (cv. Bartolo), *T. hirtum* (cv. Hykon), *B. pelecinus* (cv. Casbah), *T. glanduliferum* (cv. Prima), *O. compressus* (cv. Santorini) and *T. subterraneum* (cv. Narrikup) failed to establish successfully again due to invasion by *J. bufonius*, despite an early season application of Spinnaker (Nufarm Australia, Laverton North, Vic, Australia; 700 g kg⁻¹ imazethapyr) at 70 g ha⁻¹. As with the Yass site, a *Sclerotinia* sp. was identified on *O. sativus* (cv. Margarita) and controlled with Prosaro 420 SC (210 g L⁻¹ prothioconazole and 210 g L⁻¹ tebuconazole) at 300 mL ha⁻¹.

Beckom and Belfrayden 2014

All species sown at these sites produced sufficient herbage in spring to enable them to be harvested. However, the spring was relatively dry with only approximately half the average rainfall at Belfrayden and two-thirds of the average rainfall at Beckom in September and October (Table 1; table S2).

Rainfall

The rainfall received over the May–October period at all sites over the 2012–2014 seasons was below average (Table 1; table S2). For example, at Yass the May–October rainfall was 93, 52.9 and 73.5 mm below the long-term (1889–2015) average for 2012, 2013 and 2014 respectively (table S2). Similarly, Burrinjuck received 65.7 and 63.5 mm below the long-term average rainfall for May–October during 2013 and 2014 respectively, whereas Belfrayden and Beckom received 89.3 and 83.3 mm below average respectively for 2014.

Although drier than average conditions were experienced over the May–October period, there was also transient waterlogging present at the Yass and Burrinjuck sites during 2013 and 2014 as a consequence of the higher than average June rainfall for these years (table S2). Waterlogging was more evident at Burrinjuck, and particularly in 2014.

Measurements

Herbage dry matter

Shoots were cut at ground level from three quadrats (200 mm × 500 mm) in each plot on: 6 November 2012, 2 October 2013 and 9 October 2014 at Yass; 23 October 2013 and 24 October 2014 at Burrinjuck; 1 October 2014 at Belfrayden; and 18 September 2014 at Beckom. These harvests were intended to assess the approximate peak period for spring growth rate. Shoots were dried at 70°C for 72 h and weighed to provide an estimate of the dry matter (DM) production for each plot.

Soil sampling

Soil P levels were determined at the time of the peak spring harvest by taking eight soil cores (depth 0–10 cm) from each of the three areas cut for DM assessment (total 24 soil cores per plot). Soil cores were broken up, mixed and dried at 40°C. Once

dry, the soil samples were mixed thoroughly again and subsampled to determine the Colwell- and Olsen-extractable P concentrations (Olsen *et al.* 1954; Colwell 1963).

Herbage P concentrations

Herbage DM (i.e. whole shoots) harvested from four of the six P treatments (the treatment receiving no P and three other treatments selected to span the critical soil test P level of each pasture variety) was used for determination of whole-shoot P concentrations at all sites in 2014. The dry shoot material was milled and ~50 mg subsamples were ashed at 550°C. The ash was then dissolved in 2 M HCl to facilitate the colourimetric determination of P concentration using malachite green (Irving and McLaughlin 1990).

Colonisation of roots by mycorrhizal fungi

Colonisation of roots by arbuscular mycorrhizal fungi (AMF) was measured on topsoil roots of *O. sativus*, *O. compressus* and *T. subterraneum* grown in the unfertilised treatment at all four field sites in spring 2014 at the time of the peak spring harvest. Ten cores (diameter 2.5 cm, depth 10 cm) were taken per plot and combined to wash the roots from the soil. The roots were cleared (10% w/v KOH for 2–4 days, followed by rinsing in water and 1% v/v HCl), stained (5% v/v Schaeffer blue ink–white vinegar solution for 1 h; Vierheilig *et al.* 1998) and colonisation was measured using the grid-line intersect method (Giovannetti and Mosse 1980).

Root hair length

Root hair length was measured on topsoil roots (depth 0–10 cm) of *O. sativus*, *O. compressus* and *T. subterraneum* grown in the unfertilised treatment (i.e. 0 kg P ha⁻¹) and 15 kg P ha⁻¹ treatments at the Yass, Burrinjuck and Beckom field sites in May 2016. Assessments could not be made at Belfrayden because the site had been resumed for cropping. Two cores (diameter 6.5 cm, depth 10 cm) were taken per plot and combined. Roots were washed from the soil and root hairs were measured on five lengths of root selected at random from each sample. Each length of root was photographed using a Leica MZFLIII fluorescence microscope (Leica Microsystems, Sydney, NSW, Australia) fitted with a Zeiss AxioCam camera (Zeiss, Sydney, NSW, Australia). Fifteen root hairs perpendicular to the line of vision were selected on each root sample for length measurement using ImageJ v. 1.46r (U.S. National Institutes of Health, Bethesda, MD; <https://imagej.nih.gov/ij/docs/guide>, accessed 22 August 2018).

Experiment design and statistical analysis

The experimental design software DiGger (Coombes 2009) was used to produce randomised complete block designs that avoided row, column and diagonal treatment duplicates. Treatments comprised the factorial of six P rates and 12 cultivars and were replicated three times.

Linear mixed-model technology was used to fit a statistical model to herbage DM measurements. The linear mixed model can be formulated in such a way that it is analogous to an analysis of variance (ANOVA). For a single trial this would be an ANOVA for a randomised complete block design where the sums of squares are partitioned into sums of squares for the

overall mean, sums of squares for blocks and sums of squares for treatments (being the factorial of P rates and cultivars). Unlike ANOVA, the linear mixed model has the ability to accommodate missing values in the response and explanatory variables and can accommodate a wide range of variance models, in particular spatial models.

Spatial models for each trial were considered following the process described by Gilmour *et al.* (1997) for identifying and accounting for sources of field spatial variability. Spatial variability can be divided into three sources: local, global and extraneous. Local variation is based on the notion that plots closer together will be more similar than plots further apart regardless of treatment (or block) effects. The approach considered by Gilmour *et al.* (1997) to model local variation is based on the first-order separable autoregressive models described by Cullis and Gleeson (1991). Global variation, such as soil fertility trends, is modelled by considering the statistical significance of centred linear covariates for field row and field column. Extraneous variation, such as variation attributable to trial management operations, is modelled by considering additional terms to fit in the model, such as random field column and row effects.

Plot herbage DM outliers and poor-quality data were determined using a combination of field notes and statistical model diagnostics. Field notes included observations on grazing by wild vertebrate pests, large subsurface rocks, sclerotinia damage, root rot, waterlogged hollows and/or establishment failure. Plot values were only rejected if adverse observations coincided with a plot residual, produced after linear mixed model analysis, that was >3 .

For herbage DM, all models were fitted using the statistical software package ASRemL (Butler 2009) within the R computing environment (R Core Team 2018). This analysis showed that effects of P rate and cultivar were significant ($P < 0.05$), but the interaction of P rate and cultivar was not. Graphs of herbage DM response to soil P fertility partitioned according to cultivar were drawn using fitted data from ASRemL (VSN International, Hemel Hempstead, UK). Plot-level fitted data from ASRemL were used initially to fit the Mitscherlich diminishing returns equation ($y = A + B \times R^c$) using GENSTAT (16th edition; VSN International, Hemel Hempstead, UK), where y is the plot-level shoot dry matter (kg ha^{-1}) and c is the plot-level soil test P concentration (mg kg^{-1}) measured during spring at the time the herbage was harvested. The aim was to estimate critical soil test P requirements, maximum yields (i.e. the asymptote value, A) and the standard errors associated with the equation parameters (see table S3).

Phosphorus fertiliser application rates had been planned to exceed the anticipated P requirement of *T. subterraneum*, but the P requirements of the other species were unknown. We encountered problems fitting the Mitscherlich equation to species with shallow curvature (i.e. varieties with very low critical P requirements) and to species that achieved maximum yields only at the highest rates of P supply because, under these circumstances, the Mitscherlich equation overestimates maximum yield, critical P requirement and/or the error associated with these parameters. Therefore, we adopted the method of Dyson and Conyers (2013) to fit a yield response function to the plot-level data (i.e. the fitted

data from ASRemL for herbage yields and the corresponding Colwell P concentrations). Critical soil test P concentrations were then determined using these yield response functions. Dyson and Conyers (2013) recognised that herbage yield and soil test values (STV) are both inexact and unknown in datasets of crop yield response to soil fertility. The relationship between plot-level fitted herbage yield data and plot-level fitted Colwell soil test P values (STV) was straightened by plotting $\ln(\text{STV})$ against $\arcsin([RY\%/100]^{0.5})$, where RY is relative herbage yield (i.e. yield expressed as a proportion of the maximum or asymptote value for herbage DM when P is non-limiting). The $\ln(\text{STV})$ was plotted along the vertical axis, with $\arcsin([RY\%/100]^{0.5})$ as the horizontal axis to permit determination of the standard error associated with a predicted y -value (i.e. the critical Colwell P concentration) using Excel 2013 (Microsoft, Redmond, WA, USA). This process required the initial determination of maximum yield and the subsequent exclusion of RY values associated with very high STV values, which may otherwise bias estimates of critical P concentrations (Dyson and Conyers 2013). The yield response of each variety was determined independently at each site in each year. Where the yield of a variety clearly plateaued at high STV, maximum yield was estimated as the average of yield values along the response plateau (e.g. varieties of *Ornithopus* spp.; Figs 1–3). However, when a clear yield plateau was not observed, maximum yield was assumed to be the average yield obtained from replicate plots to which the highest rates of P fertiliser had been applied (e.g. varieties of *T. subterraneum*; Figs 1–3). The only species where there was any doubt that this procedure would give a reasonable approximation of maximum yield was *M. sativa*, for which the growth response to P was often linear and did not plateau below the highest rates of P application on four of the six occasions that it was grown (Figs 1–3). When this was the case, no attempt was made to estimate maximum yield or the critical Colwell P concentration. RY values associated with very high STV were excluded as prescribed by Dyson and Conyers (2013), who used an arbitrary cut-off of twice the initial critical STV estimate. Our yield response data were considerably less variable than those of Dyson and Conyers (2013) and we reduced the cut-off to 1.5-fold the initial critical STV estimate, after determining this was likely to be the minimum multiplier without effect on the predicted critical STV.

Critical Colwell P concentrations corresponding to 95% and 90% of maximum yield were estimated from the linear relationships to enable comparisons with published data that adopt different critical criteria (e.g. Gourley *et al.* 2007, 2019 (95%); Moody 2007 (90%)). The 95% confidence interval (CI) associated with each critical Colwell P value was estimated as the sum of twice the standard error on the high side of the estimate plus twice the standard error on the low side of the estimate (note, standard errors for values close to the asymptote are asymmetric). Critical Olsen P concentrations were subsequently determined using relationships between Colwell P and Olsen P determined using the soil samples taken in spring at each site in each year.

For each replicate of each pasture variety, herbage P concentrations were regressed against their corresponding Colwell soil test P fitted data from ASRemL to assess response of herbage P concentration to soil P fertility. The mean $\pm$ s.e.m. ($n = 3$) P concentration of herbage that

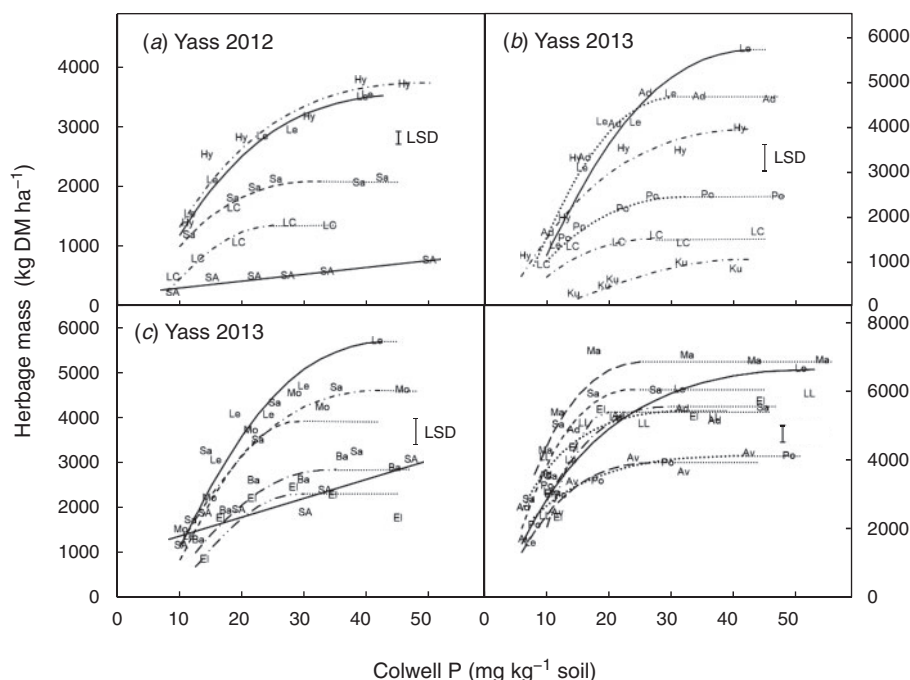


Fig. 1. Peak spring herbage dry matter (DM) at Yass in (a) 2012, (b, c) 2013 and (d) 2014 in response to increased concentrations of Colwell phosphorus (P) in the 0- to 10-cm layer. Lines show the Dyson and Conyers (2013) yield response equation back-fitted to the data for all varieties except *Medicago sativa* cv. SARDI 10 because its response to increased soil P was linear. The equation of Dyson and Conyers (2013) addresses yield response to P below the maximum yield. Fine dotted lines indicate the implied maximum yield plateau. Bars indicate the largest l.s.d. ($P = 0.05$) from an unbalanced ANOVA comparing the maximum herbage yield of the pasture varieties. (b, c) Varieties grown at Yass in 2013 are shown in two panels for clarity and *Trifolium subterraneum* (cv. Leura) is shown in both these panels for easy reference. Also for reasons of clarity, the symbols represent the average P treatment values for each pasture variety. However, the yield response functions were fitted using plot-level data for herbage yield and Colwell-extractable P. Le with solid line, *T. subterraneum* cv. Leura; Ad with dotted line, *Phalaris aquatica* cv. Advanced AT; Av with long dashed line, *Ornithopus compressus* cv. Avila; Ba with long dash plus dot line, *Trifolium spumosum* cv. Bartolo; El with long dash plus two dots line, *Trifolium purpureum* cv. Electra; Hy with short dash plus dot line, *Trifolium hirtum* cv. Hykon; Ku with short dash plus dot line, *Trifolium ambiguum* cv. Kuratas; LC with short dash plus dot line, *Lotus corniculatus* line LC07AUYF; LL with dotted line, *Lupinus albus* cv. Luxor and *T. subterraneum* cv. Leura mixed planting; Ma with long dashed line, *Ornithopus sativus* cv. Margurita; Mo with short dash plus dot line, *Biserrula pelecinus* cv. Mauro; Po with dotted line, *Dactylis glomerata* cv. Porto; Sa with short dashed line, *Ornithopus compressus* cv. Santorini; SA with solid line, *Medicago sativa* cv. SARDI 10.

corresponded with the critical soil test P level was estimated using these relationships.

Results

Critical soil test P concentrations

The Mitscherlich function was initially fitted to the yield response data (table S3). However, for the reasons outlined above, the Mitscherlich function was occasionally unable to adequately estimate asymptotic yield maxima and critical soil test P requirements. Consequently, the critical soil test P requirement for 90% or 95% of maximum yield was determined using the approach proposed by Dyson and Conyers (2013; Fig. 4, Table 2). Because there are regional preferences for the use of alternative soil test P methods, the corresponding critical Olsen P concentrations were derived using

the relationships between Colwell P and Olsen P determined for each site in each year (Table 3). However, unless stated otherwise, results in this paper refer primarily to the critical Colwell P concentrations that correspond with a 95% maximum yield threshold.

Yass

In 2012, the critical external Colwell P requirement of *T. subterraneum* (cv. Leura) was $34 \text{ mg P kg}^{-1} \text{ soil}$ (Fig. 4). *O. compressus* (cv. Santorini) and *L. corniculatus* (line LC07AUYF) had significantly ($P < 0.05$) lower critical Colwell P requirements, but their maximum yields were only half or less that of *T. subterraneum* (Fig. 1). *T. hirtum* (cv. Hykon) had a similar critical P requirement and yield to that of *T. subterraneum*. The critical P requirement of *M. sativa* (cv. SARDI 10) could not be determined because a yield asymptote

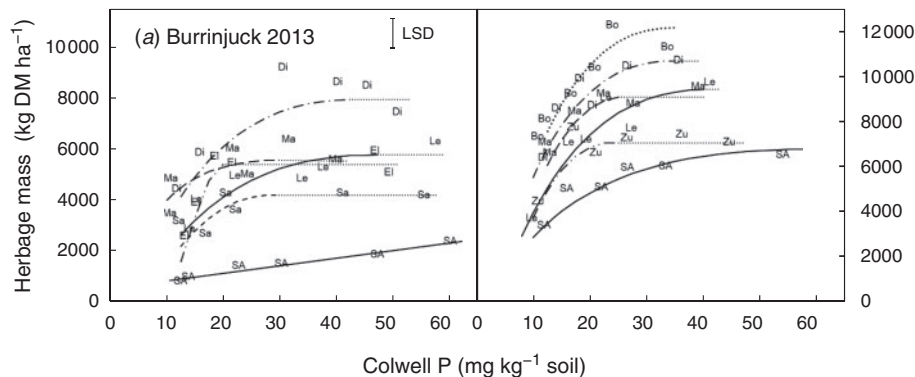


Fig. 2. Peak spring herbage dry matter (DM) at Burrinjuck in (a) 2013 and (b) 2014 in response to increased concentrations of Colwell phosphorus (P) in the 0- to 10-cm layer. Lines show the Dyson and Conyers (2013) yield response equation back-fitted to the data for all varieties except *Medicago sativa* cv. SARDI 10 when its response to increased soil P was linear. The equation of Dyson and Conyers (2013) addresses yield response to P below the maximum yield. Fine dotted lines indicate the implied maximum yield plateau. Bars indicate the largest l.s.d. ($P = 0.05$) from an unbalanced ANOVA comparing the maximum herbage yield of the pasture varieties. The reference cultivar of *Trifolium subterraneum* grown at this site was cv. Leura. For clarity, the symbols represent the average P treatment values for each pasture variety. However, the yield response functions were fitted using plot-level data for herbage yield and Colwell-extractable P. Le with solid line, *T. subterraneum* cv. Leura; Bo with dotted line, *Trifolium michelianum* cv. Bolta; Di with long dash dot line, *Trifolium incarnatum* cv. Dixie; El with long dash plus two dots line, *Trifolium purpureum* cv. Electra; Ma with long dash line, *Ornithopus sativus* cv. Margurita; Sa with short dash line, *Ornithopus compressus* cv. Santorini; SA with solid line, *M. sativa* cv. SARDI 10; Zu with long dash dot line, *Trifolium vesiculosum* cv. Zulu II.

was not achieved within the P treatment range (0–80 kg P applied per hectare).

In 2013, *T. subterraneum* was the highest yielding species and had a critical Colwell P requirement of 34 mg P kg⁻¹. *Ornithopus compressus* (cv. Santorini), *T. purpureum* (cv. Electra) and *L. corniculatus* (line LC07AUYF) had critical Colwell P requirements approximately half to two-thirds that of *T. subterraneum*. The yield of *O. compressus* was approximately 70% of that of *T. subterraneum*, but the other P-efficient species were again low yielding. The perennial grasses *D. glomerata* (cv. Porto) and *P. aquatica* (cv. Advanced AT) also had low critical Colwell P requirements, often equivalent to those of the P-efficient legumes (*O. compressus*, *T. purpureum*, *L. corniculatus*). However, the critical Colwell P requirements of *T. hirtum* (cv. Hykon), *B. pelecinus* (cv. Mauro), *T. spumosum* (cv. Bartolo) and *T. ambiguum* (cv. Kuratas) did not differ significantly from that of *T. subterraneum*. As in 2012, the critical P requirement of *M. sativa* could not be determined. No attempt was made to estimate the critical P requirement of *B. bituminosa* (line 27) because of the high loss of seedlings to frost.

In 2014, the critical Colwell P requirement of *T. subterraneum* was 36 mg P kg⁻¹. *O. compressus* (cv. Avila, Santorini), *O. sativus* (cv. Margurita) and *T. purpureum* (cv. Electra) had critical Colwell P requirements that were 45–55% of that of *T. subterraneum*. *O. sativus* (cv. Margurita) and *O. compressus* (cv. Santorini) yielded as well as *T. subterraneum*, but the yield of *O. compressus* (cv. Avila) was only half that of *T. subterraneum*. *P. aquatica* (cv. Advanced AT) again had a low critical P requirement, similar to that of the more P-efficient legumes. However, *D. glomerata* (cv. Porto) yielded poorly and had a

critical P requirement that was not significantly lower than that of *T. subterraneum*. The experimental mixture of *L. albus* and *T. subterraneum* had a low critical Colwell P requirement (similar in magnitude to that of the *Ornithopus* spp.), but also had a maximum yield that was 18% lower than that of *T. subterraneum* in monoculture.

Burrinjuck

In 2013, the critical Colwell P requirement of *T. subterraneum* (cv. Leura) was 34 mg P kg⁻¹ (Fig. 2). *Trifolium purpureum* (cv. Electra), *O. sativus* (cv. Margurita) and *O. compressus* (cv. Santorini) had critical Colwell P requirements that were numerically approximately two-thirds that of *T. subterraneum* (Fig. 4). However, only the critical P requirement of *T. purpureum* was significantly ($P < 0.05$) lower. The maximum herbage yields of *T. purpureum* and *O. sativus* equalled that of *T. subterraneum*, but the yield of *O. compressus* (cv. Santorini) was only approximately 70% of *T. subterraneum*. The forage legume *T. incarnatum* yielded at least 30% more than any other species. The yield of *M. sativa* (cv. SARDI 10) did not plateau within the P treatment range (0–80 kg P applied per hectare) and the critical P requirement could not be determined.

In 2014, spring DM yields were considerably higher than those measured in 2013. Although 2014 was below average for rainfall, the soil at the Burrinjuck was relatively wet during spring. *J. bufonius* is favoured by wet soil conditions and germinated in high density in several plots, causing establishment failures for all but the most competitive species. The critical Colwell P requirement of *T. subterraneum* was 30 mg P kg⁻¹. *O. sativus* (cv. Margurita) again yielded as well as

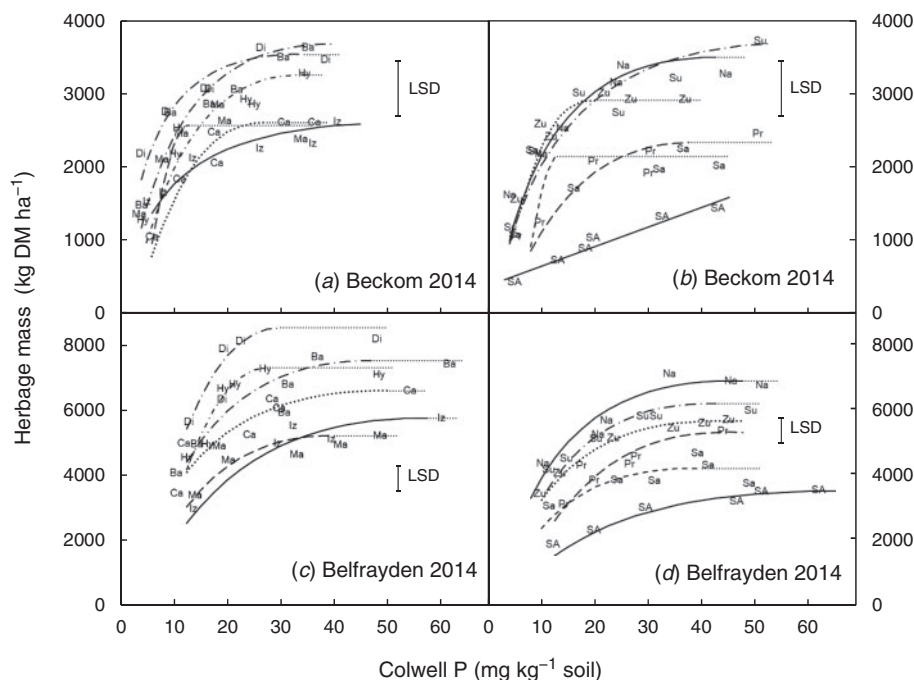


Fig. 3. Peak spring herbage dry matter (DM) at (a, b) Beckom in 2014 and (c, d) Belfrayden in 2014 in response to increasing levels of soil phosphorus (P) measured as Colwell P in the 0- to 10-cm layer. Lines show the Dyson and Conyers (2013) yield response equation back-fitted to the data for all varieties except *Medicago sativa* cv. SARDI 10 when its response to increased soil P was linear. The equation of Dyson and Conyers (2013) addresses yield response to P below the maximum yield. Fine dotted lines indicate the implied maximum yield plateau. Bars indicate the largest l.s.d. ($P = 0.05$) from an unbalanced ANOVA comparing the maximum herbage yield of the pasture varieties. For clarity, the symbols represent the average P treatment values for each pasture variety. However, the yield response functions were fitted using plot-level data for herbage yield and Colwell-extractable P. The varieties grown at both sites are shown in two panels for clarity. The reference *Trifolium subterraneum* varieties grown at these sites were cv. Izmir and cv. Narrikup. Iz with solid line, *T. subterraneum* cv. Izmir; Na with solid line, *T. subterraneum* cv. Narrikup; Ba with long dash plus dot line, *Trifolium spumosum* cv. Bartolo; Ca with dotted line, *Biserrula pelecinus* cv. Casbah; Di with long dash dot line, *Trifolium incarnatum* cv. Dixie; Hy with short dash dot line, *Trifolium hirtum* cv. Hykon; Ma with long dash line, *Ornithopus sativus* cv. Margurita; Pr with long dash line, *Trifolium glanduliferum* cv. Prima; Sa with short dash line, *Ornithopus compressus* cv. Santorini; SA with solid line, *M. sativa* cv. SARDI 10; Su with long dash dot line, *Medicago truncatula* cv. Sultan SU; Zu with dotted line, *Trifolium vesiculosum* cv. Zulu II.

T. subterraneum, with a significantly lower critical Colwell P requirement of 20 mg P kg⁻¹. *T. vesiculosum* (cv. Zulu II) also had a significantly lower critical P requirement (19 mg P kg⁻¹), but yielded relatively poorly. The critical P requirements of *T. michelianum* and *T. incarnatum* (cv. Dixie) were not significantly different from that of *T. subterraneum*. However, *T. michelianum* (cv. Bolta) exceeded the yield of *T. subterraneum* by 16%. *M. sativa* (cv. SARDI 10) achieved a yield plateau in this season. The critical Colwell P requirement of *M. sativa* was 45 mg kg⁻¹, significantly greater than that of any of the other legumes.

Belfrayden and Beckom

The late-maturing *T. subterraneum* cv. Leura was not grown at Beckom and Belfrayden because it is not adapted to the climate at these locations. The better-adapted (mid-season) cultivars Izmir and Narrikup were grown instead.

At Beckom, the critical Colwell P estimates for the *T. subterraneum* cultivars Narrikup and Izmir were 28 and 32 mg P kg⁻¹, respectively, but the two values were not significantly different (Fig. 4). *O. sativus* (cv. Margurita), *O. compressus* (cv. Santorini) and *T. vesiculosum* (Zulu II) had critical P requirements half or less than that of the *T. subterraneum* cultivars. *T. subterraneum* cv. Narrikup out-yielded cv. Izmir (Fig. 3). *T. vesiculosum* was high yielding. The maximum yields of *O. sativus* and *O. compressus* were not significantly different to that of *T. subterraneum* (cv. Izmir), but were substantially less than that of cv. Narrikup. *T. spumosum* (cv. Bartolo), *T. hirtum* (cv. Hykon), *M. truncatula* (Sultan-SU) and *B. pelecinus* (cv. Casbah) had critical P requirements and maximum yields that were not different from those of the high-yielding cv. Narrikup. The yield of *M. sativa* did not plateau, and its critical P requirement could not be determined.

At Belfrayden, the critical Colwell P requirements of the *T. subterraneum* cultivars Narrikup and Izmir were 30 and 40 mg

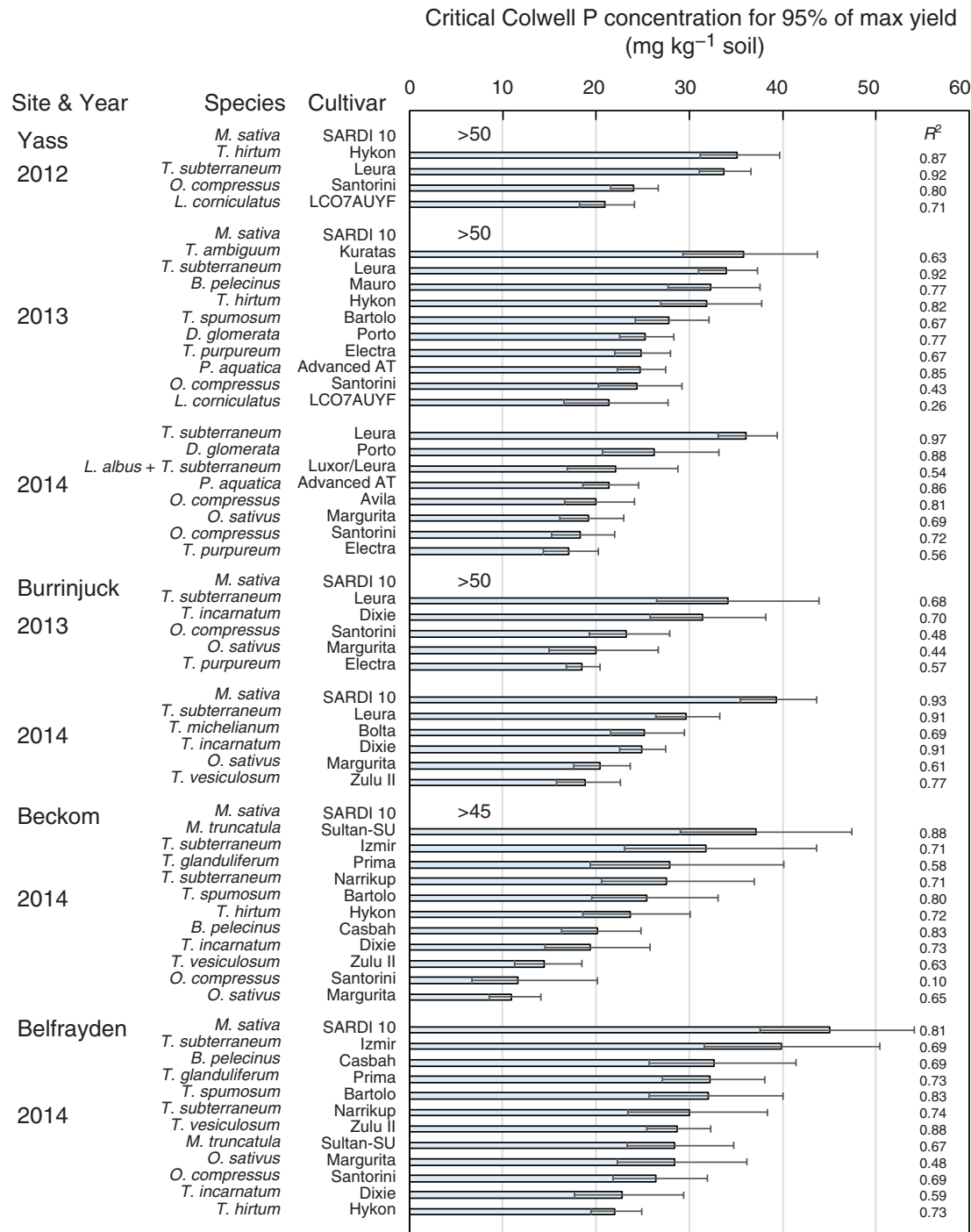


Fig. 4. Critical Colwell phosphorus (P) concentrations of topsoil in the 0- to 10-cm layer that correspond to 95% of maximum herbage yield. Data show the means and their 95% confidence intervals (i.e. $\pm 2 \times \text{s.e.m.}$) for each critical soil test P estimate and are used for comparison among varieties within each site-year experiment. Coefficients of determination (R^2) are provided for the Dyson and Conyers (2013) relationships between $\ln(\text{soil test P})$ and $\arcsine([\text{relative herbage yield}\%/100]^{0.5})$, which were used to estimate the critical Colwell P concentrations.

Table 2. Mean critical soil test phosphorus (P) values for those species that had established and grown well in the experiments

Each site $\times$ year result is regarded as independent for the reasons given in the text. Standard deviations are provided in parentheses as a measure of the repeatability of the critical P determinations because standard deviation is independent of sample size

Species	Cultivar(s) tested	Sample size ^A	Critical Colwell P ^B (mg kg ⁻¹)		Critical Olsen P ^C (mg kg ⁻¹)	
			For 95% maximum yield	For 90% maximum yield	For 95% maximum yield	For 90% maximum yield
<i>Medicago sativa</i>	SARDI 10	6	>45–50	>40–50	>15.5–21.4	>13.8–21.4
<i>Trifolium ambiguum</i>	Kuratas	1	36	33	12.5	12
<i>Trifolium subterraneum</i>	5 $\times$ Leura, 2 $\times$ Izmir, 2 $\times$ Narrikup	9	33 (4)	28 (4)	13.2 (1.8)	11.1 (1.4)
<i>Medicago truncatula</i>	Sultan-SU	2	33 (6)	27 (4)	14.5 (4.3)	11.7 (3.2)
<i>Trifolium glanduliferum</i>	Prima	2	30 (3)	26 (3)	13.1 (0)	11.2 (0)
<i>Trifolium spumosum</i>	Bartolo	3	28 (3)	24 (3)	11.6 (1.6)	9.8 (0.9)
<i>Biserrula pelecinus</i>	2 $\times$ Casbah, 1 $\times$ Mauro	3	28 (7)	24 (6)	11.3 (2.0)	9.7 (1.4)
<i>Trifolium hirtum</i>	Hykon	4	28 (6)	24 (5)	11.0 (2.0)	9.6 (1.7)
<i>Dactylis glomerata</i>	Porto	2	26 (1)	22 (1)	9.3 (0.3)	7.9 (0.3)
<i>Trifolium michelianum</i>	Bolta	1	25	22	9.0	7.9
<i>Trifolium incarnatum</i>	Dixie	4	25 (5)	21 (5)	9.8 (1.8)	8.3 (1.8)
<i>Phalaris aquatica</i>	Advanced AT	2	23 (2)	20 (3)	8.3 (0.8)	7.3 (1.1)
<i>Lotus corniculatus</i>	Breeders line LCO7AUYF	2	21 (0)	19 (0)	8.2 (0.5)	7.5 (0.6)
<i>Ornithopus compressus</i>	6 $\times$ Santorini, 1 $\times$ Avila	7	21 (5)	19 (4)	8.2 (1.9)	7.3 (1.5)
<i>Ornithopus sativus</i>	Margurita	5	20 (6)	17 (5)	7.7 (2.4)	6.7 (1.9)
<i>Trifolium vesiculosum</i>	Zulu II	3	21 (7)	18 (6)	8.3 (2.8)	7.1 (1.9)
<i>Trifolium purpureum</i>	Electra	3	20 (4)	19 (4)	7.5 (1.3)	7.5 (1.3)

^ASample size refers to the number of 'cultivar–site–year' instances that yielded a critical soil test P result for each of the species. For specific 'cultivar–site–year' results, refer to Fig. 4.

^BThe critical Colwell P concentrations were derived from experiments grown in soils with a phosphorus buffering index range of 40–80.

^CCritical Olsen P values were determined from critical Colwell P values using relationships between Olsen P and Colwell P determined for each site in each year of the experiment (Table 3).

Table 3. Relationships between Olsen P (y) and Colwell P (x) concentrations (mg P kg⁻¹ soil) of topsoil (depth 0–10 cm) sampled in spring at the Yass, Burrinjuck, Beckom and Belfrayden sites during the years in which P response experiments were conducted

Site	Year	Relationship	R ²
Yass	2012	$y = 0.342x + 1.3449$	0.93
Yass	2013	$y = 0.3275x + 0.7723$	0.95
Yass	2014	$y = 0.3601x + 0.0646$	0.96
Burrinjuck	2013	$y = 0.3909x + 0.2264$	0.94
Burrinjuck	2014	$y = 0.3363x + 0.5457$	0.97
Beckom	2014	$y = 0.4858x - 0.4622$	0.97
Belfrayden	2014	$y = 0.4572x - 1.5644$	0.95

P kg⁻¹, respectively, but the two values were not significantly different (Fig. 4). However, cv. Narrikup again out-yielded cv. Izmir (by ~23%; Fig. 3). Only *T. incarnatum* (cv. Dixie) and *T. hirtum* (cv. Hykon) had critical P requirements that were significantly lower than that of *T. subterraneum* cv. Izmir, but their critical P requirements were not significantly lower than that of *T. subterraneum* cv. Narrikup. The maximum yield of *O. sativus* (cv. Margurita) was equivalent to that of *T. subterraneum* cv. Izmir. *O. compressus* (cv. Santorini) yielded relatively poorly. The critical P requirement of *M. sativa* was 45 mg P kg⁻¹ and significantly ($P < 0.05$) higher than that of *T. vesiculosum* (Zulu II), *M. truncatula* (Sultan-SU), *O. sativus* (cv. Margurita), *O. compressus* (cv. Santorini), *T. incarnatum* (cv. Dixie) and *T. hirtum* (cv. Hykon), but not significantly higher than that of either of the *T. subterraneum* cultivars.

Repeatability of critical P estimates

The repeatability of critical soil test P estimates for the pasture species was examined by assuming that each 'cultivar–site–year' experiment was an independent assessment of a species' critical P requirement (Table 2). This was clearly the case when considering critical P estimates among sites, but was also considered to be reasonable when considering critical P estimates among years and within sites because the weather differed from year to year, and the experiment plots were oversown each year with additional seed to ensure plant densities did not decline and confound yield responses to soil P fertility. The number of 'site–year' experiments in which each species was represented varied for the reasons described above, so repeatability of the critical P estimate was determined by calculating the standard deviation associated with each estimate of average critical P value. By this measure, the critical P estimates for some pasture varieties (e.g. *L. corniculatus*, *D. glomerata*, *P. aquatica*) were highly repeatable, whereas the repeatability of critical P estimates for some other varieties (e.g. *B. pelecinus*, *T. vesiculosum*) was relatively poor.

Herbage P concentrations

Herbage P concentrations were determined for all species grown in the final year of the experiments (2014). Natural log functions described the positive relationships between topsoil Colwell P and herbage P concentrations (Table 4) and were used to estimate the herbage P concentration of plants when grown at their critical external P supply level. Within each site, critical herbage P concentrations ranged up to 1.5-fold among the legumes.

Table 4. P concentrations of herbage achieved at the critical Colwell soil test P level (depth 0–10 cm) for 95% of maximum yield. Values were derived by interpolation of the relationships developed between Colwell P concentrations (mg kg⁻¹ soil; x) and the corresponding herbage P concentrations (y) in whole shoot samples harvested at peak spring yield. Different lowercase letters indicate significant differences in critical herbage P concentration between pasture legume varieties at the same site. DM, dry matter

Species	Cultivar	Critical herbage P concentration (mg g ⁻¹ DM)	Relationship between Colwell soil test P concentration and herbage P concentration
Yass 2014			
<i>Trifolium subterraneum</i>	Leura	3.22 a	$y = 1.304 \times \ln(x) - 1.472$; $R^2 = 0.93$
<i>Ornithopus compressus</i>	Avila	2.68 b	$y = 1.105 \times \ln(x) - 0.653$; $R^2 = 0.90$
<i>Dactylis glomerata</i>	Porto	2.57 bc	$y = 1.055 \times \ln(x) - 1.015$; $R^2 = 0.89$
<i>Ornithopus compressus</i>	Santorini	2.38 bc	$y = 1.322 \times \ln(x) - 1.440$; $R^2 = 0.88$
<i>Ornithopus sativus</i>	Margurita	1.91 d	$y = 1.344 \times \ln(x) - 2.048$; $R^2 = 0.91$
<i>Phalaris aquatica</i>	Advanced AT	1.81 d	$y = 0.528 \times \ln(x) + 0.211$; $R^2 = 0.91$
<i>Trifolium purpureum</i>	Electra	1.80 d	$y = 1.437 \times \ln(x) - 2.216$; $R^2 = 0.83$
<i>Lotus albus</i> + <i>Trifolium subterraneum</i>	Luxor, Leura	2.58 bc	$y = 1.404 \times \ln(x) - 1.781$; $R^2 = 0.92$
l.s.d. ($P = 0.05$)		0.34	
Burrinjuck 2014			
<i>Trifolium subterraneum</i>	Leura	2.50 a	$y = 0.903 \times \ln(x) + 0.605$; $R^2 = 0.84$
<i>Trifolium michelianum</i>	Bolta	2.16 b	$y = 1.129 \times \ln(x) - 1.526$; $R^2 = 0.79$
<i>Trifolium incarnatum</i>	Dixie	1.99 bc	$y = 0.461 \times \ln(x) + 0.865$; $R^2 = 0.51$
<i>Ornithopus sativus</i>	Margurita	1.91 c	$y = 0.606 \times \ln(x) + 0.961$; $R^2 = 0.76$
<i>Trifolium vesiculosum</i>	Zulu II	1.57 d	$y = 0.807 \times \ln(x) + 0.895$; $R^2 = 0.68$
l.s.d. ($P = 0.05$)		0.18	
Beckom 2014			
<i>Trifolium subterraneum</i>	Narrakup	2.04 a	$y = 0.497 \times \ln(x) + 0.393$; $R^2 = 0.91$
<i>Ornithopus sativus</i>	Margurita	1.90 ab	$y = 0.670 \times \ln(x) + 0.300$; $R^2 = 0.74$
<i>Trifolium incarnatum</i>	Dixie	1.87 bc	$y = 0.552 \times \ln(x) + 0.235$; $R^2 = 0.92$
<i>Medicago truncatula</i>	Sultan-SU	1.86 bc	$y = 0.364 \times \ln(x) + 0.545$; $R^2 = 0.67$
<i>Trifolium spumosum</i>	Bartolo	1.77 bcd	$y = 0.321 \times \ln(x) + 0.735$; $R^2 = 0.82$
<i>Trifolium subterraneum</i>	Izmir	1.73 cd	$y = 0.381 \times \ln(x) + 0.409$; $R^2 = 0.84$
<i>Biserrula pelecinus</i>	Casbah	1.60 de	$y = 0.246 \times \ln(x) + 0.860$; $R^2 = 0.68$
<i>Trifolium vesiculosum</i>	Zulu II	1.57 e	$y = 0.516 \times \ln(x) + 0.198$; $R^2 = 0.78$
<i>Trifolium hirtum</i>	Hykon	1.49 f	$y = 0.261 \times \ln(x) + 0.662$; $R^2 = 0.70$
<i>Ornithopus compressus</i>	Santorini	1.46 f	$y = 0.445 \times \ln(x) + 0.367$; $R^2 = 0.80$
<i>Trifolium glanduliferum</i>	Prima	1.36 f	$y = 0.211 \times \ln(x) + 0.660$; $R^2 = 0.75$
l.s.d. ($P = 0.05$)		0.16	
Belfrayden 2014			
<i>Ornithopus sativus</i>	Margurita	1.93 a	$y = 0.670 \times \ln(x) + 0.300$; $R^2 = 0.74$
<i>Biserrula pelecinus</i>	Casbah	1.75 ab	$y = 0.246 \times \ln(x) + 0.860$; $R^2 = 0.68$
<i>Trifolium incarnatum</i>	Dixie	1.70 abc	$y = 0.552 \times \ln(x) + 0.235$; $R^2 = 0.92$
<i>Trifolium spumosum</i>	Bartolo	1.64 bc	$y = 0.321 \times \ln(x) + 0.735$; $R^2 = 0.82$
<i>Trifolium subterraneum</i>	Izmir	1.62 bc	$y = 0.381 \times \ln(x) + 0.409$; $R^2 = 0.84$
<i>Medicago sativa</i>	SARDI 10	1.57 bcd	$y = 0.339 \times \ln(x) + 0.541$; $R^2 = 0.90$
<i>Trifolium subterraneum</i>	Narrakup	1.56 bcd	$y = 0.497 \times \ln(x) + 0.393$; $R^2 = 0.91$
<i>Medicago truncatula</i>	Sultan-SU	1.46 cde	$y = 0.364 \times \ln(x) + 0.545$; $R^2 = 0.67$
<i>Trifolium vesiculosum</i>	Zulu II	1.46 cde	$y = 0.516 \times \ln(x) + 0.198$; $R^2 = 0.78$
<i>Trifolium glanduliferum</i>	Prima	1.37 de	$y = 0.211 \times \ln(x) + 0.660$; $R^2 = 0.75$
<i>Ornithopus compressus</i>	Santorini	1.36 de	$y = 0.445 \times \ln(x) + 0.367$; $R^2 = 0.80$
<i>Trifolium hirtum</i>	Hykon	1.30 e	$y = 0.261 \times \ln(x) + 0.660$; $R^2 = 0.70$
l.s.d. ($P = 0.05$)		0.24	

However, the rankings of many genotypes were often not consistent among the sites. Plant biomass P concentrations are promoted as potentially indicative of plant P status. For example, biomass P concentrations have been most extensively studied in *T. subterraneum* and the consensus critical range for whole shoots near flowering is expected to be 2.8–3.2 mg P g⁻¹ DM (Pinkerton *et al.* 1997). The P concentrations of whole shoots of *T. subterraneum* fell within or close to this range at Yass and

Burrinjuck, but values were lower at Beckom and Belfrayden, prompting us to examine whether dry late spring weather conditions were associated with relatively low herbage P concentrations. Only *T. subterraneum* and the *Ornithopus* spp. were grown at all four sites in 2014. Their critical (internal) herbage P concentrations were negatively correlated with the prevailing October rainfall at each site (Fig. 5a). However, critical (external) soil test P concentrations were not

affected consistently by dry late-season conditions (Fig. 5b). Interpretation of critical herbage P concentrations is subject to many qualifications, because biomass P concentrations vary with cultural conditions, plant age, prevailing environment and genotype (Smith and Loneragan 1997). The present results serve to reinforce the caution that must be used when relying on biomass P testing as an indicator of soil fertility.

Mycorrhizal colonisation

In 2014, colonisation of roots by AMF was measured on topsoil roots (depth 0–10 cm) of *O. sativus*, *O. compressus* and *T. subterraneum* grown in the unfertilised treatment at all four field sites. The roots of all three species were heavily colonised by AMF (Table 5). Colonisation ranged from 44% to 57% of root length at the Yass, Beckom and Belfrayden sites, and from 27% to 46% at the Burrinjuck site.

Root hair length

In 2016, root hair length was measured on topsoil roots (depth 0–10 cm) of *O. sativus*, *O. compressus* and *T. subterraneum* from the unfertilised (i.e. 0 kg P ha⁻¹) and 15 kg P ha⁻¹ treatments at the Yass, Burrinjuck and Beckom sites. Root hairs on the *Ornithopus* spp. were typically 0.73–0.97 mm long, whereas those on *T. subterraneum* were typically 0.37–0.45 mm long

(Table 6). There was no significant effect ($P > 0.05$) of P treatment on root hair length. The density of root hairs per unit root length was higher on the *Ornithopus* spp. than on *T. subterraneum* (data not shown).

Discussion

Critical external P requirement and yield potential of pasture legumes

Critical soil test P concentrations in the top 10 cm of soil profiles that indicate 90% (Moody 2007) or 95% (Gourley *et al.* 2007, 2019) of maximum yield have been developed for pasture management in the temperate Mediterranean climatic areas of southern Australia based on analysis of empirical data from numerous field experiments. The experiments were conducted during spring, when growth rates of clover-based pastures are usually at or near their maximum, so that the soil test P benchmark represents the requirement for high-yielding pasture. The definition of critical soil test P requirements at this time of year also fits well with the common farm practice of soil testing in spring to determine fertiliser application rates for late summer–autumn, before the opening seasonal rainfall. Widespread application of the P benchmarks assumes that pasture legumes are present and fixing nitrogen, that temperatures and soil moisture conditions during spring are

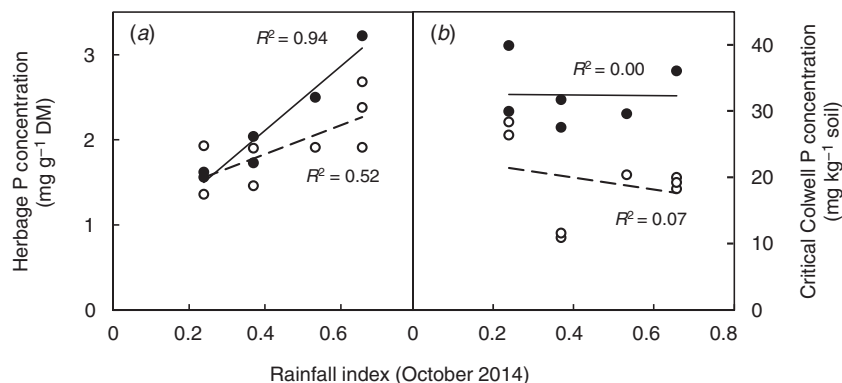


Fig. 5. Relationships between the incidence of low rainfall in October 2014 (expressed as a the rainfall index: October rainfall 2014/average October rainfall) and (a) the phosphorus (P) concentrations of herbage achieved at the critical Colwell soil test P concentration for each cultivar in 2014 and (b) the estimated critical Colwell P concentrations of topsoil (depth 0–10 cm) in 2014. The data represent all cultivars of *Trifolium subterraneum* (closed circles) and *Ornithopus* spp. (open circles) grown at the Yass (rainfall index 0.66), Burrinjuck (rainfall index 0.53), Beckom (rainfall index 0.37) and Belfrayden (rainfall index 0.24) sites in 2014.

Table 5. Colonisation by arbuscular mycorrhizal fungi (AMF) of the roots of three pasture legume species grown at four field sites

Roots were sampled in spring 2014 from the 0- to 10-cm layer. Values are the mean $\pm$ s.e.m. ($n = 3$) of the unfertilised (i.e. 0 kg P ha⁻¹) treatment

Species	Cultivar	AMF colonisation (% root length)			
		Yass	Burrinjuck	Beckom	Belfrayden
<i>Ornithopus sativus</i>	Margurita	57 $\pm$ 14	27 $\pm$ 2	55 $\pm$ 6	49 $\pm$ 2
<i>Ornithopus compressus</i>	Santorini	50 $\pm$ 9	46 $\pm$ 15	45 $\pm$ 12	44 $\pm$ 9
<i>Trifolium subterraneum</i>	Leura	46 $\pm$ 20	27 $\pm$ 9	Not sown	Not sown
<i>Trifolium subterraneum</i>	Izmir	Not sown	Not sown	40 $\pm$ 5	50 $\pm$ 8

Table 6. Root hair length of three pasture legume species grown at the Yass, Burrinjuck and Beckom field sites

Roots were sampled in May 2016 from the 0- to 10-cm layer. Values are the mean $\pm$ s.e.m. ($n = 3$) of the unfertilised (i.e. 0 kg P ha⁻¹) and 15 kg P ha⁻¹ treatments. At each of the three sites, the root hair length was in the order *Ornithopus sativus* $\geq$ *Ornithopus compressus* $>$ *Trifolium subterraneum*. There was no significant effect ($P > 0.05$) of P treatment on root hair length

Species	Cultivar	Root hair length (mm)					
		Yass		Burrinjuck		Beckom	
		P applied (kg ha ⁻¹)		P applied (kg ha ⁻¹)		P applied (kg ha ⁻¹)	
		0	15	0	15	0	15
<i>Ornithopus sativus</i>	Margurita	0.82 $\pm$ 0.07	0.81 $\pm$ 0.02	0.88 $\pm$ 0.03	0.89 $\pm$ 0.05	0.85 $\pm$ 0.03	0.92 $\pm$ 0.07
<i>Ornithopus compressus</i>	Santorini	0.87 $\pm$ 0.02	0.73 $\pm$ 0.01	0.75 $\pm$ 0.03	0.82 $\pm$ 0.01	0.95 $\pm$ 0.01	0.97 $\pm$ 0.02
<i>Trifolium subterraneum</i>	Leura	0.45 $\pm$ 0.08	0.40 $\pm$ 0.13	0.37 $\pm$ 0.02	0.35 $\pm$ 0.02	Not sown	
<i>Trifolium subterraneum</i>	Narrikup	Not sown		Not sown		0.37 $\pm$ 0.08	0.45 $\pm$ 0.02

conductive for rapid growth rates (e.g. Cullen *et al.* 2008), that other nutrients and soil physical and/or chemical conditions are non-limiting for pasture growth or will be corrected concurrently (e.g. Trotter *et al.* 2014) and that plant-available P is predominantly concentrated in the uppermost layer of the soil profile. The latter assumption is reasonable for many southern Australian soils with moderate to high P sorption capacity. For example, Simpson *et al.* (2015) found, after 14 years of optimal and supra-optimal P fertiliser applications at a site near Canberra in the Australian Capital Territory (phosphorus-buffering index (PBI) = 50), that 70% of change in soil total P had occurred in the top 10 cm of the soil, with the remaining change confined to the 10- to 20-cm layer. Similarly, after 100 years of P fertiliser use near Rutherglen in Victoria (PBI = 120), all the increase in soil total P concentration was confined to the top 20 cm of the soil, with 80–84% of the change occurring in the topmost 10 cm of the soil profile (Scheffe *et al.* 2015). However, in light-textured soils with very low P-buffering capacity (e.g. PBI < 15), where soil P can leach from the uppermost soil layer (e.g. Lewis *et al.* 1981; Ritchie and Weaver 1993), it is likely that critical topsoil P benchmarks will be less reliable indicators of pasture yield potential.

Different soil test protocols extract different amounts of P. Consequently, critical P concentrations vary with the soil test being used, and may vary among soil types. Two bicarbonate extracts of topsoil (depth 0–10 cm) are the most widely used extractable soil P tests in southern Australia (Olsen *et al.* 1954; Colwell 1963). The critical Olsen P value for pasture production is claimed to be independent of soil type (Gourley *et al.* 2007, 2019), whereas the critical Colwell P value varies with a soil's P-buffering capacity (Helyar and Spencer 1977). Consequently, different critical Colwell P concentrations are recommended for soils based on results of the PBI test (Burkitt *et al.* 2002, 2008) of the soil (Gourley *et al.* 2007, 2019; Moody 2007), and the two tests are typically offered concurrently by soil testing services.

In the present study we determined the critical external P requirement and yield potential of a range of pasture legumes that are used, or are of potential use, in southern Australia at four sites (PBI range 40–80; Table 1) where we can expect that surface applications of P fertiliser will not be leached from the uppermost soil layers (e.g. Scheffe *et al.* 2015; Simpson *et al.* 2015).

At all sites and in every season, a range in critical soil test P requirements was observed among the pasture varieties. We surmise that the reference species *T. subterraneum* would

have a critical P requirement that reflected the critical soil test P guidelines for soil fertility management of clover-based pastures (Gourley *et al.* 2007, 2019), because they were derived in many cases from experiments with pastures based on *T. subterraneum* and *T. repens*, and *T. subterraneum* and *T. repens* have similar critical P requirements (e.g. Helyar and Anderson 1970, 1971). The current recommended critical P concentration for 95% of maximum yield falls within the range 28–32 mg Colwell P kg⁻¹ (i.e. for soils with PBI 40–80), or is 15 mg Olsen P kg⁻¹ soil (all soil types). The average critical P concentrations from all 'site-year' measurements for *T. subterraneum* in the present study were close to these benchmark values (e.g. 33 mg Colwell P kg⁻¹ and 13.2 mg Olsen P kg⁻¹; Fig. 4; Table 2).

Differences in the maximum yield of cultivars within a species were observed on the few occasions that such a comparison was made (Figs 1–4). However, there were no significant differences in the critical P requirements among cultivars from a single species. In contrast, significant differences in critical P concentrations were found among some legume species. Nine of the legumes were compared with *T. subterraneum* on three or more 'cultivar-site-year' occasions (Fig. 4). Of this group, five species had a significantly lower critical P requirement than *T. subterraneum* in most of the comparisons: *O. compressus* in five of seven comparisons; *O. sativus* in three of five comparisons; *T. purpureum* in three of three comparisons; and *T. incarnatum* and *T. vesiculosum* in two of three comparisons (Fig. 4). In every instance, the exceptions occurred at Burrinjuck in 2013 or Belfrayden in 2014. On these occasions, the species putatively identified as having a lower critical P requirement always ranked below *T. subterraneum*, and the inability to demonstrate differences in these specific 'site-year' instances suggests there was higher variance due to site or seasonal conditions. The average critical P concentrations of the low critical-P legumes was 20–21 mg Colwell P kg⁻¹ (7.5–8.2 mg Olsen P kg⁻¹) for *O. compressus*, *O. sativus*, *T. purpureum* and *T. vesiculosum*, and 25 mg Colwell P kg⁻¹ (9.8 mg Olsen P kg⁻¹) for *T. incarnatum*. The results for *Ornithopus* spp. are consistent with those of other studies that have reported *O. compressus* and *O. sativus* to have critical applied P requirements that are less than that of *T. subterraneum*, and more comparable to those of grasses (Blair and Cordero 1978; de Ruiter 1981; Bolland and Paynter 1992, 1994; Moir *et al.* 2014).

L. corniculatus was only tested twice because it did not persist over the dry summer periods at the Yass site, and resowing became impossible due to the low availability of seed. However, it also appeared to have a low critical P requirement, similar to that of the *Ornithopus* spp. A low applied P requirement has been noted previously (e.g. Acuña 2008), and further assessment, in a perennial growing season environment, may be justified.

We deduced that *M. sativa* is likely to have a critical P requirement that is substantially higher than that of *T. subterraneum* (Fig. 4). The highest P application rate used in the experiments was not high enough on four of six occasions to observe a plateau in the growth response of *M. sativa* to soil P fertility. On one of the two occasions that a yield plateau was achieved, the critical P requirement of *M. sativa* was significantly higher than that of *T. subterraneum*. Consequently, we suspect that the critical P requirement for 95% maximum yield of *M. sativa* will be greater than 45–50 mg Colwell P kg⁻¹ (greater than 16–21 mg Olsen P kg⁻¹). *M. sativa* is a globally important forage species prized for its high yields and high-quality forage (Annicchiarico *et al.* 2015), yet we are unaware of any study that has determined its critical soil test P requirement under field conditions. Limited data are available from glasshouse experiments, and they support the hypothesis that *M. sativa* has a substantially higher critical P requirement than *T. subterraneum* (Helyar and Anderson 1970, 1971). The high P requirement of *M. sativa* may also be exacerbated by the plant's susceptibility to aluminium toxicity, even in moderately acid soils (pH 5.0–5.5; Munns 1965; Crocker *et al.* 1985).

Implications for management of soil P status

Superficially, there appeared to be a wide range in critical P requirements among the varieties tested in the field experiments (Table 2), as found in glasshouse and controlled environment experiments (Haling *et al.* 2016a; Sandral *et al.* 2018). However, in the glasshouse, a much wider range in critical P application rates was found than observed for critical soil test P concentrations in the field. The glasshouse experiments (Sandral *et al.* 2018) also identified five statistically significant ($P < 0.05$) critical P groupings among many of the species examined in the present field experiments, namely *T. subterraneum* = *T. spumosum* > *T. hirtum* = *M. truncatula* = *T. purpureum* = *T. incarnatum* > *T. michelianum* = *T. vesiculosum* = *T. glanduliferum* > *B. pelecinus* > *O. compressus* = *O. sativus*. However, the present field experiments only supported the notion that the critical P requirement of *T. subterraneum* was significantly greater than that of *T. incarnatum*, *T. purpureum*, *T. vesiculosum*, *O. compressus* and *O. sativus* in most instances. The experiments have also indicated that *M. sativa* has a substantially higher critical P requirement than *T. subterraneum*.

We expect that seasonal conditions in the field will not always permit the expression of differences in critical P requirements. For example, dry soil conditions such as experienced at Belfrayden in 2014, where rainfall received during September and October was only half the average for that time of year, are likely to restrict P acquisition (e.g. Pinkerton and Simpson 1986). This is because the diffusion path for P in a drying surface soil is attenuated and/or broken as soil moisture content declines. We

found that dry late spring conditions were associated with lower herbage P concentrations and assume that this was a reflection of the increased need for P mobilisation from older leaves to support the growth of young leaves as P acquisition became constrained by a drying topsoil. We contend that the general compression of the range in critical external P requirement among these species in the field (i.e. compared with that in the well-watered and pasteurised soil conditions of a glasshouse experiment) may also be a consequence of the many challenges to root growth in a natural soil environment, such as hard soils (Passioura 2002), soil acidity (Munns 1965) and root diseases (Simpson *et al.* 2011). In addition, high levels of colonisation of roots by AMF in the field (Table 5) may have a similar effect. Schweiger *et al.* (1995) have shown, in glasshouse experiments, that *T. subterraneum* gains a greater P acquisition benefit when colonised by AMF than do the *Ornithopus* spp. These authors hypothesised that this was because *T. subterraneum* has short root hairs, whereas the root hairs of *Ornithopus* spp. are long. Indeed, the lengths of root hairs on these two species in the field (Table 6) were short (0.40 mm) and long (0.86 mm) respectively, and similar to their contrasting lengths of root hairs observed in glasshouse experiments (Haling *et al.* 2016b; Yang *et al.* 2017; Sandral *et al.* 2018).

The critical soil test P concentrations recommended for pasture management in southern Australia (Gourley *et al.* 2007) do not recognise that there may be differences in P requirements among legumes that underpin different pasture types. The data from the present field experiments confirm the suitability of current recommendations for *T. subterraneum* pastures, and indicate that the benchmark critical P concentrations for *T. subterraneum* are suitable for most of the pasture legumes that were examined. However, a lower soil P availability benchmark is feasible for pastures based on species such as *O. compressus* and *O. sativus*, and forage crops using *T. incarnatum*, *T. purpureum* or *T. vesiculosum*. A much higher soil test P benchmark is potentially needed for *M. sativa* pastures. However, further field experiments will be necessary to determine the appropriate critical soil test P concentration for this legume.

Reducing the requirement for P fertiliser in grass-legume pastures

O. compressus and *O. sativus* showed the greatest potential to improve P efficiency in pasture systems because of their low critical P requirements. Their use, as alternatives to *T. subterraneum*, would reduce the critical soil test P concentration for pasture production to a P level similar to that required by key companion grasses (e.g. *P. aquatica*, *D. glomerata*), but it may still be greater than that required for other P-efficient grasses (e.g. *Lolium rigidum* Gaudin, *Vulpia* spp., *Rytidosperma richardsonii* (Cashmore) Connor and Edgar, *Holcus lanatus* L.; Hill *et al.* 2005). Compared with *T. subterraneum*, the *Ornithopus* spp. have higher specific root lengths and longer root hairs that allow them to explore the soil for P more effectively (Haling *et al.* 2016b).

It has been estimated previously that if the soil test P benchmark for pasture production were reduced from 15 mg

Olsen P kg⁻¹ (i.e. that recommended for *T. subterraneum*) to 10 mg Olsen P kg⁻¹ (i.e. suitable for *Ornithopus* spp.), the annual P fertiliser input for soil fertility maintenance may be reduced by approximately 30% (Simpson *et al.* 2014). In moderate to high P-sorbing soils, P accumulates after fertiliser application at a rate that is positively correlated with the soil test P concentration at which the soil is being maintained. Reducing the soil test P concentration of a soil is expected to drive efficiency in P use because the rate at which P accumulates in the soil would also be reduced.

The *Ornithopus* spp. are already used successfully on sandy, acid soils in parts of Western Australia, northern New South Wales and as a pasture ley in crop rotations in Western Australia and the Riverina region of New South Wales (Freebairn 1996; Nichols *et al.* 2007; Hackney *et al.* 2013). Further work is now required to investigate the potential to use *Ornithopus* spp. in pastures across a wider area of southern Australia. There is limited evidence to suggest that they may not persist well in some pasture environments (Hayes *et al.* 2015); consequently, there is a need to investigate the factors that may limit wider use (e.g. flowering time, hard-seededness, seed softening, waterlogging tolerance, sensitivity to aluminium or manganese in acid soils).

An experimental mixture of *L. albus* and *T. subterraneum* cv. Leura was sown at Yass in 2014 to determine whether *T. subterraneum* could benefit from companion planting with a species that releases high amounts of citrate from its roots and has been shown to mobilise 'sparingly available' P (Gardner *et al.* 1982; Johnson *et al.* 1996; Neumann *et al.* 2000). *T. subterraneum* relies on 'nutrient foraging' to acquire P by proliferating root length in nutrient patches when growing in low-P soil (Haling *et al.* 2016b) and has very low amounts of organic anions, such as citrate, in its rhizosphere compared with *L. albus* (Kidd *et al.* 2016). Combining species with complementary P acquisition strategies such as these may result in an improved yield outcome (Gardner and Boundy 1983; Hinsinger *et al.* 2011). Indeed, the combined species did achieve a significantly lower critical soil test P requirement than *T. subterraneum* cv. Leura alone. On this basis, the treatment seemed surprisingly successful, because the critical soil test P of the mixture was as low as that achieved by the *Ornithopus* spp. However, the maximum yield of the mixture was also 18% lower than that of the *T. subterraneum* monoculture. It is unclear whether the lower maximum yield was due to competitive interference within the mixture, or an artefact of the experimental protocol (e.g. *Lupinus* seed was sod seeded into established *T. subterraneum* pasture plots, inevitably causing a certain amount of disturbance). Although the mixed sward did appear to have some yield advantage in moderately P-deficient soil, the main factor reducing the estimate of critical P concentration was the lower maximum yield. Consequently, the value or otherwise of mixing these species with complementary root traits for P acquisition was not resolved and warrants further examination.

Conclusions

The series of field experiments reported in this paper demonstrates differences in the critical soil test P

requirements among pasture legume species that are either used currently or are of potential use for southern Australia. The results indicate that for practical P fertiliser management of grass-legume pastures on soils with PBI 40–80, pastures based on *T. subterraneum*, *T. spumosum*, *T. hirtum*, *M. truncatula*, *T. michelianum*, *T. glanduliferum* and *B. pelecinus* should be fertilised to the current recommended soil P fertility benchmarks (e.g. Gourley *et al.* 2007, 2019). However, the forage species *T. incarnatum*, *T. purpureum* and *T. vesiculosum* and two pasture species *O. compressus* and *O. sativus* had lower critical soil test P requirements than *T. subterraneum* in most instances, suggesting they may be fertilised to a lower soil test P concentration without compromising yield. The data also indicate that *M. sativa* has a substantially higher P requirement than *T. subterraneum*, but further work is required to define the soil test P concentration to which this species should be fertilised for maximum yield. The *Ornithopus* spp., which already have some use as permanent pasture varieties (Loi *et al.* 2005; Nichols *et al.* 2012), may have the potential to underpin development of productive pasture systems that require less P fertiliser than is presently needed for pastures based on *T. subterraneum* in southern Australia.

Conflicts of interest

The authors have no conflicts of interest to declare.

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