Final report

Phosphorus management and requirements of tropical legume pasture swards

Project code: P.PSH.1050

Prepared by: Jonathan McLachlan, Chris Guppy, Richard Flavel
University of New England

Date published: 26th May 2023

PUBLISHED BY
Meat & Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

This is an MLA Donor Company funded project.

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Tropical pasture legumes often lack persistence in the extensive grazing systems of northern Australia. However, their inclusion in these systems is expected to improve productivity through atmospheric nitrogen (N) fixation and increased pasture quality. Because the soils of northern Australia are often low in available phosphorus (P), it is expected that a greater understanding of legume P requirements and fertiliser application strategies will lead to better legume productivity and persistence. Numerous controlled-environment growth experiments were conducted to examine the P requirements of a range of tropical grasses and legumes. Subsequent experimentation focused on understanding the mechanisms behind P acquisition and identifying appropriate fertiliser application strategies for better legume growth. The results demonstrated that there are significant differences in yield potential and critical P requirements among tropical pasture species (including between grasses and legumes, and between different legume species). This indicates there is potential to select and use P-efficient species in soil that has inherently low P levels. The results also demonstrated that banded applications of P fertiliser can improve legume productivity in mixed pasture swards. These results have direct implications for the management of legumes and soil fertility in the extensive grazing systems of northern Australia.

Executive summary

Background

The extensive grazing systems of northern Australia are dominated by C_4 grasses. It is expected that the inclusion of tropical pasture legumes in these systems will improve pasture productivity and quality. However, legumes are often outcompeted by pasture grasses which could be due to differences in palatability (i.e. preferential grazing of the legume component) or differences in nutrient requirements (i.e. higher requirement for P by legumes). Further research into P requirements and fertiliser application strategies is expected to improve the management of legumes for better productivity. This will directly benefit the extensive grazing industries of northern Australia, which are based on pastures dominated by C_4 grasses that can be highly productive but are generally low quality. The results of this project will be used to inform species selection in nutrient-deficient soils and encourage/improve fertiliser application practices.

Objectives

This research sought to quantify the critical P requirements of numerous tropical grasses and legumes, identify grass/legume combinations that compete effectively for P, investigate the root traits associated with P acquisition among these species, increase our understanding of fertiliser application strategies to improve legume productivity, and elucidate the effect of factors such as nutrient interactions, soil water and soil temperature on P acquisition in tropical pasture species.

Methodology

Controlled-environment growth experiments were used to investigate P requirements and fertiliser application strategies in a range of tropical pasture species. Soils that had low available P were collected from various sites, ranging from northern NSW to northern QLD. These soils were amended with P fertiliser, using different rates and application strategies. Plants were harvested to investigate parameters including shoot yield, root traits, fertiliser recovery and N fixation.

Results/key findings

Tropical pasture legumes generally had lower yield potentials and higher critical P requirements than the grasses with which they are grown. This means that the legume component of a mixed pasture sward has an increased risk of being outcompeted, particularly in nutrient-deficient soil that receives minimal nutrient input. Fertiliser application improved the productivity of both components, but the grasses remained highly competitive. Preferential placement of P below the legume component at planting was an appropriate strategy for providing legumes with an early advantage.

Benefits to industry

The results of this project will inform species selection and fertiliser application practices in the extensive grazing industries of northern Australia, which are based on nutrient-deficient soils and are dominated by C_4 grasses.

Future research and recommendations

Future research should focus on the effectiveness of N fixation by tropical pasture legumes, to ensure that the benefit of this pasture component is fully realised.

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1. Background

Legume productivity and persistence in phosphorus (P) deficient soils are often compromised because the P requirements for adequate growth of legumes are generally higher than that of grasses. Although some legumes (e.g. *Stylosanthes* spp.) persist in low-P soils, the majority of tropical legumes either have high, or unknown, P requirements. There is evidence that differences in P efficiency can represent as much as 200% in yield gains by some species under P-limiting conditions. Meat and Livestock Australia (MLA) recently funded a review of P fertiliser strategies for legume production in the Brigalow bioregion (B.NBP.0769) (Peck et al., 2015). The review concluded that P fertilisation of existing grass/legume swards, preferably starting with soils of higher P fertility, would deliver returns on investment of between 12-24% due to higher pasture productivity and better protein in the sward.

The key gaps identified in that review concerned the following:

1. Demonstrating the animal production response and economic impact of P fertiliser applied to legume-based pastures at the paddock scale in the sub-tropics and developing recommendations for important legume species.
2. Pot trials to rapidly develop comparative response curves for adapted legumes to determine critical P requirements and the rate of response to applied P for legumes.
3. Test the field responses of legumes to applied fertiliser (rate and application method). Key measures to be quantified include pasture yield, N fixation response and pasture composition changes.
4. Understand Brigalow clay soil responses to fertiliser P (i.e. how much fertiliser and how often does it need to be applied to achieve critical P levels).
5. Develop a better understanding of the extent and impact of P deficiency on animal production (i.e. screen herds for their P status).
6. Test the impact of other nutrient deficiencies (e.g. sulfur and potassium) on the productivity of pasture legumes.

The use of P fertiliser in northern Australia currently remains low because either i) producer perceptions are that soils are fertile enough for pastures, or ii) that the economic benefits of fertiliser application have not yet been demonstrated. In temperate pastures, legumes are the first component to drop out of the pasture because legume root system architecture is generally not adapted for efficient P foraging. Despite producer perceptions of P fertility, maintaining legumes in tropical pasture swards remains challenging and there is independent evidence that soil P fertility is currently low across the northern region. Productivity (both plant and livestock) from tropical swards is therefore lower than it otherwise needs to be.

The review identified a lack of knowledge of the extent of animal P deficiency, and little understanding of the P requirements of tropical legumes and the best way to manage and incorporate them into productive grazing systems. Alternatives to P nutrition of pastures include direct animal supplementation to address animal nutrition constraints. However, this is unlikely to address the additional production benefits possible from more productive pastures that have higher legume components. Concerning fertiliser application, standard P application strategies are based upon surface broadcasting due to the ease and cost of application. Recent research at the University of New England (UNE) has demonstrated that shifts in grass and legume competitiveness and productivity can be achieved by changing P placement strategies (e.g. subsurface banding) and by varying the volume of P-enriched soil (e.g. McLachlan et al., 2019a). These small projects have demonstrated proof-of-concept for banding/injecting P fertiliser to depths that encourage legume persistence and increase P acquisition/utilisation efficiency relative to grasses. This raises the

prospect of developing fertiliser application strategies to increase legume content and persistence in mixed pasture swards.

The MLA-funded review undertook user-group surveys (graziers and mixed farmers), collated existing soil test data (past government surveys and soil test company databases), and consulted agronomists and experts in the region to develop model inputs concerning the economic response of P fertiliser application strategies. Current research in this area is limited to two P trials using six species (three *Medicago* spp., two *Desmanthus* spp. and one *Stylosanthes* spp.) and five P rates in southern QLD. While this is an excellent start, the two sites are constrained by inherent soil fertility and seasonal climatic patterns, so are unlikely to have the same basal P responses as other areas. Complementing this work with detailed research is essential.

The value proposition for investment in understanding the P requirements and fertiliser application strategies for the establishment and persistence of tropical legumes was outlined in the aforementioned MLA-funded review. In more fertile soil, the return on investment to the red meat industry of targeted and successful P nutrition of legumes in pastures was estimated at between 12-24%. In less fertile soils, as higher P inputs are required to bring production levels to profitable levels and species with lower critical P requirements need to be identified, return on investment was estimated at between 9-15%. Details of how these values were calculated are presented in MLA project B.NBP.0769 (Peck et al., 2015).

2. Objectives

1) Deliver critical P requirements of twelve legumes and grasses in soil with a PBI <100, and the effect of PBI and pH on the determined critical P requirements of selected species. Species potentially examined include Desmanthus (3–4 species), Stylos (3–4 species) and agreed elite Stylo lines, Burgundy Bean, Butterfly Pea, Centro and common grass species (Premier Digit, Bambatsi Panic, Purple Pigeon, Green Panics and Rhodes grasses) and others in discussion with producers and LPP partners.

2) Deliver critical P requirements of a range of Desmanthus genotypes in partnership with Agrimix in soil with a PBI <100, and identify any intraspecific variation in basic root morphological traits associated with P acquisition.

3) Identify a mechanism to translate detailed P uptake dynamics from pot trials to critical soil test P values for field plots of tropical legumes.

4) Identify the effect of soil temperature on the P acquisition of selected species, to determine the suitability of growing tropical species in the relatively cooler soils of northern NSW.

5) Deliver recommendations on P fertiliser application strategies to increase P acquisition by legumes relative to grasses through either banding granules, injecting fluid P at different row-spacings or broadcast application.

6) Identify the effect of soil water draw-down by selected species (Digit and Desmanthus, Rhodes and Centro) on the relative availability of a banded application of P fertiliser.

7) Identify combinations of grass/legume that partner effectively with respect to P acquisition and critical soil test ranges in collaboration with LPP partners, and measure recovery of applied P fertiliser efficiently and the effects of competition with grasses on the N fixation of the legumes (constraints in this area may be associated with appropriate rhizobium application).

8) Identify root P acquisition strategies of key tropical legumes (and grasses), particularly architectural structure, root hair lengths, and if possible, organic acid exudates.

9) Deliver interactions of P nutrition with S and K status in partnership with soil constraints to legume persistence project (Hayes (DPI)).

10) Four honours projects, 1–2 PhD students, and one post-doc trained in pasture nutrition and persistence.

3. Methodology

3.1 Introduction

The project was achieved by conducting numerous controlled-environment growth experiments and one field trial in Armidale, NSW, Australia. This Methodology section provides a summary of the general procedures that were used in the controlled-environment growth experiments. The precise methodology that was used in each experiment is outlined in the Appendices, which are listed in Table 1.

Table 1. The numerous experiments that were conducted in the project, the project objectives which each experiment was designed to address, and the Appendix in which the methodology and results/discussion of the experiments are located.

Experiment	Objective*	Appendix
Root morphology and phosphorus requirements of twelve tropical pasture species	1, 8	Appendix 1
Evaluation of the root traits and phosphorus requirements of several *Desmanthus* and *Stylosanthes* genotypes	1, 8	Appendix 2
The effect of pH and PBI on the critical phosphorus requirements of two tropical pasture species	1	Appendix 3
Differences in phosphorus acquisition and critical phosphorus requirements among nine *Desmanthus* spp. genotypes	2	Appendix 4
Premier Digit and Progardes Desmanthus compete effectively for applied phosphorus under mixed sward conditions	1, 7	Appendix 5
Mixed sward plantings influence the shoot yield and phosphorus requirements of tropical pasture species	1, 7, 8	Appendix 6
Mycorrhizal fungi influence phosphorus acquisition in mixed tropical pasture swards	7, 8	Appendix 7
Warm-season pasture species respond to subsurface placement of phosphorus fertiliser	5, 8	Appendix 8
Spatial and temporal development of roots by tropical pasture species	5, 7, 8	Appendix 9
Emergence and establishment of four *Desmanthus* spp. genotypes in three alkaline clay soils	5, 9	Appendix 10
Starter fertiliser reduces the critical phosphorus requirements of two tropical pasture legumes	1, 5	Appendix 11
Starter fertiliser applied at planting improves the productivity of tropical pasture species	5, 7	Appendix 12

Preferential phosphorus placement improves the productivity and competitiveness of tropical pasture legumes	5, 7	Appendix 13
Placement of seed and fertiliser at planting influences phosphorus acquisition in a mixed tropical pasture sward	5, 7	Appendix 14
Legume productivity is improved by preferential fertiliser application at planting in mixed pasture swards	5, 7	Appendix 15
Interactions between phosphorus, potassium and sulfur in tropical pasture species	9	Appendix 16
Sulfur response curves for Premier Digit and two *Desmanthus* spp. genotypes	9	Appendix 17
Phosphorus acquisition by tropical pasture species in response to watering method	6	Appendix 18
Preferential water drawdown in the vicinity of banded fertiliser reduces legume productivity	6	Appendix 19
Soil temperature influences the root growth and phosphorus acquisition of tropical pasture species	4	Appendix 20
Translating between controlled-environment studies and field-relevant recommendations for tropical pasture legumes	3	Appendix 21

*objective 10 is not listed in this table because it was not of an experimental nature

3.2 Plant material

The following species/genotypes were used in the experiments:

- **Tropical grasses** – Bambatsi Panic (*Panicum coloratum*), Floren Bluegrass (*Dichanthium aristatum*), Gatton Panic (*Panicum maximum*), Katambora Rhodes (*Chloris gayana* cv. Katambora) and Premier Digit (*Digitaria eriantha* cv. Premier).
- **Tropical legumes** – Bundey Centro (*Centrosema pascuorum* cv. Bundey), Butterfly Pea (*Clitoria ternatea*), Caatinga Stylo (*Stylosanthes seabrana* cvv. Primar and Unica), Cardillo Centro (*Centrosema pubescens* cv. Cardillo), Common Stylo (*Stylosanthes guianensis* var. V8), Marc Desmanthus (*Desmanthus virgatus* cv. Marc), and Shrubby Stylo (*Stylosanthes scabra* cv. Seca).
- **Agrimix *Desmanthus* spp. genotypes** – JCU 1 (*D. leptophyllus*), JCU 2 (*D. virgatus*), JCU 3 (*D. virgatus*), JCU 4 (*D. bicornutus*), JCU 5 (*D. virgatus*), JCU 6 (*D. bicornutus*), JCU 7 (*D. leptophyllus*), JCU 8 (*D. virgatus*) and JCU 9 (*D. pernambucanus*).
- **Elite/Experimental *Stylosanthes* lines** – Stylo lines #9, #25, #36, #39 and #40.

3.3 Soil

Soil was collected from various sites, ranging from northern NSW to northern QLD (Table 2). Each of the soils had a low Colwell extractable P concentration, which enabled responses to the application of P fertiliser to be investigated. The two sandy soils were primarily used when plants were grown to determine P response curves and root traits, due to the ease of washing roots from the soil. The clay soils were primarily used when plants were grown to investigate fertiliser application strategies, as these soils are more representative of the soils found where the tropical pasture species are commonly grown. The basic properties of the soils that were used in the controlled-environment growth experiments are listed in Table 2.

Table 2. The soils that were collected and used for the controlled-environment growth experiments, including information about the location of collection and basic soil properties.

Soil	Collection Location	pH_{CaCl2}	Colwell P (mg/kg)	PBI
Sandy, Grey Tenosol	Armidale, NSW	5.0	3	29
Sandy Loam, Brown Chromosol	Armidale, NSW	4.7	7	51
Clay, Grey Vertosol	Delungra, NSW	5.0	13	180
Clay, Red Ferrosol	Kingaroy, QLD	4.6	12	385
Clay, Black Vertosol	Mantuan Downs, QLD	7.2	13	90
Clay Loam, Brown Dermosol	Gregory, QLD	7.4	3	68
Clay, Grey Vertosol	Burketown, QLD	7.3	3	78

The soils were prepared by crushing and/or sieving before they were amended with basal nutrients (including N, K, S and micronutrients). The amended soils were added to either pots, boxes or rhizoscanners (see examples shown in Fig. 1). Phosphorus treatments (e.g. different levels of soil P supply and fertiliser application strategies) were prepared by applying P fertiliser to the soil, either before or after the soil was added to the pots/boxes/rhizoscanners.

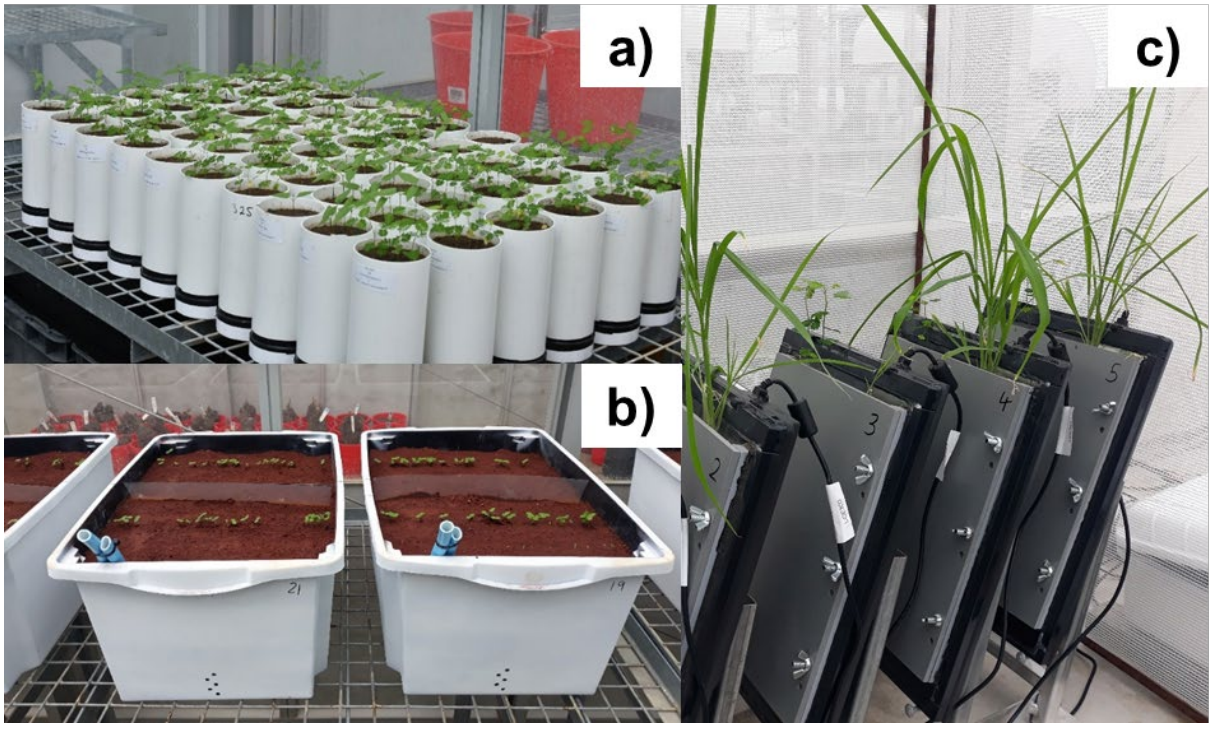

Figure 1. Examples of the pots (a), boxes (b) and rhizoscanners (c) that were used in the controlled-environment growth experiments.

3.4 Growth conditions

Micro-swards of the tropical pasture species were established in the amended soils. There were between two and five replicates of each treatment combination, depending on the experiment and the required statistical analyses. Plants were grown in a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. Pots/boxes/rhizoscanners were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006). Soil moisture was maintained at 80–100% field capacity by regularly watering, either to a pre-determined weight or from a subsoil water reservoir.

3.5 Harvest and measurements

Plants were harvested between four and twelve weeks after planting. Depending on the experiment, the following general procedures were conducted:

- **Emergence** – Seedling emergence was monitored and recorded regularly.
- **Shoot yield** – Shoots were cut at the soil surface, oven-dried at 70°C for three to seven days (until a stable weight was achieved) and weighed.
- **Tissue P** – Shoot samples were finely cut before a subsample was digested in nitric acid. The P concentration of the digested samples was determined either colorimetrically using Malachite green reagent or by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Shoot P content was calculated by multiplying shoot tissue P concentration and shoot dry mass.
- **Root traits** – Roots were washed from the soil over 2 mm sieves to assess traits including root length and diameter (using WinRHIZO), root hair length (by microscopy), and mycorrhizal colonisation (by staining and microscopy). Roots were oven-dried at 70°C and weighed. Root traits (e.g. length and branching) were also assessed using the rhizoscanners.
- **Phosphorus recovery** – Digested shoot samples were analysed for ^{32}P and/or ^{33}P radioactivity using Liquid Scintillation Counting (LSC).

3.6 Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models or linear mixed-effects models. When appropriate, the effect of 'replicate' was included in the most parsimonious model. Means were compared using either least significant difference (LSD) or Tukey's honest significant difference (HSD). Correlations were analysed using linear regression (Crawley, 2013). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Additional statistical analyses included:

- **Critical external P requirements** – Shoot yield responses to P application were determined by fitting a self-starting Weibull growth function ($y = a – b * \exp(–\exp(c) * x^d)$, where x is the P application rate and y is the shoot dry mass) as described by Crawley (2013). Critical external P requirements were calculated as the amount of P applied to achieve 90% of maximum yield based on the fitted Weibull growth functions, and the 95% confidence intervals of the critical P requirements were determined by 'bootstrapping' residuals as described by Crawley (2013).
- **Critical internal P requirements** – Critical internal P requirements were calculated as the shoot P concentration that corresponded with the critical external P application rate.
- **Time to emergence** – Seedling emergence was assessed by fitting a self-starting Weibull growth function ($y = a – b * \exp(–\exp(c) * x^d)$, where x is days and y is emergence), as described by Crawley (2013). Time to 90% emergence was calculated as the days taken to achieve 90% of maximum emergence based on the fitted Weibull growth functions, and the 95% confidence intervals of the time to emergence were determined by 'bootstrapping' residuals as described by Crawley (2013).

4. Results

Key results from the experiments that were conducted in the project are provided in Table 3. Detailed results, including all figures and tables, are provided in the Appendices (see Table 1 for the complete list of experiments and appendices).

Table 3. The key results from each experiment conducted in the project. The numbers in parentheses after each experiment title indicate the objective/s that the experiment was designed to address, while the relevant Appendix which includes the detailed results is also listed in parentheses.

Experiment	Key Results
Root morphology and phosphorus requirements of twelve tropical pasture species (1, 8) (see Appendix 1)	The shoot yield of each species increased in response to soil P supply, although there were differences in maximum shoot yield (1.7–9.8 g DM pot^{-1}) and critical external P requirements (12.8–38.0 mg P kg^{-1} soil) among the species. In general, the tropical grasses were more productive than the legumes when supplied with adequate P, and also had equivalent or lower critical P requirements. Differences in shoot P content (an indication of plant P uptake) were associated with differences in root length (R^2 = 0.38–0.40 in the 15–30 mg P kg^{-1} soil treatments).
Evaluation of the root traits and phosphorus requirements of several *Desmanthus* and *Stylosanthes* genotypes (1, 8) (see Appendix 2)	Each of the legumes produced larger shoot yields in response to increasing levels of soil P supply. However, there were differences in maximum shoot yield (1.5–3.7 g DM pot^{-1}) and critical external P requirements (12.4–43.0 mg P kg^{-1} soil) among the legumes. In general, the three *Desmanthus* spp. genotypes utilised acquired P efficiently even when grown in soil with higher P levels, whereas the eight *Stylosanthes* spp. genotypes tended to accumulate excess tissue P in these treatments.
The effect of pH and PBI on the critical phosphorus requirements of two tropical pasture species (1) (see Appendix 3)	Critical external P requirements were generally lowest when Digit (14.3–18.7 mg P kg^{-1} soil) and Desmanthus (39.9–42.6 mg P kg^{-1} soil) were grown in soil with a pH of either 6 or 7, and increased in response to lower soil pH levels. The critical external P requirements of Digit and Desmanthus generally increased in response to higher soil PBI levels.
Differences in phosphorus acquisition and critical phosphorus requirements among nine *Desmanthus* spp. genotypes (2) (see Appendix 4)	There were differences in maximum shoot yield (1.5–3.5 g DM pot^{-1}) and critical external P requirements (29.4–64.0 mg P kg^{-1} soil) among the Agrimix *Desmanthus* spp. genotypes. Root length was found to be important for P acquisition (R^2 = 0.37–0.62 in the 10–30 mg P kg^{-1} soil treatments).

Premier Digit and Progardes Desmanthus compete effectively for applied phosphorus under mixed sward conditions (1, 7) (see Appendix 5)	The shoot yield and tissue P concentrations of Premier Digit and Progardes Desmanthus increased in response to soil P supply. Both species competed effectively for applied P, with the legume representing between 33–47% of the total yield of the mixed sward plantings.
Mixed sward plantings influence the shoot yield and phosphorus requirements of tropical pasture species (1, 7, 8) (see Appendix 6)	Digit/Desmanthus and Rhodes/Centro partnered effectively and the legumes made up 30–60% of the mixed swards at the first harvest, but these legume contents were lower at the second harvest because the grasses recovered quicker than the legumes after being harvested.
Mycorrhizal fungi influence phosphorus acquisition in mixed tropical pasture swards (7, 8) (see Appendix 7)	Desmanthus was more competitive than Stylo when grown with either Bambatsi Panic, Katambora Rhodes or Premier Digit. However, the grasses generally had higher shoot P concentrations when grown with Desmanthus, indicating that the grass yield penalty was not associated with nutrition but with other factors such as canopy space and soil moisture. Mycorrhizal fungi were estimated to have contributed to the P acquisition of the grasses and legumes by between 1% and 34%.
Warm-season pasture species respond to subsurface placement of phosphorus fertiliser (5, 8) (see Appendix 8)	The grasses (Bambatsi Panic and Premier Digit) respond to banded P by proliferating roots whereas the legumes (Haymaster Lucerne and Progardes Desmanthus) did not (at least within the relatively short timeframe of the experiment). Nevertheless, the grasses and the legumes all derived more P from the zone of P enrichment when P fertiliser was banded (31% in the banded high-P treatment, compared to 3% and 9% in the uniform low-P and high-P treatments, respectively).
Spatial and temporal development of roots by tropical pasture species (5, 7, 8) (see Appendix 9)	Digit foraged the soil more effectively for applied P than Desmanthus, with the highly productive grass component quickly overwhelming the legume component due to the speed at which roots were produced. Nevertheless, Digit and Desmanthus both proliferated roots in the relatively small volume of soil in response to a banded application of P fertiliser, which resulted in higher shoot yields in response to P application.
Emergence and establishment of four *Desmanthus* spp. genotypes in three alkaline clay soils (5, 9) (see Appendix 10)	There were differences in the emergence of viable seed among the *Desmanthus* spp. genotypes (JCU 2 = JCU 4 > JCU 9 = JCU 7). On average across the three soils, gypsum increased seedling emergence by 15% but did not affect shoot yield. In contrast, starter

	fertiliser did not influence seedling emergence but increased shoot yield in two of the three soils (the soils with relatively low Colwell extractable P concentrations of ~3 mg P kg^{-1} soil).
Starter fertiliser reduces the critical phosphorus requirements of two tropical pasture legumes (1,5) (see Appendix 11)	An application of starter P fertiliser increased the shoot yield of Centro and Desmanthus in soil that had previously received between 5–20 mg P kg^{-1}. Without starter fertiliser, the critical P requirement of Centro (23.0 mg P kg^{-1} soil) was lower than that of Desmanthus (52.8 mg P kg^{-1} soil). The addition of starter fertiliser reduced the critical P requirements of both legumes so that they were comparable (16.0 and 13.6 mg P kg^{-1} soil for Centro and Desmanthus, respectively).
Starter fertiliser applied at planting improves the productivity of tropical pasture species (5, 7) (see Appendix 12)	Starter fertiliser that included both N and P increased the shoot yield of Digit and Desmanthus in two clay soils. In one of the soils, Digit was highly productive and outcompeted Desmanthus (critical external P requirements were 21.2 and 40.7 mg P kg^{-1} soil, respectively). In the other soil, Digit growth was constrained and Desmanthus was more productive (critical external P requirements were 37.2 and 33.3 mg P kg^{-1} soil, respectively).
Preferential phosphorus placement improves the productivity and competitiveness of tropical pasture legumes (5, 7) (see Appendix 13)	Preferential placement of P fertiliser below Centro and Desmanthus, when grown with Rhodes and Digit respectively, increased legume growth (e.g. the average legume content increased from 29% to 66% when P was banded below the legume only). Across the treatments, between 20–77% of legume shoot P was derived from applied P. Both grasses foraged and acquired a small amount of P that had been applied below the legumes.
Placement of seed and fertiliser at planting influences phosphorus acquisition in a mixed tropical pasture sward (5, 7) (see Appendix 14)	Preferential placement of P fertiliser below the Desmanthus resulted in the highest yields. When P fertiliser was applied below both components, legume growth was not influenced by either fertiliser application (broadcast vs. banded) or sowing configuration (separate vs. together). However, across the treatments, the proportion of legume declined from an average of 61% to an average of 40% between the first and second harvests.
Legume productivity is improved by preferential fertiliser application at planting in mixed pasture swards (5, 7) (see Appendix 15)	Preferential placement of starter fertiliser, which included both N and P, increased the shoot yield (avg. +63%) and shoot P content (avg. + 87%) of Lucerne and Desmanthus without having a detrimental impact on the

	grasses with which they were grown (Tall Fescue and Digit, respectively). However, the apparent recovery of applied P fertiliser by the legumes was low (2.3% for Lucerne and 0.4% for Desmanthus).
Interactions between phosphorus, potassium and sulfur in tropical pasture species (9) (see Appendix 16)	The shoot yields of Digit and four *Desmanthus* spp. genotypes were constrained by P, but not by S or K, in a nutrient-deficient clay soil collected from northern Australia. Although the addition of P improved shoot yields and shoot P concentrations, it negatively affected shoot S concentrations. The addition of both P and S was therefore important for increasing legume productivity and maintaining legume quality.
Sulfur response curves for Premier Digit and two *Desmanthus* spp. genotypes (9) (see Appendix 17)	The shoot yields of Digit and two *Desmanthus* spp. genotypes increased in response to the application of S, but only between the 0 and 4 mg S kg^{-1} soil treatments. The critical external S requirements of the species were comparable (1.5–2.7 mg S kg^{-1} soil), but the shoot S concentrations of the *Desmanthus* spp. genotypes were, on average, 2.1-fold higher than that of Digit.
Phosphorus acquisition by tropical pasture species in response to watering method (6) (see Appendix 18)	Centro, Desmanthus, Digit and Rhodes all responded to the application of P fertiliser. In general, there was no effect of watering method (i.e. from the surface simulating rainfall, or from the base simulating stored moisture) on shoot yield. The exception was Centro, which was more productive in the surface high-P and banded high-P treatments when watered from the surface.
Preferential water drawdown in the vicinity of banded fertiliser reduces legume productivity (6) (see Appendix 19)	Proliferation of roots in the vicinity of banded P fertiliser led to water drawdown that constrained the growth of Desmanthus but not Digit when water movement was restricted by the coarse sand capillary breaks.
Soil temperature influences the root growth and phosphorus acquisition of tropical pasture species (4) (see Appendix 20)	Digit and Desmanthus grew well in the cooler soils, but not the warmer soils, possibly due to lower respiration rates or because the soil profile was slower to dry down.
Translating between controlled-environment studies and field-relevant recommendations for tropical pasture legumes (3) (see Appendix 21)	Comparison of controlled-environment and field-collected data indicates that the growth of tropical pasture legumes would be constrained in soil with a Colwell extractable P concentration <10 mg P kg^{-1}, but not in soil with a Colwell P between 10–15 mg P kg^{-1} and above. This validates many of the responses seen in the controlled-environment experiments.

5. Conclusion

The project has successfully quantified the critical internal and external P requirements of numerous tropical grasses and legumes, identified suitable grass/legume combinations, characterised important root traits among tropical pasture species, and identified P fertiliser application strategies that result in benefits for legume productivity. The ten project objectives, the overall outcome of each objective, and brief summaries of the objective outcomes are listed in Table 4.

Table 4. The ten project objectives, including the overall outcome of each objective (achieved, partially achieved, not achieved) and brief summaries of the objective outcomes.

Objective	Outcome
1. Deliver critical P requirements of twelve legumes and grasses in soil with a PBI <100, and the effect of PBI and pH on the determined critical phosphorus requirements of selected species. Species potentially examined include Desmanthus (3–4 species), Stylos (3–4 species) and agreed elite Stylo lines, Burgundy Bean, Butterfly Pea, Centro and common grass species (Premier Digit, Bambatsi Panic, Purple Pigeon, Green Panics and Rhodes grasses) and others in discussion with producers and LPP partners.	**ACHIEVED** (see Appendices 1, 2, 3, 5, 6, 11) The critical P requirements of the following species/genotypes, when grown as monocultures, have been determined in soil with a PBI <100: Bambatsi Panic, Gatton Panic, Floren Bluegrass, Katambora Rhodes, Premier Digit, Bundey Centro, Cardillo Centro, Marc Desmanthus, JCU 7 Desmanthus, JCU 9 Desmanthus, Butterfly Pea, Caatinga Stylo, Common Stylo, Shrubby Stylo, and five elite/experimental Stylo lines. The critical P requirements of some of the species/genotypes listed above, when grown under mixed sward conditions (i.e. one grass and one legume grown together), have also been determined. The effect of pH and PBI on the critical P requirements of Digit and Desmanthus have been determined.
2. Deliver critical P requirements of a range of Desmanthus genotypes in partnership with Agrimix in soil with a PBI <100, and identify any intraspecific variation in basic root morphological traits associated with P acquisition.	**ACHIEVED** (see Appendix 4) The critical P requirements and basic root morphological traits (including root length and root hair parameters) of the Agrimix *Desmanthus* spp. genotypes (JCU 2–JCU 9) have been determined in soil with a PBI <100.
3. Identify a mechanism to translate detailed P uptake dynamics from pot trials to critical soil test P values for field plots of tropical legumes.	**ACHIEVED** (see Appendix 21) Numerous controlled-environment growth experiments have been completed, each of which provided shoot yield and tissue P data that was used to calculate critical internal and external P requirements.

	Paired plant and soil samples were collected from five sites across southern QLD, which were analysed for tissue P and Colwell P. The data described above was used to translate between the controlled-environment studies and field-relevant recommendations.
4. Identify the effect of soil temperature on the P acquisition of selected species, to determine the suitability of growing tropical species in the relatively cooler soils of northern NSW.	**PARTIALLY ACHIEVED** (see Appendix 20) The effect of soil temperature on shoot yield has been determined for Digit and Desmanthus.
5. Deliver recommendations on P fertiliser application strategies to increase P acquisition by legumes relative to grasses through either banding granules, injecting fluid P at different row-spacings or broadcast application.	**ACHIEVED** (see Appendices 8, 9, 10, 11, 12, 13, 14, 15) Several fertiliser application strategies have been investigated, including fertiliser source (finely ground fertiliser vs. nutrient solutions) and fertiliser placement (broadcast vs. banded). The effectiveness of these strategies was investigated by assessing shoot yield, tissue P and P acquisition/recovery (using radioisotope).
6. Identify the effect of soil water draw-down by selected species (Digit and Desmanthus, Rhodes and Centro) on the relative availability of a banded application of P fertiliser.	**ACHIEVED** (see Appendices 18, 19) The effect of soil water draw-down on shoot yield, root growth and P acquisition/recovery (using radioisotope) has been determined for Digit and Desmanthus.
7. Identify combinations of grass/legume that partner effectively with respect to P acquisition and critical soil test ranges in collaboration with LPP partners and measure recovery of applied phosphorus fertiliser efficiently and the effects of competition with grasses on the N fixation of the legumes (constraints in this area may be associated with appropriate rhizobium application).	**ACHIEVED** (see Appendices 5, 6, 7, 9, 12, 13, 14, 15) The effect of sward dynamics on yield and critical P requirements has been determined for numerous tropical pasture species. From this work, effective/suitable combinations of tropical grasses and legumes have been identified. Radioisotope has also been used to quantify competition for applied P fertiliser by grasses and legumes. Several *Desmanthus* spp. genotypes were treated with commercially available peat inoculant, but nodules were not observed on the roots of these plants (even when soil with a history of Desmanthus growth was used). For this reason, N-fixation was not calculated from the experiments in this project. This highlights the need for effective rhizobia inoculant.

8. Identify root P acquisition strategies of key tropical legumes (and grasses), particularly architectural structure, root hair lengths, and if possible, organic acid exudates.	**ACHIEVED** (see Appendix 1, 2, 6, 7, 8, 9) The root traits and P acquisition strategies of most species/genotypes were investigated. Measured traits included root length and specific root length, root architecture/branching, root hair length, mycorrhizal colonisation, and P acquisition/recovery (using radioisotope).
9. Deliver interactions of P nutrition with S and K status in partnership with soil constraints to legume persistence project (Hayes (DPI)).	**ACHIEVED** (see Appendix 10, 16, 17) The interaction of P with S and K was investigated in monocultures of Digit and several *Desmanthus* spp. genotypes.
10. Four honours projects, 1–2 PhD students, and one post-doc trained in pasture nutrition and persistence.	**ACHIEVED** Five honours and one masters project were completed. Two post-doctoral research fellows were trained in pasture nutrition and persistence.

5.1 Key findings

The key findings of this project are:

- Tropical pasture legumes generally have lower yield potentials and higher critical P requirements than the grasses with which they are grown. This means that the legume component of a mixed pasture sward is at risk of being outcompeted, particularly in nutrient-deficient soils that receive minimal nutrient input. Nevertheless, there were differences in the critical P requirements of the legumes, which suggests that P-efficient varieties can be selected for growth in inherently low-P soils.
- Fertiliser application improves the productivity of both grasses and legumes, which indicates that there is potential to improve the feedbase in extensive grazing systems that are based on nutrient-deficient soil. However, the benefit derived by grasses from any application of fertiliser means they are highly competitive. If there is minimal grazing management, the legume component is still expected to be outcompeted and may fail to persist.
- Select tropical grasses and legumes proliferate roots in response to P fertiliser application, although grasses generally produce roots that are longer and thinner, so they are more effective at foraging the soil for P than legumes.
- Preferential placement of P below the legume component at planting is an appropriate strategy for providing legumes with an early advantage. This fertiliser application strategy would be useful in soils of moderate fertility, where grasses are still relatively productive and do not require additional P fertiliser. Nevertheless, banded applications of P fertiliser will also be beneficial in low-P soils, because they will improve legume productivity and provide an initial advantage over constituent pasture grasses. It is expected that fertiliser applied below the legume component will eventually benefit the grasses as they forage for available P.

5.2 Benefits to industry

The results of the project directly benefit the grazing industries of northern Australia which are based on C_4 grasses. The critical P requirements determined in this research will be published in peer-reviewed journals (e.g. journal articles and short communications) and distributed to industry (e.g. incorporated into existing factsheets), to inform species selection and fertiliser application rates when tropical pasture species are to be grown in nutrient-deficient soil. Furthermore, guidelines about fertiliser application strategies for the best use of fertiliser will also be published in peer-reviewed journals (e.g. journal articles and short communications) and distributed to industry (primarily through the publication of this report), although further field work is warranted to determine if outcomes seen in the control-environment studies accurately reflect the potential for improvement in the field. Through the selection of appropriate pasture species and effective fertiliser application techniques, the legume content of tropical pastures could be improved with consequent benefits for animal production and enterprise profitability.

6. Future research and recommendations

Future research should focus on understanding the benefit of legumes to northern Australian grazing systems. The inclusion of legumes is expected to improve pasture productivity and quality, because of atmospheric N-fixation and the higher protein content of legumes. However, it is not known whether tropical pasture legumes are actively fixing N in the hostile conditions of northern Australia. Indeed, there were very few nodules observed on the roots of the tropical pasture legumes in the controlled-environment growth experiments, even when the legumes were grown in soil that had a history of growing tropical pasture legumes. This raises the question whether the benefit of pasture legumes is yet to be fully realised in northern Australia. There is a need to understand the impact of soil fertility and soil type on rhizobia. Quantification of N-fixation and associated requirements would be beneficial for the red meat industry.

The critical P requirements of the tropical pasture species were generally lower than that of temperate pasture species (e.g. subterranean clover). This difference warrants further investigation, particularly if P dynamics in northern grazing systems (based on tropical pasture species) are substantially different to that of southern grazing systems (based on temperate species). Currently, the MLA Soil Phosphorus Tool is based on the 'Five Easy Steps' to optimise soil P fertility, following research in temperate pastures across southern Australia. With some additional research, the results from the current project could be used to inform the MLA Soil Phosphorus Tool; by confirming whether or not the P fertility of northern and southern systems can be managed in a similar way. There is also potential to present project findings in conjunction with other extension activities (e.g. 'Overcoming the Knowledge Gaps' which is a project encouraging the adoption of legumes such as *Desmanthus* and *Stylosanthes* in northern Australia).

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8. Appendices

8.1 Appendix 1 – Root morphology and phosphorus requirements of twelve tropical pasture species

Materials and Methods

Plant material

Twelve tropical pasture species were grown to investigate shoot yield and root morphology. There were five grasses, including Bambatsi Panic (*Panicum coloratum*), Floren Bluegrass (*Dichanthium aristatum*), Gatton Panic (*Panicum maximum*), Katambora Rhodes (*Chloris gayana* cv. Katambora) and Premier Digit (*Digitaria eriantha* cv. Premier). There were seven legumes, including Bundey Centro (*Centrosema pascuorum* cv. Bundey), Butterfly Pea (*Clitoria ternatea*), Cardillo Centro (*Centrosema pubescens* cv. Cardillo), Common Stylo (*Stylosanthes guianensis* var. V8), JCU 7 Desmanthus (*Desmanthus leptophyllus* cv. JCU 7), JCU 9 Desmanthus (*Desmanthus pernambucanus* cv. JCU 9) and Shrubby Stylo (*Stylosanthes scabra* cv. Seca).

Soil and nutrient treatments

A sandy soil (Grey Tenosol; Isbell 1996) was collected from the upper 2–15 cm soil layer of a field at Newholme SMART Farm, Armidale, NSW, Australia (30°26’21.4”S 151°39’55.5”E). The soil had a Colwell extractable P concentration of 3 mg P kg^{-1} (as measured by the method of Colwell (1963)), a Phosphorus Buffering Index (PBI) of 29 (as measured by the modified method of Burkitt et al. (2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of ~5.3. The soil was passed through a 5 mm sieve and a basal nutrient solution was applied which included 34 mg kg^{-1} soil $MgSO_4.7H_2O$, 36 mg kg^{-1} $CaSO_4.2H_2O$, 141 mg kg^{-1} KNO_3, 23 mg kg^{-1} $(NH_4)_2SO_4$, 14 mg kg^{-1} NH_4NO_3, 99 µg kg^{-1} H_3BO_3, 635 µg kg^{-1} $MnCl_2.4H_2O$, 301 µg kg^{-1} $ZnSO_4.7H_2O$, 28 µg kg^{-1} $CuSO_4.5H_2O$, 60 µg kg^{-1} $(NH_4)_2MoO_4$, 17 µg kg^{-1} $CoCl_2{\cdot}6H_2O$ and 1283 µg kg^{-1} FeNa-EDTA. Six P-amended soils (0, 7.5, 15, 30, 60 and 120 mg P kg^{-1}, hereafter referred to as P0, P7.5, P15, P30, P60 and P120, respectively) were prepared by adding KH_2PO_4 to the nutrient solution before it was applied to the soil. KCl was also added to the nutrient solution that was applied to the P0–P30 treatments, to balance the K so that it was equivalent to that of the P60 treatment. Based on previous experimentation, the P120 treatment was expected to allow maximum growth whereas the P0–P60 treatments were predicted to be within the P-responsive range of the legumes (McLachlan et al., 2021). After the addition of all nutrients, the Colwell extractable P concentrations of the P0–P120 soils were: 3, 7, 14, 25, 38 and 96 mg Colwell P kg^{-1}. Cylindrical PVC pots (87 mm internal diameter; 200 mm height) were filled with 1.3 kg (oven-dry basis) of the P-amended soils. The total depth of soil was ~190 mm and the bulk density was ~1.15 g cm^{-3}.

Plant growth conditions and experimental design

Micro-swards of each species were established by sowing seed (~5 mm depth) to achieve a population of 6 plants pot^{-1}. The seed of JCU 7 Desmanthus and JCU 9 Desmanthus was heat treated, while the seed of Gatton Panic was mechanically scarified, to break seed dormancy prior to sowing. Four replicate pots of each species in each P treatment were prepared. After planting, the pots were watered and moved to a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25ºC, day/night) in Armidale, NSW, Australia. Humidifiers were used to increase the humidity within the glasshouse to ~50%. Pots were arranged in a randomised complete block design (blocks comprised

the different replicates) which was generated using DiGGer (Coombes, 2006). Soil moisture was maintained at 80–100% field capacity by watering daily to a predetermined weight. An additional 50 mg N kg soil^{-1} as CH_4N_2O was applied to the surface of each pot four weeks after planting due to signs of N deficiency.

Harvest and measurements

Plants were harvested after five weeks of growth. Shoots were cut at the soil surface, oven-dried at 75°C for 72 h and weighed. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass.

The soil from each pot was removed as an intact core and cut into three vertical sections; 1) one-quarter for measurement of root hair length and mycorrhizal colonisation, 2) one-half for drying, and 3) one-quarter for scanning and drying. The roots were then washed from the soil over 2 mm sieves. Root samples for the measurement of root hair length and mycorrhizal colonisation were stored in 70% (v/v) ethanol at 4°C. Root samples for drying were oven-dried at 75°C for 72 h and weighed. Root samples for scanning were scanned using an Epson Perfection V700 Photo flatbed scanner (Seiko Epson Corporation, Suwa, Japan) at 600 dpi. When a root sample was too large for scanning, a representative subsample was scanned and the remaining roots were dried. Root length and average root diameter were determined using WinRHIZO^{TM} software (Regent Instruments Inc., Quebec, Canada) (Bouma et al., 2000). Scanned root samples were then oven-dried at 75°C for 72 hr and weighed. The estimated total mass of roots in the entire soil core was calculated as 4/3 multiplied by the combined dry mass of the unscanned half and scanned quarter. Total root mass fraction was calculated as the estimated total mass of roots divided by total plant mass (i.e. combined dry mass of shoots and roots). Total root length was calculated by multiplying the specific root length of roots from the scanned quarter by the estimated total mass of roots. Root length densities were calculated as root length per unit soil volume; the total soil volume was 1129 cm^3.

Root hairs were measured on two lengths of root selected at random from each of the stored samples. Roots were imaged using a Nikon SMZ25 stereomicroscope fitted with a high-resolution Nikon DS-Ri2 digital camera (Nikon Corporation, Tokyo, Japan). Root hairs approximately perpendicular to the line of vision were randomly selected and measured using ImageJ 1.52 (FIJI) (Schindelin et al., 2012). The lengths of five root hairs per image (i.e. 10 root hairs per pot) were measured.

The root hair cylinder volume (RHCV) was calculated as follows:

$$RHCV = \pi \times ((ARD/2) + RHL)^2 \times RL \quad (1)$$

where: ARD = average root diameter, RHL = root hair length, and RL = root length.

Root length colonisation by mycorrhizal fungi was measured on the plants grown in the P7.5 treatment. Roots were cleared in 10% (w/v) KOH for 50 min at 90°C, rinsed in water and 0.1 M (v/v) HCl, and stained using a 5% (v/v) Schaeffer black ink/white vinegar solution for 2 h (Vierheilig et al., 1998). Colonisation % was determined using the gridline intersect method, by examining the presence/absence of mycorrhizal fungi at 100 intersects on the stained root samples (Giovannetti and Mosse, 1980).

Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'species' and 'P treatment' as predictor variables. When appropriate, the effect of 'replicate' from the randomised complete block design were included in the most parsimonious model. The effect of 'replicate' accounted for the error associated with spatial variation in the glasshouse. The means and standard errors for root hair length and mycorrhizal colonisation were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). Shoot yield responses to P application were determined by fitting a self-starting Weibull growth function ($y = a – b * \exp(–\exp(c) * x^d)$, where x is the P application rate and y is the shoot dry mass) as described by Crawley (2013). Critical external P requirements were calculated as the amount of P applied to achieve 90% of maximum yield based on the fitted Weibull growth functions, and the 95% confidence intervals of the critical P requirements were determined by bootstrapping residuals as described by Crawley (2013). Critical internal P requirements were calculated as the shoot P concentration that corresponded with the critical external P application rate. Correlations were analysed using linear regression (Crawley, 2013). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield and critical external P requirements

The shoot dry mass of each pasture species increased in response to soil P supply, with the largest shoot yields generally produced in the P30–P120 treatments ($P < 0.001$; Fig. 2). The exceptions were Shrubby Stylo and Floren Bluegrass, which produced lower shoot yields when grown above the P30 and P60 treatments, respectively. Although soil P supply generally had a positive effect on shoot yield, there were substantial differences in how the species responded to P ($P < 0.001$; Fig. 2). In particular, there was a 5.9-fold difference in maximum shoot yield (1.6–9.5 g DM pot^{-1}) (Fig. 2) and a 3.0-fold difference in critical external P requirements (12.8–38.0 mg P kg^{-1} soil) (Table 5). The most productive species was Gatton Panic and the species with the lowest critical external P requirement was Katambora Rhodes.

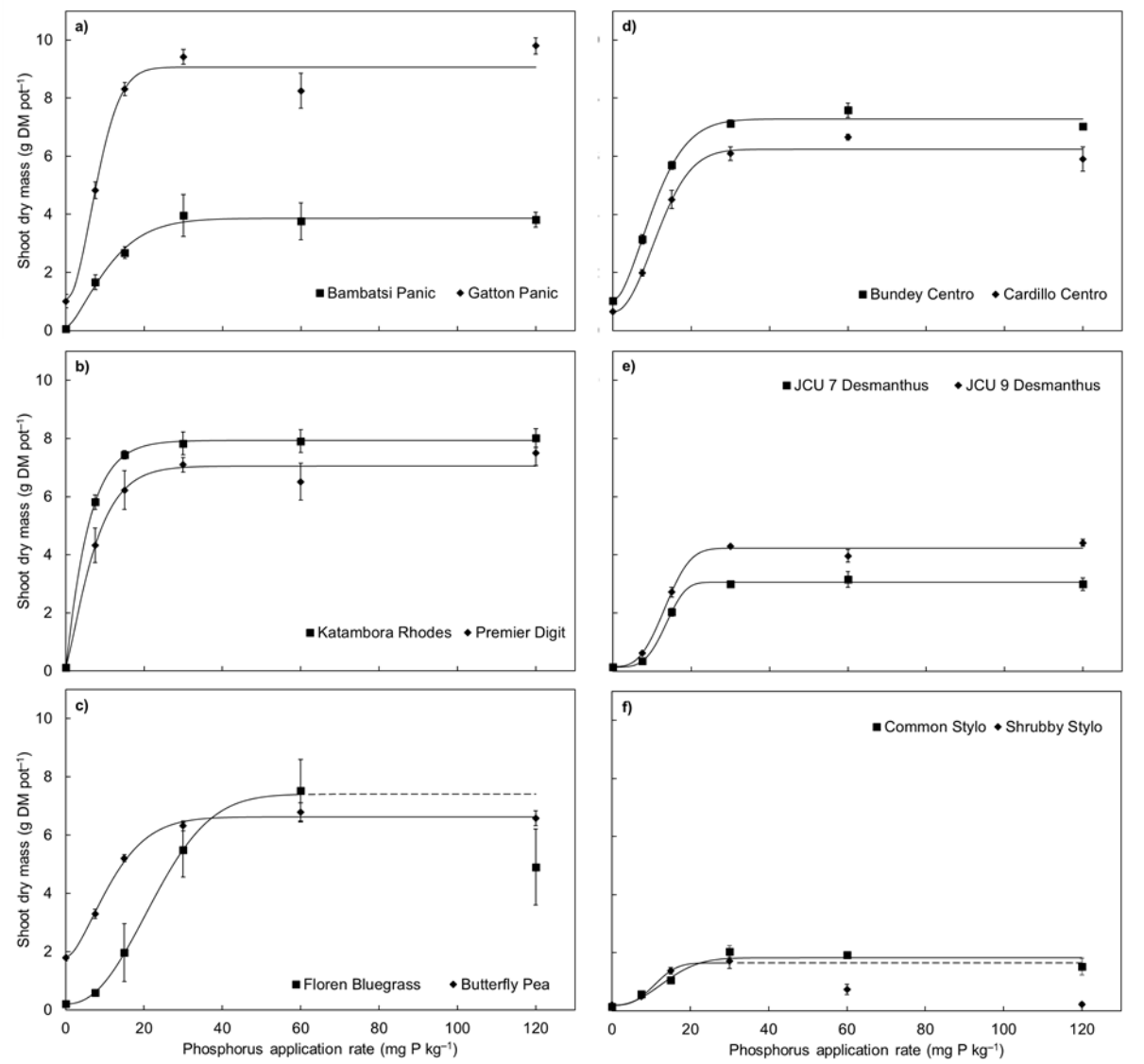

Figure 2. The shoot dry mass of twelve tropical pasture species when grown in response to six rates of applied P (0, 7.5, 15, 30, 60 and 120 mg P kg^{-1} soil). Values show the mean ± s.e. (n = 4). The curves that were fitted to the shoot yield data show Weibull growth functions (Crawley, 2013). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001. The yield achieved by the Shrubby Stylo in the P60 and P120 treatments was significantly lower than in the P30 treatment. To enable the Weibull growth function to be fitted, the shoot yield achieved in the P60 and P120 treatments was assumed to be equivalent to that of the P30 treatment. Similarly, the yield achieved by Floren Bluegrass in the P120 treatment was significantly lower than in the P30 treatment. To enable the Weibull growth function to be fitted, the shoot yield achieved in the P120 treatment was assumed to be equivalent to that of the P60 treatment. The dashed lines represent the assumed P response curves.

Table 5. The critical external and internal P requirements of twelve tropical pasture species when grown in response to six rates of applied P (0, 7.5, 15, 30, 60 and 120 mg P kg^{-1} soil). The critical external P requirements were calculated as the amount of P applied to achieve 90% of maximum yield based on the self-starting Weibull growth functions that were fitted to the shoot yield data, with the value range in parentheses showing the 95% confidence intervals determined using bootstrap analysis (Crawley, 2013). The critical internal P requirements were determined as the shoot P concentration that corresponded with the critical external P application rate. * indicates the values that could not be determined due to variable plant growth.

Species	Critical external P requirement (mg P kg^{-1} soil)	Critical internal P requirement (mg P g^{-1} DM)
Bambatsi Panic	**22.6** (14.7–*)	**1.31**
Gatton Panic	**14.4** (11.9–20.4)	**0.67**
Katambora Rhodes	**12.8** (10.2–16.9)	**0.90**
Premier Digit	**15.7** (11.2–29.1)	**1.28**
Floren Bluegrass	**38.0** (29.5–*)	**1.55**
Butterfly Pea	**21.2** (17.6–25.0)	**1.41**
Bundey Centro	**19.7** (17.7–22.0)	**1.63**
Cardillo Centro	**20.4** (17.7–25.4)	**1.77**
JCU 7 Desmanthus	**18.4** (16.5–22.0)	**1.53**
JCU 9 Desmanthus	**19.7** (17.7–22.5)	**1.81**
Common Stylo	**22.5** (16.8–45.6)	**2.80**
Shrubby Stylo	**17.1** (14.8–20.1)	**2.46**

Shoot P concentration and critical internal P requirements

The shoot P concentration of each pasture species increased in response to soil P supply, with the highest concentrations achieved in the P120 treatment ($P < 0.001$; Fig. 3). Across the entire P treatment range, there were significant differences among the pasture species (3.1-fold in the P0 treatment to 4.7-fold in the P120 treatment) ($P < 0.001$). There were also differences in the critical internal P requirement of the species (Table 5). The grasses ranged between 0.67–1.55 mg P g^{-1} DM, while the legumes ranged between 1.41–2.80 mg P g^{-1} DM. Shoot P content reflected the shoot yield and shoot P concentration results, with significant differences between P treatments ($P < 0.001$) and among pasture species ($P < 0.001$) (data not shown).

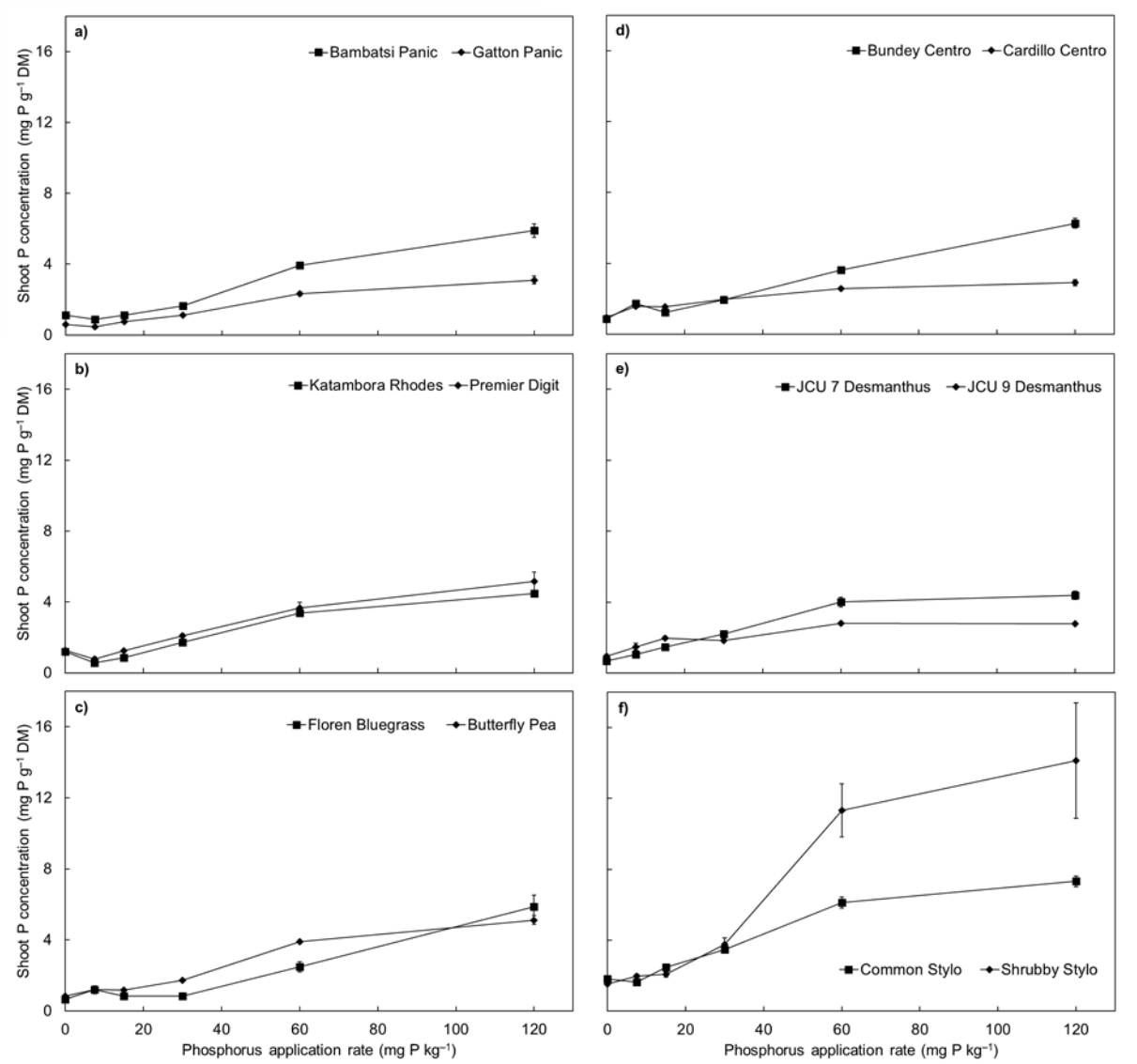

Figure 3. The shoot P concentration of twelve tropical pasture species when grown in response to six rates of applied P (0, 7.5, 15, 30, 60 and 120 mg P kg^{-1} soil). Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001.

Root traits

Root mass fraction was lowest when the pasture species were grown with an adequate supply of P that allowed maximum growth (i.e. the P30–P120 treatments) (Fig. 4). At lower rates of soil P supply, the proportion of plant biomass allocated to roots increased significantly ($P < 0.001$) so that most of the species allocated the largest proportion of plant biomass to roots when grown in the P0 treatment. On average across the P treatments, there was a substantial difference in the proportion of biomass allocated to roots by the different pasture species ($P < 0.001$). In general, the grasses allocated more biomass to roots than the legumes.

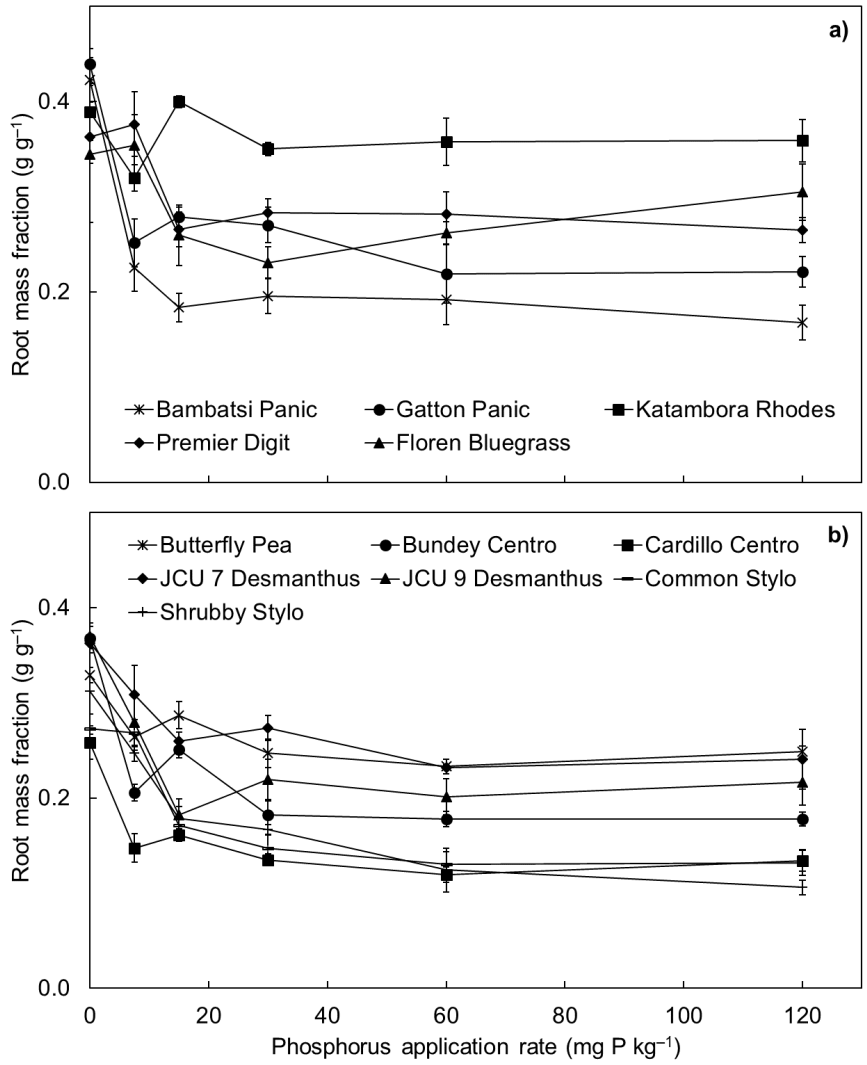

Figure 4. The root mass fraction of five tropical grasses (a) and seven tropical legumes (b) when grown in response to six rates of applied P (0, 7.5, 15, 30, 60 and 120 mg P kg soil^{-1}). Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001.

Specific root length differed according to soil P supply ($P < 0.001$) and pasture species ($P < 0.001$; Fig. 5). On average, the pasture species achieved the highest specific root lengths in the low P treatments (i.e. P0–P7.5 treatments). However, this was clearly not the case for each of the species, hence the significant interaction between species*P treatment ($P < 0.001$). For example, the higher specific root lengths in response to low P were clear in some of the grasses (e.g. Gatton Panic, Katambora Rhodes and Premier Digit) whereas the specific root lengths of Stylos generally declined when grown in the lower P soil treatments.

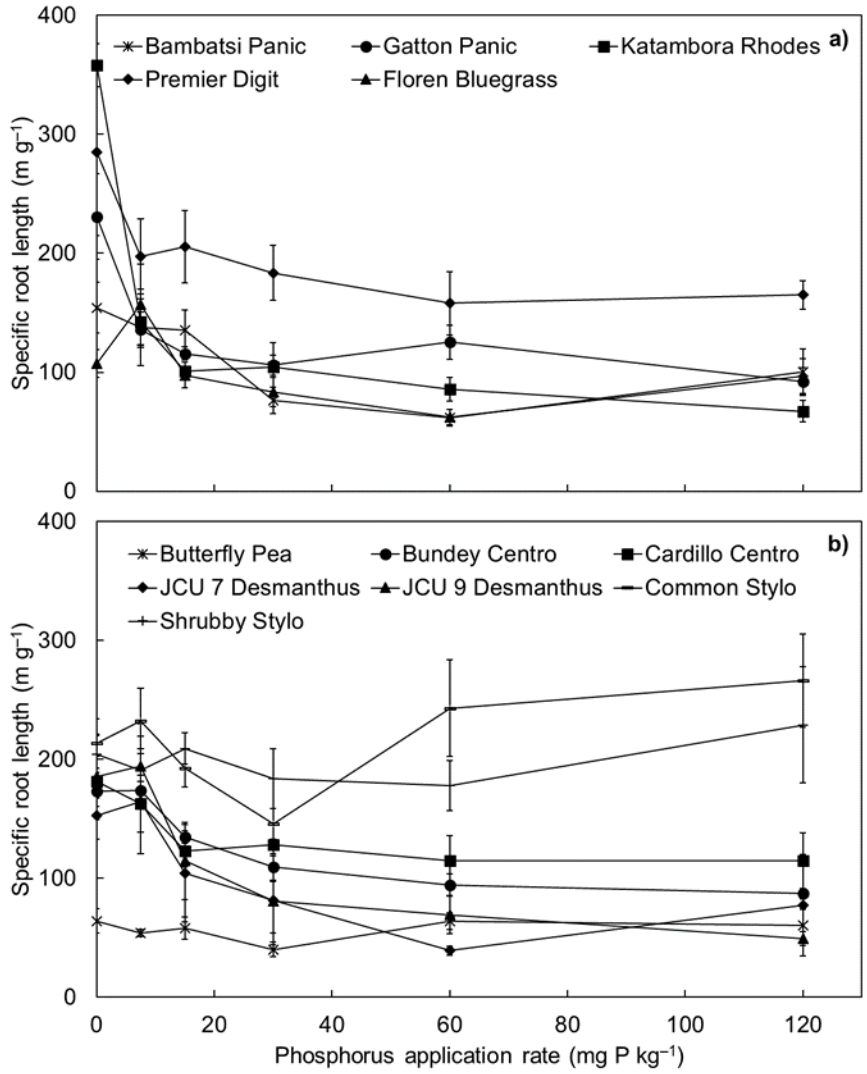

Figure 5. The specific root length of five tropical grasses (a) and seven tropical legumes (b) when grown in response to six rates of applied P (0, 7.5, 15, 30, 60 and 120 mg P kg soil^{-1}). Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001.

Root length density (i.e. root length per unit soil volume) was generally lowest in the P0–P7.5 treatments and increased in response to soil P supply, with the longest roots often achieved in the P15–P120 treatments ($P < 0.001$; Fig. 6). The development of root length varied significantly among the pasture species ($P < 0.001$). For example, Gatton Panic, Katambora Rhodes and Premier Digit produced relatively long roots in the P7.5–P120 treatments. In contrast, the Stylos produced relatively short roots in each of the P treatments.

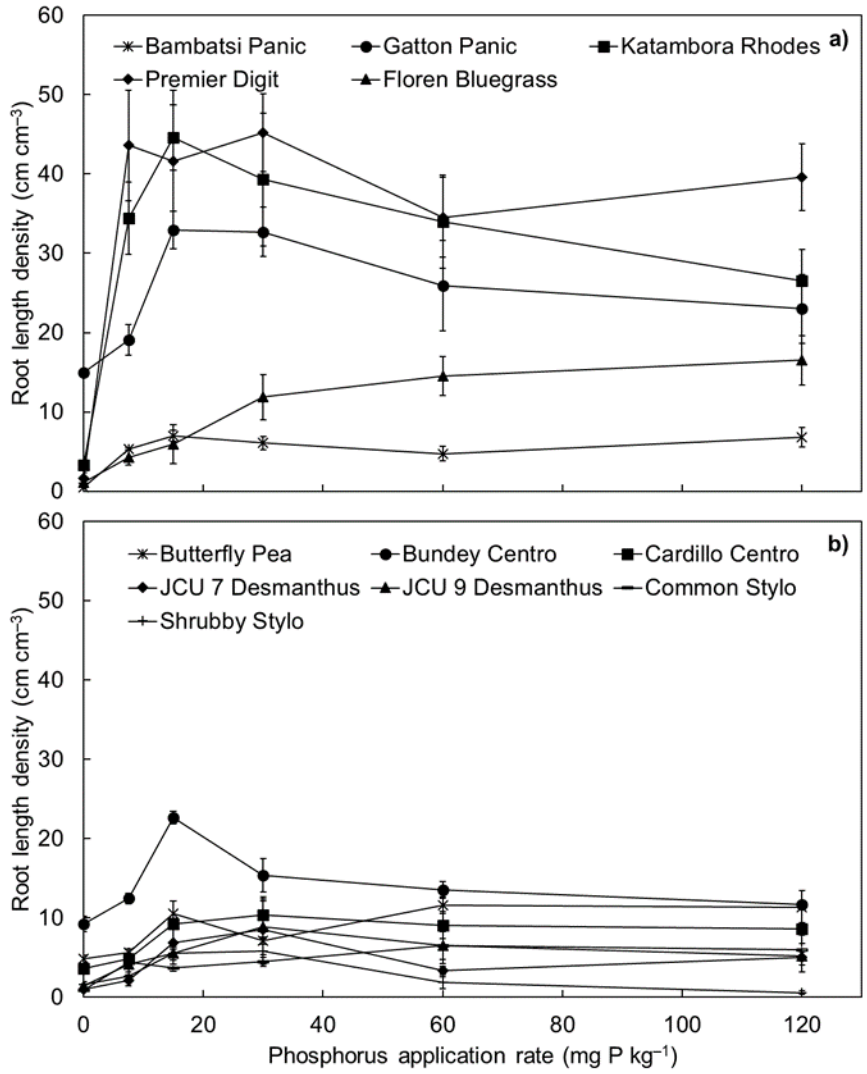

Figure 6. The root length density of five tropical grasses (a) and seven tropical legumes (b) when grown in response to six rates of applied P (0, 7.5, 15, 30, 60 and 120 mg P kg soil^{-1}). Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001.

Root hair length was relatively constant across the soil P treatments (P = 0.043). Consequently, the root hair lengths of the species were averaged across the P treatments (Table 6). Root hair length varied by 3.4-fold among the pasture species (P < 0.001). The longest root hairs were produced by the two Stylo cultivars and the shortest were produced by the two Desmanthus cultivars. Mycorrhizal colonisation ranged between 3–51% among the pasture species (Table 6).

Table 6. Average root hair length (RHL) and root length colonised by mycorrhizal fungi (AMF) of twelve tropical pasture species. Root hair length was averaged across the six P treatments (0, 7.5, 15, 30, 60 and 120 mg P kg^{-1} soil) because the main effect of soil P supply was minimal (P = 0.043). Mycorrhizal colonisation was only assessed in the 7.5 mg P kg^{-1} soil treatment. Different letters denote significant differences at P = 0.05.

Species	Average RHL (mm)	AMF Colonisation (%)
Bambatsi Panic	0.27 c	43.3 c
Gatton Panic	0.28 c	16.5 a
Katambora Rhodes	0.35 d	12.8 a
Premier Digit	0.24 bc	14.3 a
Floren Bluegrass	0.19 ab	37.5 bc
Butterfly Pea	0.18 ab	20.3 ab
Bundey Centro	0.16 a	51.0 c
Cardillo Centro	0.18 ab	38.2 c
JCU 7 Desmanthus	0.14 a	5.8 a
JCU 9 Desmanthus	0.15 a	16.0 a
Common Stylo	0.38 d	6.7 a
Shrubby Stylo	0.48 e	2.8 a

Phosphorus acquisition

Root length density was correlated with shoot P content at each level of soil P supply (Fig. 7). In soil that was below the critical external P requirements of the species (i.e. P0–P7.5 treatments), the R^2 coefficients of determination were relatively weak (R^2 = 0.24 and 0.14, respectively). At higher levels of soil P supply, the correlations were stronger (R^2 = 0.38–0.45 in the P15–P120 treatments). Nevertheless, the R^2 coefficients of determination indicated that there were significant differences in shoot P content (an indicator of plant P uptake) per unit root length. Indeed, shoot P content per unit root length increased in response to soil P supply ($P < 0.001$) and varied significantly among the pasture species ($P < 0.001$) (data not shown).

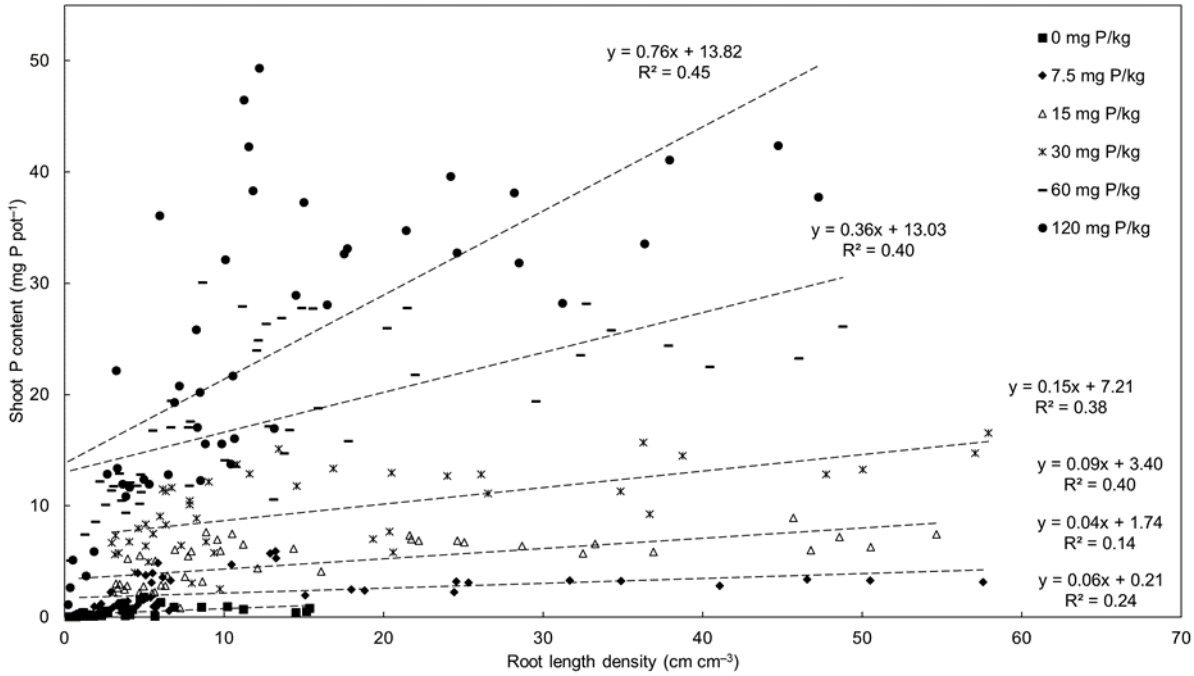

Figure 7. The relationship between root length and shoot P content among twelve tropical pasture species that were grown in response to six rates of applied P (0, 7.5, 15, 30, 60 and 120 mg P kg soil^{-1}). The regression lines and R^2 coefficients of determination were fitted separately for each P treatment and include all data points of each genotype.

Additional observations

Average canopy height increased in response to soil P supply ($P < 0.001$), and reflected the shoot yields of the pasture species ($R^2 = 0.62$, $P < 0.001$) (data not shown). In general, the grasses produced taller canopies than the legumes, particularly at lower levels of soil P supply.

Practical implications

The tropical pasture grasses were generally more productive and had comparable or lower critical external P requirements than the tropical pasture legumes. This means that if the legumes were grown in mixed pasture swards with the grasses, the legume component could be outcompeted particularly in nutrient-deficient soil when selective grazing cannot be managed easily. Nevertheless, differences in critical external P requirements among the tropical pasture species indicate that there is potential to use P-efficient varieties in inherently low-P soil. These differences in P efficiency were associated, to varying degrees, with the development of root length. Future improvements in P acquisition efficiency could be achieved by selecting and breeding for plants with longer roots that forage the soil for available nutrients. Indeed, the efficient development of root length is likely to be important in northern Australia where soils are nutrient deficient and also seasonally dry, meaning that surface-foraging roots for nutrient acquisition and deeper roots for water acquisition will drive productivity and persistence.

8.2 Appendix 2 – Evaluation of the root traits and phosphorus requirements of several *Desmanthus* and *Stylosanthes* genotypes

Materials and Methods

Plant material

Three *Desmanthus* spp. genotypes and eight *Stylosanthes* spp. genotypes were grown to investigate shoot yield and root traits in response to soil P supply. The genotypes (selections) included Marc Desmanthus (*Desmanthus virgatus* cv. Marc), JCU 7 Desmanthus (*Desmanthus leptophyllus* cv. JCU 7), JCU 9 Desmanthus (*Desmanthus pernambucanus* cv. JCU 9), Caatinga Stylo (*Stylosanthes seabrana* cvv. Primar and Unica), Common Stylo (*Stylosanthes guianensis* var. V8), Shrubby Stylo (*Stylosanthes scabra* cv. Seca), and five experimental Stylo lines (referred to hereafter as Stylo lines 9, 25, 36, 39 and 40).

Soil preparation

A sandy soil (Grey Tenosol; Isbell 1996) was collected from the upper 2–15 cm soil layer of a field at Newholme SMART Farm, Armidale, NSW, Australia (30°26’21.4”S 151°39’55.5”E). The soil had a Colwell extractable P concentration of 3 mg P kg^{-1} (as measured by the method of (Colwell, 1963)), a Phosphorus Buffering Index (PBI) of 29 (as measured by the modified method of (Burkitt et al., 2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of ~5.3. The soil was passed through a 5 mm sieve and a basal nutrient solution was applied which included 34 mg kg^{-1} soil $MgSO_4.7H_2O$, 36 mg kg^{-1} $CaSO_4.2H_2O$, 141 mg kg^{-1} KNO_3, 23 mg kg^{-1} $(NH_4)_2SO_4$, 14 mg kg^{-1} NH_4NO_3, 99 µg kg^{-1} H_3BO_3, 635 µg kg^{-1} $MnCl_2.4H_2O$, 301 µg kg^{-1} $ZnSO_4.7H_2O$, 28 µg kg^{-1} $CuSO_4.5H_2O$, 60 µg kg^{-1} $(NH_4)_2MoO_4$, 17 µg kg^{-1} $CoCl_2{\cdot}6H_2O$ and 1283 µg kg^{-1} FeNa-EDTA. Six P-amended soils (0, 5, 10, 20, 40 and 80 mg P kg^{-1}, hereafter referred to as P0, P5, P10, P20, P40 and P80, respectively) were prepared by adding KH_2PO_4 to the nutrient solution before it was applied to the soil. KCl was also applied to the P0–P20 treatments, to balance the K so that it was equivalent to that of the P40 treatment. Cylindrical PVC pots (87 mm internal diameter, 200 mm height) were filled with 1.3 kg (oven-dry basis) of the P-amended soils. The total depth of soil was ~190 mm.

Plant growth conditions and experimental design

Micro-swards of each genotype were established by sowing seed (~5 mm depth) to achieve a population of 8 plants pot^{-1}. Four replicate pots of each genotype at each P application rate were prepared. After planting, the pots were moved to a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. Plants were grown between August–October 2022. Plants were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006). Soil moisture was maintained at 80–100% field capacity by watering daily to a predetermined weight using distilled water.

Harvest and measurements

Plants were harvested after eight weeks’ growth. Shoots were cut at the soil surface, oven-dried at 70°C for 72 h and weighed. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the

malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass.

The soil from each pot was removed as an intact core and cut into two vertical sections; 1) one-half for drying, and 2) one-half for scanning and drying. Root samples for drying were oven-dried at 70°C for 72 h and weighed. Root samples for scanning were scanned using an Epson Perfection V700 Photo flatbed scanner (Seiko Epson Corporation, Suwa, Japan) at 600 dpi. When a root sample was too large for scanning, a representative subsample was scanned and the remaining roots were dried. Root length and average root diameter were determined using WinRHIZO^{TM} software (Regent Instruments Inc., Quebec, Canada) (Bouma et al., 2000). Scanned root samples were then oven-dried at 70°C for 72 hr and weighed. Total root mass fraction was calculated as the total mass of roots divided by total plant mass (i.e. combined dry mass of shoots and roots). Total root length was calculated by multiplying the specific root length of roots from the scanned half by the total mass of roots in the entire soil core. Root length densities were calculated as root length per unit soil volume; the total soil volume was 1129 cm^3.

Statistical analysis

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'genotype' and 'P treatment' as the predictor variables. When appropriate, the effect of 'replicate' was included in the most parsimonious model. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). Shoot yield responses to P application were determined by fitting a self-starting Weibull growth function ($y = a - b * \exp(-\exp(c) * x^d)$, where x is the P application rate and y is the shoot dry mass) as described by Crawley (2013). Critical external P requirements were calculated as the amount of P applied to achieve 90% of maximum yield based on the fitted Weibull growth functions, and the 95% confidence intervals of the critical P requirements were determined by bootstrapping residuals as described by Crawley (2013). Critical internal P requirements were calculated as the shoot P concentration that corresponded with the critical external P application rate. Four pots were removed from analysis due to low plant numbers that influenced shoot yield. Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield and critical external P requirements

The shoot dry mass of each legume increased in response to soil P supply, with the largest shoot yields generally produced in the P40–P80 treatments ($P < 0.001$; Fig. 8). The exception was Stylo Line #9, which produced a lower shoot yield when grown above the P40 treatment. Although soil P supply generally had a positive effect on shoot yield, there were substantial differences in how the species responded to P ($P < 0.001$; Fig. 8). In particular, there was a 2.4-fold difference in maximum shoot yield (1.5–3.7 g DM pot^{-1}) (Fig. 8) and a 3.5-fold difference in critical external P requirements (12.4–43.0 mg P kg^{-1} soil) (Table 7). The most productive legumes were JCU 7 and JCU 9 Desmanthus, while the legumes with the lowest critical external P requirements were Stylo Lines #25, #36 and #39.

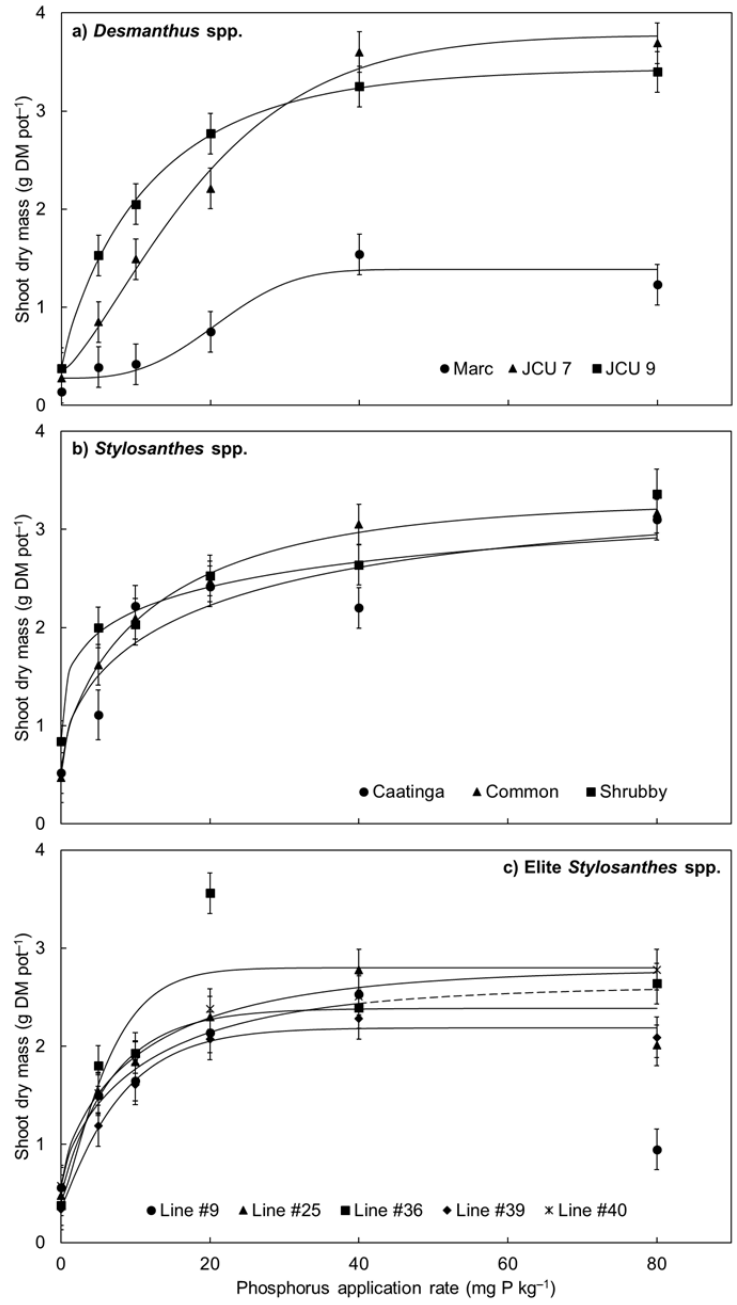

Figure 8. The shoot dry mass of three Desmanthus and eight Stylosanthes spp. genotypes when grown in response to six rates of applied P (0, 5, 10, 20, 40 and 80 mg P kg^{-1} soil). Values show the mean ± s.e. (n = 3). The curves that were fitted to the shoot yield data show Weibull growth functions (Crawley, 2013). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001. The yield achieved by Stylo Line #9 in the P80 treatment was significantly lower than in the P40 treatment. To enable the Weibull growth function to be fitted, the shoot yield achieved in the P80 treatment was assumed to be equivalent to that of the P40 treatment. The dotted line represents the assumed P response curve.

Table 7. The critical external and internal P requirements of three Desmanthus and eight Stylosanthes spp. genotypes when grown in response to six rates of applied P (0, 5, 10, 20, 40 and 80 mg P kg^{-1} soil). The critical external P requirements were calculated as the amount of P applied to achieve 90% of maximum yield based on the self-starting Weibull growth functions that were fitted to the shoot yield data, with the value range in parentheses showing the 95% confidence intervals determined using bootstrap analysis (Crawley, 2013). The critical internal P requirements were determined as the shoot P concentration that corresponded with the critical external P application rate. * indicates the values that could not be determined due to variable plant growth.

Species	Critical external P requirement (mg P kg^{-1} soil)	Critical internal P requirement (mg P g^{-1} DM)
Marc Desmanthus	**30.2** (22.4–*)	**2.31**
JCU 7 Desmanthus	**38.4** (30.8–49.2)	**1.56**
JCU 9 Desmanthus	**29.9** (21.6–52.0)	**1.19**
Caatinga Stylo	**43.0** (20.2–*)	**3.63**
Common Stylo	**34.0** (21.7–50.4)	**2.86**
Shrubby Stylo	**35.3** (20.1–77.9)	**2.92**
Stylo Line #9	**29.6** (17.6–57.5)	**1.70**
Stylo Line #25	**14.6** (8.1–*)	**1.06**
Stylo Line #36	**12.4** (7.6–*)	**0.96**
Stylo Line #39	**16.6** (9.6–*)	**1.21**
Stylo Line #40	**29.1** (13.9–*)	**1.82**

Shoot P concentration and critical internal P requirements

The shoot P concentration of each legume increased in response to soil P supply, with the highest concentrations generally achieved in the P80 treatment ($P < 0.001$; Fig. 9). There were substantial differences among the legumes across the entire P treatment range ($P < 0.001$). However, the largest differences were observed in the P80 treatment, in which there was a 7.8-fold range in shoot P concentration. This was because the *Desmanthus* spp. genotypes mainly low shoot P concentrations in the high P treatments, whereas the *Stylosanthes* spp. genotypes continued to accumulate shoot P. There were also differences in the critical internal P requirement of the species (Table 7). The *Desmanthus* spp. genotypes ranged between 1.19–2.31 mg P g^{-1} DM, the *Stylosanthes* spp. genotypes ranged between 2.86–3.63 mg P g^{-1} DM, and the elite *Stylosanthes* spp. lines ranged between 0.96–1.82 mg P g^{-1} DM. Shoot P content reflected the shoot yield and shoot P concentration results, with significant differences between P treatments ($P < 0.001$) and among pasture species ($P < 0.001$) (data not shown).

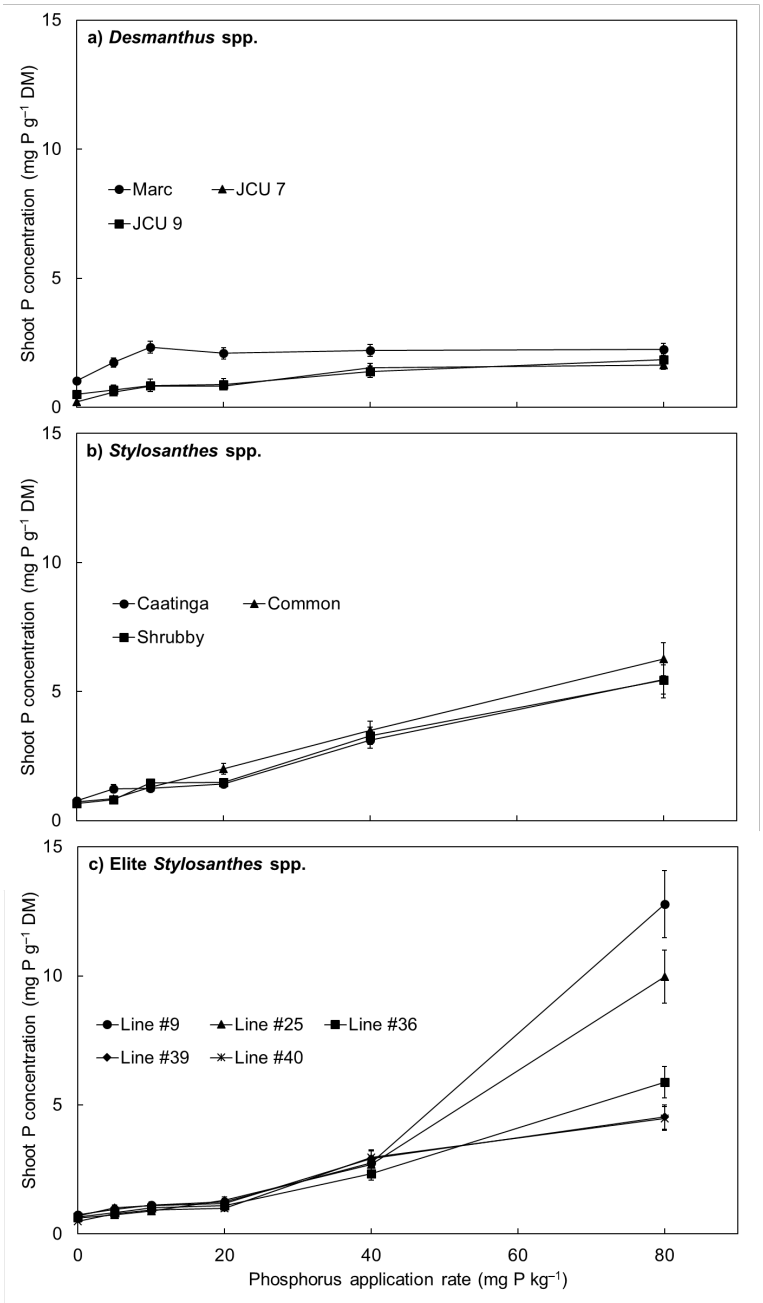

Figure 9. The shoot P concentration of three Desmanthus and eight Stylosanthes spp. genotypes when grown in response to six rates of applied P (0, 5, 10, 20, 40 and 80 mg P kg^{-1} soil). Values show the mean ± s.e. (n = 3). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001.

Root traits

Root mass fraction was lowest when the legumes were grown with sufficient P (i.e. the P40–P80 treatments) and generally increased at lower levels of soil P supply ($P < 0.001$; Fig. 10). In the P0 treatment, the legumes allocated between 0.32–0.40 g g^{-1} of plant biomass to roots. There were substantial differences in root mass fraction among the legumes, particularly in the higher P treatments ($P < 0.001$; Fig. 10). For example, *Desmanthus* spp. genotypes JCU 7 and JCU 9 allocated more biomass to roots than the other legumes. These genotypes generally allocated the same proportion of biomass to roots across the entire P treatment range.

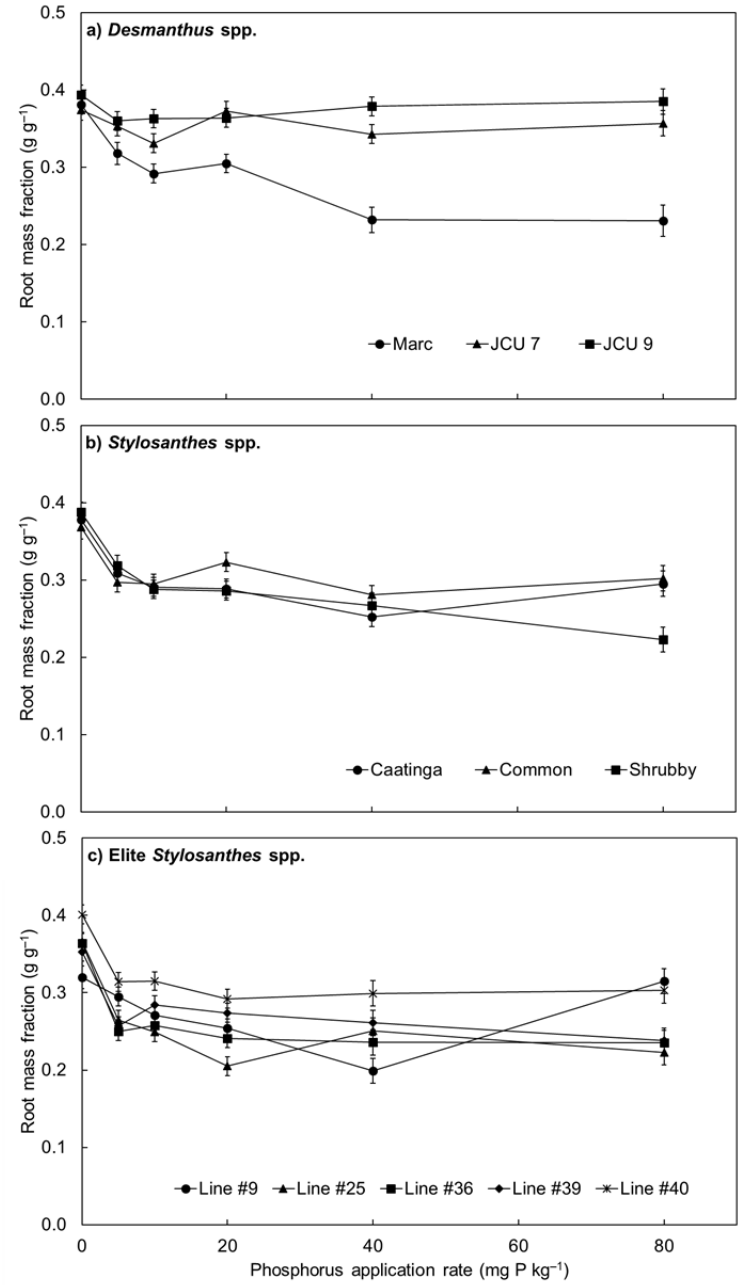

Figure 10. The root mass fraction of three Desmanthus and eight Stylosanthes spp. genotypes when grown in response to six rates of applied P (0, 5, 10, 20, 40 and 80 mg P kg^{-1} soil). Values show the mean ± s.e. (n = 3). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001.

Specific root length was generally lowest in the P20–P80 treatments and increased in response to P deficiency so that the most efficient production of root length occurred in the P0 treatment (P < 0.001; Fig. 11). There were significant differences in specific root length among the legumes (P < 0.001). Across the P treatment range, the *Stylosanthes* genotypes generally produced root length more efficiently than the *Desmanthus* genotypes. Differences in specific root length were associated with differences in average root diameter (R^2 = 0.48, P < 0.001) (data not shown).

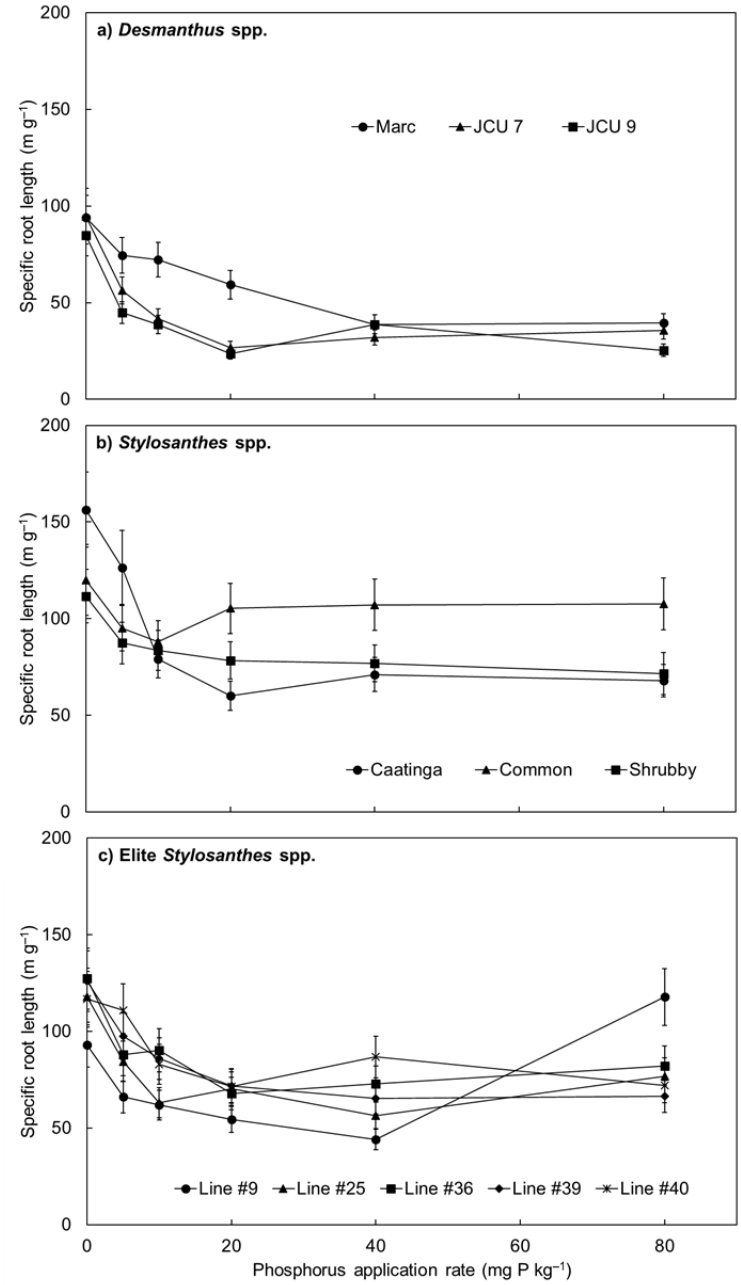

Figure 11. The specific root length of three Desmanthus and eight Stylosanthes spp. genotypes when grown in response to six rates of applied P (0, 5, 10, 20, 40 and 80 mg P kg^{-1} soil). Values show the mean ± s.e. (n = 3). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001.

Root length density (i.e. root length per unit soil volume) generally increased in response to soil P supply to be highest in the P80 treatment ($P < 0.001$; Fig. 12). Across the entire P treatment range, there were substantial differences in the production of root length among the legumes ($P < 0.001$). For example, *Desmanthus* spp. genotypes Marc and JCU 7 produced relatively short roots whereas the Common and Shrubby *Stylosanthes* produced relatively long roots.

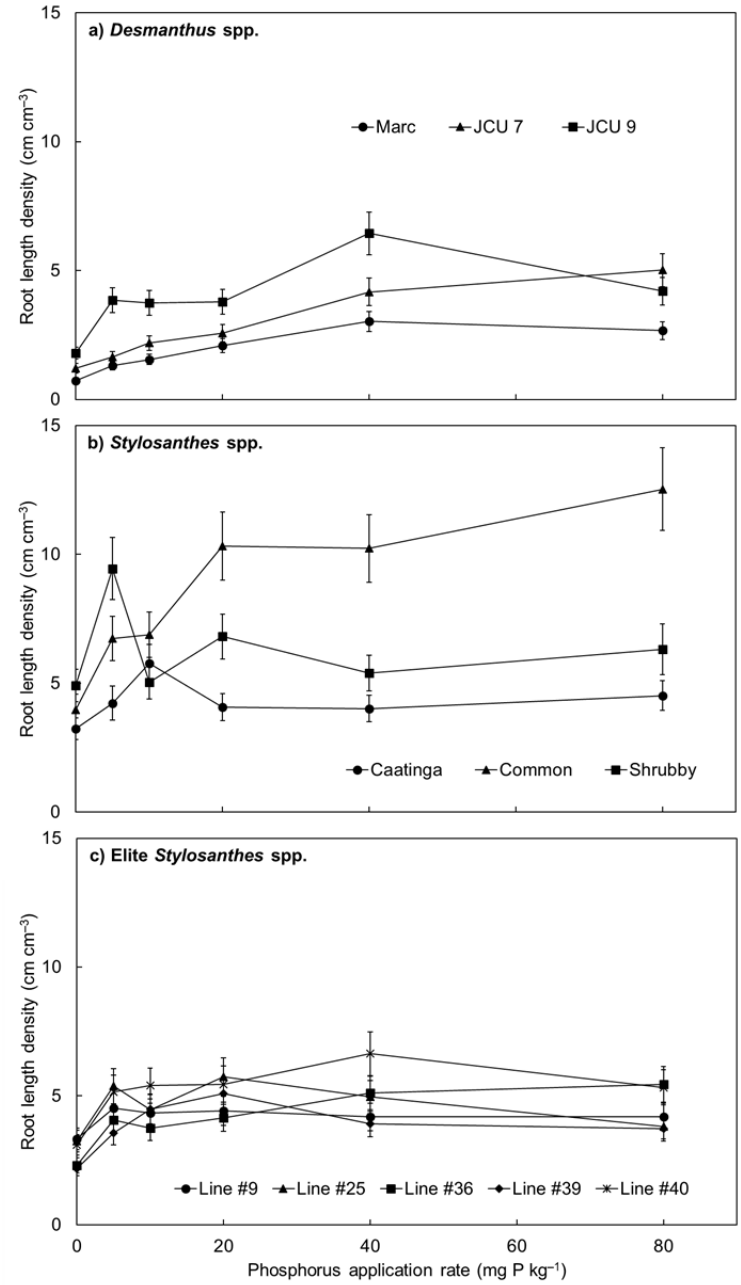

Figure 12. The root length density of three Desmanthus and eight Stylosanthes spp. genotypes when grown in response to six rates of applied P (0, 5, 10, 20, 40 and 80 mg P kg^{-1} soil). Values show the mean ± s.e. (n = 3). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001.

Phosphorus acquisition

Phosphorus acquisition was associated with the development of root length (Fig. 13). In the P0–P20 treatments, when the legumes were grown at or below their critical external P requirements, the correlations between shoot P content (an indicator of plant P uptake) and root length density were generally strong (e.g. R^2 = 0.65, 0.57 and 0.52 in the P0, P10 and P20 treatments, respectively). The exception was in the P5 treatment, where the R^2 = 0.20 and was more similar to the P40–P80 treatments. The R^2 coefficients of determination indicated that there were differences in shoot P

content per unit root length (Fig. 14). Across the legumes, shoot P content per unit root length increased in response to soil P supply ($P < 0.001$; Fig. 14). However, there were substantial differences in how the species responded to soil P supply ($P < 0.001$). For example, the shoot P content per unit root length of the three *Desmanthus* spp. genotypes was relatively stable above the P20 treatment, whereas that of the eight *Stylosanthes* spp. genotypes increased significantly.

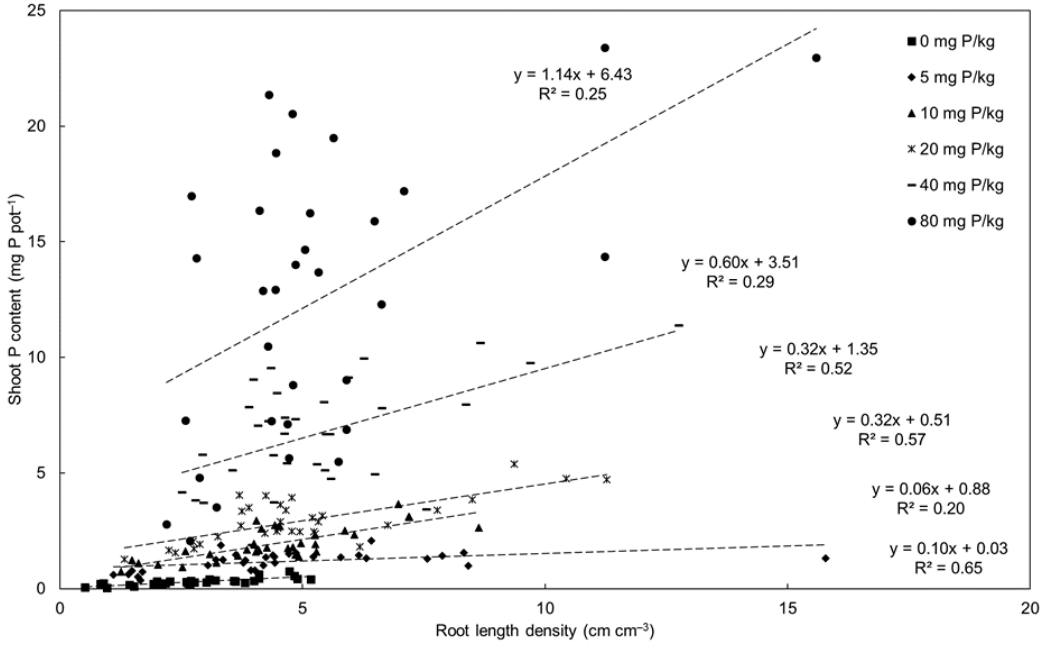

Figure 13. The relationship between root length and shoot P content for three Desmanthus and eight Stylosanthes spp. genotypes when grown in response to six rates of applied P (0, 5, 10, 20, 40 and 80 mg P kg^{-1} soil). The regression lines and R^2 coefficients of determination were fitted separately for each P treatment and include all data points of each genotype.

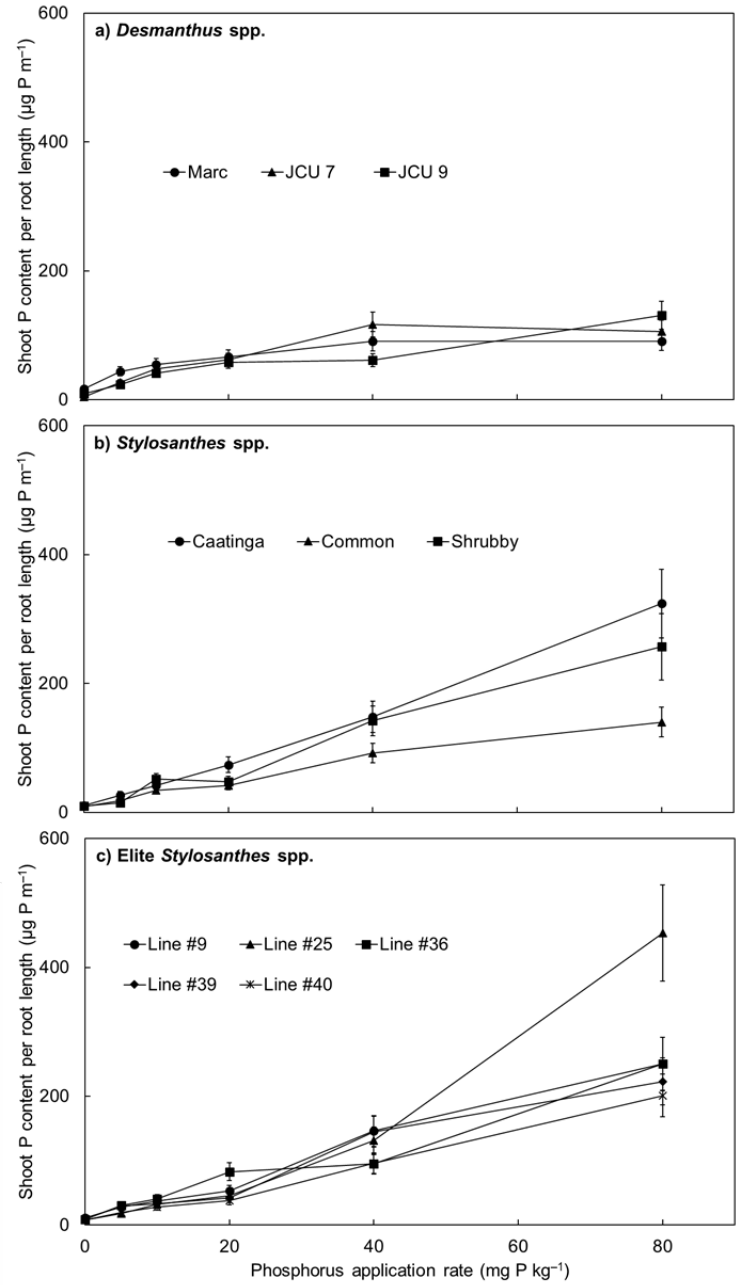

Figure 14. Shoot P content per unit root length of three Desmanthus and eight Stylosanthes spp. genotypes when grown in response to six rates of applied P (0, 5, 10, 20, 40 and 80 mg P kg^{-1} soil). Values show the mean ± s.e. (n = 3). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, species × P treatment interaction P < 0.001.

Practical implications

There were differences in maximum shoot yield and critical external P requirements among the *Desmanthus* and *Stylosanthes* genotypes. The *Desmanthus* spp. genotypes JCU 7 and JCU 9 were more productive than the other legumes but generally had relatively high critical external P requirements. These species may be more suited to moderately fertile soils, which have higher native Colwell P levels or receive applications of P fertiliser. Similarly, some of the *Stylosanthes* spp. genotypes were more productive in the higher P soil. In contrast, three of the elite *Stylosanthes* spp.

genotypes had very low critical external P requirements. These genotypes would be suited to low-P soils where nutrient inputs are limited. Genotypes of *Desmanthus* and *Stylosanthes* could therefore be selected based on P-deficiency. Nevertheless, legumes of the two genera are often suited to different soil types. For example, *Desmanthus* spp. genotypes are suited to neutral to alkaline clay soils, with a medium to heavy texture. *Desmanthus* is more likely to be productive and persistent when grown in these conditions. In contrast, *Stylosanthes* spp. genotypes are more suited to sandy to loam soils, and can tolerant acidic to alkaline soil conditions. These two genera of legumes are still likely to be selected based upon suitability to climate and soil, before consideration is given to P efficiency.

Shoot P concentrations were similar when the legumes were grown in the P0–P20 treatments. However, substantial differences occurred in the higher P treatments. In particular, there was a large difference between the *Desmanthus* and *Stylosanthes* genotypes. The relatively low shoot P concentrations of the *Desmanthus* spp. genotypes in the high P soil indicates that these genotypes use acquired P efficiently to produce dry matter. This is consistent with the findings of McLachlan et al. (2021), who reported that *Desmanthus* genotype JCU 9 efficiently produced shoot dry matter even when grown in high P soil. In the present experiment, the *Stylosanthes* genotypes continued to accumulate shoot P when grown with increasing levels of soil P supply. This difference indicates that although *Desmanthus* genotypes may be more productive in higher P soil, *Stylosanthes* genotypes are likely to provide a higher quality diet for grazing animals when soil P levels are higher.

Differences in shoot yield and shoot P concentration were associated with differences in root traits, indicating that the different genotypes used different strategies to be most productive. For example, the *Desmanthus* spp. genotypes achieved relatively low specific root lengths but high root mass fractions (i.e. the relatively inefficient production of roots was overcome by allocating more plant biomass to roots). This was possible because the *Desmanthus* spp. genotypes continued to use acquired P relatively efficiently. Furthermore, the *Desmanthus* spp. genotypes generally did not acquire more P per unit root length, even when grown in the higher P soil treatments. This indicates that the ability of *Desmanthus* spp. genotypes to upregulate P acquisition when soil P concentrations are higher is limited. The opposite was true for the *Stylosanthes* genotypes, which produced root length relatively efficiently and could upregulate the acquisition of P in the higher P soil treatments.

8.3 Appendix 3 – The effect of pH and PBI on the critical phosphorus requirements of two tropical pasture species

Materials and Methods

Plant growth conditions

Digit (*Digitaria eriantha* cv. Premier) and Desmanthus (*Desmanthus pernambucanus* cv. JCU 9) were grown to determine shoot yield and tissue P concentrations in response to soil pH and PBI. There were two components to the study.

Soil pH component: A clay loam soil was collected from Gregory Downs Station, Gregory, QLD, Australia. The soil had a Colwell extractable P concentration of 3, a Phosphorus Buffering Index (PBI) of 65, and a pH ($CaCl_2$) of ~7.0. The soil was crushed and amended with a basal nutrient solution that included 34 mg kg^{-1} soil $MgSO_4.7H_2O$, 36 mg kg^{-1} $CaSO_4.2H_2O$, 141 mg kg^{-1} KNO_3, 23 mg kg^{-1} $(NH_4)_2SO_4$, 14 mg kg^{-1} NH_4NO_3, 99 µg kg^{-1} H_3BO_3, 635 µg kg^{-1} $MnCl_2.4H_2O$, 301 µg kg^{-1} $ZnSO_4.7H_2O$, 28 µg kg^{-1} $CuSO_4.5H_2O$, 60 µg kg^{-1} $(NH_4)_2MoO_4$, 17 µg kg^{-1} $CoCl_2{\cdot}6H_2O$ and 1283 µg kg^{-1} FeNa-EDTA. Based on previous soil incubations, five soil pH treatments (4, 5, 6, 7 and 8) were prepared by either adding sulfuric acid (2M H_2SO_4, to reduce the pH to 4–6) or lime ($CaCO_3$, to increase the pH to 8). Cylindrical PVC pots (50 mm internal diameter, 115 mm height) were filled with 300 g (oven-dry basis) of the five amended soils. Ten P solutions were prepared using KH_2PO_4 (K was balanced with KCl) which were applied to the soil surface, resulting in ten different soil P treatments: 0, 2.5, 5, 10, 15, 20, 40, 60, 80 and 120 mg P kg^{-1} (hereafter referred to as P0, P2.5, P5 etc.). The soil was watered, dried and incorporated to distribute the applied P within the top ~5 cm of the profile.

Soil PBI component: A clay soil was collected from Kingaroy, QLD, Australia. The soil had a Colwell extractable P concentration of 12, a PBI of 385, and a pH ($CaCl_2$) of ~4.6. The soil was amended with the basal nutrient solution outlined above and lime (5 g $CaCO_3$ kg^{-1} soil) was added to raise the pH ($CaCl_2$) to ~6.0. Five soil PBI treatments (65, 145, 225, 305 and 385) were prepared by mixing different amounts of the amended Kingaroy soil with different amounts of the amended Gregory Downs soil described above.

Micro-swards of Digit and Desmanthus were established in the pots prepared in the 'pH' and 'PBI' components, by sowing seed (~5 mm depth) to achieve a population of 3 plants pot^{-1}. Two replicate pots of each species at each pH/PBI level and P application rate were prepared. After planting, the pots were watered and moved to a glasshouse (natural daylight, ~1800 mmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. The pH component of the study occurred in October–November 2022 while the PBI component occurred in November–December 2022. Pots were arranged in a randomised complete block design (blocks comprised the different replicates). Soil moisture was maintained between 80-100% field capacity by watering daily to a predetermined weight using distilled water.

Harvest and analysis

Plants were harvested after four weeks' growth. Shoots were cut at the soil surface, oven-dried at 70°C for 72 h and weighed. Shoot samples were then finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colourimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by

multiplying shoot P concentration and shoot dry mass. Measured parameters were analysed using R (R Core Team, 2020). Critical external P requirements were calculated as the amount of P required to achieve 90% of maximum yield based on a Weibull growth function, with the 95% confidence intervals determined by bootstrapping the residuals. Critical internal P requirements were calculated as the shoot P concentrations that corresponded with the critical external P requirements.

Results and Discussion

Shoot yield and critical external P requirements

The shoot dry mass of Digit and Desmanthus increased in response to soil P supply ($P < 0.001$; Fig. 15). In the soil pH component (Fig. 15a,b), both species were most productive in the pH 5–8 treatments (Digit produced much less biomass in the pH 4 treatment while Desmanthus did not grow at all). In general, the critical external P requirements of Digit and Desmanthus were lowest in the pH 7 treatment and increased at lower (i.e. pH 4–6 treatments) or higher (i.e. pH 8 treatment) pH levels (Table 8). In the soil PBI component (Fig. 15c,d), the species had the lowest critical external P requirements when soil PBI was lowest (i.e. PBI 65 treatment) (Table 8). The critical requirements of the species increased with soil PBI. In both the pH and PBI components, Digit consistently out-yielded Desmanthus and had lower critical external P requirements.

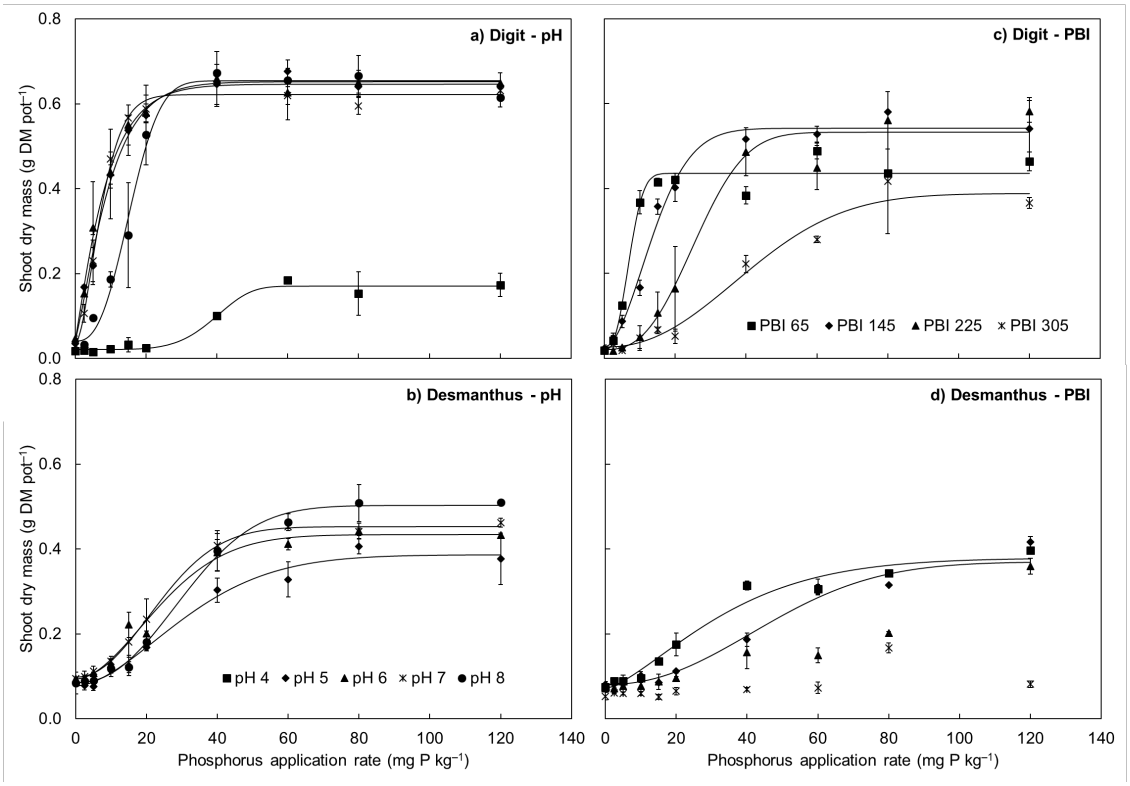

Figure 15. The shoot dry mass of Digit (a, c) and Desmanthus (b, d) when grown in soils with different pH (a, b) and PBI (c, d) levels. Values show the mean ± s.e. (n = 2). Fitted curves show Weibull growth functions. Curves could not be fitted for Desmanthus in the PBI 225–385 treatments due to limited but highly variable growth

Table 8. The critical external and internal P requirements of Digit and Desmanthus when grown in soils with different pH and PBI levels. Critical external P requirements were calculated as the amount of P applied to achieve 90% maximum yield, with 95% confidence intervals shown in parentheses. Critical internal P requirements were the shoot P concentrations that corresponded with the critical external P requirements. * shows the values that could not be calculated.

Species pH or PBI	**Critical external P requirement (mg P kg^{-1} soil)**	**Critical internal P requirement (mg P g^{-1} DM)**
Digit – pH		
pH 4	48.6 (44.7–68.7)	1.74
pH 5	20.1 (17.0–24.7)	1.02
pH 6	18.7 (14.7–25.5)	0.98
pH 7	14.3 (12.4–16.8)	1.14
pH 8	24.0 (20.8–32.5)	0.91
Desmanthus – pH		
pH 4	*	*
pH 5	56.3 (44.8–75.3)	1.33
pH 6	42.6 (33.6–50.0)	1.54
pH 7	39.9 (33.0–46.7)	1.28
pH 8	50.2 (44.3–56.1)	1.43
Digit – PBI		
PBI 65	11.0 (9.5–12.9)	0.97
PBI 145	25.7 (22.0–31.6)	0.86
PBI 225	40.1 (25.9–54.4)	1.22
PBI 305	68.9 (50.0–*)	1.20
PBI 385	*	*
Desmanthus – PBI		
PBI 65	62.7 (52.2–74.8)	1.81
PBI 145	75.4 (68.3–83.5)	1.49
PBI 225	*	*
PBI 305	*	*
PBI 385	*	*

Shoot P concentration and critical internal P requirements

Shoot P concentrations increased in response to soil P supply for both Digit and Desmanthus ($P < 0.001$; Fig. 16). Although there were some differences across treatments, shoot P concentrations were generally not influenced strongly by pH or PBI level, as demonstrated by the relatively consistent critical internal P requirements of the species (Table 8).

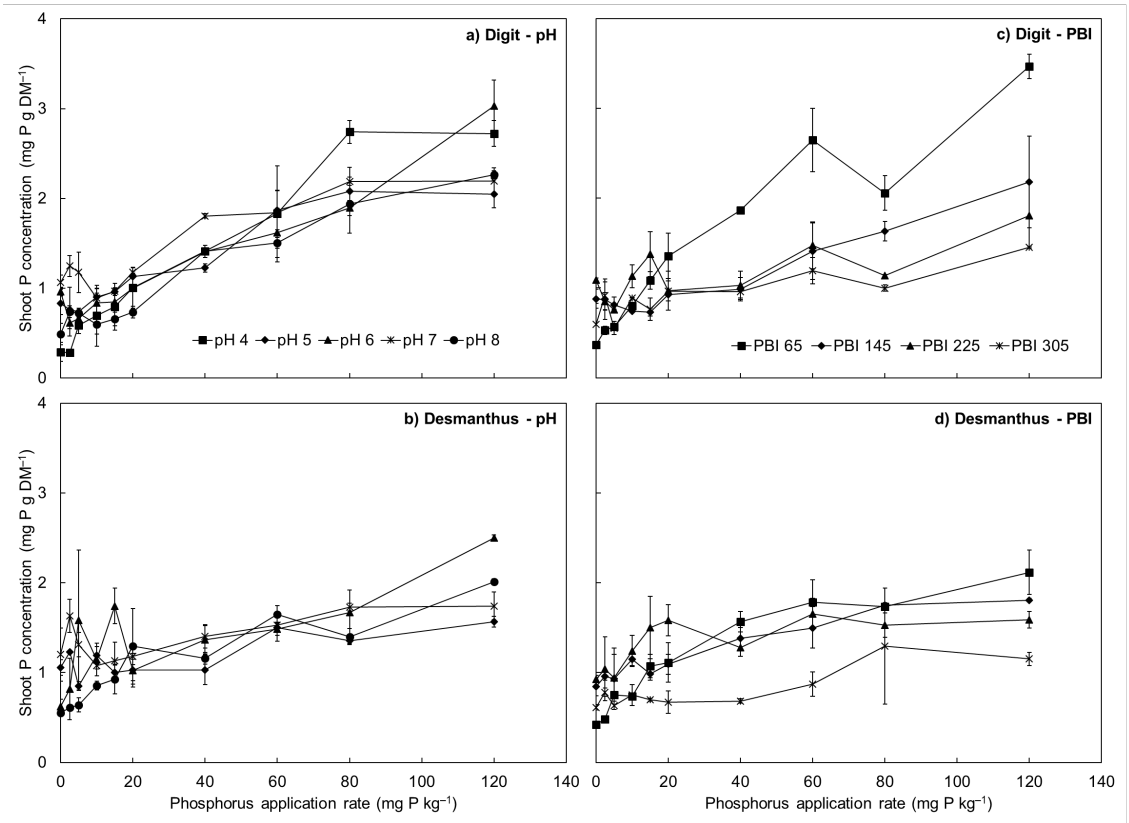

Figure 16. The shoot P concentration of Digit (a, c) and Desmanthus (b, d) when grown in soils with different pH (a, b) and PBI (c, d) levels. Values show the mean ± s.e. (n = 2).

Practical implications

Soil pH and PBI are likely to influence the response of tropical pasture species to P application. However, this may be due to varietal differences in preferred soil type rather than just P uptake dynamics. For example, Desmanthus is suited to neutral to alkaline clay soils, with a medium to heavy texture. It is expected that this species would perform poorly in lighter soils with a relatively low pH. Nevertheless, critical internal P requirements were relatively consistent regardless of soil pH and PBI (Digit = 0.86–1.22 mg P g^{-1} DM, and Desmanthus = 1.28–1.81 mg P g^{-1} DM). Tissue P tests may therefore be a useful way to determine likely responses of tropical pasture species to P fertiliser application across a range of soil types.

8.4 Appendix 4 – Differences in phosphorus acquisition and critical phosphorus requirements among nine *Desmanthus* spp. genotypes

Materials and Methods

Plant material

Nine genotypes from four *Desmanthus* spp. were grown to determine varietal differences in shoot yield and root morphology that were associated with P-acquisition efficiency. The genotypes (selections) were JCU 1 (*D. leptophyllus*), JCU 2 (*D. virgatus*), JCU 3 (*D. virgatus*), JCU 4 (*D. bicornutus*), JCU 5 (*D. virgatus*), JCU 6 (*D. bicornutus*), JCU 7 (*D. leptophyllus*), JCU 8 (*D. virgatus*) and JCU 9 (*D. pernambucanus*). The nine genotypes are commercially available and can be combined into enviro-specific blends of Progardes® Desmanthus.

Soil and nutrient treatments

A sandy soil (Grey Tenosol; Isbell 1996) was collected from the upper 2–15 cm soil layer of a field at Newholme SMART Farm, Armidale, NSW, Australia (30°26'21.4"S 151°39'55.5"E). The soil had a Colwell extractable P concentration of 5 mg P kg^{-1} (as measured by the method of Colwell (1963)), a Phosphorus Buffering Index (PBI) of 29 (as measured by the modified method of Burkitt et al. (2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of ~5.3. The soil was passed through a 5 mm sieve and a basal nutrient solution that contained 150 mg kg^{-1} soil CH_4N_2O, 100 mg kg^{-1} K_2SO_4, 100 mg kg^{-1} $MgSO_4.7H_2O$, 0.4 mg kg^{-1} $MnCl_2.4H_2O$, 0.4 mg kg^{-1} $CuCl_2.2H_2O$, 0.4 mg kg^{-1} $ZnCl_2.2H_2O$, 0.4 mg kg^{-1} Na_2MoO_4 and 0.4 mg kg^{-1} H_3BO_3 was applied to the soil. The amended soil was then weighed into individual plastic bags (1.0 kg oven-dry equivalent). Phosphorus was applied to the individually bagged soil at rates of 0, 10, 30, 60 and 100 mg P kg^{-1} soil (hereafter referred to as P0, P10, P30, P60 and P100, respectively) by adding $Ca(H_2PO_4)_2.H_2O$ salt to the soil surface, before the soil was thoroughly mixed in the bags to evenly distribute the applied P. The P100 treatment was expected to allow maximum shoot growth whereas the P0–P60 treatments were predicted to be within the P responsive range of the *Desmanthus* spp. genotypes based on preliminary work in this soil type. A $CaCl_2.2H_2O$ solution was used to balance the calcium that was applied in the P0–P60 treatments to be equivalent to the calcium applied in the P100 treatment. Five weeks after the addition of all nutrients, the Colwell extractable P concentrations of the P0, P30, P60 and P100 treatments were 5, 20, 41 and 63 mg Colwell P kg^{-1}, respectively. The Colwell extractable P concentration of the P10 treatment was not measured but was estimated to be ~11 mg Colwell P kg^{-1}, based on the Colwell extractable P concentrations of the other P treatments that closely matched the known response to applied P that was determined for this particular soil during preliminary soil characterisation ($y = 0.001x^2 + 0.582x + 4.950$, $R^2 = 1.000$, $P < 0.001$). The bagged soil was placed in cylindrical pots (110 mm internal diameter; 125 mm height) with a total soil depth of ~100 mm and a bulk density of ~1.05 g cm^{-3}.

Plant growth conditions and experimental design

Micro-swards of each *Desmanthus* spp. genotype were established by sowing seed (~5 mm depth) to achieve a density of 3 plants pot^{-1}, which was equivalent to 316 plants m^{-2}. To increase germination rates, the seed of each genotype was mechanically scarified and heat-treated using hot water prior to sowing (Hopkinson and English, 2004). Three replicate pots of each genotype at each P application rate were prepared. After planting, the pots were watered and moved to a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia.

Humidifiers were used to increase the humidity within the glasshouse to ~50%. Plants were grown between 26th September 2019 and 5th November 2019. Pots were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006). Soil moisture was maintained at 80–100% field capacity by watering daily to a predetermined weight using distilled water (100% field capacity was equivalent to ~16% soil water content in the sandy soil).

Harvest and measurements

Plants were harvested after five weeks' growth. The height of individual plants was measured and the number of shoot nodes (defined as the points on the stem from which branches originated) were counted. These measured parameters were used to determine the average canopy height and the average branching frequency of the micro-swards in each pot. Shoots were then cut at the soil surface and oven-dried at 75°C for 72 h and weighed. The soil from each pot was removed intact and washed over 2 mm sieves. The roots from the entire core were washed for root scanning. The samples were scanned using an Epson Perfection V700 Photo flatbed scanner (Seiko Epson Corporation, Suwa, Japan) at 600 dpi. When the root samples were too large for scanning, a representative subsample was scanned and the remaining roots were dried. Root length and average root diameter were determined using WinRHIZO™ software (Regent Instruments Inc., Quebec, Canada) (Bouma et al., 2000). Roots were then oven-dried at 75°C for 72 hr and weighed. When the entire root sample was scanned, the scanned root length was equivalent to the total root length of the sample. When a representative subsample of the root sample was scanned, total root length was determined by multiplying the specific root length (i.e. length per unit root mass) of roots from the scanned subsample by the total mass of roots in the entire core (combined dry mass of scanned and unscanned roots). Root mass fraction was calculated as the mass of roots divided by total plant mass (i.e. shoot and root dry mass combined). Root length density was calculated as root length per unit soil volume; the total volume of soil was 950 cm^3.

Root hair parameters were also determined because root hairs were visible on the scanned root samples. Root hair length was determined by measuring root hairs at 10 random locations using ImageJ 1.52 (FIJI) (Schindelin et al., 2012). Root hair coverage (i.e. the proportion of root length covered with root hairs) (McLachlan et al., 2019b) was determined by examining the presence/absence of root hairs at 100 gridline intersects (Giovannetti and Mosse, 1980), by overlaying the scanned root images with a grid using ImageJ 1.52 (FIJI). The root hair cylinder volume (RHCV), adapted from Haling et al. (2016) to include root hair coverage, was then calculated as follows:

$$RHCV = ((\pi \times ((ARD/2) + RHL)^2 \times RL) \times RHC) + ((\pi \times (ARD/2)^2 \times RL) \times (1 - RHC)) \quad (1)$$

where: ARD = average root diameter, RHL = root hair length, RL = root length, and RHC = root hair coverage.

Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass.

Statistical analyses

Parameters of yield, root morphology and P acquisition were analysed in R Version 4.0.2 (R Core Team, 2020). Measured parameters were analysed by fitting linear models and using an analysis of variance with 'genotype' and 'P treatment' as predictor variables. When appropriate, the effects of 'rep' and 'row' from the randomised complete block design were included in the most parsimonious model. The effects of 'rep' and 'row' accounted for the error associated with spatial variation. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). Correlations and R^2 coefficients of determination between measured parameters were determined using linear regression (Crawley, 2013). Shoot yield responses to P application were determined by fitting a self-starting Weibull growth function ($y = a - b * \exp(-\exp(c) * x^d)$, where x is the P application rate and y is the shoot dry mass) as described by Crawley (2013). Critical external P requirements were calculated as the amount of P applied to achieve 90% of maximum yield based on the fitted Weibull growth functions, and the 95% confidence intervals of the critical P requirements were determined by bootstrapping residuals as described by Crawley (2013). Critical internal P requirements were calculated as the shoot P concentration that corresponded with the critical external P application rate. The critical P requirements of *Desmanthus* genotype JCU 1 could not be determined because the Weibull growth function did not fit the highly variable growth of this genotype. Consequently, the data associated with *Desmanthus* genotype JCU 1 was removed from subsequent analysis (the shoot yield data for this genotype is shown in Figure 17). A further nine pots from among the remaining genotypes were removed from analysis due to low plant numbers that influenced shoot yield. Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. The linear models that were fitted to shoot branching frequency, root mass fraction and shoot P content were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

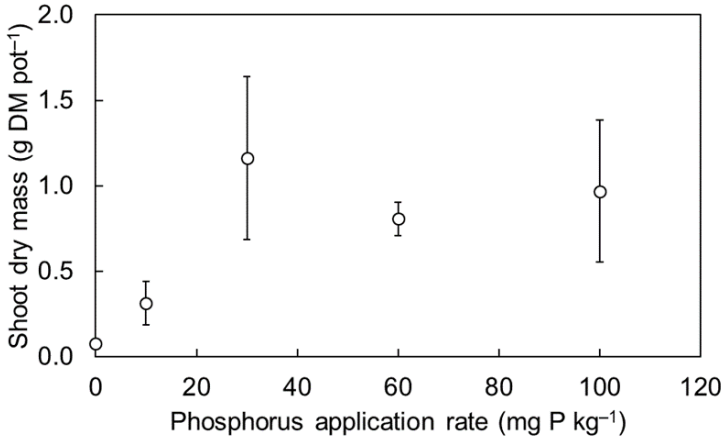

Figure 17. The shoot dry mass of Desmanthus genotype JCU 1 grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). Values show the measured mean ± s.e. (n = 3). The Weibull growth function, which shows the relationship between P application rate and shoot dry mass, could not be fitted to the highly variable growth of this genotype.

Results and Discussion

Shoot yield and critical external P requirements

Shoot dry mass increased in response to increased soil P supply for each *Desmanthus* spp. genotype ($P < 0.001$; Fig. 18). The largest shoot yields were generally achieved when plants were grown in the P100 treatment, except for *Desmanthus* genotype JCU 8 which produced 50% less shoot dry mass in the P100 treatment than in the P60 treatment. Among the genotypes, there was a 2.7-fold range in shoot yield when plants were grown in the P100 treatment. There were also 2.2- to 4.0-fold differences in shoot yield among the genotypes at lower levels of soil P supply ($P < 0.001$; Fig. 18). On average across the P treatments, *Desmanthus* genotype JCU 9 was the most productive ($P < 0.05$); it produced shoot yields that were between 1.1- to 1.8-fold larger than the next most productive genotype across the P treatment range (Fig. 18).

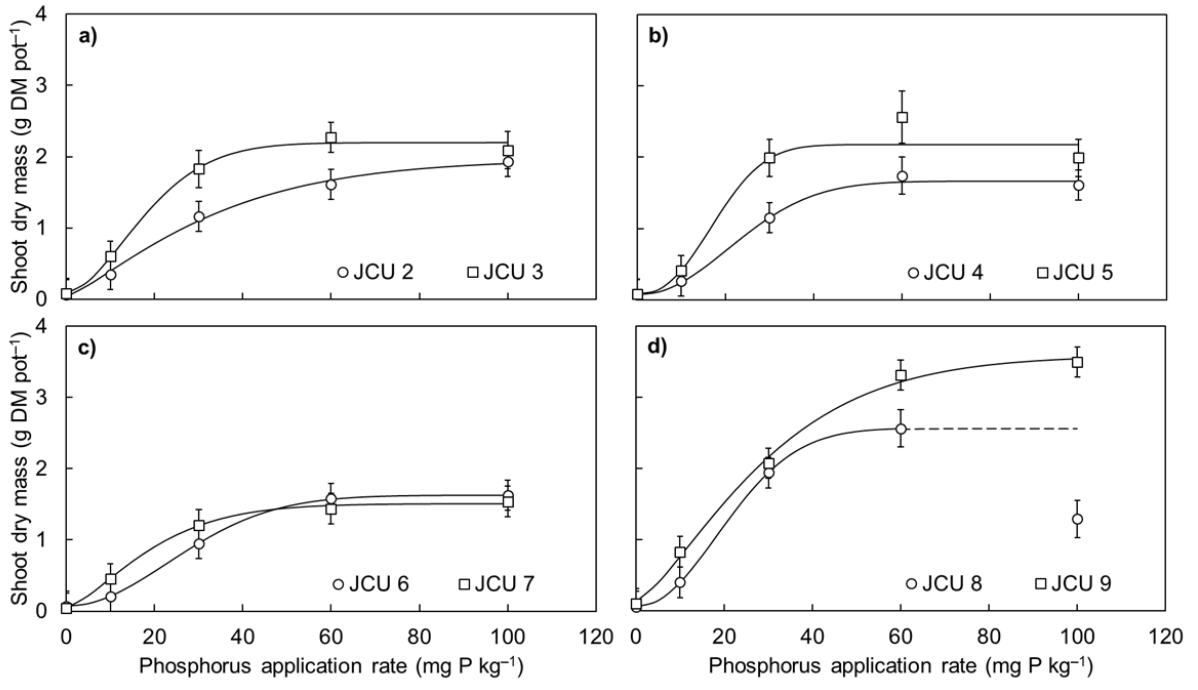

Figure 18. The shoot dry mass of eight genotypes of Desmanthus spp. grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). Values show the calculated mean ± s.e. (n = 3). The curves that were fitted to the shoot yield data show Weibull growth functions (Crawley, 2013). Analysis of variance results were: genotype P < 0.001, P treatment P < 0.001, genotype × P treatment interaction P = 0.004. The shoot yield achieved by Desmanthus genotype JCU 8 in the P100 treatment was significantly lower than in the P60 treatment. To enable the Weibull growth function to be fitted, the shoot yield achieved in the P100 treatment was assumed to be equivalent to that of the P60 treatment. The dashed line represents the assumed P response curve above the P60 treatment.

Varietal differences in shoot yield meant that the critical external P requirements of the genotypes varied by up to 2.2-fold (Table 9). The lowest critical external P requirement was 29.4 mg P kg^{-1} soil for *Desmanthus* genotype JCU 5 and the highest was 64.0 mg P kg^{-1} soil for *Desmanthus* genotype JCU 2. However, these differences in critical external P requirement did not reflect the P efficiency of dry matter production due to substantial differences in maximum yield potential (Fig. 18). In

particular, the highly productive *Desmanthus* genotype JCU 9 out-yielded the other genotypes in each P treatment (Fig. 18), yet had a relatively high critical external P requirement of 58.5 mg P kg^{-1} soil (Table 9).

Table 9. The critical external and internal P requirements of eight genotypes of Desmanthus spp. grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). The critical external P requirements were calculated as the amount of P applied to achieve 90% of maximum yield based on the self-starting Weibull growth functions that were fitted to the shoot yield data, with the value range in parentheses showing the 95% confidence intervals determined using bootstrap analysis (Crawley, 2013). The critical internal P requirements were determined as the shoot P concentration that corresponded with the critical external P application rate. * indicates the values that could not be determined due to highly variable plant growth.

Desmanthus spp. Genotype	Critical external P requirement (mg P kg^{-1} soil)	Critical internal P requirement (mg P g^{-1} DM)
JCU 1	*	*
JCU 2	**64.0** (51.4–79.7)	5.2
JCU 3	**34.5** (23.1–*)	2.5
JCU 4	**41.6** (33.9–66.1)	3.2
JCU 5	**29.4** (24.2–36.8)	2.3
JCU 6	**49.5** (37.7–68.2)	4.1
JCU 7	**39.3** (24.0–*)	3.5
JCU 8	**38.2** (31.2–54.7)	3.1
JCU 9	**58.5** (48.4–69.5)	2.5

Shoot P concentration and critical internal P requirements

Shoot P concentration increased in response to increased soil P supply for each *Desmanthus* spp. genotype ($P < 0.001$; Fig. 19), although it remained similar between the P10 and P30 treatments. Nevertheless, the genotypes responded differently to increasing concentrations of P fertiliser (as indicated by the significant genotype*P treatment interaction; $P < 0.001$). This resulted in large differences in shoot P concentration among the genotypes when plants were grown at higher levels of soil P supply ($P < 0.001$; Fig. 19); 1.8-fold in the P60 treatment and 2.5-fold in the P100 treatment. These differences in shoot P concentration, in conjunction with the 2.2-fold range in critical external P requirements, meant that there were substantial differences in the critical internal P requirements of the genotypes (Table 9); *Desmanthus* genotype JCU 5 (2.3 mg P g^{-1} DM) had the lowest critical internal P requirement and *Desmanthus* genotype JCU 2 (5.2 mg P g^{-1} DM) had the highest. There was a strong relationship between critical external and internal P requirements for the *Desmanthus* spp. genotypes (data not shown), except for *Desmanthus* genotype JCU 9 which had a relatively high critical external P requirement but a relatively low critical internal P requirement (Table 9).

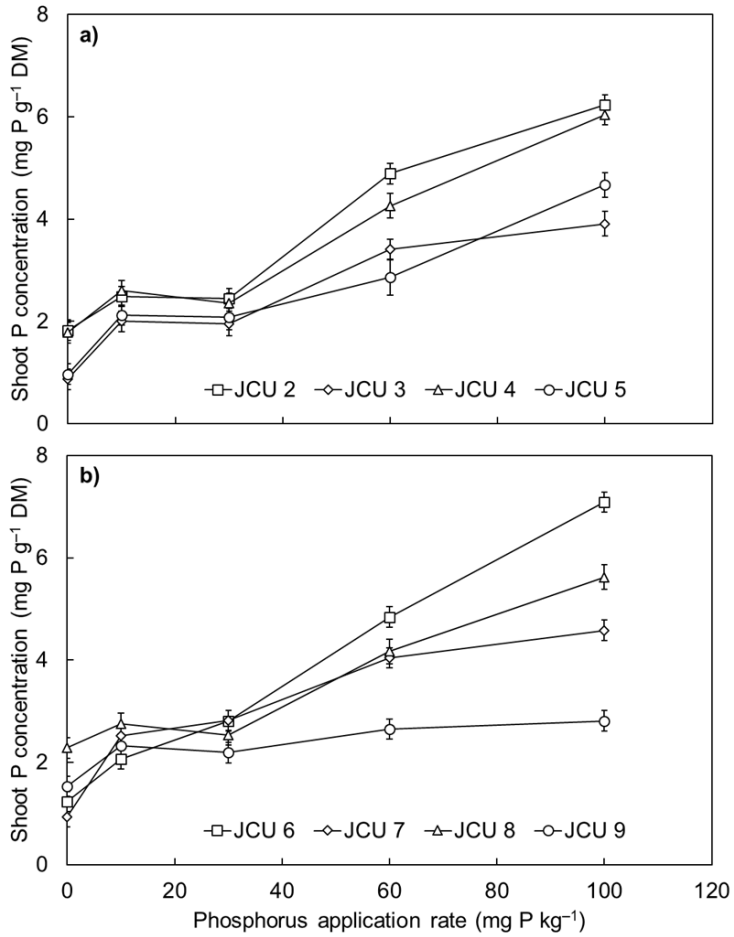

Figure 19. The shoot P concentration of eight genotypes of Desmanthus spp. grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). Values show the calculated mean ± s.e. (n = 3). Analysis of variance results were: genotype P < 0.001, P treatment P < 0.001, genotype × P treatment interaction P < 0.001.

Shoot morphology

Average canopy height increased in response to increased soil P supply ($P < 0.001$; Fig. 20) and largely reflected the shoot yields that were achieved by the *Desmanthus* spp. genotypes ($R^2 = 0.72$, $P < 0.001$; data not shown). Consequently, the tallest canopies were generally produced when plants were grown with an adequate supply of P that allowed maximum growth (i.e. the P100 treatment). In this P-sufficient treatment, there was a 2.1-fold range in average canopy height among the genotypes; *Desmanthus* genotype JCU 9 produced the tallest canopy whereas *Desmanthus* genotype JCU 3 produced the shortest (35 cm c.f. 17 cm). Varietal differences in average canopy height were also apparent when plants were grown in the lower-P soil treatments ($P < 0.001$; Fig. 20). These differences in average canopy height occurred in conjunction with differences in the average branching frequency of shoots (data not shown). Branching frequency generally increased with increased soil P supply ($P < 0.001$) but varied substantially among the genotypes ($P = 0.001$).

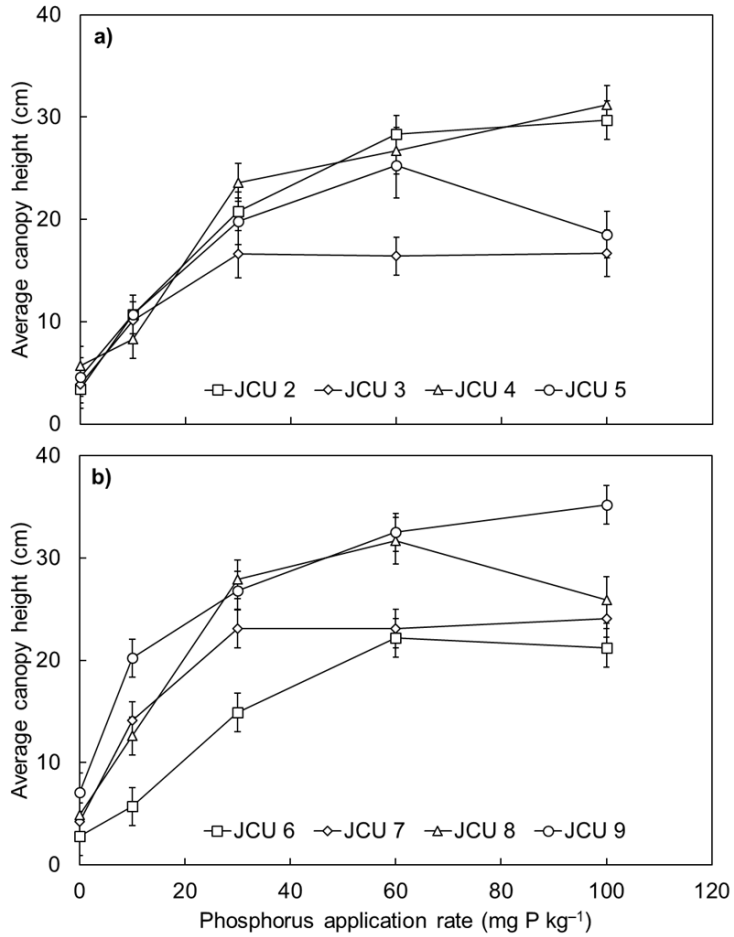

Figure 20. The average canopy height of eight genotypes of Desmanthus spp. grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). Values show the calculated mean ± s.e. (n = 3). Analysis of variance results were: genotype P < 0.001, P treatment P < 0.001, genotype × P treatment interaction P = 0.001.

Root mass fraction

Root mass fraction was lowest and most comparable among the *Desmanthus* spp. genotypes when plants were grown with an adequate supply of P that allowed maximum growth (i.e. the P100 treatment) (Fig. 21). As soil P supply was reduced, the proportion of plant biomass allocated to roots increased substantially ($P < 0.001$) so that most *Desmanthus* spp. genotypes allocated the largest proportion of their total plant biomass to roots when grown in the P0 treatment. On average across the P treatments, there were significant differences in the proportion of biomass allocated to roots among the genotypes ($P = 0.003$; Fig. 21). These differences were most pronounced when plants were grown in the P0 and P10 treatments. In particular, *Desmanthus* genotypes JCU 7, JCU 8 and JCU 9 had relatively low root mass fractions in the P10 treatment compared to the other genotypes, but increased their biomass allocation to roots substantially when grown in the P0 treatment (as indicated by the significant genotype*P treatment interaction; $P = 0.036$).

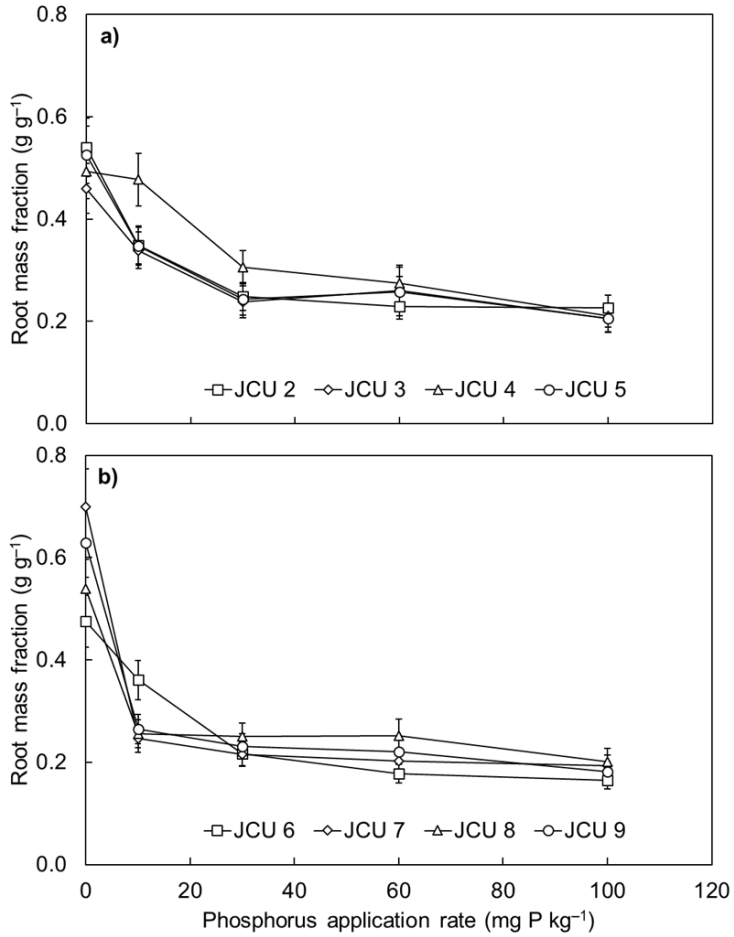

Figure 21. The root mass fraction of eight genotypes of Desmanthus spp. grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). Values show the calculated mean ± s.e. (n = 3). Analysis of variance results were: genotype P = 0.003, P treatment P < 0.001, genotype × P treatment interaction P = 0.036.

Root morphology

Specific root length was highly variable and not significantly different among the *Desmanthus* spp. genotypes across the five soil P treatments (P = 0.094; Table 10). On average across the genotypes, the highest specific root length was achieved when plants were grown in the P0 treatment (avg. 106 m g^{-1}) and the lowest when plants were grown in the P30 treatment (avg. 76 m g^{-1}) (P < 0.001). Among the genotypes, there was a weak correlation between specific root length and average root diameter (R^2 = 0.16, P < 0.001; data not shown). Average root diameter varied by ~1.2-fold among the genotypes when plants were grown in the P10–P100 treatments (P < 0.001; Table 10). On average across the genotypes, average root diameter was lowest in the P60–P100 treatments and highest in the P0 treatment (P < 0.001).

Table 10. The specific root length and average root diameter of eight genotypes of Desmanthus spp. grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). Values show the calculated mean ± s.e. (n = 3). Analysis of variance results are given for genotype, P treatment and the genotype × P treatment interaction.

***Desmanthus* spp.**	**Specific root length (m g^{-1})**					**Average root diameter (mm)**				
Genotype	**0 mg P kg^{-1}**	**10 mg P kg^{-1}**	**30 mg P kg^{-1}**	**60 mg P kg^{-1}**	**100 mg P kg^{-1}**	**0 mg P kg^{-1}**	**10 mg P kg^{-1}**	**30 mg P kg^{-1}**	**60 mg P kg^{-1}**	**100 mg P kg^{-1}**
JCU 2	103 ± 11	76 ± 11	69 ± 11	73 ± 11	80 ± 11	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
JCU 3	127 ± 11	91± 11	80 ± 14	85 ± 11	87 ± 14	0.30 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01
JCU 4	126 ± 11	68 ± 11	79 ± 11	66 ± 14	75 ± 11	0.28 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01
JCU 5	114 ± 11	91 ± 11	71 ± 14	69 ± 19	93 ± 14	0.29 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.27 ± 0.02	0.28 ± 0.01
JCU 6	99 ± 11	95 ± 11	77 ± 11	82 ± 11	93 ± 11	0.29 ± 0.01	0.27 ± 0.01	0.29 ± 0.01	0.28 ± 0.01	0.29 ± 0.01
JCU 7	83 ± 11	112 ± 11	87 ± 11	104 ± 11	93 ± 11	0.29 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
JCU 8	118 ± 11	102 ± 11	73 ± 11	69 ± 14	100 ± 14	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.24 ± 0.01
JCU 9	76 ± 11	105 ± 11	75 ± 11	66 ± 11	73 ± 11	0.30 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.28 ± 0.01
			ANOVA					ANOVA		
Genotype			*P* = 0.094					***P* < 0.001**		
P treatment			***P* < 0.001**					***P* < 0.001**		
Genotype × P treatment			*P* = 0.152					*P* = 0.129		

Table 11. The root hair length and root hair coverage of eight genotypes of Desmanthus spp. grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). Values show the calculated mean ± s.e. (n = 3). Analysis of variance results are given for genotype, P treatment and the genotype × P treatment interaction.

***Desmanthus* spp.**	**Root hair length (mm)**					**Root hair coverage (% root length)**				
Genotype	**0 mg P kg^{-1}**	**10 mg P kg^{-1}**	**30 mg P kg^{-1}**	**60 mg P kg^{-1}**	**100 mg P kg^{-1}**	**0 mg P kg^{-1}**	**10 mg P kg^{-1}**	**30 mg P kg^{-1}**	**60 mg P kg^{-1}**	**100 mg P kg^{-1}**
JCU 2	0.14 ± 0.01	0.20 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	18.3 ± 1.5	23.3 ± 1.5	13.3 ± 1.5	14.7 ± 1.5	16.3 ± 1.5
JCU 3	0.12 ± 0.01	0.17 ± 0.01	0.15 ± 0.02	0.14 ± 0.01	0.14 ± 0.01	12.0 ± 1.5	15.7 ± 1.5	7.5 ± 1.9	13.7 ± 1.5	16.0 ± 1.9
JCU 4	0.14 ± 0.01	0.17 ± 0.01	0.14 ± 0.01	0.15 ± 0.02	0.13 ± 0.01	12.3 ± 1.5	11.7 ± 1.5	12.0 ± 1.5	14.0 ± 1.9	13.3 ± 1.5
JCU 5	0.14 ± 0.01	0.17 ± 0.01	0.15 ± 0.02	0.15 ± 0.02	0.13 ± 0.01	23.0 ± 1.5	11.0 ± 1.5	12.0 ± 1.9	20.0 ± 2.6	16.5 ± 1.9
JCU 6	0.12 ± 0.01	0.18 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	13.0 ± 1.5	11.0 ± 1.5	12.0 ± 1.5	12.7 ± 1.5	13.7 ± 1.5
JCU 7	0.16 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.15 ± 0.01	19.7 ± 1.5	12.3 ± 1.5	22.7 ± 1.5	21.0 ± 1.5	16.0 ± 1.5
JCU 8	0.16 ± 0.01	0.18 ± 0.01	0.14 ± 0.01	0.17 ± 0.02	0.15 ± 0.01	16.7 ± 1.5	13.7 ± 1.5	17.7 ± 1.5	10.5 ± 1.9	20.5 ± 1.9
JCU 9	0.13 ± 0.01	0.20 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	11.0 ± 1.5	8.0 ± 1.5	10.7 ± 1.5	7.3 ± 1.5	9.7 ± 1.5
			ANOVA					ANOVA		
Genotype			***P* = 0.012**					***P* < 0.001**		
P treatment			***P* < 0.001**					***P* = 0.019**		
Genotype × P treatment			*P* = 0.781					***P* < 0.001**		

Root length densities varied by 1.9- to 4.4-fold among the *Desmanthus* spp. genotypes across the five soil P treatments ($P < 0.001$; Fig. 22). The highest root length densities were achieved when plants were grown in the P60–P100 treatments; the root length densities of some genotypes peaked in the P60 treatment and declined at higher P supply, whereas other genotypes continued to increase their root length densities as soil P supply was increased. The lowest root length densities were achieved by each genotype when plants were grown in the P0 treatment (Fig. 22). The difference between minimum and maximum root length densities provided a measure of root length proliferation in response to soil P supply. *Desmanthus* genotype JCU 6 produced the largest proliferation response (9.2-fold difference) whereas *Desmanthus* genotype JCU 9 produced the smallest (4.2-fold difference) (Fig. 22).

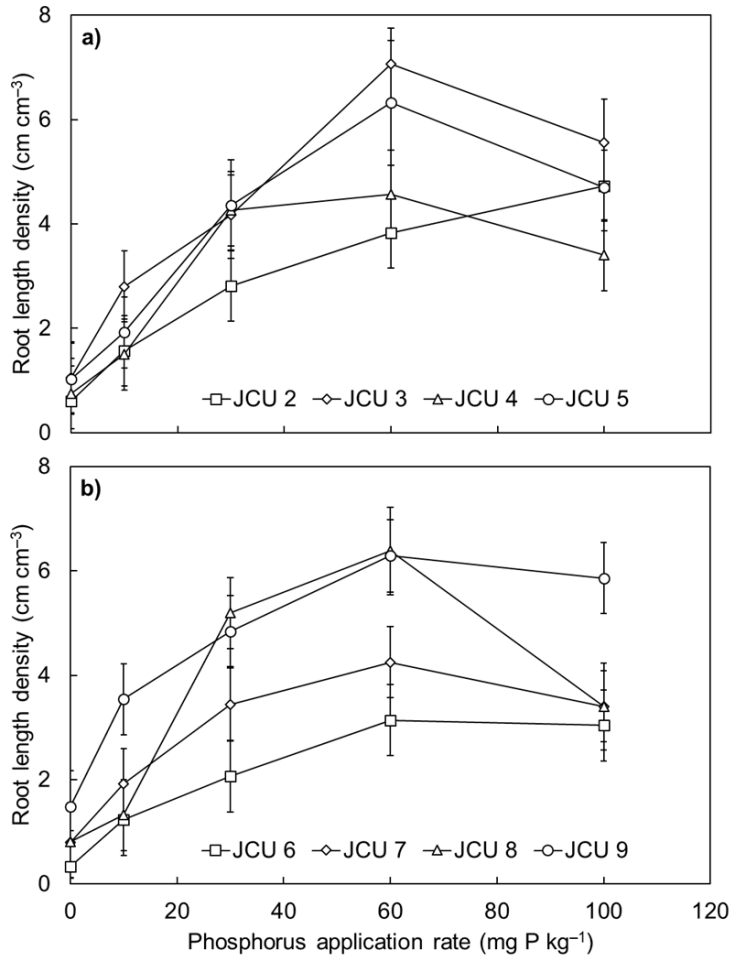

Figure 22. The root length density of eight genotypes of Desmanthus spp. grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). Values show the calculated mean ± s.e. (n = 3). Analysis of variance results were: genotype P < 0.001, P treatment P < 0.001, genotype × P treatment interaction P = 0.598.

The root hairs of the *Desmanthus* spp. genotypes were relatively short in each of the soil P treatments yet, on average, they responded to soil P supply (Table 11; $P < 0.001$); root hairs were longest in the P10 treatment (avg. 0.18 mm) and shortest in the P0 and P100 treatments (avg. 0.14 mm). On average across the P treatments, *Desmanthus* genotype JCU 2 produced the longest root hairs (avg. 0.17 mm) whereas *Desmanthus* genotype JCU 6 produced the shortest (avg. 0.14 mm) (*P*

= 0.012). Root hairs were absent from a large proportion of the root length of each *Desmanthus* spp. genotype (Table 11). On average across the P treatments, there were significant varietal differences in root hair coverage ($P < 0.001$); *Desmanthus* genotype JCU 7 achieved the highest coverage (~18% of root length) whereas *Desmanthus* genotype JCU 9 achieved the lowest (~9% of root length). The combined influence of short root hairs and low root hair coverage meant that no more than ~0.5% of the soil volume was explored by the *Desmanthus* spp. genotypes (data not shown), based on root hair cylinder volume compared to soil volume.

Phosphorus acquisition

Shoot P content increased with increased soil P supply for each *Desmanthus* spp. genotype ($P < 0.001$; Fig. 23). Among the genotypes, shoot P content varied by 1.8- to 4.4-fold across the five soil P treatments ($P < 0.001$). Shoot P content, which generally reflects the total amount of plant P acquired from soil, was influenced by the development of root length. This was apparent in each of the P treatments, although positive correlations between shoot P content and root length were strongest in the P10–P60 treatments ($R^2 = 0.30–0.62$, $P < 0.001$; Fig. 24). These correlations indicated that shoot P content was also influenced by P acquisition per unit root length (data not shown). Shoot P content per unit root length increased with increased soil P supply ($P < 0.001$) and varied significantly among the *Desmanthus* spp. genotypes ($P < 0.001$).

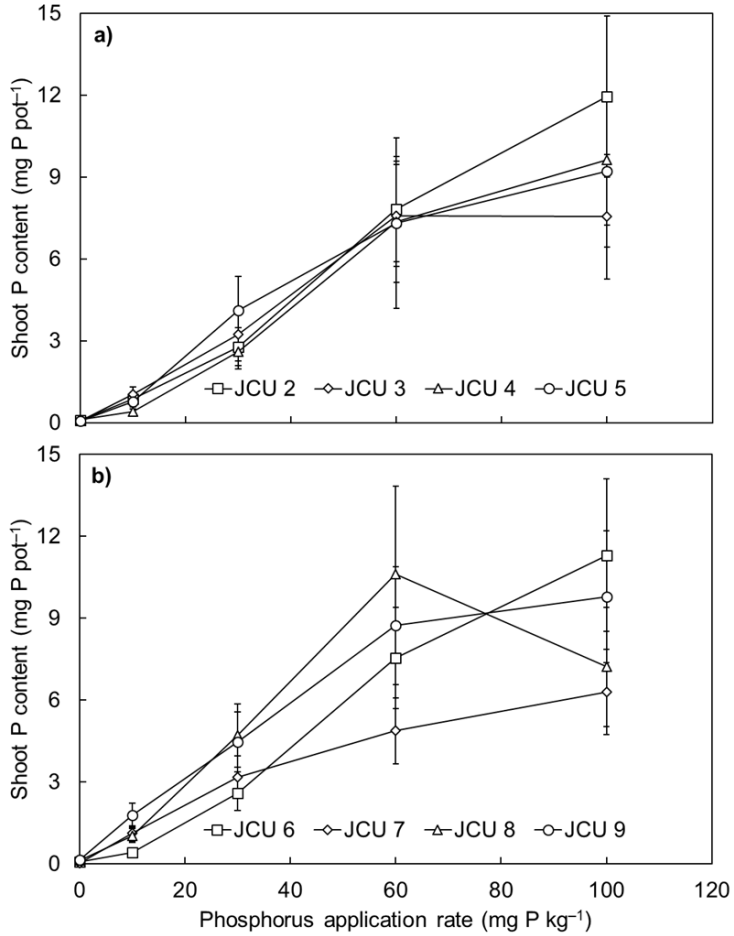

Figure 23. The shoot P content of eight genotypes of Desmanthus spp. grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). Values show the calculated mean ± s.e. (n = 3). Analysis of variance results were: genotype P < 0.001, P treatment P < 0.001, genotype × P treatment interaction P = 0.100.

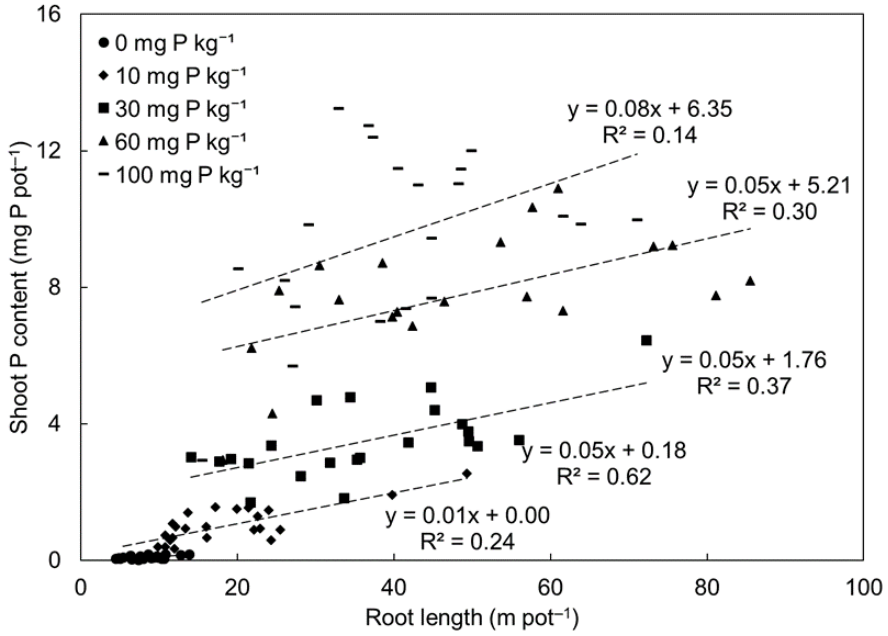

Figure 24. The relationship between root length and shoot P content among eight genotypes of Desmanthus spp. that were grown in response to five rates of applied P (0, 10, 30, 60 and 100 mg P kg^{-1} soil). The regression lines and R^2 coefficients of determination were fitted separately for each P treatment and include all data points of each genotype.

Additional observations

Desmanthus genotype JCU 7 dropped its lower leaves when grown in the P30–P100 treatments, whereas the other *Desmanthus* spp. genotypes did not.

Practical implications

There were substantial differences in shoot yield and critical external P requirements among the *Desmanthus* spp. genotypes. These differences were primarily associated with P-acquisition efficiency, because genotypes that produced longer roots generally acquired more P. However, differences in shoot yield were also associated with P-utilisation efficiency. In particular, *Desmanthus* genotype JCU 9 was efficient at using acquired P even at higher levels of soil P supply. In terms of root development/distribution, there may be trade-offs between P-acquisition efficiency and water acquisition for legume persistence. Nevertheless, our study indicates that there is potential to identify P-efficient *Desmanthus* spp. genotypes, such as *Desmanthus* genotype JCU 9, that may produce more shoot biomass when grown in the low-P soils of northern Australia. These P-efficient genotypes may have greater persistence in mixed pasture swards.

8.5 Appendix 5 – Premier Digit and Progardes Desmanthus compete effectively for applied phosphorus under mixed sward conditions

Materials and Methods

Plant growth conditions

Premier Digit (*Digitaria eriantha*) and Progardes Desmanthus (*Desmanthus* spp., cvv. JCU 1–5) were grown to determine shoot yield and tissue P concentrations under monoculture and mixed-sward conditions. These pasture species are considered to be suitable companion species (Boschma et al., 2021). Both species were grown in a sandy soil (Grey Tenosol; Isbell 1996) that was collected from the upper 2–15 cm soil layer of a field at Armidale, NSW, Australia. The soil had a Colwell extractable P concentration of 5 mg P kg^{-1} soil, a Phosphorus Buffering Index (PBI) of 29, and a pH ($CaCl_2$) of ~5.3. The soil was passed through a 5 mm sieve before 2 kg of oven-dry soil was weighed into plastic bags. Basal nutrients were applied to the individually bagged soil as a solution, and included 15 mg N kg^{-1} (as CH_4N_2O), 5.5 mg S kg^{-1} and 13.5 mg K kg^{-1} (as K_2SO_4), and 1 mg kg^{-1} Liberal BMX (which included boron, copper, iron, manganese, molybdenum and zinc). Five P treatments were established by adding P fertiliser (as KH_2PO_4) to the basal solution at rates of 0, 10, 30, 45 and 90 mg P kg^{-1}. After the nutrient solution was applied, the soil was mixed thoroughly and packed into plastic pots (height = 15 cm, diameter = 13 cm) with a total soil depth of ~13 cm and a bulk density of ~1.2 g cm^{-3}.

Micro-swards of Premier Digit and Progardes Desmanthus were established by sowing seed to achieve a target density of ~15 plants pot^{-1}. Prior to sowing, the Progardes Desmanthus seeds were heat-treated by immersing in 85°C water for 8 sec to break seed dormancy. Four replicate pots of both species, as monocultures and mixed plantings, were prepared for each P treatment. Transparent mylar screens (0.5 mm thick) were used to separate the species when grown in mixed-sward conditions, to minimise above-ground light competition. After planting, the pots were moved to a glasshouse (natural daylight; 35/25°C, day/night) in Armidale, NSW, Australia. Plants were grown between August–September 2019. Pots were arranged in a randomised complete block design (blocks comprised the different replicates). Soil moisture was maintained by watering daily and by watering to 90% field capacity once per week.

Harvest and analysis

Plants were harvested after 35 days' growth. Shoots were cut at the soil surface and were oven-dried at 60°C for 72 h and weighed. Shoot samples were then ground to <2 mm before a ~0.5 g subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 2 h. Samples were digested using a Milestone UltraWAVE 640. The P concentration of the digested samples was determined using ICP-OES. Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. Measured parameters were analysed using R (R Core Team, 2020). Critical external P requirements were calculated as the amount of P required to achieve 90% of maximum yield based on a Weibull growth function, with the 95% confidence intervals determined by bootstrapping the residuals. Critical internal P requirements were calculated as the shoot P concentrations that corresponded with the critical external P requirements.

Results and Discussion

Shoot yield

Shoot dry mass increased with P fertiliser application for both Premier Digit and Progardes Desmanthus ($P < 0.001$; Fig. 25). Under both monoculture and mixed-sward conditions, Premier Digit out-yielded Progardes Desmanthus. Nevertheless, the shoot yield of Progardes Desmanthus represented 33–47% of the total yield in the mixed swards.

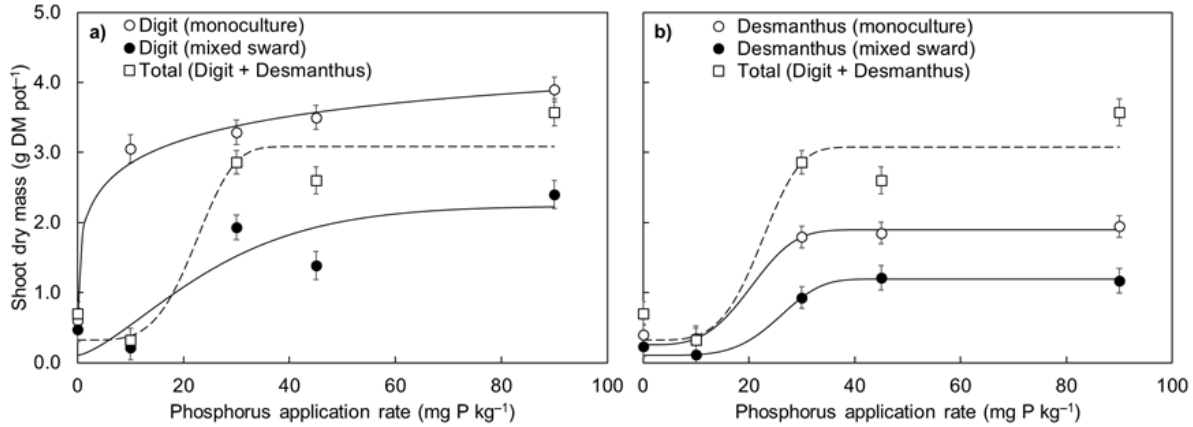

Figure 25. The shoot dry mass of Premier Digit (a) and Progardes Desmanthus (b) grown in response to five P application rates (0, 10, 30, 45 and 90 mg P kg^{-1} soil), under both monoculture and mixed-sward conditions. The total shoot dry mass of Digit and Desmanthus in the mixed swards is also shown in both panels (dashed line). Values show the mean ± s.e. (n = 4). Fitted curves show Weibull growth functions.

Critical external P requirements

The critical external P requirements of Progardes Desmanthus were either equivalent to or lower than that of Premier Digit, under both monoculture and mixed-sward conditions (Table 12). Mixed-sward conditions did not change the critical external P requirements of either species significantly.

Table 12. The critical external and internal P requirements of Premier Digit and Progardes Desmanthus. Critical external P requirements were calculated as the amount of P applied to achieve 90% maximum yield, with 95% confidence intervals shown in parentheses. Critical internal P requirements were the shoot P concentrations that corresponded with the critical external P requirements. * shows the values that could not be calculated.

Species	Critical external P requirement (mg P kg^{-1} soil)	Critical internal P requirement (mg P g^{-1} DM)
Premier Digit (monoculture)	**38.2** (24.6–50.8)	**4.30**
Progardes Desmanthus (monoculture)	**28.0** (24.7–38.2)	**2.38**
Premier Digit (mixed sward)	**49.2** (33.3–*)	**7.44**
Progardes Desmanthus (mixed sward)	**33.0** (30.2–*)	**2.02**
Total (Digit + Desmanthus)	**29.0** (24.6–34.7)	*

Shoot P content

Shoot P content increased with P fertiliser application for both species ($P < 0.001$; Fig. 26). However, the shoot P content of Premier Digit was generally larger than that of Progardes Desmanthus.

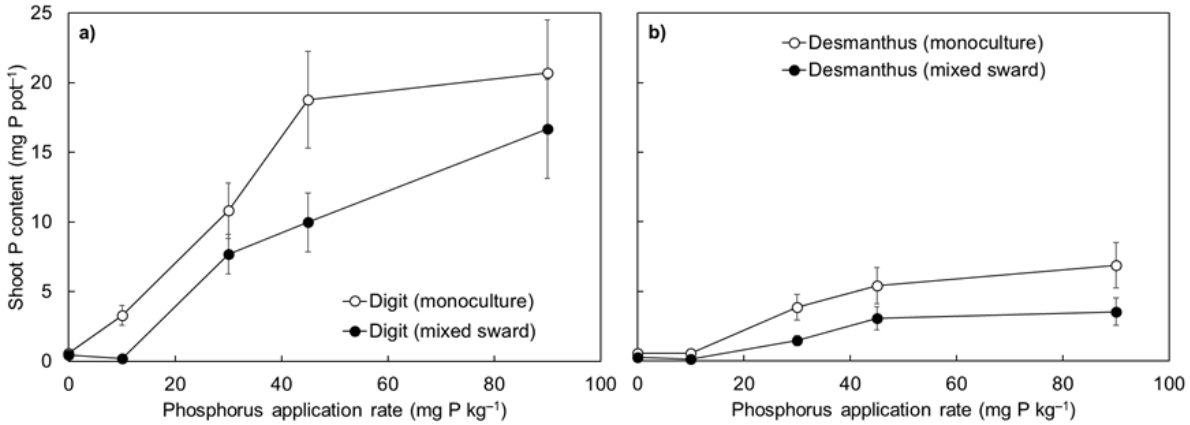

Figure 26. The shoot P content of Premier Digit (a) and Progardes Desmanthus (b) grown in response to five P application rates (0, 10, 30, 45 and 90 mg P kg^{-1} soil), under both monoculture and mixed-sward conditions. Values show the mean ± s.e. (n = 4).

Shoot P-use efficiency

Shoot P-use efficiency declined in response to P fertiliser application for both species ($P < 0.001$; Fig. 27). Premier Digit was particularly efficient at producing dry matter in the P0–P10 treatments. In contrast, Progardes Desmanthus used acquired P relatively efficiently in the higher P treatments as well. On average, Progardes Desmanthus used acquired P more efficiently under mixed-sward conditions than under monoculture conditions; no difference was observed for Premier Digit.

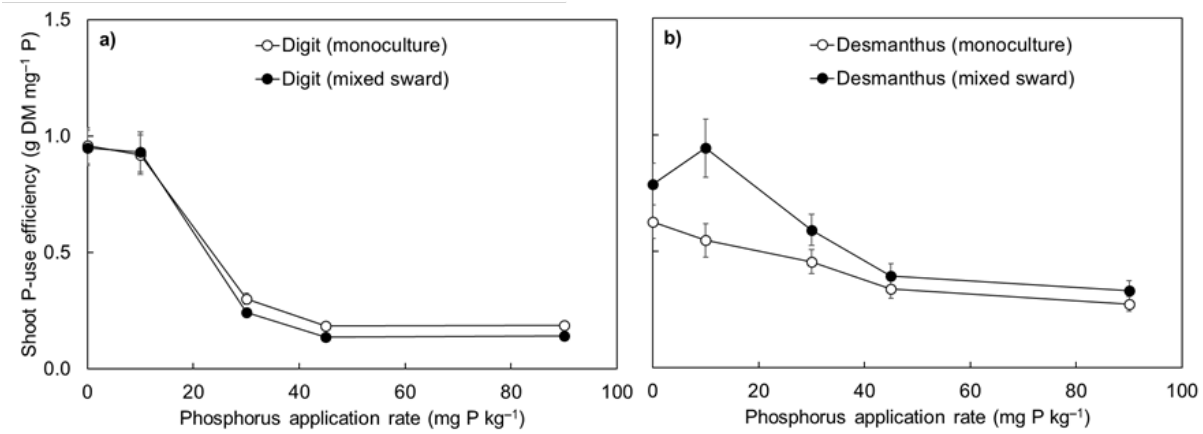

Figure 27. The shoot P-use efficiency of Premier Digit (a) and Progardes Desmanthus (b) grown in response to five P application rates (0, 10, 30, 45 and 90 mg P kg^{-1} soil), under both monoculture and mixed-sward conditions. Values show the mean ± s.e. (n = 4).

Practical implications

Premier Digit and Progardes Desmanthus were both responsive to P fertiliser when grown in the low-P soil. In general, the critical external P requirements of Progardes Desmanthus were equal to or lower than that of Premier Digit, under both monoculture and mixed-sward conditions. This indicates that Progardes Desmanthus is relatively efficient at acquiring P, and maintains this efficiency when grown with a constituent pasture grass. The shoot P-use efficiency of Progardes Desmanthus was also comparatively high across the P treatment range and increased in response to mixed-sward conditions. Nevertheless, Premier Digit had a much larger biomass potential which indicates that the grass component is likely to out-yield the legume component regardless of soil P supply, provided adequate N is available (although this is unlikely in many pastures where the relationship will be determined by legume N-fixation). This difference in productivity may be associated with varietal differences in root morphology, because grasses generally forage the soil more efficiently than legumes (Evans, 1977). Indeed, the root morphology of *Desmanthus* spp. genotypes is known to be relatively poor because roots are short and thick while root hairs are short and mostly absent (McLachlan et al., 2021).

8.6 Appendix 6 – Mixed sward plantings influence the shoot yield and phosphorus requirements of tropical pasture species

Materials and Methods

Plant material

Four pasture species were grown as monocultures and as mixed swards to determine the effect of plant competition on shoot yield and tissue P concentration. The pasture species were Digit (*Digitaria eriantha* cv. Premier), Rhodes (*Chloris gayana* cv. Katambora), Desmanthus (*Desmanthus pernambucanus* cv. JCU 9) and Centro (*Centrosema pubescens* cv. Cardillo). These species are commonly grown in the grazing systems of northern Australia.

Soil and nutrient treatments

A sandy loam soil (Brown Chromosol; Isbell 1996) was collected from the upper 2–15 cm soil layer of a field at Kirby SMART Farm, Armidale, NSW, Australia (30°25'35.0"S 151°39'08.4"E). The soil had a Colwell extractable P concentration of 2 mg P kg^{-1} (as measured by the method of Colwell (1963)), a Phosphorus Buffering Index (PBI) of 51 (as measured by the modified method of Burkitt et al. (2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of ~4.6. The soil was passed through a 5 mm sieve and lime (1.5 g $CaCO_3$ kg^{-1}) was mixed through the soil to raise the pH to ~5.2. While mixing the soil, basal nutrients were applied as a solution including 34 mg kg^{-1} soil $MgSO_4.7H_2O$, 36 mg kg^{-1} $CaSO_4.2H_2O$, 141 mg kg^{-1} KNO_3, 23 mg kg^{-1} $(NH_4)_2SO_4$, 14 mg kg^{-1} NH_4NO_3, 99 µg kg^{-1} H_3BO_3, 635 µg kg^{-1} $MnCl_2.4H_2O$, 301 µg kg^{-1} $ZnSO_4.7H_2O$, 28 µg kg^{-1} $CuSO_4.5H_2O$, 60 µg kg^{-1} $(NH_4)_2MoO_4$, 17 µg kg^{-1} $CoCl_2 \cdot 6H_2O$ and 1283 µg kg^{-1} FeNa-EDTA. Six P-amended soils (0, 7.5, 15, 30, 60 and 300 mg P kg^{-1}, hereafter referred to as P0, P7.5, P15, P30, P60 and P300, respectively) were prepared by adding KH_2PO_4 to the nutrient solution before the soil was amended. KCl was also applied to the P0–P15 treatments to balance the K to be equivalent to that of the P30 treatment. After the addition of lime and nutrients, the Colwell extractable P concentration of the P0–P300 soils were: 2, 7, 12, 20, 40 and 165 mg Colwell P kg^{-1}. The P0–P60 soils were used to prepare five uniformly distributed soil P treatments. Cylindrical PVC pots (87 mm internal diameter; 200 mm height) were filled with 1.3 kg (oven-dry basis) of these soils. The total depth of soil was ~190 mm. The P0 and P300s soil was used to prepare one localised soil P treatment, by adding three layers of soil to the PVC pots; a low-P 'subsoil' layer (840 g oven-dry soil; 123 mm soil height; P0), a high-P 'band' layer (140 g oven-dry soil; 20 mm soil height; P300) and a low-P 'topsoil' layer (320 g oven-dry soil; 47 mm soil height; P0). This treatment was prepared to investigate shoot yield and root morphology in response to a banded application of P fertiliser. The total available Colwell P in this treatment was expected to be similar to the uniformly distributed P30 treatment. Alkathene beads were placed around the interior edge of these pots to mark the interface between each of the soil layers.

Plant growth conditions

Micro-swards of each species were established by sowing seed (~5 mm depth) to achieve a population of 4 plants pot^{-1}. The species were established both as monocultures (4 plants pot^{-1} of either grass or legume) and mixed swards (2 plants pot^{-1} each of a grass and legume). The grass/legume combinations were Digit/Desmanthus and Rhodes/Centro. Prior to sowing, the Desmanthus seeds were heat-treated to break seed dormancy and increase germination rates. Four replicate pots of each species in each P treatment (i.e. P0–P60 and banded high-P) and each planting combination (i.e. monoculture and mixed sward) were prepared. After planting, the pots were

watered and moved to a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25ºC, day/night) in Armidale, NSW, Australia. Humidifiers were used to increase the June–September 2020. Pots were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006). Soil moisture was maintained at 80–100% field capacity by watering daily to a predetermined weight. Additional N as CH_4N_2O was applied to the surface of each pot six weeks (50 mg N kg $soil^{-1}$) and nine weeks (30 mg N kg $soil^{-1}$) after planting due to signs of N deficiency

Harvest and measurements

Plants were initially harvested after 7 weeks of growth. Shoots were cut ~5 cm above the soil surface, to maintain ~1–2 true leaves on the legume plants for regrowth of the P0–P60 treatments. Shoots were then oven-dried at 75°C for 72 h and weighed. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass.

The soil from the banded high-P treatments was removed as an intact core and cut at the interfaces of the three soil layers. The roots were then washed from the soil over 2 mm sieves. Roots from the topsoil, band and subsoil layers were washed from their respective sections as a whole for scanning and drying. Root samples were scanned using an Epson Perfection V700 Photo flatbed scanner (Seiko Epson Corporation, Suwa, Japan) at 600 dpi. When a root sample was too large for scanning, a representative subsample was scanned and the remaining roots were dried. Root length and average root diameter were analysed using WinRHIZO™ software (Regent Instruments Inc., Quebec, Canada) (Bouma et al., 2000). Roots were then oven-dried at 75°C for 72 hr and weighed. When the entire root sample was scanned, the scanned root length was equivalent to the total root length. When a subsample of the root sample was scanned, total root length was determined by multiplying the specific root length (i.e. length per unit dry mass) of roots from the scanned subsample by the total mass of roots in the entire core (combined dry mass of scanned and unscanned roots). Topsoil, band, subsoil and total root mass fractions were calculated as the mass of roots in either the topsoil, band, subsoil or whole pot divided by total plant dry mass (i.e. combined dry mass of shoots and roots), respectively. Root length densities were calculated as root length per unit soil volume; soil volumes were 279, 119 and 731 cm^3 in the topsoil, band and subsoil layers, respectively.

Plants in the P0–P60 treatments were regrown for a further 4 weeks' before being harvested again. Shoots were cut at the soil surface and were analysed for dry mass and tissue P concentration as outlined above.

Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'species' and 'P treatment' as predictor variables. Linear mixed effects models were fitted to root traits that were measured in each soil layer (i.e. topsoil, band and subsoil) (R package: nlme) (Pinheiro et al., 2020). The models included 'species', 'P treatment' and 'depth' as fixed effects, and 'pot' as a random effect. When appropriate, the effects of 'rep' and 'row' from the randomised complete block design were included in the most parsimonious model. The effects of 'rep' and 'row' accounted for the error associated with spatial variation. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020)

and means were compared using Tukey's honest significant differences (HSD). Shoot yield responses to P application were determined by fitting a self-starting Weibull growth function ($y = a - b * \exp(-\exp(c) * x^d)$), where x is the P application rate and y is the shoot dry mass) as described by Crawley (2013). Critical external P requirements were calculated as the amount of P applied to achieve 90% of maximum yield based on the fitted Weibull growth functions, and the 95% confidence intervals of the critical P requirements were determined by bootstrapping residuals as described by Crawley (2013). Critical internal P requirements were calculated as the shoot P concentration that corresponded with the critical external P application rate. Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield and critical external P requirements

The shoot yield of each species (as monocultures and as mixed swards) increased in response to soil P supply ($P < 0.001$; Fig. 28). As monocultures, the grasses were generally more productive than the legumes (Fig. 28). Under mixed sward conditions, the grasses made up a larger proportion of the mixed swards than the legumes (data not shown). The competitive growth of the grasses was most pronounced at higher levels of soil P supply. Yields were generally higher at the second harvest.

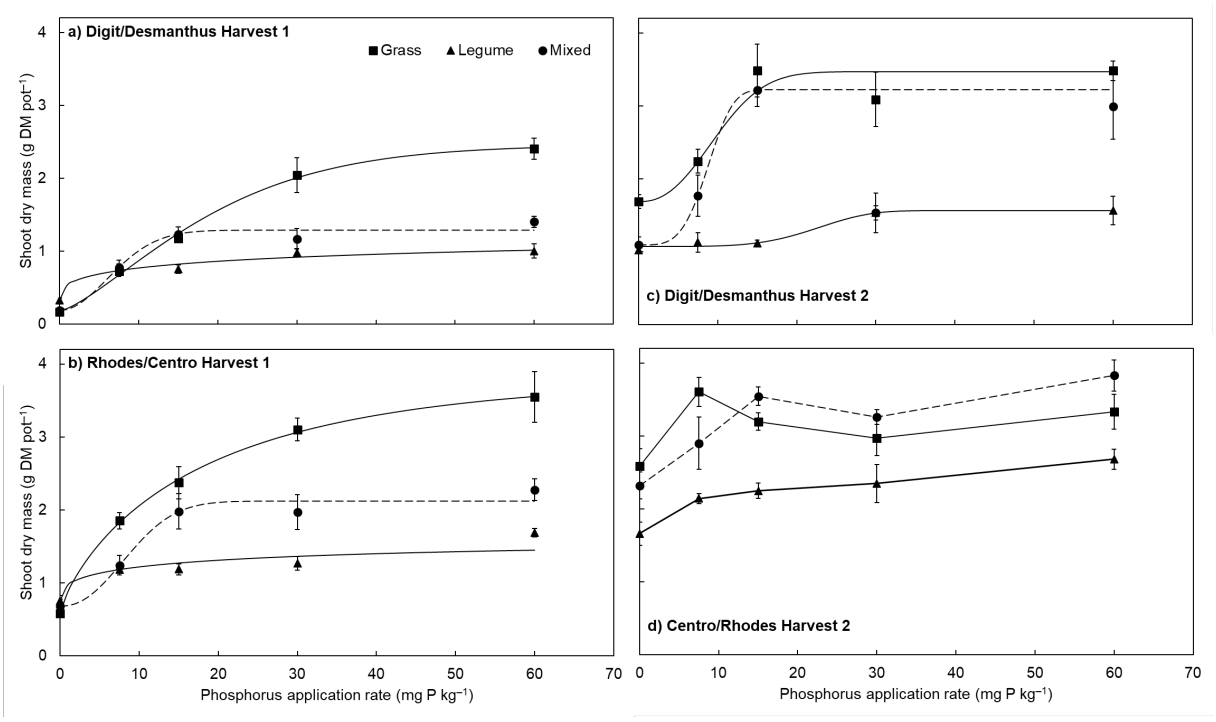

Figure 28. The shoot dry mass of Digit/Desmanthus (a, c) and Rhodes/Centro (b, d) grown as monocultures and mixed swards in response to five P application rates (0, 7.5, 15, 30 and 60 mg P kg^{-1} soil) at two harvests. Values show the mean ± s.e. (n = 4). The curves that were fitted to the shoot yield data show Weibull growth functions (Crawley, 2013), except for Centro/Rhodes at the second harvest 2 because curves could not be fitted to the highly variable data.

Critical external P requirements were higher for the grasses than the legumes at the first harvest (Table 13). The mixed swards had lower critical external P requirements than when the grasses and

legumes were grown as monocultures. The critical external P requirement of Desmanthus was consistent across the two harvests, whereas that of Digit was significantly lower.

Table 13. The critical external P requirements of Digit/Desmanthus and Rhodes/Centro when grown as monocultures and mixed swards. Critical external P requirements were calculated as the amount of P applied to achieve 90% maximum yield, with 95% confidence intervals shown in parentheses. * shows the values that could not be calculated.

Species	Harvest 1 critical P requirement (mg P kg^{-1} soil)	Harvest 2 critical P requirement (mg P kg^{-1} soil)
Digit (monoculture)	**36.3** (27.3–*)	**14.1** (11.6–19.1)
Desmanthus (monoculture)	**28.8** (17.1–56.1)	**25.0** (19.7–32.4)
Digit/Desmanthus (mixed sward)	**12.6** (10.8–23.0)	**11.3** (11.3–16.7)
Rhodes (monoculture)	**35.3** (22.6–*)	*
Centro (monoculture)	**19.0** (11.8–25.1)	*
Rhodes/Centro (mixed sward)	**13.9** (11.1–29.6)	*

Benefit of banded P fertiliser application

When compared to the maximum shoot yields achieved in the P0–P60 treatments, the shoot yields of Digit/Desmanthus and Rhodes/Centro were comparable or higher when grown in soil with a banded layer of P fertiliser (Table 14). The greatest benefit of the banded layer of P occurred in the two mixed swards, when the grasses and legumes were actively competing for applied P fertiliser.

Table 14. The modelled yield potential (i.e. the maximum shoot yield based on the Weibull growth functions), the banded yield potential (i.e. the shoot yield achieved in the P300 treatment when P fertiliser was only applied in the band layer), and the benefit of banded P fertiliser (i.e. comparison of the banded yield potential and the modelled yield potential).

Species	Modelled yield potential (g DM pot^{-1})	Banded yield potential (g DM pot^{-1})	Banded P benefit (%)
Digit (monoculture)	2.42	2.54	+5.2
Desmanthus (monoculture)	1.02	1.08	+5.5
Digit/Desmanthus (mixed sward)	1.29	1.95	+51.4
Rhodes (monoculture)	3.56	3.47	-2.4
Centro (monoculture)	1.45	1.72	+18.3
Rhodes/Centro (mixed sward)	2.12	2.66	+25.3

The grasses responded to the banded layer of P fertiliser by proliferating root length in that layer, whereas the legumes generally did not respond (Fig. 29). The proliferation response seen in the mixed swards was most likely associated with the development of roots by the grass component (grass and legume roots could not be separated).

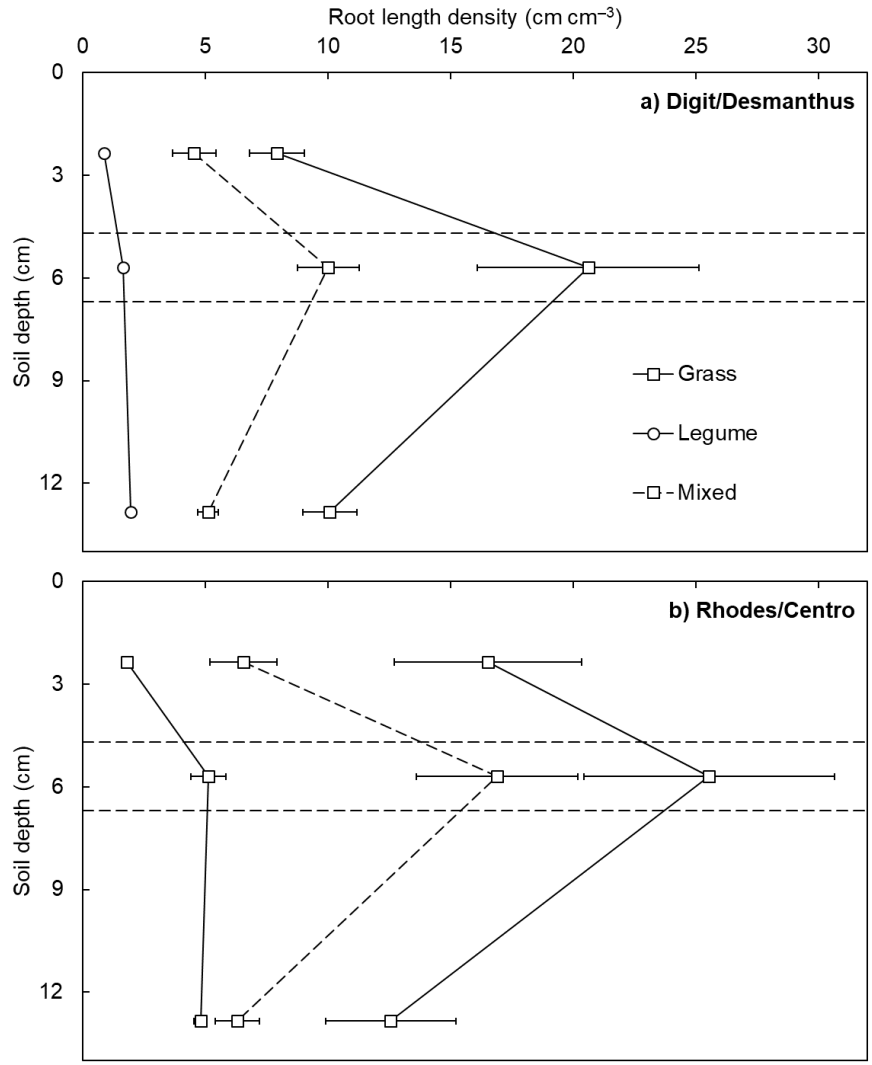

Figure 29. Root length density in the topsoil, band and subsoil layers of Digit/Desmanthus and Rhodes/Centro when grown in response to a banded layer of highly concentrated P fertiliser. Soil depth values correspond to the depth below the soil surface of the mid-point of each soil layer (i.e. topsoil = 2.4 cm, band = 5.7 cm and subsoil = 12.9 cm) and the horizontal lines depict the interfaces between soil layers. Data points of the species grown as monocultures are joined by solid lines whereas data points of the species grown as mixed swards are joined by dashed lines.

Practical implications

The grasses generally produced larger shoot yields than the legumes and hence made up a larger proportion of the mixed swards. Each of the species were able to maintain biomass production when P fertiliser was applied in a highly concentrated band, although it was only the grasses that responded by proliferating root length in the vicinity of the banded P fertiliser. This method of applying P fertiliser may be appropriate in mixed pasture swards (indeed the benefit of P fertiliser was highest in these treatments), although the grass component is likely to outcompete the legume component.

8.7 Appendix 7 – Mycorrhizal fungi influence phosphorus acquisition in mixed tropical pasture swards

Materials and Methods

Plant material

Five tropical pasture species were grown to investigate shoot yield, root traits and P acquisition in response to mycorrhizal colonisation. The species were Bambatsi Panic (*Panicum coloratum*), Katambora Rhodes (*Chloris gayana* cv. Katambora), Premier Digit (*Digitaria eriantha* cv. Premier), Desmanthus (*Desmanthus pernambucanus* cv. JCU 9) and Stylo (an experimental line of *Stylosanthes*, #25). These species were combined into six grass/legume mixes (i.e. Desmanthus or Stylo with either Panic or Rhodes or Digit). These species mixes are common in the pastures of northern NSW and southern QLD of Australia.

Soil preparation

A clay loam soil was collected from the upper 0–30 cm soil layer of a field at Armraynald Station, Burketown, QLD, Australia (17°57'57.1"S 139°42'51.5"E). The soil had a Colwell extractable P concentration of 3 mg P kg^{-1} (as measured by the method of (Colwell, 1963)), a Phosphorus Buffering Index (PBI) of 70 (as measured by the modified method of (Burkitt et al., 2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of ~5.9. The collected soil was dried, crushed by hand and homogenised. The soil was amended with a basal nutrient solution that included 34 mg kg^{-1} soil $MgSO_4.7H_2O$, 36 mg kg^{-1} $CaSO_4.2H_2O$, 141 mg kg^{-1} KNO_3, 23 mg kg^{-1} $(NH_4)_2SO_4$, 14 mg kg^{-1} NH_4NO_3, 99 µg kg^{-1} H_3BO_3, 635 µg kg^{-1} $MnCl_2.4H_2O$, 301 µg kg^{-1} $ZnSO_4.7H_2O$, 28 µg kg^{-1} $CuSO_4.5H_2O$, 60 µg kg^{-1} $(NH_4)_2MoO_4$, 17 µg kg^{-1} $CoCl_2 \cdot 6H_2O$ and 1283 µg kg^{-1} FeNa-EDTA. Three P-amended soils (3, 10 and 40 mg P kg^{-1}, hereafter referred to as P3, P10 and P40, respectively) were prepared by adding KH_2PO_4 to the nutrient solution before it was applied to the soil. KCl was also added to the nutrient solution that was applied to the P3–P10 treatments, to balance the K so that it was equivalent to that of the P40 treatment.

100 g (oven-dry basis) of the P-amended soils were weighed into plastic bags to be labelled with a 1 ppm solution of ^{32}P-radioisotope. The soil was then incubated for three weeks to ensure the soil P was uniformly labelled with radioisotope. After incubation, the labelled soil was added to small PVC tubes (50 mm internal diameter, 50 mm height) that had 30 µm mesh covering both ends. The mesh was small enough to exclude roots and root hairs, but large enough to allow mycorrhizal hyphae to access the labelled soil. Cylindrical PVC pots (87 mm internal diameter, 200 mm height) were filled with 0.7 kg (oven-dry basis) of the P-amended soils. The small PVC tubes with the labelled soil were then placed horizontally in the PVC pots, before a further 0.5 kg (oven-dry basis) of the P-amended soils was added to the pots. A root/soil mix of *Desmanthus virgatus* cv. JCU 2, which had been growing for ~18 months, was used as a source of mycorrhizal inoculum. 10 g of the root/soil mixture was added to the surface of each pot and incorporated to ~2 cm. The total depth of soil was ~195 mm.

Plant growth conditions and experimental design

Micro-swards of each species were established by sowing seed (~5 mm depth) to achieve a population of 8 plants pot^{-1}. The species were established as mixed swards (4 plants pot^{-1} each of the grass and legume). Four replicate pots of each species combination at each P application rate were prepared. After planting, the pots were moved to a glasshouse (natural daylight, ~1800 µmol

m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. Plants were grown between September–October 2022. Plants were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006). Soil moisture was maintained at 80–100% field capacity by watering daily to a predetermined weight using distilled water.

Harvest and measurements

Plants were harvested after six weeks' growth. Shoots were cut at the soil surface, oven-dried at 70°C for 72 h and weighed. Shoot samples were finely cut before a ~0.1–0.5 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Agilent, Mulgrave, Australia). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. Digested shoot samples were also analysed for ^{32}P-radioisotope activity using a PerkinElmer Tri-Carb 2810TR (PerkinElmer, Waltham, United States of America). A scintillation cocktail was prepared by mixing 3 mL of sample with 17 mL of scintillant (PerkinElmer UltimaGold AB). All samples were analysed by liquid scintillation counting (LSC) for 5 min and were corrected for radioactive decay and dilution. The ^{32}P-radioisotope counts were used to calculate the specific activity of the shoot material and the amount of plant P derived from applied P (to estimate P acquisition by mycorrhizal fungi):

$$Specific\ activity = \frac{radioactivity\ in\ shoots}{total\ P\ in\ shoots} \quad (1)$$

$$Plant\ P\ derived\ from\ applied\ P = \frac{specific\ activity\ of\ shoots}{specific\ activity\ of\ applied\ P} \times 100 \quad (2)$$

The soil in each pot was stored until radioactivity had decayed, at which point the soil was removed as an intact core. Roots were then washed from the soil over 2 mm sieves to assess root length colonisation by mycorrhizal fungi. Roots were cleared in 10% (w/v) KOH for 50 min at 90°C, rinsed in water and 0.1 M (v/v) HCl, and stained using a 5% (v/v) Schaeffer black ink/white vinegar solution for 2 h (Vierheilig et al., 1998). Colonisation % was determined using the gridline intersect method, by examining the presence/absence of mycorrhizal fungi at 100 intersects on the stained root samples (Giovannetti and Mosse, 1980).

Statistical analysis

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'species combination' and 'P treatment' as the predictor variables. Linear mixed effects models were fitted to measured variables where the two components (i.e. grass and legume) were compared (R package: nlme) (Pinheiro et al., 2020). The models included 'species combination', 'P treatment' and 'component' as fixed effects, and 'pot' as a random effect. When appropriate, the effect of 'replicate' was included in the most parsimonious model. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot dry mass

Shoot dry mass of the grass/legume micro-swards increased in response to the higher rates of soil P supply ($P < 0.001$; Fig. 30). However, the two components generally responded differently to the addition of P. In particular, the grasses produced more dry mass in the higher P treatments, whereas the legumes did not respond ($P < 0.001$). Consequently, the proportion of legume in the mixed micro-swards declined at higher rates of soil P supply. There were also differences in shoot dry mass among the species. The most productive grass was Rhodes, followed by Digit and then Bambatsi. For the legumes, Desmanthus was, on average, more productive than Stylo.

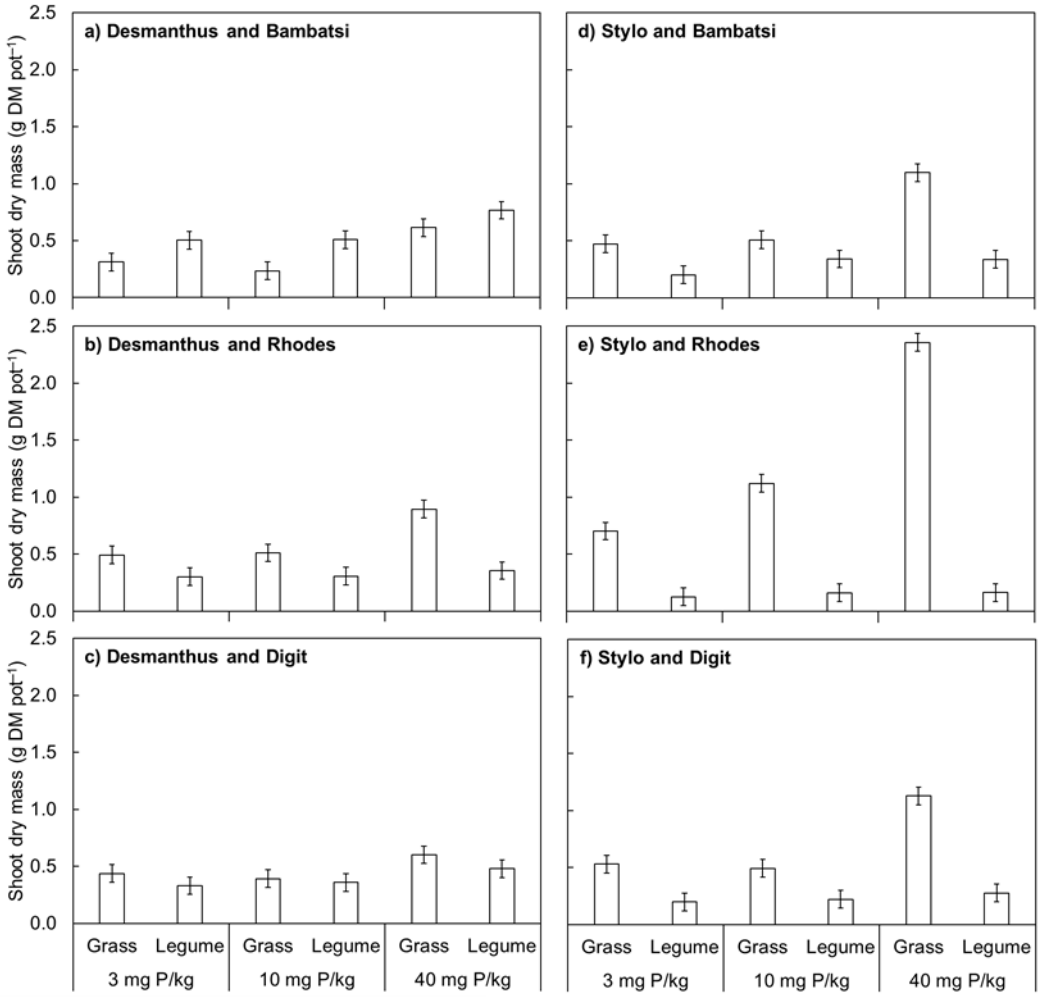

Figure 30. Shoot dry mass of six tropical pasture species combinations grown in response to three soil P treatments (3, 10 and 40 mg P kg^{-1}). Values show the mean ± s.e. (n = 4). Analysis of variance results were: species combination P < 0.001, P treatment P < 0.001, species combination × P treatment interaction P < 0.001.

Shoot P concentration

Shoot P concentrations increased in response to the higher rates of soil P supply ($P < 0.001$; Fig. 31). Both components responded to the addition of P, although the response was higher among the grasses. The shoot P concentrations of the grasses were either equivalent to (c.f. Stylo) or higher

than (c.f. Desmanthus) that of the legumes ($P < 0.001$). Although the grasses were not as productive when grown with Desmanthus, they did have higher shoot P concentrations compared to when they were grown with Stylo.

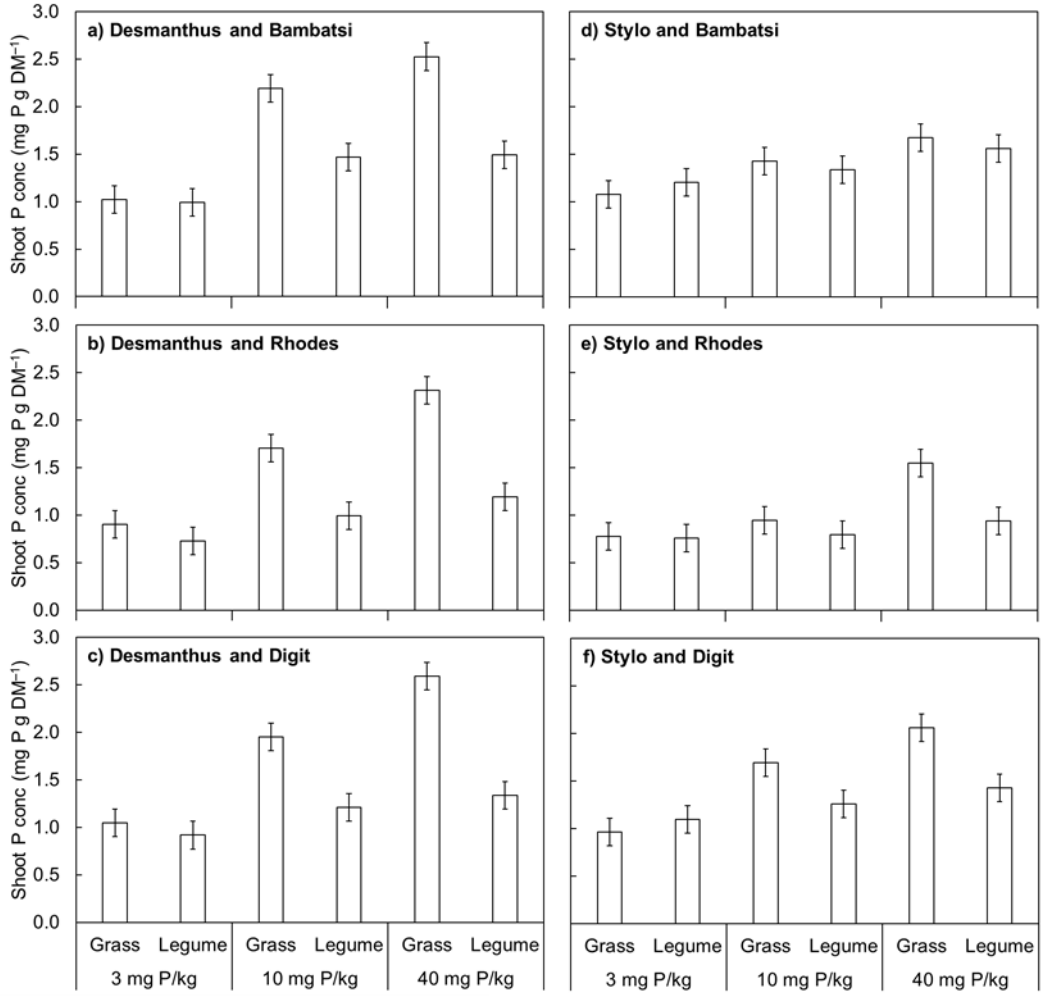

Figure 31. Shoot P concentration of six tropical pasture species combinations grown in response to three soil P treatments (3, 10 and 40 mg P kg^{-1}). Values show the mean ± s.e. (n = 4). Analysis of variance results were: species combination P < 0.001, P treatment P < 0.001, species combination × P treatment interaction P < 0.001.

Root length colonised by mycorrhizal fungi

Root samples taken from the mixed swards (i.e. combined grass and legume root samples) were colonised by mycorrhizal fungi in each of the species and P treatment combinations (Fig. 32). However, there were differences among the species combinations in the level of colonisation across the P treatments. For example, mycorrhizal colonisation of some species combinations declined in the higher soil P treatments (e.g. Desmanthus and Bambatsi, Stylo and Rhodes, Stylo and Digit) whereas in others it was consistent or increased across the soil P treatments (e.g. Desmanthus and Rhodes, Stylo and Bambatsi).

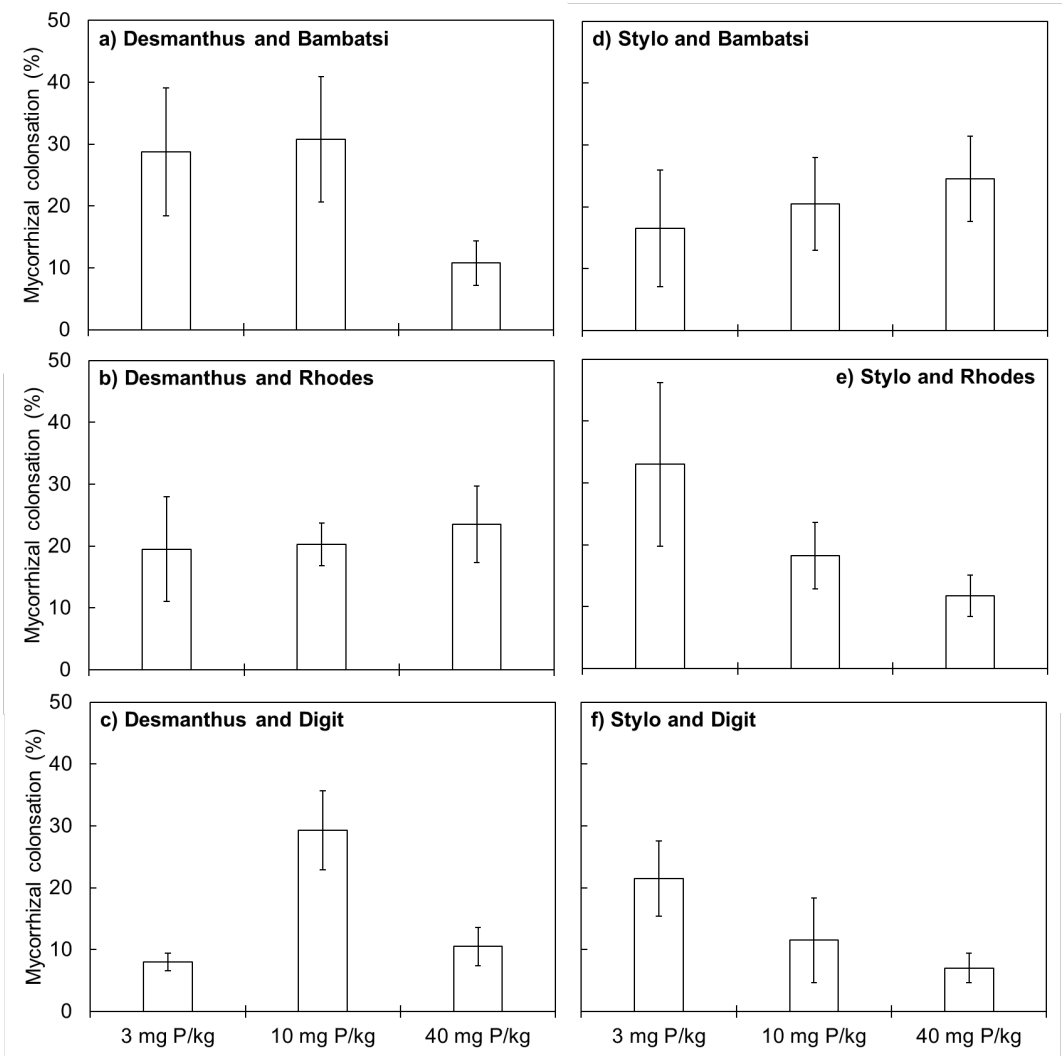

Figure 32. Mycorrhizal colonisation of six tropical pasture species combinations grown in response to three soil P treatments (3, 10 and 40 mg P kg^{-1}). Values show the mean ± s.e. (n = 4).

Phosphorus derived by mycorrhizal fungi

Phosphorus was derived by mycorrhizal fungi in each of the pasture species (Fig. 33). In general, P derived by mycorrhizal fungi was relatively low in each of the species combinations and soil P treatments, although there were some exceptions. There was no difference among the species combinations (i.e. the six grass/legume mixes) or component (i.e. grass vs. legume in each mix). Nevertheless, there were differences among the P treatments. The highest amount of P derived by mycorrhizal fungi occurred in the P40 treatments, compared to the P3 and P10 treatments which were equally low.

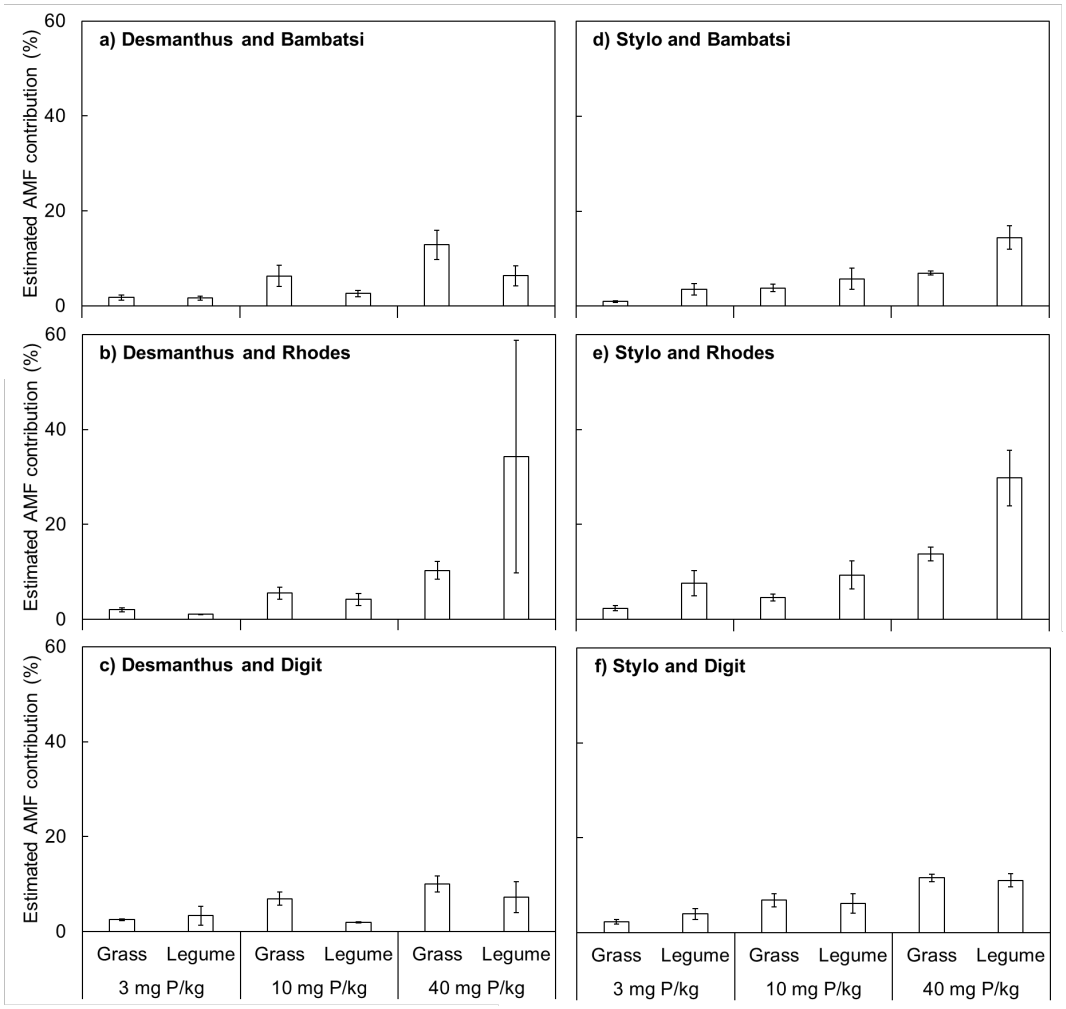

Figure 33. Estimated contribution of mycorrhizal fungi to the P acquisition of six tropical pasture species combinations grown in response to three soil P treatments (3, 10 and 40 mg P kg^{-1}). Values show the mean ± s.e. (n = 4).

Practical implications

Differences in the response of the species combinations to the addition of P suggest that tropical grasses and legumes should be paired according to potential growth. For example, Desmanthus was more competitive than Stylo when grown with each of the three tropical grasses (at least within the relatively short timeframe of the experiment). This indicates that the Desmanthus variety had better early growth than the Stylo variety. It is likely that this difference in early growth would have a longer-term impact on legume productivity, particularly if the legume component is overwhelmed by the grass component (c.f. Rhodes and Stylo). Of the six pasture species combinations, it was the Desmanthus/Bambatsi and Desmanthus/Digit mixes in which the legume was able to maintain a large proportion of the total biomass. This is important in the early stages of growth, where plants are developing their canopy to maximise light acquisition. Grazing and subsequent regrowth would likely result in a grass dominant pasture, as legumes are normally preferentially grazed and grasses regrow quickly. In each species combination, mycorrhizal fungi contributed to P acquisition, although the total contribution was generally low. This suggests that root architecture and morphology are likely to have the most significant impact on P-acquisition efficiency.

8.8 Appendix 8 – Warm-season pasture species respond to subsurface placement of phosphorus fertiliser

Materials and Methods

Plant material

Four warm-season pasture species were grown to investigate shoot and root production in response to three soil-P distribution treatments. The pasture species were Bambatsi Panic (*Panicum coloratum*), Premier Digit (*Digitaria eriantha* cv. Premier), Haymaster Lucerne (*Medicago sativus* cv. Haymaster), and Progardes® Desmanthus (a mixture of five cultivars, JCU 1–5, comprising three *Desmanthus* species). These pasture species were selected because they are commonly grown in the mixed pastures of northern, inland New South Wales, Australia.

Soil preparation

A sandy soil (Grey Tenosol; Isbell 1996) was collected from the upper 2–15 cm soil layer of a paddock at Newholme SMART Farm, Armidale, NSW, Australia (30°26'21.4"S 151°39'55.5"E). This soil was used because it enables easy recovery of roots for analysis. The soil had a Colwell extractable P concentration of 5 mg P kg^{-1}, a Phosphorus Buffering Index of 29, and a pH (1:5 w/v; 0.01 M $CaCl_2$) of ~5.3. The soil was passed through a 5 mm sieve and a basal nutrient solution that contained 150 mg kg^{-1} soil CH_4N_2O, 100 mg kg^{-1} K_2SO_4, 100 mg kg^{-1} $MgSO_4.7H_2O$, 0.4 mg kg^{-1} $MnCl_2.4H_2O$, 0.4 mg kg^{-1} $CuCl_2.2H_2O$, 0.4 mg kg^{-1} $ZnCl_2.2H_2O$, 0.4 mg kg^{-1} Na_2MoO_4 and 0.4 mg kg^{-1} H_3BO_3 was applied to the soil. Two P-amended soils (5 and 50 mg P kg^{-1}) were prepared by adding $Ca(H_2PO_4)_2.H_2O$ salt to the soil. A $CaCl_2.2H_2O$ solution was used to balance the calcium that was applied in the 5 mg P kg^{-1} soil to be equivalent to that applied in the 50 mg P kg^{-1} soil. After the addition of all nutrients, the Colwell extractable P concentrations of the 5 and 50 mg P kg^{-1} soils were 9 and 37 mg Colwell P kg^{-1}, respectively.

The two amended soils were used to prepare three contrasting soil-P distribution treatments, as shown in Figure 34. The banded high-P treatment provided a concentrated, localised source of P that mimicked a shallow band of P fertiliser below the soil surface. The uniform low-P and high-P treatments were the negative and positive controls, respectively. These soil-P distribution treatments were prepared in cylindrical PVC pots (87 mm internal diameter; 200 mm height) using three layers of soil; a 'subsoil' layer (780 g oven-dry soil; 123 mm soil height), a 'band' layer (125 g oven-dry soil; 20 mm soil height) and a 'topsoil' layer (295 g oven-dry soil; 47 mm soil height). The soil that was used in the 'band' layer was labelled with ~10.5 MBq kg^{-1} of ^{32}P-radioisotope tracer. Alkathene beads were placed around the interior edge of each pot to mark the interface between the soil layers.

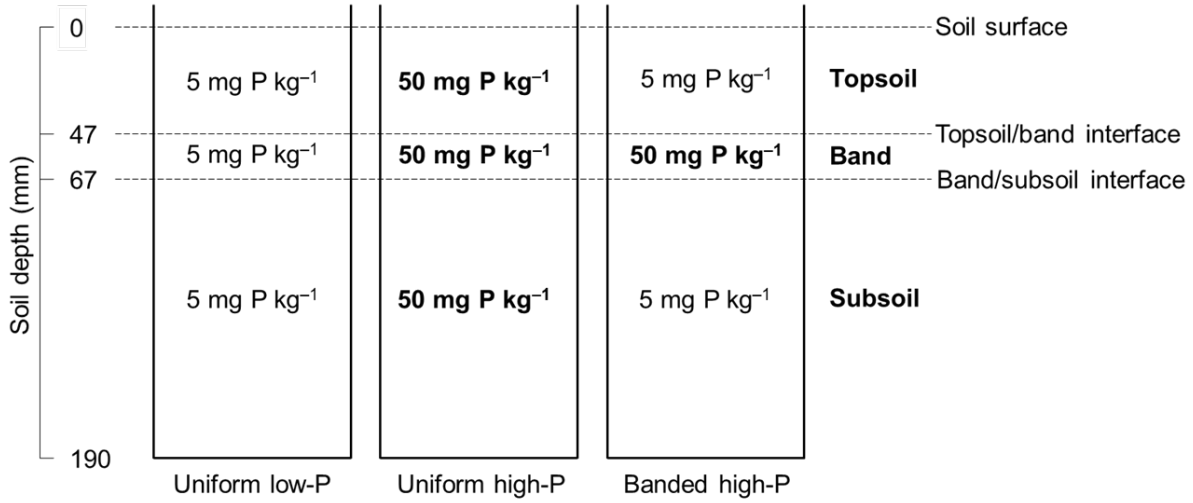

Figure 34. Diagrammatic illustration of the three soil-P distribution treatments used to investigate the root morphology and P acquisition of four warm-season pasture species. Thick lines represent the pot confine whereas dashed lines represent the soil surface and soil layer interfaces. The 5 and 50 mg P kg^{-1} labels depict the amount of P that was applied to each soil layer (i.e. topsoil, band and subsoil). The P applied to the 'band' layer was labelled with ~10.5 MBq kg^{-1} of ^{32}P-radioisotope tracer.

Plant growth conditions and experimental design

Monocultures of each pasture species were established by sowing seed to achieve a population of ~9 plants pot^{-1}. The Premier Digit and Progardes® Desmanthus seeds were immersed briefly in hot water prior to sowing to break seed dormancy (Hopkinson and English, 2004). Three replicate pots of each species in each soil-P distribution treatment were prepared. After planting, the pots were watered and moved to a glasshouse (natural daylight, 30/20°C) in Armidale, NSW, Australia. Plants were grown in July/August of 2019. Soil moisture was maintained at 80–100% field capacity by watering daily to a predetermined weight. An additional 150 mg kg^{-1} soil CH_4N_2O and 100 mg kg^{-1} K_2SO_4 were applied to each pot at the midpoint of the experiment.

Harvest and measurements

Plants were harvested after six weeks' growth. Shoots were cut at the soil surface and oven-dried at 70°C for 72 hr and weighed. The soil from each pot was removed as an intact core and cut at the interfaces of the three soil layers. The roots were then washed from the soil over 2 mm sieves. Roots were washed from each section as a whole for scanning and drying. Root samples were scanned using an Epson Perfection V700 Photo flatbed scanner (Seiko Epson Corporation, Suwa, Japan) at 600 dpi. When a root sample was too large for scanning, a representative subsample was scanned and the remaining roots were dried. Root length and average root diameter were determined using WinRHIZO^{TM} software (Regent Instruments Inc., Quebec, Canada) (Bouma et al., 2000). Roots were then oven-dried at 70°C for 72 hr and weighed. When the entire root sample was scanned, the scanned root length was equivalent to the total root length. When a subsample of the root sample was scanned, total root length was determined by multiplying the specific root length (i.e. length per unit dry mass) of roots from the scanned subsample by the total mass of roots in the entire core (combined dry mass of scanned and unscanned roots). Total root mass fraction was calculated as the mass of roots divided by total plant dry mass (i.e. combined dry mass of shoots and roots). Root

length densities were calculated as root length per unit soil volume. Soil volumes were 279, 119 and 731 cm^3 in the topsoil, band and subsoil layers, respectively.

Shoot samples were finely cut before a ~0.5 g subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 16 hr. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Agilent, Mulgrave, Australia). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. Digested shoot samples were also analysed for ^{32}P-radioisotope activity using a PerkinElmer Tri-Carb 2810TR (PerkinElmer, Waltham, United States of America). A scintillation cocktail was prepared by mixing 3 mL of sample with 17 mL of scintillant (PerkinElmer UltimaGold AB). All samples were analysed by liquid scintillation counting (LSC) for 5 min and were corrected for radioactive decay and dilution. The ^{32}P-radioisotope counts were used to calculate the specific activity of the shoot material, the amount of plant P derived from applied P, and the recovery of applied P as follows:

$$Specific\ activity = \frac{radioactivity\ in\ shoots}{total\ P\ in\ shoots} \quad (1)$$

$$Plant\ P\ derived\ from\ applied\ P = \frac{specific\ activity\ of\ shoots}{specific\ activity\ of\ applied\ P\ in\ band\ layer} \times 100 \quad (2)$$

$$Recovery\ of\ applied\ P = \frac{applied\ P\ from\ band\ layer\ in\ shoots}{total\ applied\ P\ in\ band\ layer} \times 100 \quad (3)$$

The specific activity of P applied to the 'band' layer was ~2.1 MBq mg P^{-1} in the uniform low-P treatment, and ~0.2 MBq mg P^{-1} in the uniform high-P and banded high-P treatments.

Statistical analysis

Parameters of yield, root morphology and P acquisition were analysed in R Version 4.0.2 (R Core Team, 2020). Measured parameters were analysed by fitting linear models and using an analysis of variance with 'species' and 'P treatment' as predictor variables. Linear mixed effects models were fitted to root traits that were measured in each soil layer (i.e. topsoil, band and subsoil) (R package: nlme) (Pinheiro et al., 2020). These models included 'species', 'P treatment' and 'soil layer' as fixed effects, and 'pot' as a random effect. When appropriate, the effect of 'replicate' was included in the most parsimonious model. Means and standard errors were calculated from the fitted linear models and linear mixed effects models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significance differences (HSD). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield

Shoot dry mass was influenced by soil P supply, with the largest yields generally produced when plants were grown in the uniform high-P treatment (P = 0.004; Table 15). The banded high-P treatment was also expected to improve plant growth compared to the uniform low-P treatment, but this did not occur within the six-week growth period. Because warm-season pasture species are relatively slow to establish, it is likely they would have explored more of the banded fertiliser and acquired more P if they were grown for longer. The exception to these results was Premier Digit,

which was highly productive in each of the soil-P distribution treatments. Accordingly, there were differences in shoot dry mass among the species as follows: Premier Digit > Haymaster Lucerne = Bambatsi Panic > Progardes Desmanthus ($P < 0.001$; Table 15). Known differences in growth rate and preferred soil type, along with potential differences in P-acquisition efficiency and P-utilisation efficiency, are likely to have influenced the growth of the different species. Regardless, these varietal differences in shoot yield indicate that some species may be more compatible to be grown together in mixed pasture swards than others.

Table 15. Shoot dry mass, shoot P concentration, shoot P content, and shoot P content/total root length of four warm-season pasture species grown in response to three soil-P distribution treatments. Values show the calculated mean ± s.e. (n = 3). Analysis of variance results are given for species, P treatment and the species × P treatment interaction.

P treatment & Cultivar	Shoot dry mass (g DM pot^{-1})	Shoot P concentration (mg P g^{-1} DM)	Shoot P content (mg P pot^{-1})	Shoot P content/RL (µg m^{-1})
Uniform low-P				
Bambatsi Panic	1.28 ± 0.29	2.04 ± 0.35	2.73 ± 0.52	23.9 ± 4.82
Premier Digit	4.39 ± 0.29	0.83 ± 0.14	3.64 ± 0.52	4.80 ± 0.96
Haymaster Lucerne	1.94 ± 0.29	1.38 ± 0.24	2.66 ± 0.52	15.0 ± 3.02
Progardes® Desmanthus	0.35 ± 0.29	1.76 ± 0.30	0.64 ± 0.52	27.6 ± 5.58
Uniform high-P				
Bambatsi Panic	2.34 ± 0.29	4.74 ± 0.82	10.99 ± 0.52	97.3 ± 19.7
Premier Digit	4.07 ± 0.29	3.77 ± 0.65	15.39 ± 0.52	29.0 ± 5.86
Haymaster Lucerne	2.95 ± 0.29	3.54 ± 0.61	10.44 ± 0.52	53.7 ± 10.8
Progardes® Desmanthus	1.26 ± 0.29	3.07 ± 0.53	3.89 ± 0.52	62.5 ± 12.6
Banded high-P				
Bambatsi Panic	1.81 ± 0.29	0.91 ± 0.16	1.62 ± 0.52	16.0 ± 3.23
Premier Digit	3.99 ± 0.29	1.49 ± 0.26	5.77 ± 0.52	9.20 ± 1.85
Haymaster Lucerne	1.78 ± 0.29	1.64 ± 0.28	2.93 ± 0.52	24.3 ± 4.91
Progardes® Desmanthus	0.54 ± 0.29	5.00 ± 0.86	1.98 ± 0.52	64.8 ± 13.1
		ANOVA		
Species	***P* < 0.001**	***P* = 0.003**	***P* < 0.001**	***P* < 0.001**
P treatment	***P* = 0.004**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**
Species × P treatment	*P* = 0.176	***P* < 0.001**	***P* < 0.001**	***P* = 0.004**

Shoot P

Shoot P concentrations were generally highest when plants were grown in the uniform high-P treatment ($P < 0.001$; Table 15). The exception was Progardes® Desmanthus, which also achieved a high shoot P concentration in the banded high-P treatment. The increase in shoot P concentrations associated with higher levels of soil P supply indicate that warm-season grasses and legumes will accumulate tissue P, with consequent improvements in forage quality, provided sufficient P is available in soil. There were varietal differences in shoot P concentration ($P = 0.003$; Table 15), but these were primarily due to the high shoot P concentration of Progardes® Desmanthus in the banded high-P treatment. Differences in shoot dry mass and shoot P concentration meant that shoot P content, an indicator of total plant P uptake, also differed according to soil-P distribution treatment ($P < 0.001$) and pasture species ($P < 0.001$) (Table 15).

Root traits

Total root mass fraction (i.e. the proportion of plant biomass allocated to roots) did not change in response to soil-P distribution treatment ($P = 0.168$; data not shown). However, there were differences in total root mass fraction among the pasture species ($P < 0.001$; data not shown). On average across the soil-P distribution treatments, Premier Digit (0.45 g g^{-1}) and Haymaster Lucerne (0.44 g g^{-1}) allocated more biomass to roots than Progardes® Desmanthus (0.35 g g^{-1}) and Bambatsi Panic (0.30 g g^{-1}). These differences in total root mass fraction were associated with differences in biomass allocation to the three soil layers (i.e. topsoil, band and subsoil), depending on the soil-P distribution treatment ($P = 0.065$; data not shown). Varietal differences in the allocation of biomass to roots only partly explains the shoot yield results, which suggests that other factors contributed to the efficiency of P acquisition.

Specific root length differed among the pasture species, with the grasses (i.e. Premier Digit and Bambatsi Panic) producing roots more efficiently than the legumes (i.e. Progardes® Desmanthus and Haymaster Lucerne) ($P < 0.001$; Fig. 35). This result was associated with varietal differences in average root diameter (on average across the treatments, Premier Digit = 0.14 mm, Bambatsi Panic = 0.18 mm, Progardes® Desmanthus = 0.24 mm and Haymaster Lucerne = 0.28 mm; $P < 0.001$) and is consistent with what has been observed among temperate grasses and legumes (Evans, 1977). It is likely that the efficient production of root length by the grasses influenced the efficiency of P acquisition in the current experiment, as has been found among temperate pasture species (Haling et al., 2016, Yang et al., 2017). Specific root length also differed according to soil layer ($P < 0.001$) and, to a lesser extent, soil-P distribution treatment ($P = 0.014$). On average across the soil-P distribution treatments, the specific root lengths of the grasses were highest in the 'band' layer whereas that of the legumes were highest in the 'subsoil' layer. This result may be associated with inherent differences in root development between the grasses, which produce fibrous roots that are effective for surface foraging, and the legumes, which produce taproots that are generally more effective for subsoil exploration. Further research is warranted to characterise more root traits of a wider range of pasture species.

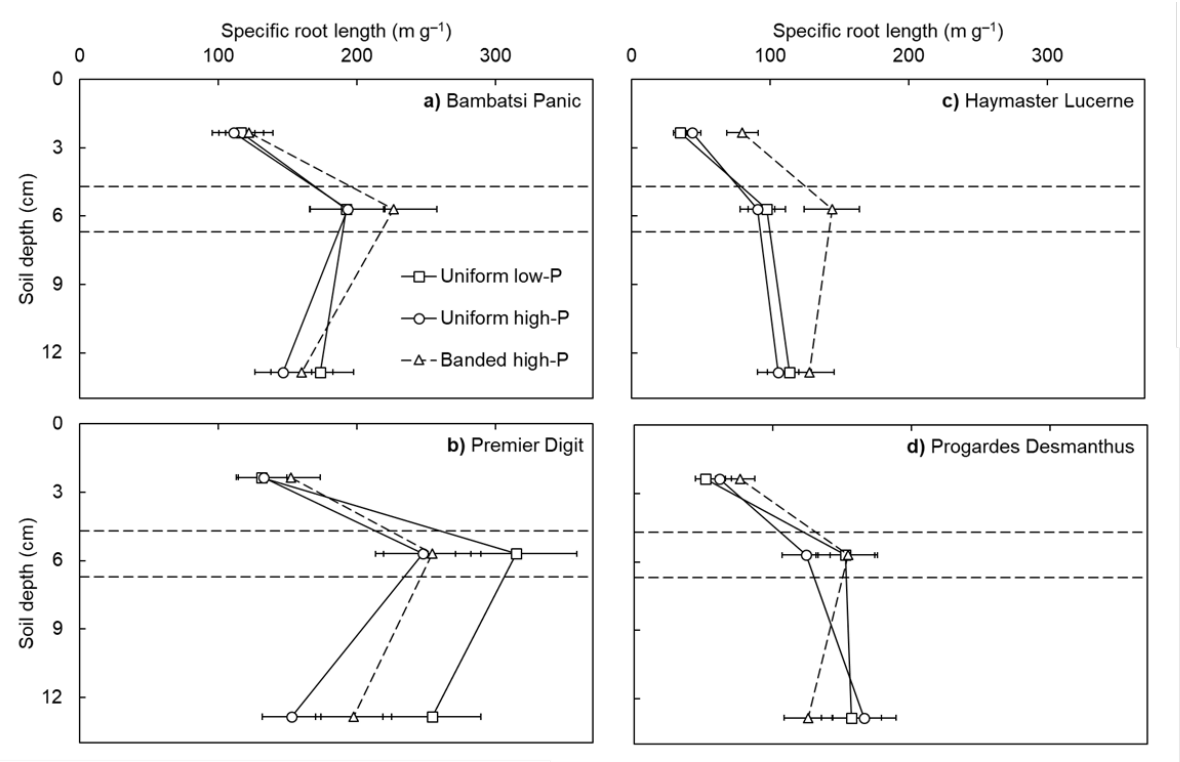

Figure 35. Specific root length in the topsoil, band and subsoil layers of four warm-season pasture species grown in response to three soil-P distribution treatments. Soil depth values correspond to the depth below the soil surface of the mid-point of each soil layer (i.e. topsoil = 2.4 cm, band = 5.7 cm and subsoil = 12.9 cm) and the horizontal lines depict the interfaces between soil layers. Values show the calculated mean ± s.e. (n = 3). Data points of the uniform low-P and uniform high-P treatments are joined by solid lines whereas data points of the banded high-P treatment are joined by dashed lines.

Root length density differed among the pasture species ($P < 0.001$: Fig. 36). The largest root length densities were achieved by Premier Digit, followed by Bambatsi Panic and Haymaster Lucerne, then Progardes® Desmanthus. These species responded differently across the three soil layers (species*soil layer interaction; $P < 0.001$). In general, the grasses produced the highest root length densities in the band layer, whereas the root length densities of the legumes were stable or increased with soil depth. Furthermore, the banded high-P treatment indicated a higher responsiveness of the grasses to the localised source of P fertiliser than the legumes. When comparing the root length densities in the topsoil and band layers (i.e. a measure of root proliferation in response to the P fertiliser), Premier Digit and Bambatsi Panic achieved a 2.1-fold and a 1.5-fold increase, respectively. In contrast, the root length densities of Haymaster Lucerne and Progardes® Desmanthus were either stable or decreased between these soil layers.

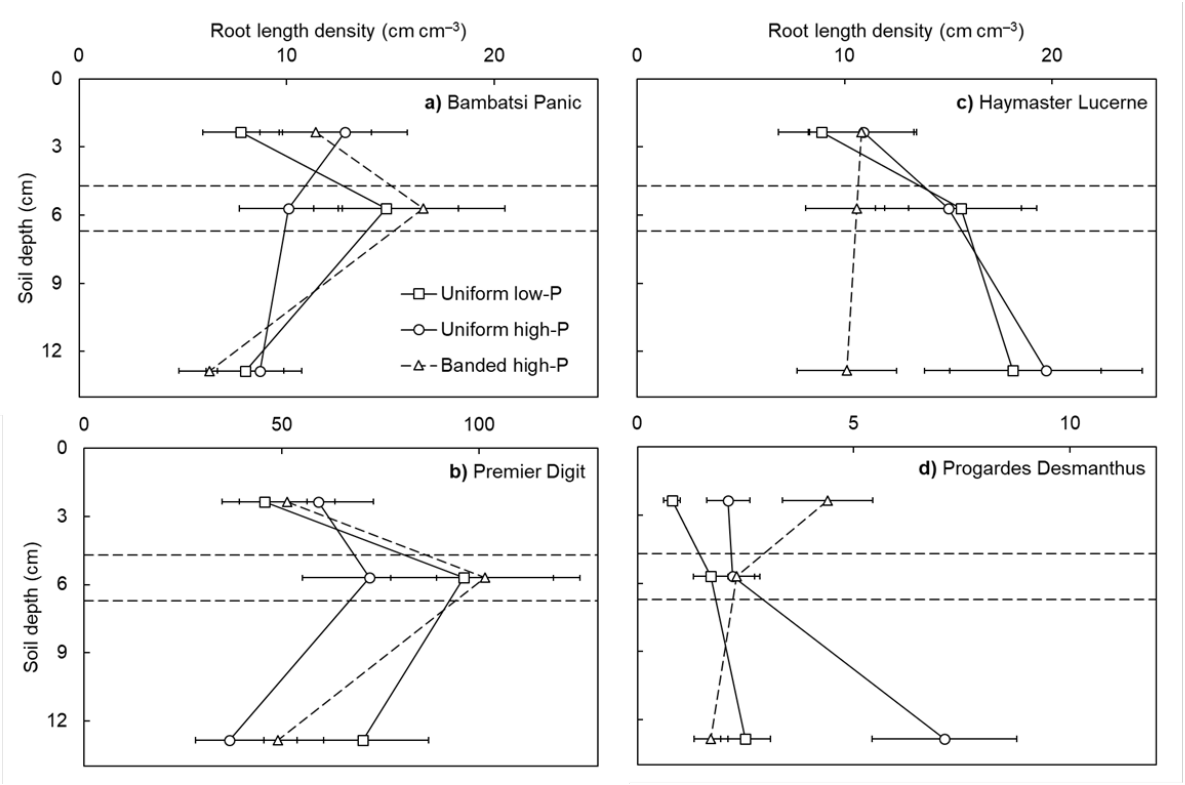

Figure 36. Root length density in the topsoil, band and subsoil layers of four warm-season pasture species grown in response to three soil-P distribution treatments. Soil depth values correspond to the depth below the soil surface of the mid-point of each soil layer (i.e. topsoil = 2.4 cm, band = 5.7 cm and subsoil = 12.9 cm) and the horizontal lines depict the interfaces between soil layers. The x-axes showing root length density are not the same for each pasture species (Premier Digit > Bambatsi Panic = Haymaster Lucerne > Progardes® Desmanthus). Values show the calculated mean ± s.e. (n = 3). Data points of the uniform low-P and uniform high-P treatments are joined by solid lines whereas data points of the banded high-P treatment are joined by dashed lines.

Phosphorus acquisition

The importance of root length for P acquisition was demonstrated by the significant correlations between total root length and shoot P content (i.e. an indicator of total plant P uptake) (Fig. 37). On average across the pasture species, the highest correlation was observed for the banded high-P treatment (R^2 = 0.72, P < 0.001), followed by the uniform high-P (R^2 = 0.67, P = 0.001) and uniform low-P (R^2 = 0.33, P = 0.029) treatments. Previous experiments have demonstrated the importance of long nutrient-foraging roots for P acquisition (Haling et al., 2016). There were also significant differences in shoot P content per unit total root length (Table 15). On average across the pasture species, P uptake per unit root length was highest in the uniform high-P treatment, followed by the banded high-P treatment then the uniform low-P treatment (P < 0.001). There were also varietal differences for this trait (P < 0.001) and differences in how the pasture species responded to the soil-P distribution treatments (species*P treatment interaction; P = 0.004). For example, most of the species had a similarly small shoot P content per unit total root length in the uniform low-P and banded high-P treatments, whereas the shoot P content per unit total root length of Progardes® Desmanthus was similarly large in the uniform high-P and banded high-P treatments.

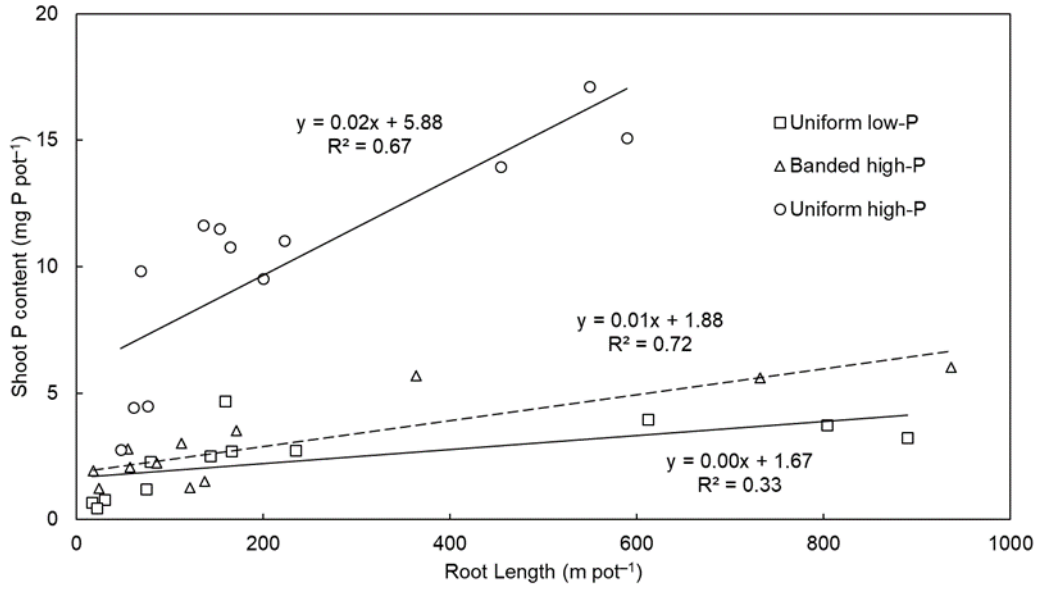

Figure 37. The relationship between total root length and shoot P content among four warm-season pasture species grown in response to three soil-P distribution treatments. The uniform low-P and uniform high-P treatments have solid regression lines whereas the banded high-P treatment has a dashed regression line. The regression lines and R^2 coefficients of determination were fitted separately for each soil-P distribution treatment and include all data points of each species.

Shoot P derived from P applied to the band layer was relatively low for each of the pasture species when P fertiliser was uniformly distributed throughout the soil profile, but was significantly higher when P fertiliser was localised in the banded high-P treatment ($P < 0.001$; Fig. 38a). Each of the pasture species therefore responded to the subsurface application of P fertiliser which indicates that this may be a useful technique to improve the productivity of warm-season pasture species in northern Australia. However, the way in which the grasses and legumes responded was different. The two grasses responded by proliferating root length in the vicinity of the applied P fertiliser. The spatial distribution of root length for nutrient foraging is important in pasture systems because soil P is generally stratified (Simpson et al., 2015, McLaughlin et al., 2011) or distributed in a heterogenous manner (Hodge, 2006, Hodge, 2004). In contrast to the grasses, the two legumes did not respond spatially so other root traits must have been important for P acquisition. For example, the legumes may have upregulated P transporters under conditions of higher P concentration. Indeed, Progardes® Desmanthus had a relatively high shoot P content per unit total root length in the uniform high-P and banded high-P treatments. Alternatively, mycorrhizal colonisation may have improved P uptake if mycorrhizal hyphae proliferated within the band layer.

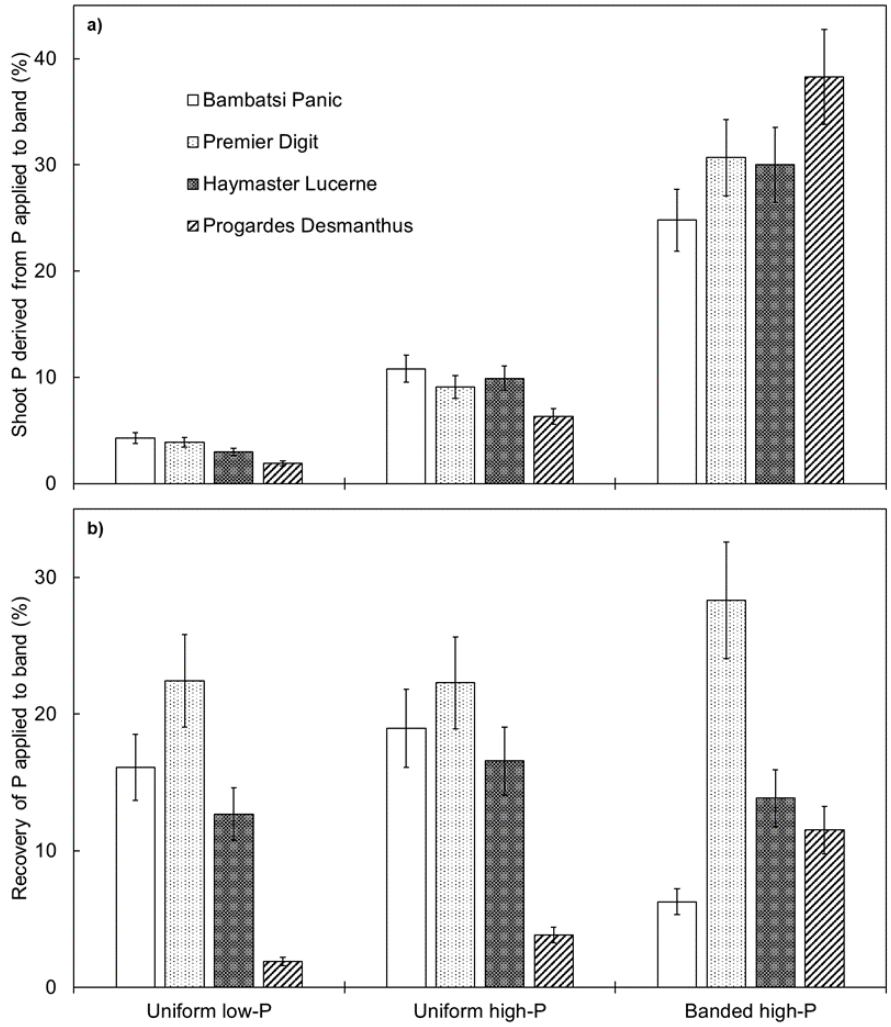

Figure 38. Shoot P derived from P applied to the band layer (a) and recovery of P applied to the band layer (b) of four warm-season pasture species grown in response to three soil-P distribution treatments. Values show the calculated mean ± s.e. (n = 3). Analysis of variance results for shoot P derived from P applied to band were: species P = 0.016 , P treatment P < 0.001, species × P treatment interaction P < 0.001. Analysis of variance results for recovery of P applied to band were: species P < 0.001, P treatment P = 0.017, species × P treatment interaction P < 0.001.

Recovery of P applied to the band layer was influenced by pasture species ($P < 0.001$) and, to a lesser extent, by soil-P distribution treatment ($P = 0.017$) (Fig. 38b). On average across the soil-P distribution treatments, the recovery of P was highest for Premier Digit, followed by Haymaster Lucerne and Bambatsi Panic, then Progardes® Desmanthus. In general, the recovery of P was similar across the three soil-P distribution treatments for Premier Digit and Haymaster Lucerne. In contrast, Bambatsi Panic recovered less P in the banded high-P treatment, whereas Progardes® Desmanthus recovered more P in this treatment, compared to when P fertiliser was uniformly distributed throughout the soil profile. This result indicates that a subsurface application of P fertiliser may be a relatively efficient way of applying P fertiliser within tropical pasture systems. This is consistent with the findings of McLachlan et al. (2019a), who suggested that banded applications of fertiliser may improve P fertiliser recovery in temperate pasture systems. Nevertheless, the recovery of P fertiliser by Premier Digit in the current experiment was substantially higher than that of Progardes® Desmanthus, suggesting that the grass component of tropical pasture swards is likely to compete more effectively for applied P fertiliser than the legume component.

Practical implications

There were significant differences in shoot yield and root morphology among the four warm-season pasture species. In particular, the two grasses responded spatially by proliferating root length in the vicinity of the banded P fertiliser whereas the two legumes did not. This may because it takes longer for legumes to proliferate roots when compared to grasses. Nevertheless, each of the species responded to the subsurface application of P fertiliser by deriving more P from the zone of P enrichment. Nutrient inputs are currently limited in the extensive grazing systems of northern Australia. Under these conditions, improved P-acquisition efficiency and overall pasture productivity may be achieved by growing plants with superior root morphologies. It is expected that under mixed sward conditions, highly productive C_4 grasses will outcompete the legume component. Nevertheless, the grasses and legumes both responded in some way to the banded application of P fertiliser in the present experiment. A subsurface application of P fertiliser, particularly below the legume component, may help improve the productivity and persistence of mixed pasture swards.

8.9 Appendix 9 – Spatial and temporal development of roots by tropical pasture species

Materials and Methods

Plant material

Digit (*Digitaria eriantha* cv. Premier) and Desmanthus (*Desmanthus pernambucanus* cv. JCU 9) were grown together to investigate shoot yield, root traits and P acquisition in response to the localised placement of P fertiliser. These pasture species are commonly grown together in the extensive grazing systems of northern Australia.

Soil preparation

A sandy loam soil (Brown Chromosol; Isbell 1996) was collected from the upper 2–15 cm soil layer of a field at Kirby SMART Farm, Armidale, NSW, Australia (30°25'35.0"S 151°39'08.4"E). The soil had a Colwell extractable P concentration of 3 mg P kg^{-1} (as measured by the method of (Colwell, 1963)), a Phosphorus Buffering Index (PBI) of 29 (as measured by the modified method of (Burkitt et al., 2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of ~5.3. The soil was passed through a 5 mm sieve and a basal nutrient solution was applied that included 34 mg kg^{-1} soil $MgSO_4.7H_2O$, 36 mg kg^{-1} $CaSO_4.2H_2O$, 141 mg kg^{-1} KNO_3, 23 mg kg^{-1} $(NH_4)_2SO_4$, 14 mg kg^{-1} NH_4NO_3, 99 µg kg^{-1} H_3BO_3, 635 µg kg^{-1} $MnCl_2.4H_2O$, 301 µg kg^{-1} $ZnSO_4.7H_2O$, 28 µg kg^{-1} $CuSO_4.5H_2O$, 60 µg kg^{-1} $(NH_4)_2MoO_4$, 17 µg kg^{-1} $CoCl_2 \cdot 6H_2O$ and 1283 µg kg^{-1} FeNa-EDTA. Two P-amended soils (0 and 40 mg P kg^{-1}) were prepared by adding KH_2PO_4 to the nutrient solution before it was applied to the soil.

A4 document scanners (Canon CanoScan LiDE 300 Flatbed Scanners) were modified so that plant roots could be scanned regularly. A hard-plastic spacer and backboard were attached to each document scanner which could contain a thin layer of soil (270 mm height, 180 mm width, 10 mm depth). 535 g (oven-dry equivalent) of the amended soils were packed into to each rhizoscanner. Following this, the backboards were removed to expose the packed soil profile. Four soil P treatments were prepared as follows:

- Uniform low-P – P0 soil packed into the rhizoscanners with no additional P applied.
- Uniform high-P – P40 soil packed into the rhizoscanners with no additional P applied.
- Banded high-P both – P0 soil packed into the rhizoscanners with two application of P solution 5 cm from the soil surface.
- Banded high-P grass/legume – P0 soil packed into the rhizoscanners with one application of P solution 5 cm from the soil surface.

Plant growth conditions and experimental design

Micro-swards of Digit and Desmanthus were established by sowing seed (~5 mm depth) to achieve a population of two grass and two legume plants pot^{-1}. There were four replicates of each treatment. The rhizoscanners were located under shade cloth, to reduce surface temperatures, in a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. Plants were grown between February–March 2021. Soil moisture was maintained at 80–100% field capacity by watering daily. The rhizoscanners were programmed to scan plant roots every 2 h, from plant emergence until the plants were harvested. Root length and average root diameter

were determined using WinRHIZO^{TM} software (Regent Instruments Inc., Quebec, Canada) (Bouma et al., 2000).

Harvest and measurements

Plants were harvested after five weeks' growth. Shoots were cut at the soil surface, oven-dried at 70°C for 72 h and weighed. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 12 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass.

The soil was removed from each rhizoscanner and washed over 2 mm sieves. The samples were then scanned using an Epson Perfection V700 Photo flatbed scanner (Seiko Epson Corporation, Suwa, Japan) at 600 dpi. Root length and average root diameter were determined using WinRHIZO^{TM} software (Regent Instruments Inc., Quebec, Canada) (Bouma et al., 2000). Roots were then oven-dried at 70°C for 72 hr and weighed. Root mass fraction was calculated as the mass of roots in each soil layer divided by total plant mass (i.e. shoot and root dry mass combined). Root length density was calculated as root length per unit soil volume; the volume of soil was ~480 cm^3.

Statistical analysis

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'soil treatment' as the predictor variable. Linear mixed effects models were fitted to measured variables where the two components (i.e. grass and legume) were compared (R package: nlme) (Pinheiro et al., 2020). The models included 'soil treatment' and 'component' as fixed effects, and 'rhizoscanner' as a random effect. When appropriate, the effect of 'replicate' was included in the most parsimonious model. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield

The shoot dry mass of Digit and Desmanthus was comparably low in the uniform low-P treatment, and increased in response to the application of P fertiliser ($P < 0.001$; Fig. 39). In particular, both the grass and the legume responded whether the P fertiliser was applied uniformly throughout the soil profile or as a concentrated band directly below the establishing plant. However, the grass was consistently more productive than the legume when both components received the same amount of P fertiliser within the root zone. When P fertiliser was placed preferentially below either the grass or the legume, it was the preferentially fertilised component that was most productive.

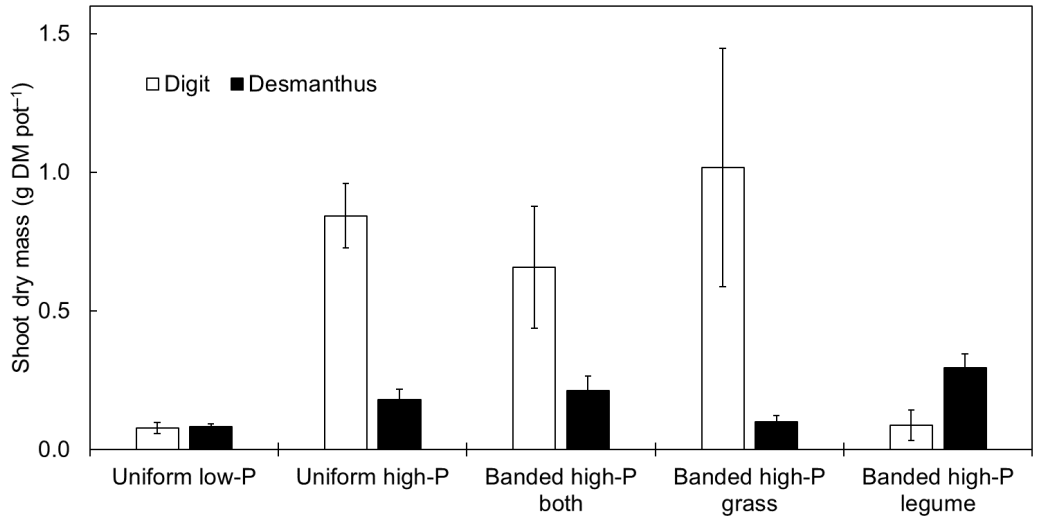

Figure 39. Shoot dry mass of Digit and Desmanthus when grown in response to five soil P placement treatments. Values show the mean ± s.e. (n = 4).

Root length development

Digit and Desmanthus both produced longer roots over time, but there were substantial differences between the species and the soil P placement treatments (Fig. 40). In general, Digit was slower to emerge and start producing roots than Desmanthus, but the grass quickly produced more roots than the legume when P fertiliser was placed within its root zone (particularly from three weeks after planting). Both species produced more roots in the uniform high-P, banded high-P and preferentially placed high-P treatments, when compared to the uniform low-P treatment.

Differences in root length were most pronounced at the end of the experiment, when roots were washed from the soil. This is because the rhizoscanners can only assess roots growing on the scanning surface, not the total length of roots in the soil profile. Similar to root length development, there were substantial differences between the species and the soil P placement treatments (Fig. 41). In particular, Digit produced longer roots than Desmanthus in each of the treatments, even when Desmanthus achieve comparable or higher shoot yields. Nevertheless, both the grass and the legume produced more roots in response to the application of P fertiliser (whether uniformly distributed throughout the soil profile or preferentially applied below the emerging plants).

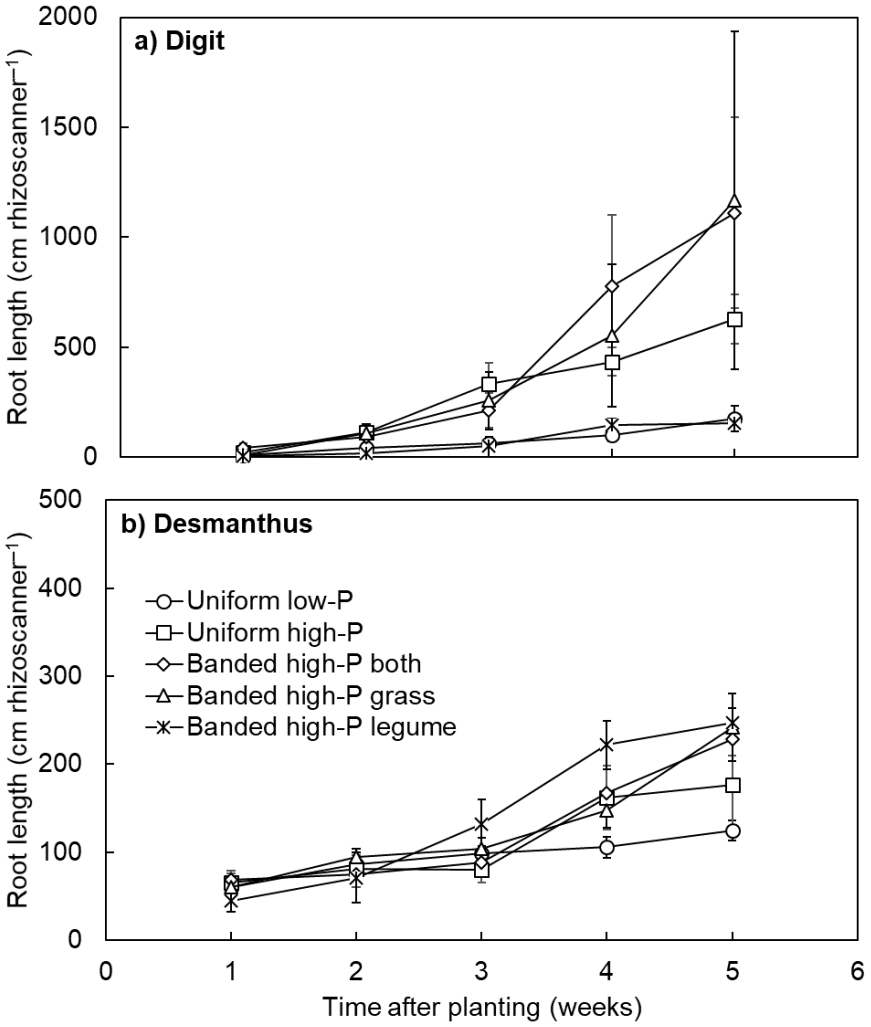

Figure 40. Root length development over time by Digit and Desmanthus when grown in response to five soil P placement treatments. Values show the mean ± s.e. (n = 4).

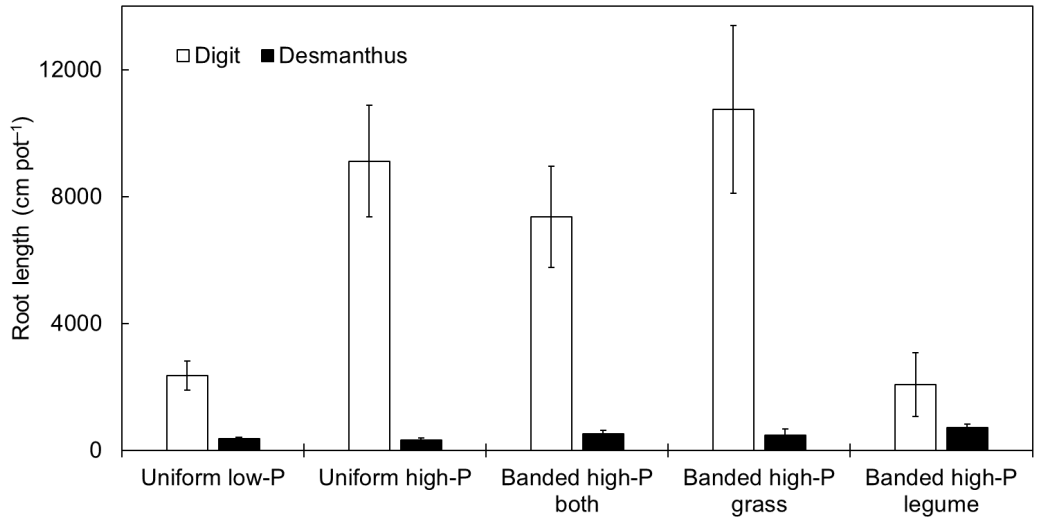

Figure 41. Final root length of Digit and Desmanthus when grown in response to five soil P placement treatments. Values show the mean ± s.e. (n = 4).

Practical implications

Digit and Desmanthus both responded to the application of P fertiliser, and both proliferated roots in response to localised band within the root zone. This suggests that tropical grasses and legumes are likely to respond to and benefit from subsurface placement of P fertiliser. However, the grass was highly competitive whenever it received P fertiliser, meaning that it quickly out-competed the legume. Banded applications of P fertiliser may need to be used cautiously when tropical grasses and legumes are grown together, otherwise the legume may still be disadvantaged.

8.10 Appendix 10 – Emergence and establishment of four *Desmanthus* spp. genotypes in three alkaline clay soils

Materials and Methods

Plant growth conditions

Four *Desmanthus* spp. genotypes were grown in three alkaline clay soils to determine differences in emergence and establishment in response to starter fertiliser and gypsum. The four genotypes were JCU 2 (*D. virgatus*), JCU 4 (*D. bicornutus*), JCU 7 (*D. leptophyllus*), and JCU 9 (*D. pernambucanus*). These cultivars are commercially available and can be combined into enviro-specific blends of Progardes® Desmanthus. The seed of each cultivar was bare and was heat-treated to break seed dormancy prior to sowing.

Three alkaline clay soils were collected from across QLD, Australia; Armraynald Station, Burketown (17°57'57.1"S 139°42'51.5"E), Cungelella Station, Mantuan Downs (24°40'13.5"S 147°9'23.8"E), and Gregory Downs Station, Gregory (18°38'51.5"S 139°9'6.4"E). The basic soil properties of each soil are shown in Table 16. The soils were dried, crushed and homogenised before 1.3 kg (oven-dry equivalent) of each was weighed into PVC pots (87 mm internal diameter, 200 mm height). A gypsum ($CaSO_4.2H_2O$) treatment was prepared by applying the equivalent of either 0 or 1 t ha^{-1} to the surface of the pots. This was followed by a wetting and drying cycle, whereby the soil was watered to 80% field capacity and then dried. A starter fertiliser treatment was prepared by applying mono-ammonium phosphate (MAP, $NH_4H_2PO_4$) to the pots at rates equivalent to either 0 or 12 kg P ha^{-1}.

Table 16. The Colwell extractable P concentration, Phosphorus Buffering Index (PBI) and pH ($CaCl_2$) of three northern Australian soils used in the experiment.

Soil	Colwell P (mg kg^{-1})	PBI	pH ($CaCl_2$)
Armraynald	3	68	7.3
Cungelella	13	90	7.2
Gregory Downs	3	78	7.4

Micro-swards of each *Desmanthus* spp. genotype were established by sowing 25 viable seeds per pot (germination percentages were determined before planting). The seeds were placed on the soil surface to mimic a broadcast application that is common in extensive grazing systems. Four replicate pots of each genotype in each soil with +/- gypsum and +/- starter fertiliser were prepared. After planting, the pots were watered and moved to a glasshouse (natural daylight, ~1800 mmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. Plants were watered every second day to reach 80% field capacity for the duration of the experiment so that the soil surface was periodically dry. Plants were grown between March–April 2022. Pots were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006).

Harvest and measurements

Seedling emergence was recorded daily for two weeks following planting. Percentage final emergence was calculated as the proportion of the number of emerged seedlings divided by the number of viable seeds sown in each pot (i.e. 25). After the first two weeks, the pots were thinned

to achieve an even population of 5 plants pot^{-1}. Average canopy height was recorded weekly. After six weeks' growth, plants were harvested to determine shoot yield and tissue P concentration. Shoots were cut at the soil surface, oven-dried at 70°C for 72 h and weighed. Shoot samples were then ground to <2 mm before a ~0.5 g subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colourimetrically at 630 nm using the malachite green method (Irving and McLaughlin 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass.

Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'soil', 'gypsum', 'starter' and 'genotype' as predictor variables. When appropriate, the effect of 'replicate' was included in the most parsimonious model. The linear models were simplified when the predictor variables were not significant. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). The rate of emergence was determined by fitting a self-starting Weibull growth function ($y = a – b * \exp(–\exp(c) * x^d)$, where x is days and y is emergence), as described by Crawley (2013). Time to 90% emergence was calculated as the days taken to achieve 90% of maximum emergence based on the fitted Weibull growth functions, and the 95% confidence intervals of the time to emergence were determined by bootstrapping residuals as described by Crawley (2013). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Seedling emergence

Final emergence was not influenced by the soil in which the legumes were sown ($P = 0.460$), so the emergence results were summarised according to *Desmanthus* spp. genotype, gypsum application and starter fertiliser application (Table 17). Across the treatments, the time to 90% emergence ranged between 7 to 11 days. On average, JCU 7 had the longest time to 90% emergence while JCU 4 had the shortest time. The percentage final emergence varied among the *Desmanthus* spp. genotypes ($P < 0.001$), with genotypes JCU 2 and JCU 4 (avg. 86%) achieving higher levels of emergence than genotypes JCU 7 and JCU 9 (avg. 65%). This difference indicates that cultivar selection may be important when cultivars of *Desmanthus* are to be sown in alkaline clay soils that are prone to surface crusting. Nevertheless, the application of gypsum increased seedling emergence by 15% for the four *Desmanthus* spp. genotypes across the starter fertiliser treatments ($P < 0.001$). Gypsum benefits may have arisen through either improved soil surface conditions and reduced hard-setting through the wetting/drying cycles, or through dissolution of gypsum to release Ca and subsequent osmopriming as water imbibed. Gypsum may therefore be a useful amendment for increasing the emergence of *Desmanthus*. In contrast, starter fertiliser did not influence seedling emergence ($P = 0.947$).

Table 17. Time to 90% emergence (T90) and percentage final emergence 16 days after planting of four Desmanthus spp. genotypes grown in response to +/- gypsum (1 t ha^{-1} equivalent) and +/- starter fertiliser (12 kg P ha^{-1} equivalent). The T90 values were calculated by fitting Weibull growth functions to the emergence data. The percentage final emergence values were calculated as the proportion of seedling emerged divided by the total number of viable seeds sown in each pot (i.e. 25). Values show the mean ± s.e. (n = 4).

Genotype Gypsum Starter	**T90 Emergence (days)**	**Final Emergence (%)**
JCU 2		
- Gypsum, - Starter	8.9 (7.5–10.6)	92 ± 6
- Gypsum, + Starter	9.5 (8.0–10.9)	76 ± 6
+ Gypsum, - Starter	8.5 (7.5–9.5)	95 ± 6
+ Gypsum, + Starter	8.1 (7.2–9.2)	84 ± 6
JCU 4		
- Gypsum, - Starter	8.3 (6.7–9.8)	77 ± 6
- Gypsum, + Starter	8.4 (7.0–9.9)	77 ± 6
+ Gypsum, - Starter	8.0 (6.7–9.8)	86 ± 6
+ Gypsum, + Starter	7.3 (6.4–8.6)	99 ± 6
JCU 7		
- Gypsum, - Starter	8.9 (7.5–10.1)	60 ± 6
- Gypsum, + Starter	10.9 (9.1–13.4)	57 ± 6
+ Gypsum, - Starter	8.0 (6.7–9.6)	68 ± 6
+ Gypsum, + Starter	9.2 (7.9–10.4)	66 ± 6
JCU 9		
- Gypsum, - Starter	8.6 (6.7–10.4)	55 ± 6
- Gypsum, + Starter	8.9 (7.5–10.2)	66 ± 6
+ Gypsum, - Starter	8.5 (7.0–9.8)	70 ± 6
+ Gypsum, + Starter	7.3 (6.4–8.2)	79 ± 6

Shoot yield

Shoot yields were influenced by the soil in which the plants were grown ($P < 0.001$). On average across the different treatment combinations, the highest yields were achieved in the Cungelella soil, followed by the Gregory Downs soil and then the Armraynald soil. In each soil, the addition of gypsum did not influence shoot yield ($P = 0.763$). Because of this, the shoot yield results were summarized according to *Desmanthus* spp. genotype and starter fertiliser application (Fig. 42). On average, the addition of starter fertiliser increased the shoot yields of the *Desmanthus* spp. genotypes in the Armraynald and Gregory downs soils, but not in the Cungelella soil. This result most likely reflects the difference in native soil fertility between the soils, as the Cungelella soil had a Colwell P concentration of ~14 mg kg^{-1} compared to the Armraynald and Gregory Downs soils which had Colwell P concentrations of ~3 mg kg^{-1}. It is expected that the addition of starter fertiliser will benefit legume establishment in low P soils (e.g. Colwell P < 10 mg kg^{-1}), as it will encourage root growth and seedling vigour. In contrast, starter fertiliser application in soils with moderate P (e.g. Colwell P >15 mg kg^{-1}) may provide a limited benefit for legume establishment. Rather, starter fertiliser that contains both N and P is likely to encourage the growth of grasses and/or weeds, which could quickly outcompete small-seeded legumes such as *Desmanthus*.

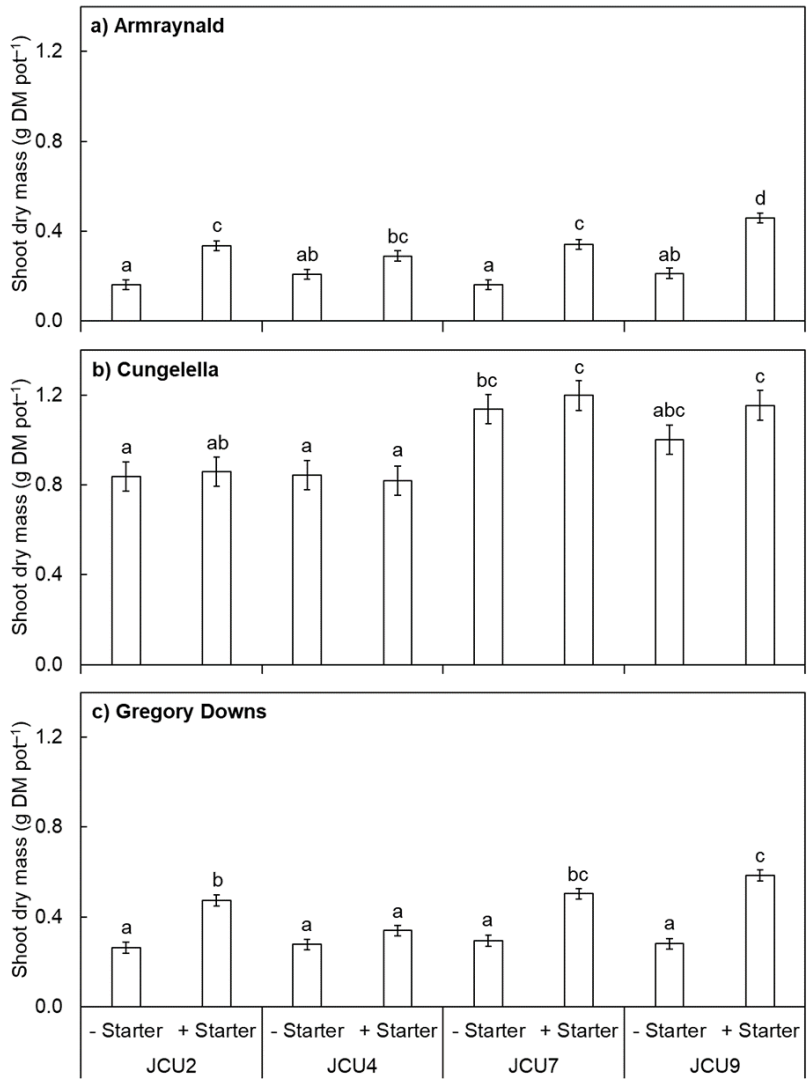

Figure 42. Shoot dry mass of four Desmanthus spp. genotypes grown in response to +/- starter fertiliser (12 kg P ha^{-1} equivalent) in three soils; Armraynald (a), Cungelella (b) and Gregory Downs (c). Values show the mean ± s.e. (n = 4). The effect of soil was significant (P < 0.001), with Cungelella > Gregory Downs > Armraynald. Different letters denote significant differences at P = 0.05 within each panel (i.e. soil).

Shoot yields differed among the *Desmanthus* spp. genotypes (Fig. 42). For example, *Desmanthus* genotypes JCU 7 and JCU 9 were more productive in the Cungelella soil than genotypes JCU 2 and JCU 4. This indicates that there are differences in yield potential among the genotypes, even in moderately fertile soil in which starter fertiliser did not increase shoot yields. Where starter fertiliser did increase shoot yields (i.e. the Armraynald and Gregory Downs soils), the starter fertiliser affected the genotypes differently. For example, starter fertiliser increase the shoot yields of *Desmanthus* genotypes JCU 2, JCU 7 and JCU 9 by ~2.1-fold in the Armraynald soil and by ~1.9-fold in the Gregory Downs soil, but did not influence the shoot yield of genotype JCU 4. This suggests that *Desmanthus* genotype JCU 4 is relatively P-efficient, but does not produce large shoot yields. This is consistent with the findings of McLachlan et al. (2021).

Shoot P

Shoot P concentrations were influenced by the soil in which the plants were grown ($P < 0.001$), with the highest concentrations achieved in the Cungelella and Gregory Downs soils. Similar to shoot yield, there was no effect of gypsum on shoot P concentrations, so the results were summarized according to *Desmanthus* spp. genotype and starter fertiliser application (Fig. 43). On average, the addition of starter fertiliser increased the shoot P concentrations of the *Desmanthus* spp. genotypes in each of the soils. In general, the shoot P concentrations of *Desmanthus* genotypes JCU 7 and JCU 9 were lower than that of genotypes JCU 2 and JCU 4. This was clearly seen in the Cungelella soil, where genotypes JCU 7 and JCU 9 had also produced higher shoot yields. This result indicates that genotypes such as JCU 7 and JCU 9 may achieve higher shoot yields through more efficient use of acquired P. This is consistent with the findings of McLachlan et al. (2021), who reported that *Desmanthus* genotype JCU 9 was highly productive because it had efficiently used acquired P even when grown in soil that had been amended with high rates of P fertiliser.

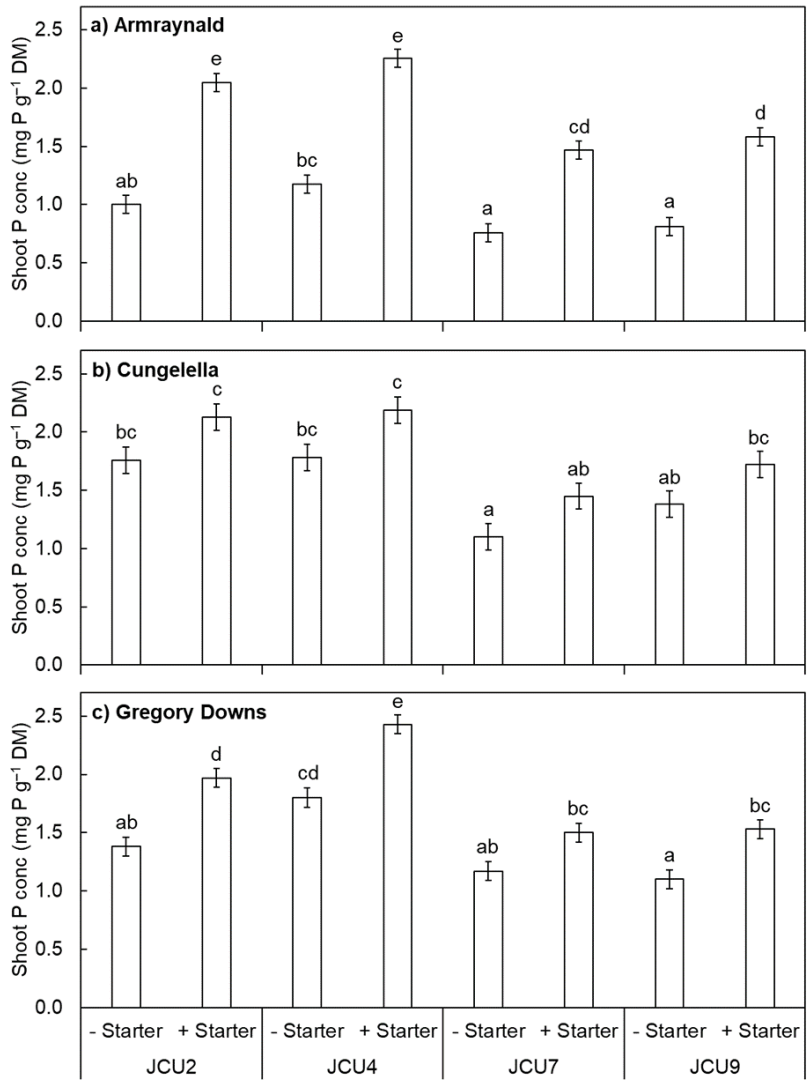

Figure 43. Shoot P concentration of four Desmanthus spp. genotypes grown in response to +/- starter fertiliser (12 kg P ha^{-1} equivalent) in three soils; Armraynald (a), Cungelella (b) and Gregory Downs (c). Values show the mean ± s.e. (n = 4). The effect of soil was significant (P < 0.001), with Cungelella = Gregory Downs > Armraynald. Different letters denote significant differences at P = 0.05 within each panel (i.e. soil).

Shoot P contents reflected differences in shoot yields and shoot P concentrations, with the largest shoot P contents achieved when the *Desmanthus* genotypes were grown in the Cungelella soil, followed by the Gregory Downs soil and then the Armraynald soil (Fig. 44). The application of starter fertiliser had a positive effect on the shoot P contents of the *Desmanthus* genotypes in each of the soils. This indicates that, even though starter fertiliser does not always increase shoot yield, it is likely to increase legume diet quality for grazing animals. This is important in the extensive grazing systems of northern Australia, which are dominated by C_4 grasses that quickly lose quality when not grazed heavily.

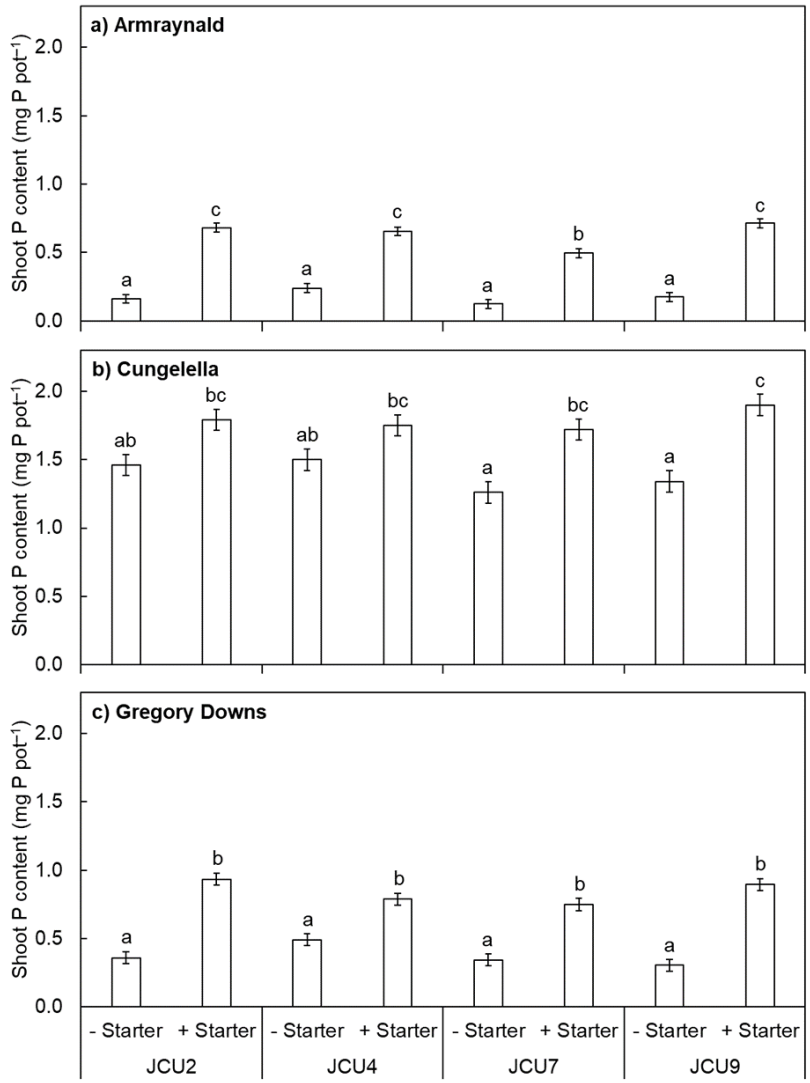

Figure 44. Shoot P content of four Desmanthus spp. genotypes grown in response to +/- starter fertiliser in three soils; Armraynald (a), Cungelella (b) and Gregory Downs (c). Values show the mean ± s.e. (n = 4). The effect of soil was significant (P < 0.001), with Cungelella > Gregory Downs > Armraynald. Different letters denote significant differences at P = 0.05 within each panel (i.e. soil).

Practical implications

There were varietal differences in emergence and early growth among the *Desmanthus* spp. genotypes. These differences could be useful when selecting cultivars to be sown in hostile soils. Gypsum had a positive impact on seedling emergence, whereas starter fertiliser had a positive impact on shoot yield. The collective results indicate that starter fertiliser will provide a benefit for legume productivity in the soils of northern Australia once seedlings have emerged. The lower levels of emergence when the *Desmanthus* spp. genotypes were grown without gypsum could be offset by increasing sowing rates, as the cost of seed is lower than that of gypsum. Furthermore, the potential to apply large rates of gypsum in extensive grazing systems is likely to be limited due to the quantity of gypsum required. Starter fertiliser could then be prioritised, particularly when soil testing indicates that Colwell P levels are below critical for optimal plant growth. Nevertheless, care must be taken when using starter fertiliser as it is likely to benefit the grass component.

8.11 Appendix 11 – Starter fertiliser reduces the critical phosphorus requirements of two tropical pasture legumes

Materials and Methods

Plant growth conditions

Centro (*Centrosema pubescens* cv. Cardillo) and Desmanthus (*Desmanthus pernambucanus* cv. JCU 9) were grown to determine shoot yield and tissue P concentrations in response to starter fertiliser.

A sandy loam soil was collected from a field at Kirby SMART Farm, Armidale, NSW, Australia. The soil had a Colwell extractable P concentration of 7 mg P kg^{-1}, a Phosphorus Buffering Index (PBI) of 51, and a pH ($CaCl_2$) of ~4.6. The soil was passed through a 5 mm sieve and lime (1 g $CaCO_3$ kg^{-1}) was mixed through the soil. While mixing, a complete basal nutrient solution (minus P) was applied to the soil. Six P-amended soils (0, 5, 10, 20, 40 and 80 mg P kg^{-1}, hereafter referred to as P0, P5, P10, P20, P40 and P80, respectively) were prepared by adding KH_2PO_4 to the nutrient solution. KCl was also applied to the P0–P20 treatments to balance the K to be equivalent to that of the P40 treatment. The P-amended soils were incubated for five weeks. After incubation, 1.3 kg (oven-dry equivalent) of the P-amended soils was added to cylindrical PVC pots (87 mm internal diameter; 200 mm height). The total depth of soil was ~190 mm and the bulk density was ~1.15 g cm^3.

Micro-swards of both legumes were established by sowing seed (~5 mm depth) to achieve a population of 4 plants pot^{-1}. Half the pots received an application of starter fertiliser (6 kg P ha^{-1} equivalent of KH_2PO_4 solution) whereas the other half did not. Five replicate pots of each legume at each native soil P level and +/- starter fertiliser were prepared. After planting, the pots were watered and moved to a glasshouse (natural daylight, ~1800 mmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. Plants were grown between April–June 2020. Pots were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006). Soil moisture was maintained between 80-100% field capacity by watering daily to a predetermined weight using distilled water.

Harvest and analysis

Plants were harvested after eight weeks' growth. Shoots were cut at the soil surface, oven-dried at 70°C for 72 h and weighed. Shoot samples were then finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colourimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. Measured parameters were analysed using R (R Core Team, 2020).

Results and Discussion

Shoot yield and critical P requirements

The shoot dry mass of both legumes increased in response to native soil P supply ($P < 0.001$; Fig. 45). Nevertheless, the addition of starter fertiliser increased shoot yields significantly ($P < 0.001$), with the largest benefit generally observed between the P5–P20 treatments. On average across the treatments, Desmanthus was more productive than Centro ($P < 0.001$).

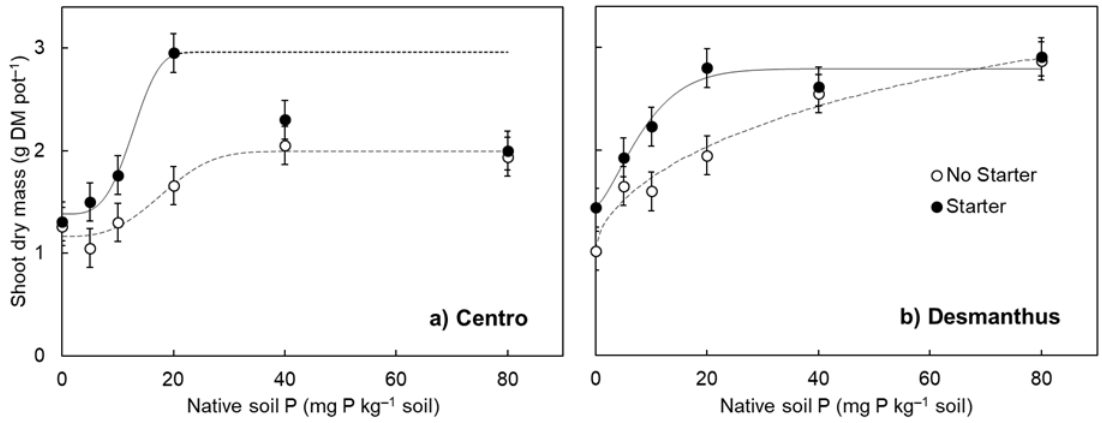

Figure 45. The shoot dry mass of Centro (a) and Desmanthus (b) when grown in response to six native soil P treatments, both with and without starter fertiliser. Values show the mean ± s.e. (n = 5). Fitted curves show Weibull growth functions. The shoot yield achieved by Centro with starter fertiliser in the P40 and P80 treatments was significantly lower than in the P20 treatment. To enable the Weibull growth function to be fitted, the shoot yield of the P40 and P80 treatments was assumed to be equivalent to that of the P20 treatment. The dotted line represents the assumed P response curve above the P20 treatment.

The critical external P requirements of both legumes decreased in response to starter fertiliser (Table 18). Centro and Desmanthus were both equally efficient when starter fertiliser was applied to the soil at planting.

Table 18. The critical external and internal P requirements of Centro and Desmanthus with and without starter fertiliser. Critical external P requirements were calculated as the amount of P applied to achieve 90% maximum yield, with 95% confidence intervals shown in parentheses. Critical internal P requirements were the shoot P concentrations that corresponded with the critical external P requirements. * shows the values that could not be calculated.

Species	Critical external P requirement (mg P kg^{-1} soil)	Critical internal P requirement (mg P g^{-1} DM)
Centro – no starter	**23.0** (15.8–56.8)	**2.21**
Centro – starter	**16.0** (12.6–22.9)	**2.13**
Desmanthus – no starter	**52.8** (36.3–*)	**2.52**
Desmanthus – starter	**13.5** (9.6–21.5)	**2.11**

Shoot P

Shoot P content increased with native soil P supply for both legumes ($P < 0.001$; Fig. 46). The addition of starter fertiliser had a positive effect on shoot P content ($P < 0.001$). Nevertheless, shoot P content was, on average across the treatments, higher for Desmanthus than Centro ($P < 0.001$).

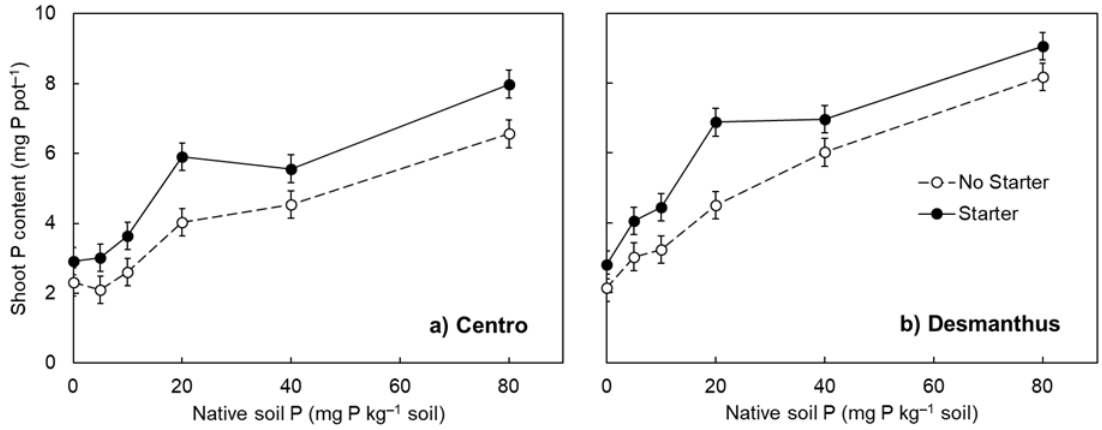

Figure 46. The shoot P content of Centro (a) and Desmanthus (b) when grown in response to six native soil P treatments, both with and without starter fertiliser. Values show the mean ± s.e. (n = 5).

Shoot P-use efficiency declined in response to native soil P supply for both legumes ($P < 0.001$; Fig. 47). There was no difference between Centro and Desmanthus in how they responded to P availability ($P = 0.311$). On average across treatments, shoot-P use efficiency was higher when starter fertiliser was not applied at planting.

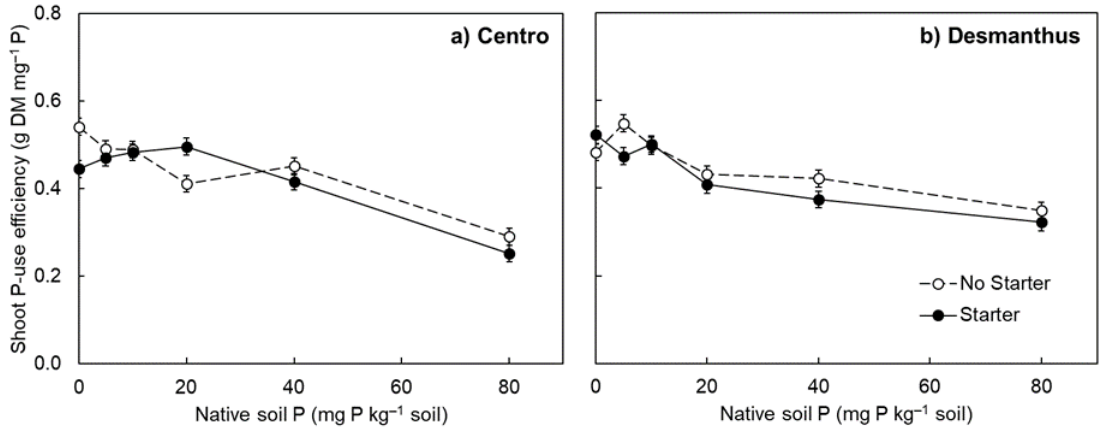

Figure 47. The shoot P-use efficiency of Centro (a) and Desmanthus (b) when grown in response to six native soil P treatments, both with and without starter fertiliser. Values show the mean ± se (n = 5).

Practical implications

Centro and Desmanthus both responded to the increasing levels of available P in the six native soil P treatments. This demonstrates that both legumes are likely to be more productive in soils with higher Colwell P levels. However, there was a substantial difference in the critical external P requirements of the species when starter fertiliser was not applied at planting. This is likely associated with differences in root traits, because both species were equally efficient at using acquired P to produce dry mass. Starter fertiliser had a positive effect on shoot yield, particularly between the P5–P20 treatments. Indeed, the addition of starter fertiliser decreased the critical external P requirements of both species, with both species having equally low critical P requirements. This indicates that starter fertiliser is likely to be useful across a range of native soil P levels for tropical pasture legumes.

8.12 Appendix 12 – Starter fertiliser applied at planting improves the productivity of tropical pasture species

Materials and Methods

Plant material

Two tropical pasture species were grown together to investigate seedling emergence, leaf area development and shoot yield in response to starter fertiliser application. The species were Digit (*Digitaria eriantha* cv. Premier) and Desmanthus (*Desmanthus pernambucanus* cv. JCU 9). These species were selected because they are important components of pastures in northern Australia.

Soil and nutrient treatments

A clay soil (Grey Vertosol; Isbell 1996) was collected from a field at Delungra, NSW, Australia. The soil had a Colwell extractable P concentration of 13 mg P kg^{-1} (as measured by the method of (Colwell, 1963)), a Phosphorus Buffering Index (PBI) of 180 (as measured by the modified method of (Burkitt et al., 2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of 5.0. Another clay soil (Red Ferrosol; Isbell 1996) was collected from a field at Kingaroy, QLD, Australia. The soil had a Colwell extractable P concentration of 12 mg P kg^{-1} (as measured by the method of (Colwell, 1963)), a Phosphorus Buffering Index (PBI) of 385 (as measured by the modified method of (Burkitt et al., 2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of 4.6.

29 kg (oven-dry basis) of either soil was placed in large plastic boxes (360 mm width, 530 mm length, 180 mm height). The boxes were split in half using mylar (0.5 mm), so there was an even amount of soil in either half of the box. The bottom 50 mm of each box was designed to act as a water reservoir, to ensure that most of the soil profile was maintained between 80–100% field capacity for the duration of the experiment. Water could be added to the reservoir by hose without wetting the soil surface, which mimics the moisture profile of soil in the field. The soil in each pasture box was amended with a broadcast application of 500 mg lime kg^{-1} (as $CaCO_3$), 50 mg K kg^{-1} and 22 mg K kg^{-1} (as K_2SO_4), and 1 mg Librel BMX kg^{-1} (which contains boron, copper, iron, manganese, molybdenum and zinc). The top 10 cm of soil was then incorporated and watered from the surface. Two rows 80 mm apart were marked in the soil to a depth of 5 mm. Finely ground mono-ammonium phosphate (MAP) was applied along the rows to achieve six starter fertiliser treatments;… . These P treatments were equivalent to 0, 6, 12, 24, 36 and 48 kg P ha^{-1}, respectively, based on the surface area of the boxes. The 0 and 6, 12 and 24, and 36 and 48 kg P ha^{-1} treatments were prepared in either side of the same box.

Plant growth conditions and experimental design

Mixed swards of Digit/Desmanthus were established by sowing seed along the marked rows to which the fertiliser had been applied. The seed of the grasses and legumes was sown in separate rows. The marked rows were then covered and the soil was again watered from the surface. Four replicates of each treatment combination were prepared. The pasture boxes were placed in a randomised complete block design in a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia.

Emergence was recorded twice daily for three weeks following planting. Photographs of plants were also taken to calculate leaf area. The emerged seedlings were then thinned to achieve a plant population of 15 grass and 15 legume plants row^{-1}. While the plants were establishing, the boxes

were watered from the surface once per week. For the duration of the experiment, sufficient water was maintained in the base of each pasture box.

Harvest and measurements

Plants were harvested after six weeks' growth. Shoots were cut at the soil surface, oven-dried at 70°C for 7 days and weighed. Shoot samples were finely cut before a 50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 16 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass.

Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'soil' and 'P treatment' as predictor variables. Linear mixed effects models were fitted to measured variables where the two components (i.e. grass and legume) were compared (R package: nlme) (Pinheiro et al., 2020). The models included 'soil', 'P treatment' and 'component' as fixed effects, and 'box' as a random effect. When appropriate, the effect of 'replicate' was included in the most parsimonious model. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). Shoot yield responses to starter fertiliser application were determined by fitting a self-starting Weibull growth function ($y = a – b * \exp(–\exp(c) * x^d)$, where x is the P application rate and y is the shoot dry mass) as described by Crawley (2013). Critical starter fertiliser requirements were calculated as the amount of starter fertiliser applied to achieve 90% of maximum yield based on the fitted Weibull growth functions, and the 95% confidence intervals of the critical starter fertiliser requirements were determined by bootstrapping residuals as described by Crawley (2013). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Seedling emergence and early vigour

Desmanthus emerged quicker than Digit in both of the soils and was not influenced by the rate of starter that was applied (Fig. 48). In contrast, the emergence of Digit was influenced by starter fertiliser; in the Delungra soil, Digit emerged quicker when it received between 12–48 kg P ha^{-1} of starter fertiliser, whereas in the Kingaroy soil, Digit emerged quicker when it received between 0–36 kg P ha^{-1}. The quicker emergence of Desmanthus meant that the legume had developed more leaf area than Digit two weeks after planting (Fig. 49). The leaf area of Desmanthus was consistently higher than that of Digit across the six starter fertiliser application rates in both soils. In the Delungra soil, Digit and Desmanthus had achieved a near maximum leaf area by the 6 kg P ha^{-1} treatment, whereas in the Kingaroy soils, this was achieved by the 24 kg P ha^{-1} treatment.

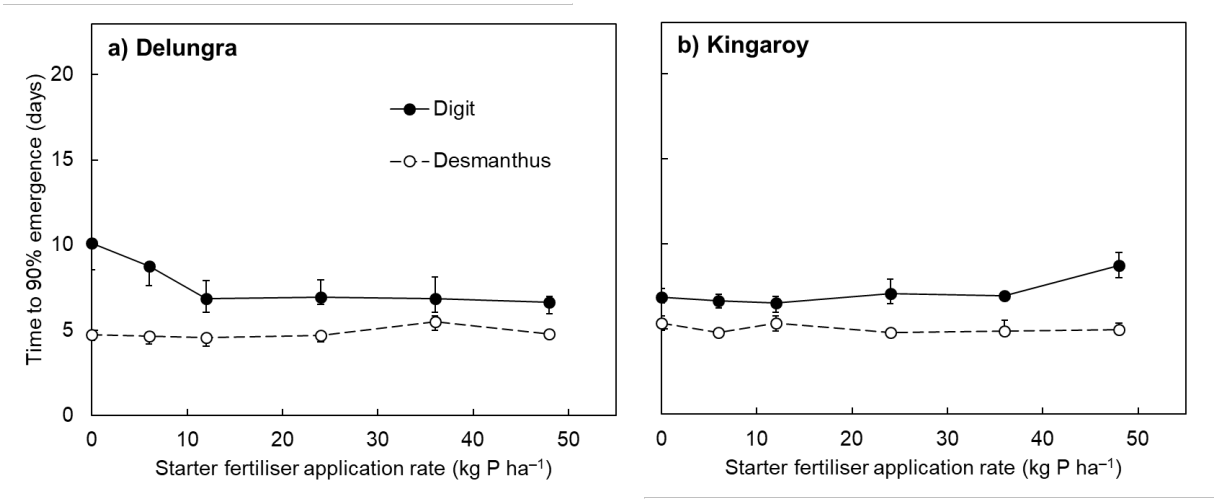

Figure 48. Time to 90% emergence of Digit and Desmanthus when grown in response to six rates of starter fertiliser (0–48 kg P ha^{-1} equivalent) in two different soils (Delungra and Kingaroy). Values show the mean ± s.e. (n = 4).

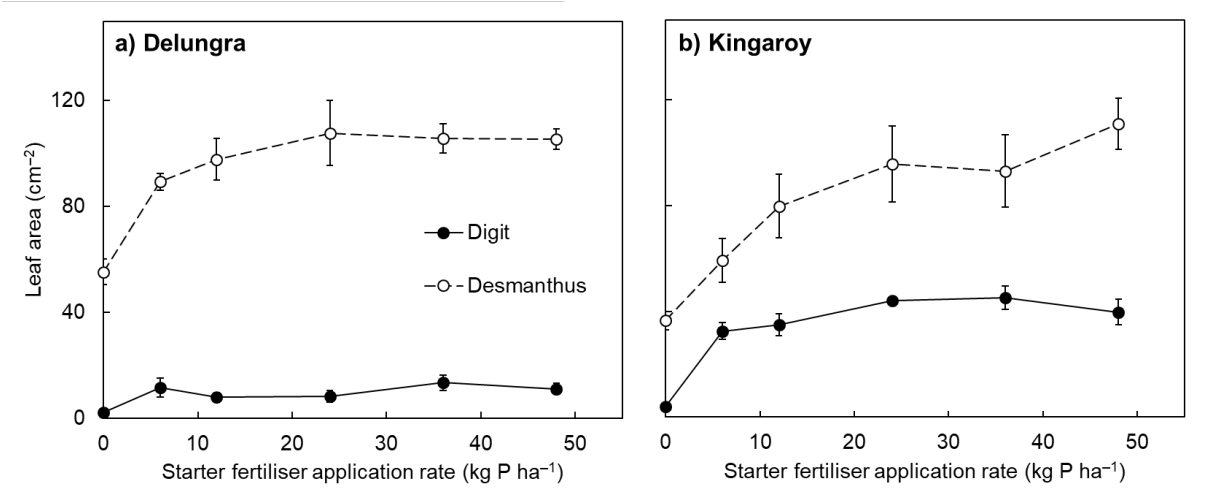

Figure 49. Leaf area of Digit and Desmanthus two weeks after planting when grown in response to six rates of starter fertiliser (0–48 kg P ha^{-1} equivalent) in two different soils (Delungra and Kingaroy). Values show the mean ± s.e. (n = 4).

Shoot yield and critical starter fertiliser requirements

Shoot yields increased in response to higher rates of starter fertiliser (Fig. 50). Desmanthus was more productive in the Delungra soil, whereas Digit was more productive in the Kingaroy soil. The critical starter fertiliser requirements were (confidence intervals are shown in parentheses):

- Delungra – Digit = 37.2 (23.9–*) and Desmanthus = 33.3 (23.0–*).
- Kingaroy – Digit = 21.2 (12.8–39.3) and Desmanthus = 40.7 (36.5–46.9).

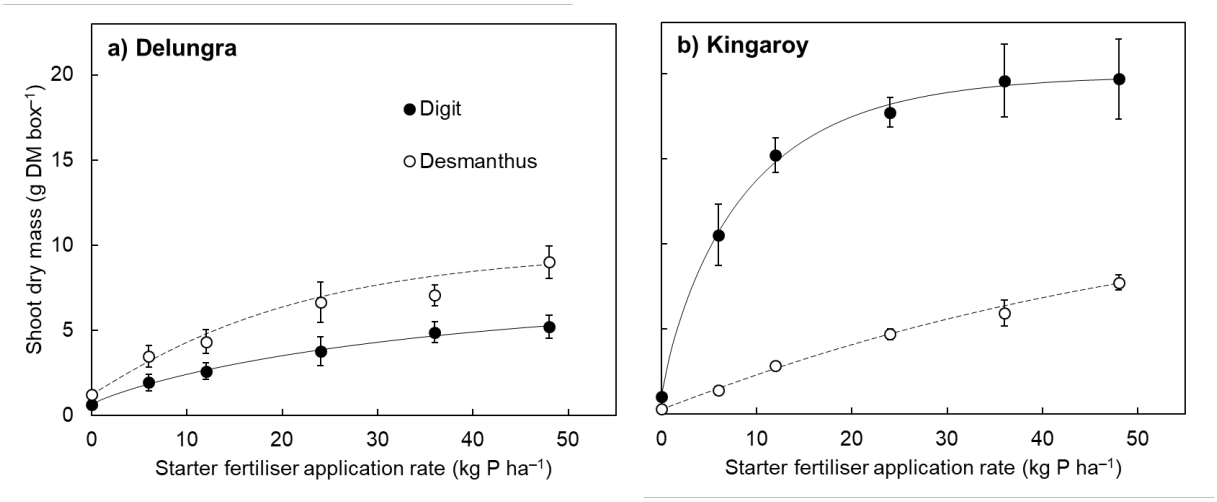

Figure 50. Shoot dry mass of Digit and Desmanthus when grown in response to six rates of starter fertiliser (0–48 kg P ha^{-1} equivalent) in two different soils (Delungra and Kingaroy). Values show the mean ± s.e. (n = 4). The curves that were fitted to the shoot yield data show Weibull growth functions (Crawley, 2013).

Practical implications

Starter fertiliser (that included both N and P) was beneficial for the early vigour and subsequent growth of both Digit and Desmanthus. For the legume, there was no negative affect of starter fertiliser on the time to 90% emergence and it had a positive impact on the development of leaf area within the first two weeks of growth. This is important for pasture establishment, because early canopy closure facilitates efficient light capture and growth compared to competing species. Nevertheless, in the Kingaroy soil, Digit still out-yielded Desmanthus by the end of the experiment, highlighting the competitiveness of this C_4 grass. Although starter fertiliser may benefit the legume component, care must be taken if there are other competitive species (e.g. both desirable and undesirable species). The experiment also highlighted the importance of soil type for plant growth, as Digit struggled to yield in the Delungra soil. However, the relatively consistent shoot yield of Desmanthus suggests that it may be suited to a range of soil types.

8.13 Appendix 13 – Preferential phosphorus placement improves the productivity and competitiveness of tropical pasture legumes

Materials and Methods

Plant material

Four tropical pasture species were grown in two mixed swards to investigate shoot yield and fertiliser recovery. The mixes were Digit (*Digitaria eriantha* cv. Premier) with Desmanthus (*Desmanthus pernambucanus* cv. JCU 9), and Rhodes (*Chloris gayana* cv. Katambora) with Centro (*Centrosema pubescens* cv. Cardillo). These species were selected because they are important components of pastures in northern Australia.

Soil and nutrient treatments

A clay loam soil was collected from the upper 0–30 cm soil layer of a field at Armraynald Station, Burketown, QLD, Australia (17°57'57.1"S 139°42'51.5"E). The soil had a Colwell extractable P concentration of 3 mg P kg^{-1} (as measured by the method of (Colwell, 1963)), a Phosphorus Buffering Index (PBI) of 70 (as measured by the modified method of (Burkitt et al., 2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of 5.9. The collected soil was dried, crushed by hand and homogenised. 30 kg (oven-dry basis) of the crushed soil was then placed in large plastic boxes (360 mm width, 530 mm length, 180 mm height) (Fig. 51). The bottom 50 mm of each box was designed to act as a water reservoir, to ensure that most of the soil profile was maintained between 80–100% field capacity for the duration of the experiment. Water could be added to the reservoir by hose without wetting the soil surface, which mimics the moisture profile of soil in the field.

Figure 51. Plastic boxes were filled with a P responsive soil to investigate the shoot yield and fertiliser recovery of two tropical grass/legume combinations. The grasses (right-hand side) and legumes (left-hand side) were planted in separate rows, and P fertiliser was applied along with the seed. The P applied below the legumes was labelled with ^{32}P-radioisotope tracer. There was a water reservoir at the bottom of each plastic box which ensured most of the soil profile was maintained between 80–100% field capacity for the duration of the experiment

The soil in each pasture box was amended with a broadcast application of 50 mg N kg^{-1} (as CH_4N_2O), 50 mg K kg^{-1} and 22 mg S kg^{-1} (as K_2SO_4), and 1 mg Librel BMX kg^{-1} (which contains boron, copper, iron, manganese, molybdenum and zinc). The top 10 cm of soil was then incorporated, watered from the surface and incubated in the plastic boxes for one week. Following the incubation period, two rows 150 mm apart were marked in the soil to a depth of 25 mm. Solutions of P fertiliser (as KH_2PO_4) were applied along the rows to achieve three treatments; 15.9 mg P box^{-1} below the grass and legume (banded low-P below both), 95.4 mg P box^{-1} below the grass and legume (banded high-P below both), and 190.7 mg P box^{-1} (banded superhigh-P below the legume only). These P treatments were equivalent to 2, 12 and 24 kg P ha^{-1}, respectively, based on the surface area of the boxes. The solutions applied below the legume component were labelled with ^{32}P-radioisotope, whereas the solutions applied below the grass component were unlabelled.

Plant growth conditions and experimental design

Mixed swards of Digit/Desmanthus and Rhodes/Centro were established by sowing seed along the marked rows to which the fertiliser had been applied. The seed of the grasses and legumes was sown in separate rows. The marked rows were then covered and the soil was again watered from the surface. Four replicates of each treatment combination were prepared. The pasture boxes were placed in a randomised complete block design in a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. Two weeks after planting, the germinated seedlings were thinned to achieve a plant population of 24 grass and 24 legume plants row^{-1}. While the plants were establishing, the boxes were watered from the surface once per week. For the duration of the experiment, sufficient water was maintained in the base of each pasture box.

Harvest and measurements

Plants were harvested after seven weeks' growth. Shoots were cut at the soil surface, oven-dried at 70°C for 7 days and weighed. Shoot samples were finely cut before a ~0.3 g subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 16 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Agilent, Mulgrave, Australia). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. Digested shoot samples were also analysed for ^{32}P-radioisotope activity using a PerkinElmer Tri-Carb 2810TR (PerkinElmer, Waltham, United States of America). A scintillation cocktail was prepared by mixing 3 mL of sample with 17 mL of scintillant (PerkinElmer UltimaGold AB). All samples were analysed by liquid scintillation counting (LSC) for 5 min and were corrected for radioactive decay and dilution. The ^{32}P-radioisotope counts were used to calculate the specific activity of the shoot material, the amount of plant P derived from applied P, and the recovery of applied P as follows:

$$Specific\ activity = \frac{radioactivity\ in\ shoots}{total\ P\ in\ shoots} \quad (1)$$

$$Plant\ P\ derived\ from\ applied\ P = \frac{specific\ activity\ of\ shoots}{specific\ activity\ of\ applied\ P} \times 100 \quad (2)$$

$$Recovery\ of\ applied\ P\ fertiliser = \frac{applied\ P\ in\ shoots}{total\ applied\ P} \times 100 \quad (3)$$

The specific activity of P applied to the soil was 45.7 MBq mg P^{-1} in the banded low-P treatment, 7.6 MBq mg P^{-1} in the banded high-P treatment, and 3.8 MBq mg P^{-1} in the banded superhigh-P treatment.

Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'species' and 'P treatment' as predictor variables. Linear mixed effects models were fitted to measured variables where the two components (i.e. grass and legume) were compared (R package: nlme) (Pinheiro et al., 2020). The models included 'species', 'P treatment' and 'component' as fixed effects, and 'box' as a random effect. When appropriate, the effect of 'replicate' was included in the most parsimonious model. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield

When P fertiliser was applied below both components of the mixed swards (i.e. the banded low-P and high-P treatments), the two grasses (Digit and Rhodes) consistently out-yielded the two legumes (Desmanthus and Centro) with which they were grown ($P < 0.001$; Fig. 52). In these treatments, the legume content of the mixed swards averaged 29%. This legume content is relatively low considering there was an equal number of grass and legume plants in each box, which highlights how tropical grasses often have a higher yield potential than tropical legumes. When P fertiliser was applied below the legumes only, grass shoot yields declined whereas legume shoot yields were either stable or higher ($P = 0.001$). This meant that the average legume content increased to 66%, compared to the when both components received P fertiliser. This legume content is higher than the desired level for optimal pasture production. However, strong legume growth following pasture establishment is expected to maximise seed-set for recruitment in subsequent years, in which grasses are expected to become progressively more competitive. On average across the banded low-P and high-P treatments (i.e. when both components received the same P fertiliser), the productivity of the species was as follows; Rhodes > Digit > Centro > Desmanthus ($P < 0.001$). These differences show that it is important to consider yield potential when pairing tropical grasses and legumes in mixed swards. Large differences in growth between grasses and legume will likely lead to poor legume persistence, particularly if pasture biomass is not carefully managed by grazing.

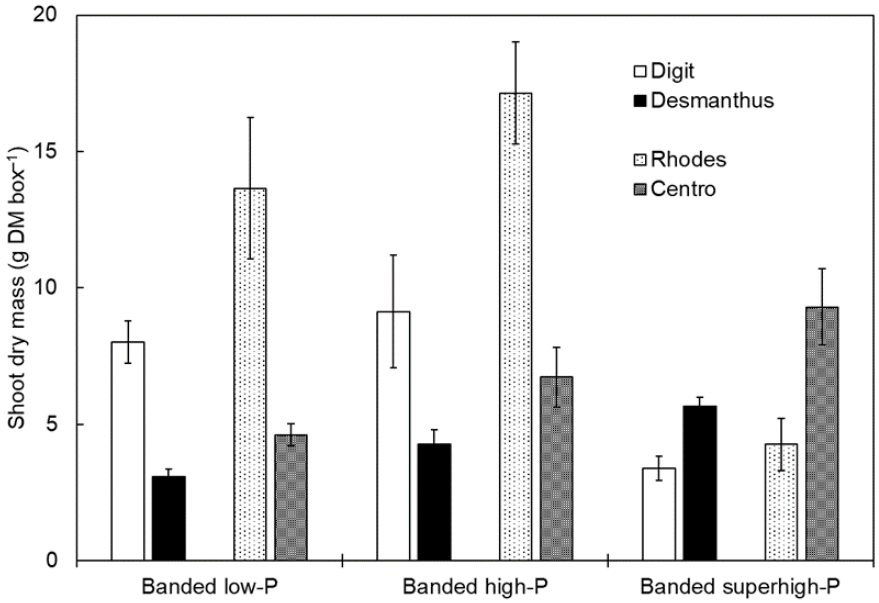

Figure 52. Shoot dry mass of two tropical pasture mixes (Digit/Desmanthus and Rhodes/Centro) grown in response to three soil-P treatments. Values show the mean ± s.e. (n = 4).

Shoot P

Shoot P concentrations were, on average, higher for the two legumes (0.12%) compared to the two grasses (0.11%) across the different treatment combinations ($P = 0.002$; data not shown). These shoot P concentrations are relatively low, which suggests that P supplementation may still be required in legume-enriched pasture swards of northern Australia from where the soil used in this experiment was collected. Regardless, the low shoot P concentrations indicate that tropical grasses and legumes produce dry matter efficiently, even when a large amount of starter fertiliser is applied at planting. There was no difference in shoot P concentration between Digit and Rhodes, or between Desmanthus and Centro ($P = 0.078$). The differences in shoot yield and shoot P concentration meant that shoot P content (an indicator of plant P uptake) differed according to species ($P < 0.001$) and component ($P < 0.001$), and, to a lesser extent, P treatment ($P = 0.012$) (Fig. 53).

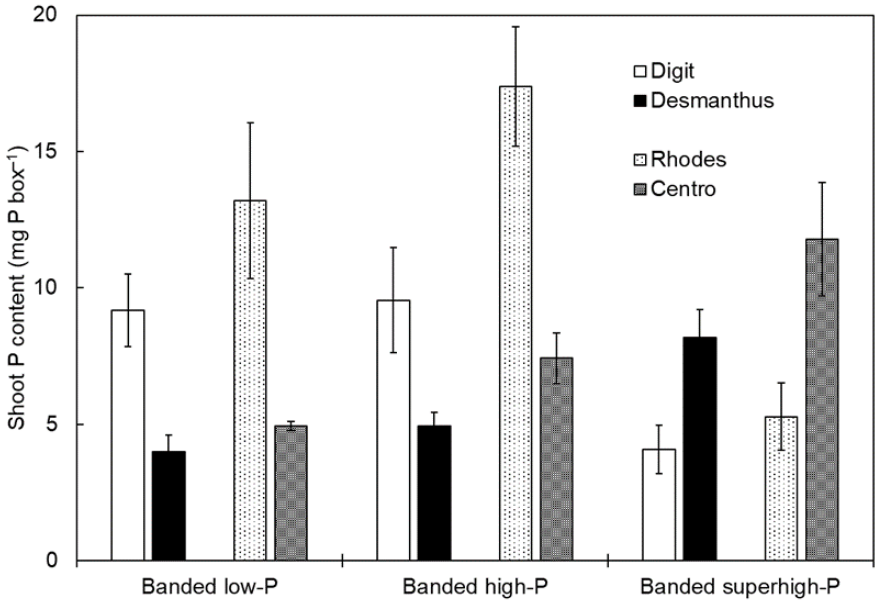

Figure 53. Shoot P content of two tropical pasture mixes (Digit/Desmanthus and Rhodes/Centro) grown in response to three soil-P treatments. Values show the mean ± s.e. (n = 4).

Plant P derived from applied P

Plant P derived from applied P is a measure of how important the applied P fertiliser was for plant growth. On average for the legumes, which were fertilised with the labelled P fertiliser, plant P derived from applied P increased from 20% to 77% as the rate of starter fertiliser below that component was increased (Fig. 54). Although shoot yield did not change between the banded low-P and high-P treatments, there was a significant increase in P derived from fertiliser. This shows that the applied P fertiliser was an important source of P for plant growth. Even though the grasses were not fertilised with the labelled P fertiliser, there was a significant amount of plant P derived from applied P in both species (5–27%). This indicates that the grasses effectively foraged the soil profile for available P, acquiring some of the starter fertiliser that was applied below the legumes. It is expected that, throughout the growing season, the grasses would explore more of the soil profile and acquire a larger amount of the P fertiliser applied below the legumes.

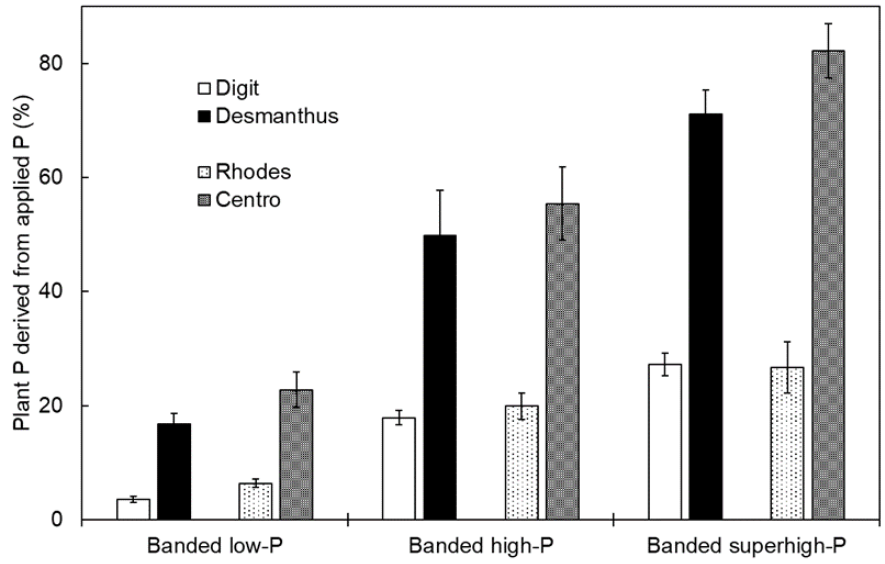

Figure 54. Plant P derived from applied P of two tropical pasture mixes (Digit/Desmanthus and Rhodes/Centro) grown in response to three soil-P treatments. Values show the mean ± s.e. (n = 4).

Recovery of applied P fertiliser

Fertiliser recoveries were relatively low across the three P treatments for each of the species. They ranged between 0.6% and 3.8% for the grasses and between 3.5% and 5.7% for the legumes (Fig. 55). Other experiments have shown that P fertiliser recoveries can be higher than this. For example, McLaren et al. (2016) reported that a temperate legume pasture recovered up to 35% in the year of application. However, plants were only grown for seven weeks in the present experiment which means that there was limited time for the plants to acquire the applied P fertiliser. This in turn means that the benefit of a banded application of P fertiliser is likely to remain for the duration of the first growing season, having a longer-term benefit for pasture productivity and persistence.

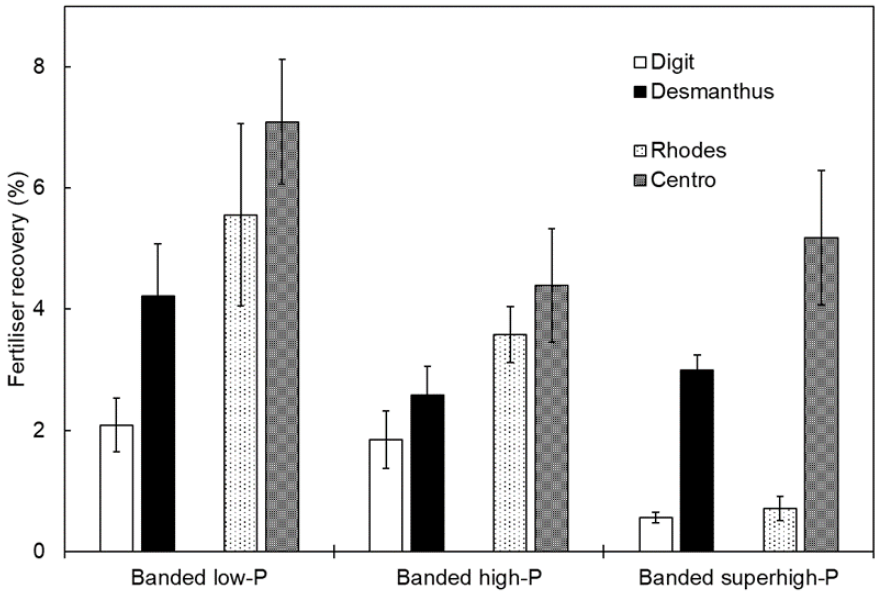

Figure 55. Phosphorus fertiliser recovery of two tropical pasture mixes (Digit/Desmanthus and Rhodes/Centro) grown in response to three soil-P treatments. Values show the mean ± s.e. (n = 4).

Practical implications

The collective results demonstrate that banded fertiliser applications are appropriate for tropical grasses and legumes. However, if both components are fertilised, the grass is likely to be highly competitive which could reduce legume productivity and persistence over time. The preferential placement of starter fertiliser below the legume component only could be a strategic way to increase legume content in the first year of growth, during which seed-set is important to ensure recruitment in subsequent years. Even when starter fertiliser is applied below the legume component only, the grasses still acquired labelled fertiliser, which suggests that, over time, the grass will also benefit from the applied fertiliser.

8.14 Appendix 14 – Placement of seed and fertiliser at planting influences phosphorus acquisition in a mixed tropical pasture sward

Materials and Methods

Plant material

Two tropical pasture species were grown in mixed swards to investigate shoot yield and fertiliser recovery. The species were Digit (*Digitaria eriantha* cv. Premier) and Desmanthus (*Desmanthus pernambucanus* cv. JCU 9). These species were selected because they are important components of pastures in northern Australia.

Soil and nutrient treatments

A clay loam soil was collected from the upper 0–30 cm (topsoil) and lower 30–50 cm (subsoil) soil layers of a field at Cungelella Station, Mantuan Downs, QLD, Australia (24°40'13.5"S 147°9'23.8"E). The topsoil had a Colwell extractable P concentration of 13 mg P kg^{-1} (as measured by the method of (Colwell, 1963)), a Phosphorus Buffering Index (PBI) of 90 (as measured by the modified method of (Burkitt et al., 2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of 7.2. The subsoil had a Colwell extractable P concentration of 5 mg P kg^{-1}. The collected soils were dried, crushed by hand and homogenised. 10 kg (oven-dry basis) of the subsoil was placed in large plastic boxes (360 mm width, 530 mm length, 180 mm height) followed by 20 kg (oven-dry basis) of the topsoil. The bottom 50 mm of each box was designed to act as a water reservoir, to ensure that most of the soil profile was maintained between 80–100% field capacity for the duration of the experiment. Water could be added to the reservoir by hose without wetting the soil surface, which mimics the moisture profile of soil in the field.

The soil in each pasture box was amended with a broadcast application of 50 mg N kg^{-1} (as CH_4N_2O), 50 mg K kg^{-1} and 22 mg S kg^{-1} (as K_2SO_4), and 1 mg Librel BMX kg^{-1} (which contains boron, copper, iron, manganese, molybdenum and zinc). The top 10 cm of soil was then incorporated and watered from the surface. Four P treatments were prepared:

- Banded low P – two rows 150 mm apart were marked in the soil to a depth of 25 mm and 15.9 mg P box^{-1} was applied along the rows as a KH_2PO_4 solution. The solution in one row was labelled with ^{32}P-radioisotope and the solution in the other row was labelled with ^{33}P-radioisotope.
- Banded high-P – two rows 150 mm apart were marked in the soil to a depth of 25 mm and 95.4 mg P box^{-1} was applied along the rows as a KH_2PO_4 solution. The solution in one row was labelled with ^{32}P-radioisotope and the solution in the other row was labelled with ^{33}P-radioisotope.
- Legume high-P – two rows 150 mm apart were marked in the soil to a depth of 25 mm and 190.7 mg P box^{-1} was applied along one row as a KH_2PO_4 solution. The solution was labelled with ^{32}P-radioisotope.
- Broadcast high-P – 1 kg (oven-dry basis) of the amended topsoil was weighed into plastic bags to which 95.4 mg P of KH_2PO_4 solution was applied. The solution was labelled with ^{32}P-radioisotope. The labelled soil was spread across the surface of the boxes.

The P applied to the banded low-P, banded/broadcast high-P and legume high-P treatments was equivalent to 2, 12 and 24 kg P ha^{-1}, respectively, based on the surface area of the boxes. After the

solutions had been applied to each treatment, a further 1 kg (oven-dry basis) of unamended soil was spread across the surface of each box to reduce exposure to radioactivity and to increase soil moisture availability where the P solutions had been applied.

Plant growth conditions and experimental design

Mixed swards of Digit/Desmanthus were established by sowing seed along the marked rows. The seed of the grasses and legumes was sown in separate rows in each of the four treatments outlined above. The grass and legume seed was also mixed and applied to both rows in the banded high-P and broadcast high-P treatments. The marked rows were then covered and the soil was again watered from the surface. Four replicates of each treatment were prepared. The pasture boxes were placed in a randomised complete block design in a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. Two weeks after planting, the germinated seedlings were thinned to achieve a plant population of 24 plants row^{-1}; 24 grass or legume plants when the species were planted separately, or 12 grass and 12 legume plants when the species were planted together. While the plants were establishing, the boxes were watered from the surface once per week. For the duration of the experiment, sufficient water was maintained in the base of each pasture box.

Harvest and measurements

Plants were initially harvested after eight weeks' growth. Shoots were cut at the soil surface, oven-dried at 70°C for 7 days and weighed. Shoot samples were finely cut before a ~0.5 g subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 16 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Agilent, Mulgrave, Australia). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. Digested shoot samples were also analysed for ^{32}P- and ^{33}P-radioisotope activity using a PerkinElmer Tri-Carb 2810TR (PerkinElmer, Waltham, United States of America). A scintillation cocktail was prepared by mixing 3 mL of sample with 17 mL of scintillant (PerkinElmer UltimaGold AB). All samples were analysed by liquid scintillation counting (LSC) for 5 min and were corrected for radioactive decay and dilution. The ^{32}P- and ^{33}P-radioisotope counts were used to calculate the specific activity of the shoot material, the amount of plant P derived from applied P, and the recovery of applied P as follows:

$$Specific\ activity = \frac{radioactivity\ in\ shoots}{total\ P\ in\ shoots} \quad (1)$$

$$Plant\ P\ derived\ from\ applied\ P = \frac{specific\ activity\ of\ shoots}{specific\ activity\ of\ applied\ P} \times 100 \quad (2)$$

$$Recovery\ of\ applied\ P\ fertiliser = \frac{applied\ P\ in\ shoots}{total\ applied\ P} \times 100 \quad (3)$$

The specific activity of P applied to the soil was 45.7 MBq mg P^{-1} in the banded low-P treatment, 7.6 MBq mg P^{-1} in the banded high-P treatment, and 3.8 MBq mg P^{-1} in the banded superhigh-P treatment.

Plants were regrown for a further four weeks before they were harvested again. Shoots were cut at the soil surface and were analysed for dry mass, tissue P concentration and radioactivity as outlined above.

Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'P treatment' as predictor variables. Linear mixed effects models were fitted to measured variables where the two components (i.e. grass and legume) were compared (R package: nlme) (Pinheiro et al., 2020). The models included 'P treatment' and 'component' as fixed effects, and 'box' as a random effect. When appropriate, the effect of 'replicate' was included in the most parsimonious model. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield

Digit and Desmanthus grew well in both the banded low-P and high-P treatments (i.e. there was no response to the higher rate of P fertiliser) (Fig. 56). At the first harvest, Desmanthus was highly productive in each of the treatments, regardless of whether it was planted in the same or separate row as Digit, or whether P fertiliser was banded or broadcast. In contrast, Digit struggled when grown in the same row as Desmanthus, and when the legume was preferentially fertilised. At the second harvest, Digit had recovered from being harvested quicker and was generally more productive than Desmanthus.

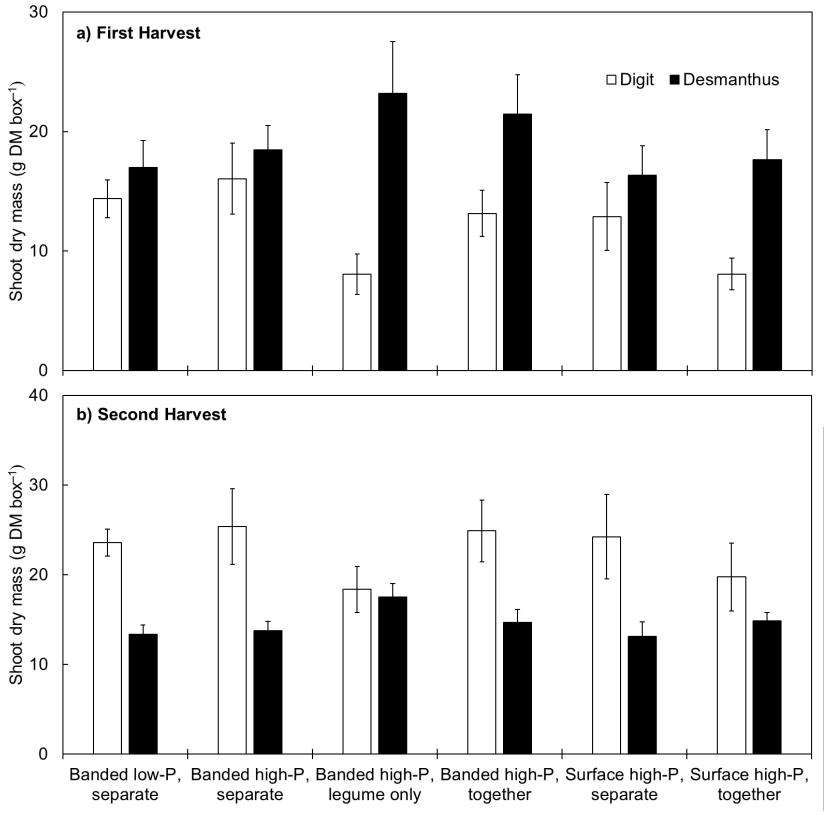

Figure 56. Shoot dry mass of Digit and Desmanthus when grown in six different seed and fertiliser placement treatments at two harvests. Values show the mean ± s.e. (n = 4).

The proportion of legume in the mixed swards was relatively high, but declined between the first and second harvested (Fig. 57). The highest legume content was maintained in the treatment where Desmanthus was preferentially fertilised.

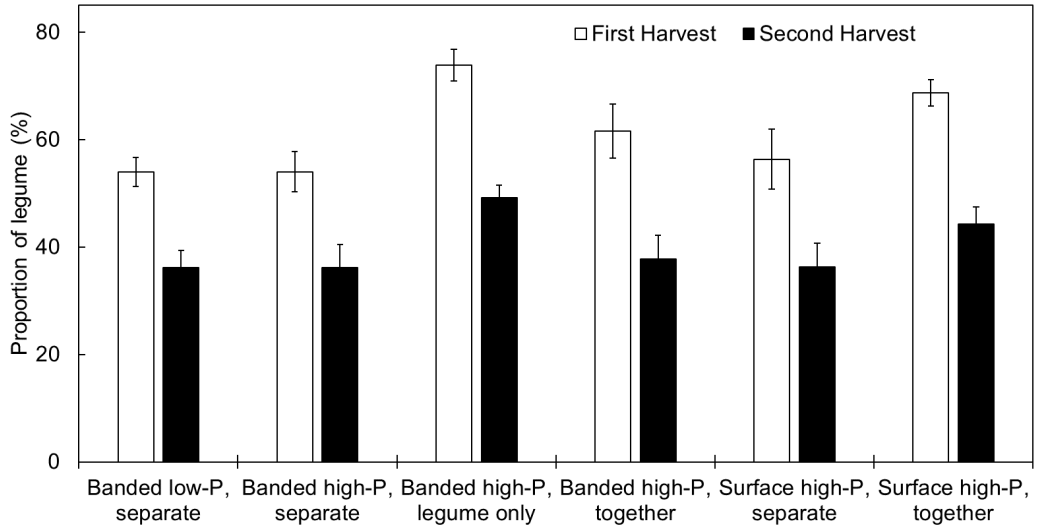

Figure 57. The proportion of Desmanthus in six different seed and fertiliser placement treatments of Digit and Desmanthus at two harvests. Values shown mean ± s.e. (n = 4).

Phosphorus acquisition

Digit and Desmanthus both acquired a significant amount of applied P fertiliser from each of the seed and fertiliser placement treatments (Fig. 58). When both components received P fertiliser, the plant P derived from applied P by Desmanthus was comparable or higher than that of Digit. When the legume was preferentially fertilised, there was a large difference between Desmanthus and Digit. Nevertheless, the small amount of plant P derived from applied P by Digit in this treatment indicates that it acquired some of the fertiliser that was applied below Desmanthus.

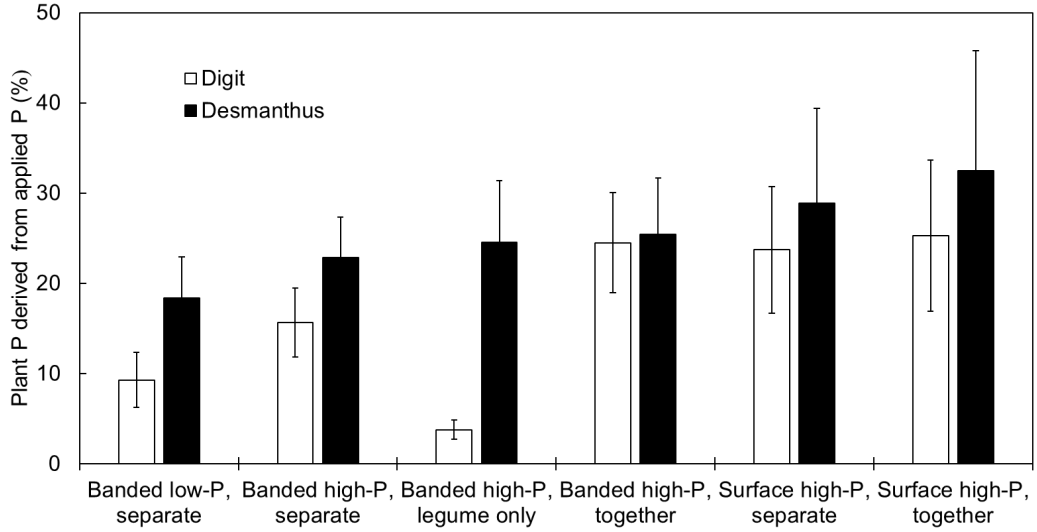

Figure 58. Plant P derived from applied P of Digit and Desmanthus when grown in six different seed and fertiliser placement treatments at the first harvest. Values show the mean ± s.e. (n = 4).

Fertiliser recovery was highest for Digit and Desmanthus in the banded low-P treatment (Fig. 59). Fertiliser recoveries were lower in the other treatments, due to the relatively large amount of fertiliser that was applied and the relatively short growing period of the experiment. In general, Desmanthus recovered more P fertiliser than Digit, due to shoot yield and plant P derived from applied P generally being higher for this component.

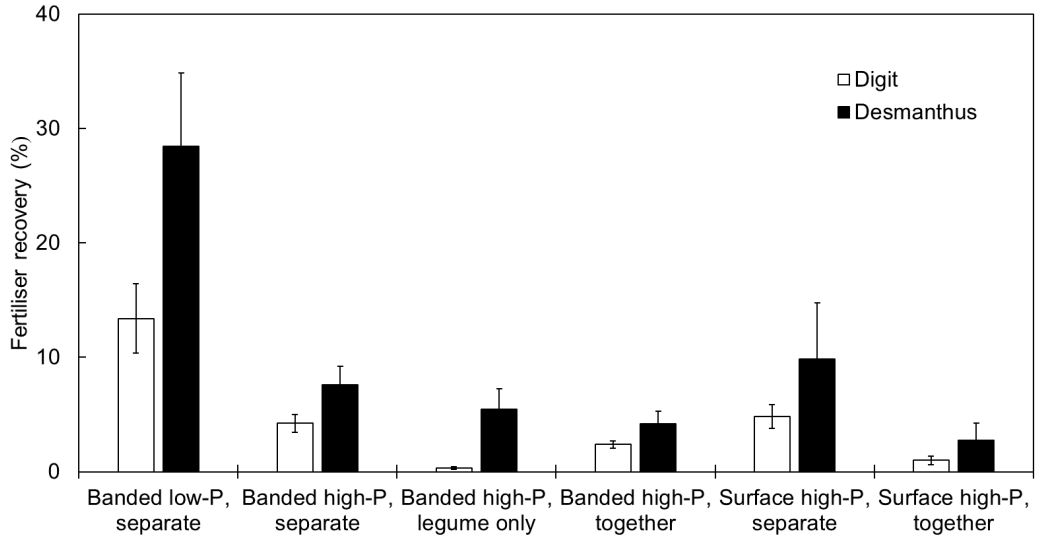

Figure 59. Phosphorus fertiliser recovery of Digit and Desmanthus when grown in six different seed and fertiliser placement treatments at the first harvest. Values show the mean ± s.e. (n = 4).

Practical implications

Digit and Desmanthus competed effectively in the moderately fertile clay soil. Desmanthus was particularly productive at the first harvest, possibly because it emerges and closes its canopy earlier than Digit. However, Digit recovered from the first harvest quicker than Desmanthus meaning that the proportion of legume declined significantly between the two harvests. This result highlights the importance of adequate rest for the legume component following grazing.

Digit and Desmanthus both acquired applied P fertiliser, even though the plants were mostly unresponsive to P fertiliser application (the topsoil had a Colwell P of 13 mg P kg^{-1} prior to fertiliser application). This result demonstrates that both species are likely to benefit from P fertiliser applications. The relatively low fertiliser recoveries were expected, given the short period of growth in the experiment. Nevertheless, they indicate that applied P will remain available for the majority of the first growing season.

8.15 Appendix 15 – Legume productivity is improved by preferential fertiliser application at planting in mixed pasture swards

Materials and Methods

Site preparation

A field trial was conducted at Kirby SMART Farm, Armidale, NSW, Australia (30°25'59.2"S 151°37'30.0"E). The soil at the site was a sandy loam (Brown Chromosol; Isbell 1996). Before any work commenced, the soil had an average Colwell extractable P concentration of ~21 mg P kg^{-1} (as measured by the method of (Colwell, 1963)) and an average pH (1:5 w/v; 0.01 M $CaCl_2$) of ~5.0. These averages were based on detailed grid-sampling of the site.

Six 'native' P fertiliser treatments were prepared approximately two years prior to the establishment of the present field trial. There were five treatments where triple superphosphate (15.7% P, 4.6% S) was broadcast onto the soil surface at rates of 0, 15, 30, 45 and 90 kg P/ha. There was one treatment where triple superphosphate was applied ~2.5 cm below the soil surface at a rate of 15 kg P/ha. The S that was applied to the P0–P30 treatments was balanced with gypsum, to be equivalent to that of the P45 treatment. The site also received a basal application of 50 kg K/ha and 22 kg S/ha (as Sulfate of Potash, 41% K, 18% S) and 1 kg/ha of micronutrients (as Librel BMX, which includes boron, copper, iron, manganese, molybdenum and zinc). Following the addition of nutrients, the site was sown with *Trifolium subterraneum*. Due to drought conditions, there was minimal growth of the *T. subterraneum* within the first six months after planting.

The site was periodically grazed after the establishment of the *T. subterraneum*, before weed control commenced in preparation for the establishment of the present experiment. The site was mown and the biomass was removed. Within a period of four months prior to planting, there were three applications of Glyphosate 450 (2.5 L/ha) to prepare a fallow. The third application included Starane Advanced (0.9 L/ha). Following weed control, there was another basal application of 50 kg K/ha and 22 kg S/ha (as Sulfate of Potash).

Trial establishment

Two grass-legume mixes were established in the plots that had been prepared with the six native P fertiliser treatments and had been amended with basal nutrients. There was a temperate pasture mix that included Tall Fescue (*Festuca arundinacea* cv. Quantum II) and Lucerne (*Medicago sativa* cv. Haymaster), and there was a tropical pasture mix that included Digit (*Digitaria eriantha* cv. Premier) and Desmanthus (*Desmanthus pernambucanus* cv. JCU 9). Sowing rates were equivalent to 15 kg/ha for Tall Fescue, 10 kg/ha for Lucerne, 10 kg/ha for Digit and 5 kg/ha for Desmanthus. These sowing rates aimed to achieve ~40% legume content based on plant population. The seed was sown using a disced coned seeder that had eight discs, with alternating rows of grass and legume sown separately (four rows of each component). Starter fertiliser (mono-ammonium phosphate, 10% N, 22% P) was applied in the rows with the seed, either as 3 kg P/ha with the grass and legume or as 6 kg P/ha with the legume only. The treatments were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006). There were five replicates of each treatment. There were a total of 120 plots, each 5 m long and 1 m wide. Plants were grown in January–April 2021, during which there was 282 mm of rainfall.

Sampling and measurements

Plants were harvested twelve weeks after planting. Two rows of grass (4 m long) and two rows of legume (4 m long), excluding 0.5 m at either end of the plots, were harvested separately. This allowed the proportion of each pasture component to be calculated. Shoots were cut at the soil surface, oven-dried at 70°C for 7 days and weighed. The harvested biomass was multiplied by 2.5 to estimate the total amount of biomass that had been produced across the entire plot. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 12 hr. All samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colourimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. Apparent fertiliser recovery was calculated as the percentage of shoot P content divided by applied P fertiliser.

Statistical analyses

Measured parameters were analysed in R Studio Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'species mix', 'fertiliser application' and 'native P treatment' as predictor variables. When appropriate, the effect of 'replicate' and 'row' from the randomised complete block design was included in the most parsimonious model. The effect of replicate and row accounted for the error associated with spatial variation. Means and standard errors were calculated from the fitted linear models (R Package: emmeans) (Lenth, 2020) and means were compared using Tukey's honest significant differences (HSD). Fourteen plots were removed from analysis due to only one component (i.e. either the grass or the legume) being present in the plot. Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals

Results and Discussion

Shoot yield

Legume shoot yield was not influenced by native soil P supply (i.e. the P0–P90 treatments) (P = 0.614), but it was influenced by the placement of banded fertiliser (i.e. below both components vs. the legume only) (P = 0.010). Because of this, the shoot yield results were summarised according to species mix and fertiliser application (Fig. 60). On average, fertiliser placed below only the legume increased the shoot yield of this component, by 63% on average. In contrast, grass shoot yield was not influenced by native soil P supply (P = 0.451) or by the placement of banded fertiliser (P = 0.702) (Fig. 60). The advantage of the preferential fertiliser application on the legume, with no yield penalty on the grass, reflects the moderate fertility of the soil where the trial was conducted. Under these conditions, it would be worthwhile prioritising the application of starter fertiliser below the legume component. Nevertheless, it is expected that starter fertiliser would have a positive impact on the yield of both grasses and legumes in highly P-responsive soils.

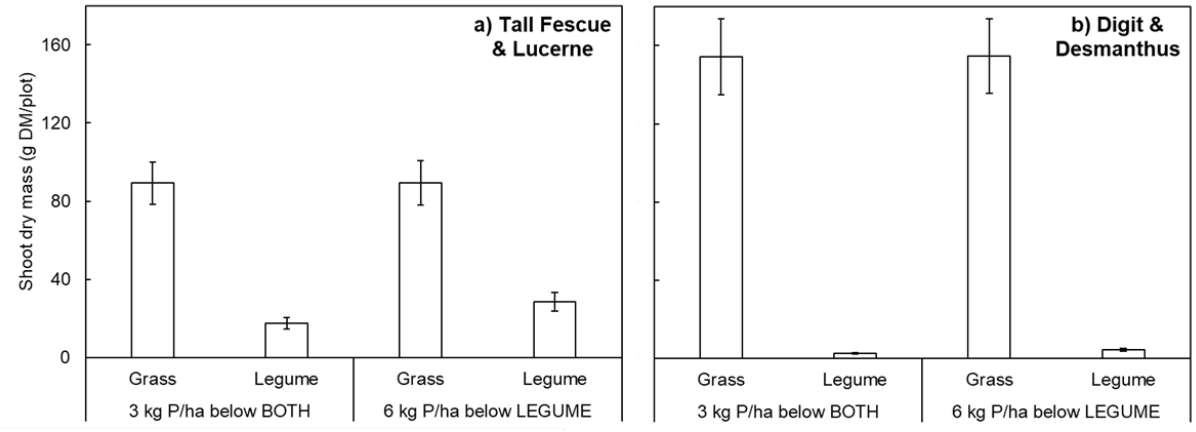

Figure 60. Shoot dry mass of two pasture mixes (Digit/Desmanthus and Tall Fescue/Lucerne) grown in response to two starter fertiliser application treatments (3 kg P/ha below both the grass and legume, or 6 kg P/ha below the legume only). Values show the mean ± s.e. (n = 4).

On average across the treatments, the yield of the different species was as follows; Digit > Tall Fescue > Lucerne > Desmanthus (Fig. 60). These differences in yield meant that both pasture mixes were grass dominant. Nevertheless, preferential placement of starter fertiliser below the legumes increased the proportion of this component, by 43% on average. The beneficial effect of starter fertiliser on the legume component indicates that this may be a viable method for increasing legume vigour at pasture establishment, provided the grass and legume components can be sown separately. Separate rows of grass and legume will provide space for both components to establish. It is expected that the grasses will forage for the starter fertiliser and, over time, will capitalise on the increased soil nutrition below the legume. Indeed, previous research has shown that within six weeks of growth, Digit had acquired P that had been applied below Desmanthus (when both components were fertilised with P and when only the legume had been fertilised with P).

Shoot P

Grass and legume shoot P concentrations were influenced by native soil P supply ($P < 0.001$ & $P = 0.001$, respectively) but not by the placement of banded fertiliser ($P = 0.861$ & $P = 0.070$, respectively). Because of this, the shoot yield results were summarised according to species mix and native P treatment (Fig. 61). In general, shoot P concentrations were lowest in the P0 treatment and increased to achieve the highest shoot P concentration in the P90 treatment. Shoot P content reflected the differences in shoot yield and, to a lesser extent, the differences in shoot P concentration (Fig. 62). In particular, grass shoot P contents were not influenced by the placement of banded fertiliser ($P = 0.680$) whereas the shoot P contents of the legumes were ($P = 0.006$). On average, fertiliser placed below only the legume component increased the shoot P content of this component, by 87% on average. Again, the advantage to the legume, with no penalty on the grass, suggests that this is an appropriate fertiliser application strategy in soil with moderate fertility.

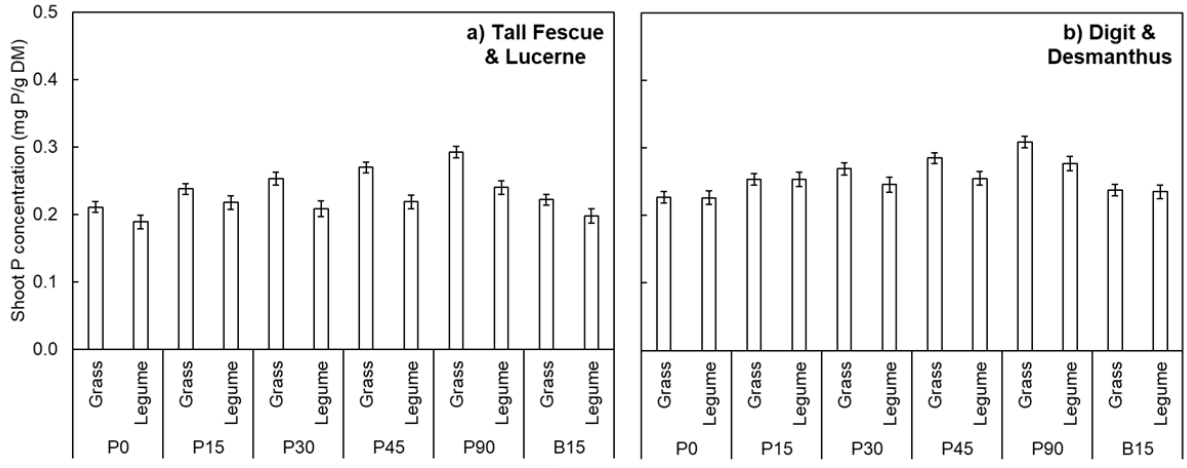

Figure 61. Shoot P concentration of two pasture mixes (Digit/Desmanthus and Tall Fescue/Lucerne) grown in response to six native soil P treatments (0, 15, 30, 45 and 90 kg P ha^{-1} broadcast and 15 kg P ha^{-1} banded). Values show the mean ± s.e. (n = 4).

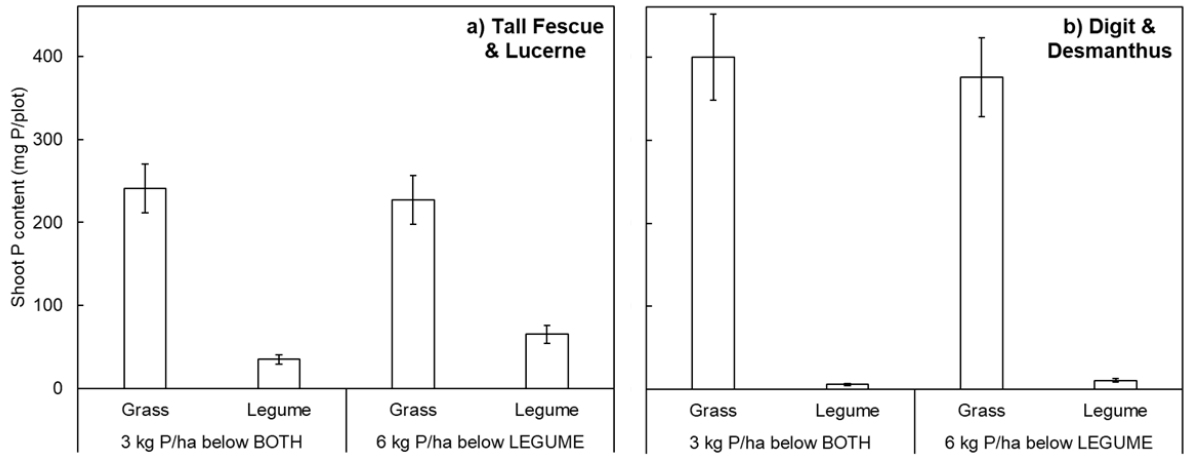

Figure 62. Shoot P content of two pasture mixes (Digit/Desmanthus and Tall Fescue/Lucerne) grown in response to two starter fertiliser application treatments (3 kg P/ha below both the grass and legume, or 6 kg P/ha below the legume only). Values show the mean ± s.e. (n = 4).

Apparent fertiliser recovery

Apparent fertiliser recovery (i.e. shoot P content as a proportion of applied P fertiliser) was not influenced by the method of fertiliser application and was relatively low for both legumes (Lucerne avg. = 2.3% and Desmanthus avg. = 0.4%) (data not shown). These low recoveries indicate that there was ample starter fertiliser available for plant uptake and growth, and that the benefit of the starter fertiliser was not fully realised. It is expected that the benefit of starter fertiliser will increase in soils of lower fertility. Nevertheless, the low apparent fertiliser recoveries indicate that starter fertiliser applications may remain beneficial for the duration of the first growing season.

Practical implications

Preferential fertiliser application, below the legume component at planting, increased the productivity of this component without having a detrimental impact on overall pasture production. This method of fertiliser application may be an appropriate way to increase the success of legume establishment, particularly when legumes are planted with highly-productive grasses. Further research is required to determine if any difference in early growth influences longer-term legume productivity and persistence. The low apparent recovery of P fertiliser indicates that any application of starter fertiliser will remain available for the duration of the first growing season.

8.16 Appendix 16 – Interactions between phosphorus, potassium and sulfur in tropical pasture species

Materials and Methods

Plant material

Five tropical pasture species were grown to investigate shoot yield and tissue nutrient concentrations in response to P, K and S. The pasture species were JCU 2 Desmanthus (*Desmanthus virgatus* cv. JCU 2), JCU 4 Desmanthus (*D. bicornutus* cv. JCU 4), JCU 7 Desmanthus (*D. leptophyllus* cv. JCU 7), JCU 9 (*D. pernambucanus* cv. JCU 9) and Digit (*Digitaria eriantha* cv. Premier).

Soil and nutrient treatments

A clay loam soil was collected from the upper 0–30 cm soil layer of a field at Armraynald Station, Burketown, QLD, Australia (17°57'57.1"S 139°42'51.5"E). The soil had a Colwell extractable P concentration of 3 mg P kg^{-1}, a KCl-40 of 2 mg S kg^{-1}, a Colwell extractable K concentration of 88 mg K kg^{-1}, and a pH (1:5 w/v; 0.01 M $CaCl_2$) of 5.9. The collected soil was dried, crushed by hand and homogenised. The soil was then amended with a basal nutrient solution that included 30 mg N kg^{-1} (as CH_4N_2O) and 1 mg kg^{-1} Librel BMX (which contains boron, copper, iron, manganese, molybdenum and zinc). Eight nutrient treatments were prepared by adding different combinations of the following solutions; 25 mg P kg^{-1} (as $Ca(H_2PO_4)_2 \cdot H_2O$), 15 mg S kg^{-1} (as $CaSO_4.2H_2O$) or 30 mg K kg^{-1} (as KCl) to the soil. The eight treatments were nil, +P, +K, +S, +P/K, +P/S, +K/S and +P/K/S. Cylindrical PVC pots (87 mm internal diameter, 200 mm height) were filled with 1.3 kg (oven-dry basis) of the amended soils. The total depth of soil was ~190 mm.

Plant growth conditions and experimental design

Micro-swards of each species were established by sowing seed (~5 mm depth) to achieve a population of 6 plants pot^{-1}. Four replicate pots of each species in each nutrient treatment were prepared. After planting, the pots were watered and moved to a glasshouse (natural daylight, ~1800 mmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia). Plants were grown between May–June 2022. Pots were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes 2006). Soil moisture was maintained at 80–100% field capacity by watering daily to a predetermined weight using distilled water.

Harvest and measurements

Plants were harvested after seven weeks' growth. Shoots were cut at the soil surface and oven-dried at 70°C for 72 h and weighed. Shoot samples were then finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). Tissue P, K and S concentrations were determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Agilent, Mulgrave, Australia). Shoot P, K and S contents were calculated by multiplying the tissue concentrations and shoot dry mass.

Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'species' and 'nutrient treatment' as the predictor variables.

When appropriate, the effect of 'replicate' was included in the most parsimonious model. Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot dry mass

The shoot dry mass of each pasture species was relatively low in the nil treatment that received no addition of P, K or S (Fig. 63). Each of the species responded to the addition of P (i.e. the P, P/K, P/S and P/K/S treatments) ($P < 0.001$). For example, between the nil and +P treatments there was a 4.6-fold increase in yield for Digit and a 2.1–3.0-fold increase in yield for the *Desmanthus* spp. genotypes. In contrast to P, the pasture species did not respond to the addition of either K or S. On average across the nutrient treatments, the largest shoot yields were produced by JCU 9 Desmanthus and Digit ($P < 0.001$).

Shoot P

Shoot P concentrations increased in response to the addition of P, but not in response to the addition of either K or S ($P < 0.001$; Fig. 64). Between the nil and +P treatments, there was a 1.6-fold increase in shoot P concentration for Digit and a 2.0–2.3-fold increase in shoot P concentration for the *Desmanthus* spp. genotypes. On average across the nutrient treatments, the shoot P concentrations of the species were; JCU 4 Desmanthus > JCU 2 Desmanthus > JCU 9 Desmanthus > Digit = JCU 7 Desmanthus. Shoot P contents reflected differences in shoot dry mass and shoot P concentration, with differences across nutrient treatments ($P < 0.001$) and between the pasture species ($P < 0.001$) (data not shown).

Shoot K

Shoot K concentrations did not respond to any of the nutrient treatments ($P = 0.372$; Fig. 65). Nevertheless, there were significant differences in shoot K concentration among the pasture species ($P < 0.001$). On average across the treatments, the shoot K concentrations of the species were; Digit > JCU 4 Desmanthus > JCU 2 Desmanthus > JCU 9 Desmanthus > JCU 7 Desmanthus. Shoot K contents reflected differences in shoot dry mass and shoot K concentration, with differences across nutrient treatments ($P < 0.001$) and between the pasture species ($P < 0.001$) (data not shown).

Shoot S

Shoot S concentrations either increased or decreased, compared to the nil treatment, in response to the addition of the different nutrient treatments ($P < 0.001$; Fig. 66). The shoot P concentrations of the nutrient treatments were; S = K/S > P/S = P/K/S > K = nil > P/K = P. On average across the nutrient treatments, the shoot S concentrations of the species were; JCU 4 Desmanthus > JCU 2 Desmanthus > JCU 9 Desmanthus > JCU 7 Desmanthus > Digit. Shoot S contents reflected differences in shoot dry mass and shoot S concentration, with differences across nutrient treatments ($P < 0.001$) and between the pasture species ($P < 0.001$) (data not shown).

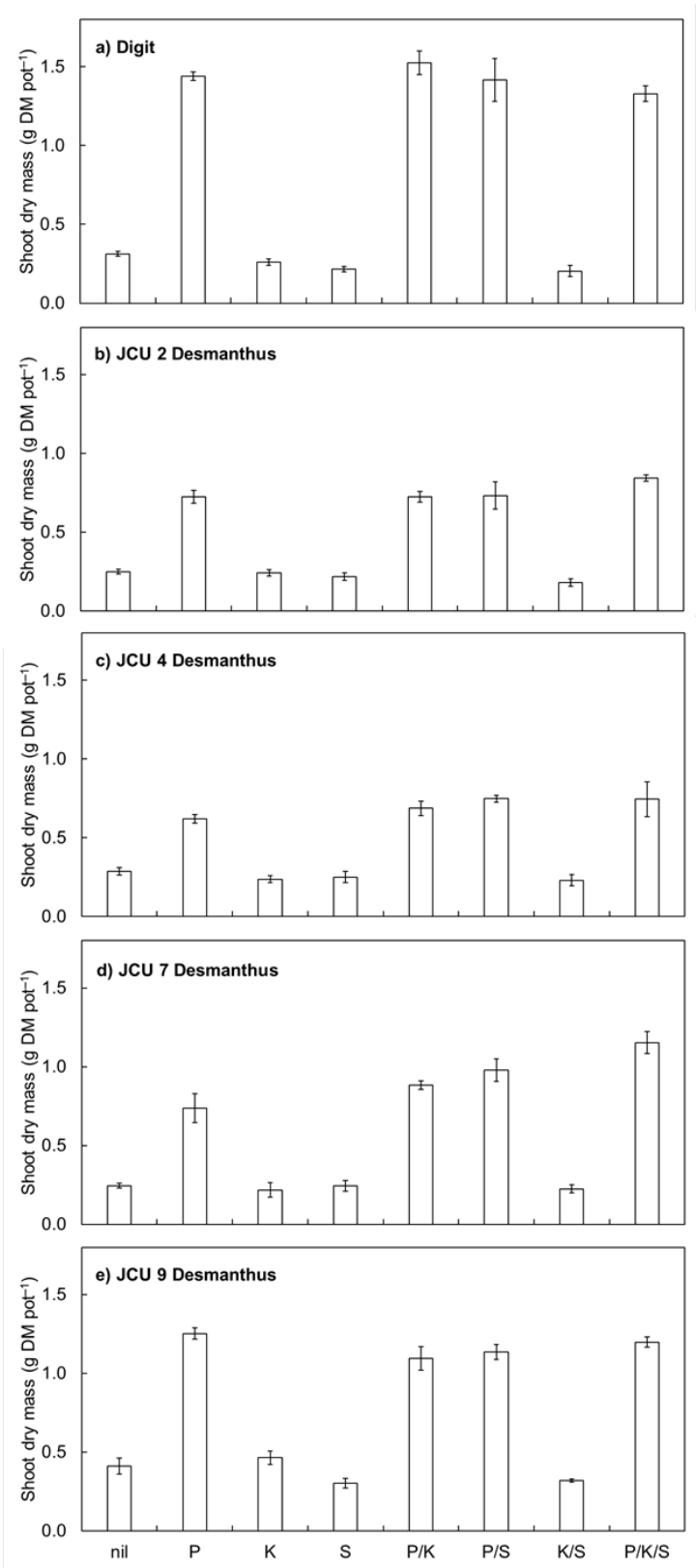

Figure 63. Shoot dry mass of five tropical pasture species grown in response to eight nutrient treatments that were combinations of P, K and S. Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, nutrient treatment P < 0.001, species × nutrient treatment interaction P < 0.001.

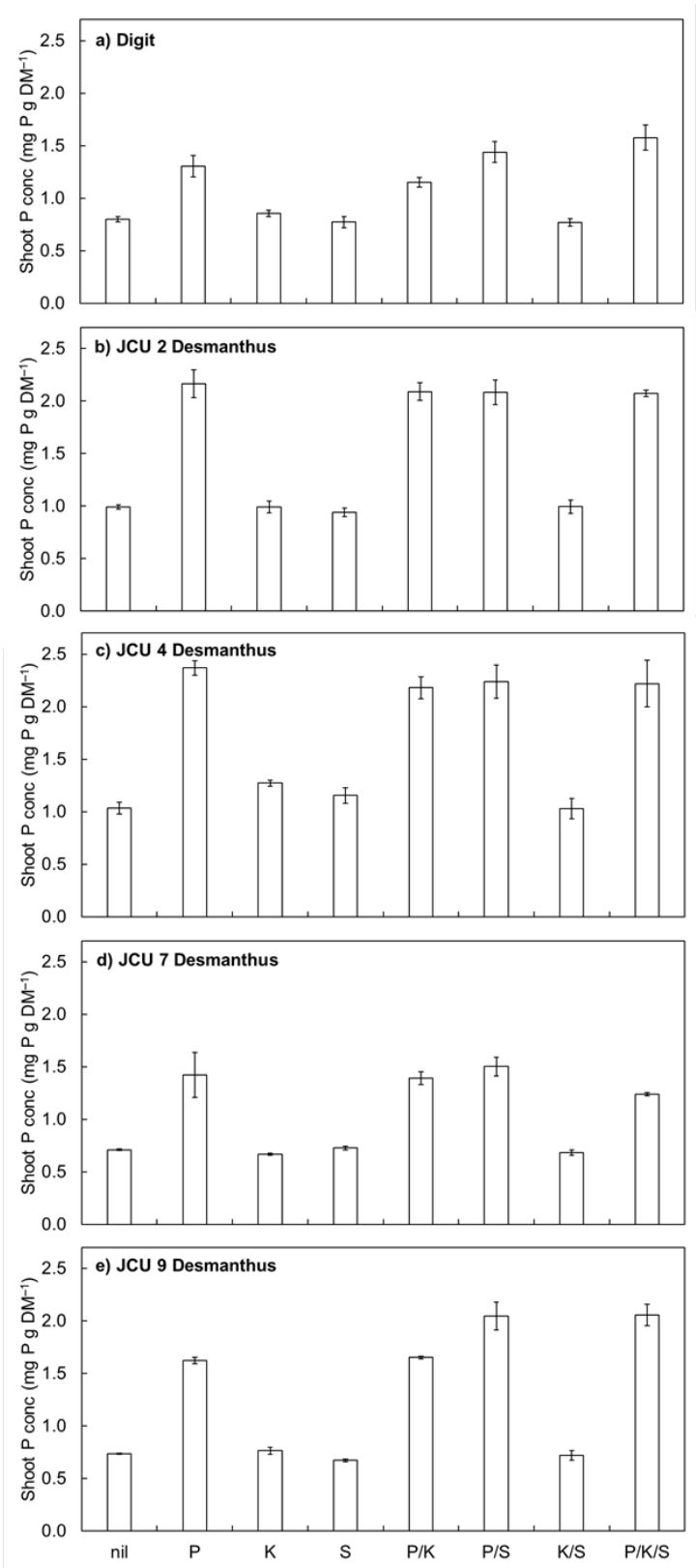

Figure 64. Shoot P concentrations of five tropical pasture species grown in response to eight nutrient treatments that were combinations of P, K and S. Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, nutrient treatment P < 0.001, species × nutrient treatment interaction P < 0.001.

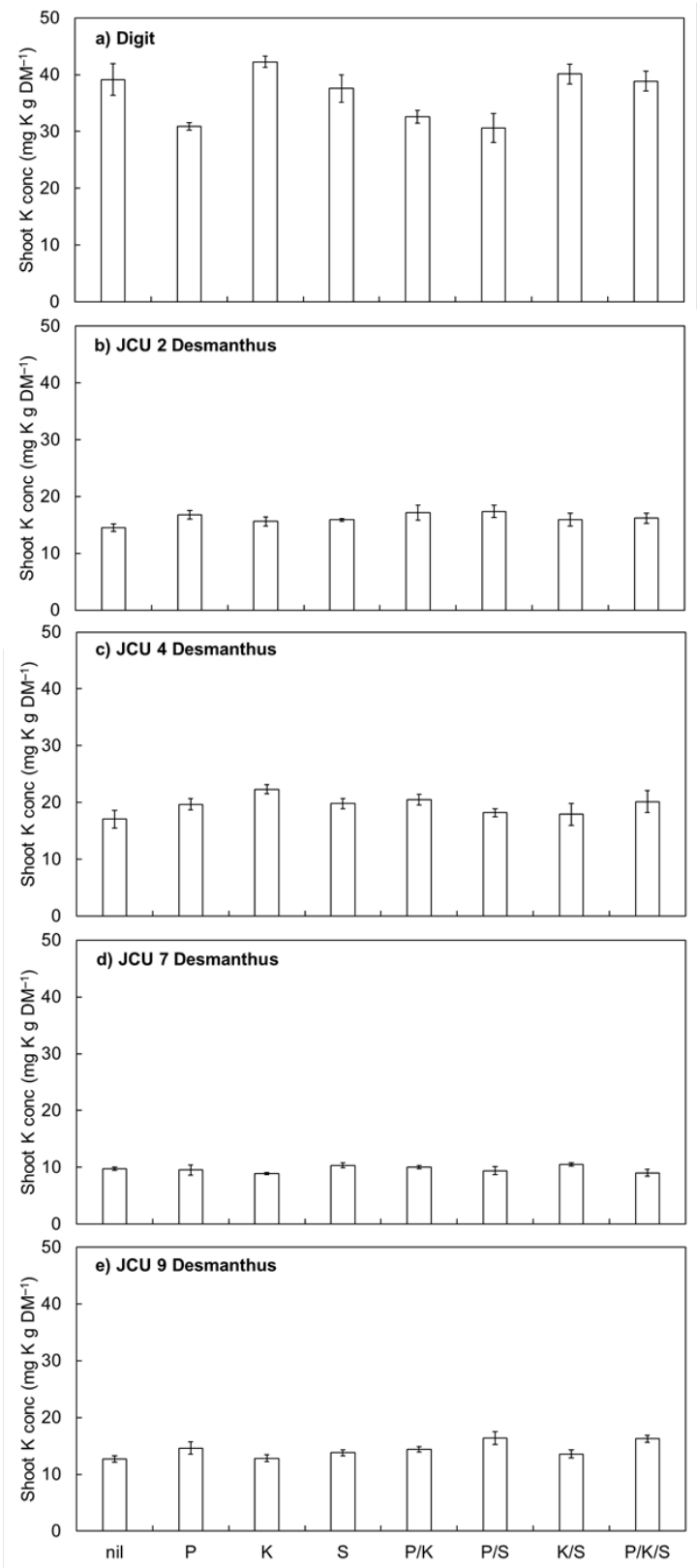

Figure 65. Shoot K concentrations of five tropical pasture species grown in response to eight nutrient treatments that were combinations of P, K and S. Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, nutrient treatment P = 0.372, species × nutrient treatment interaction P < 0.001.

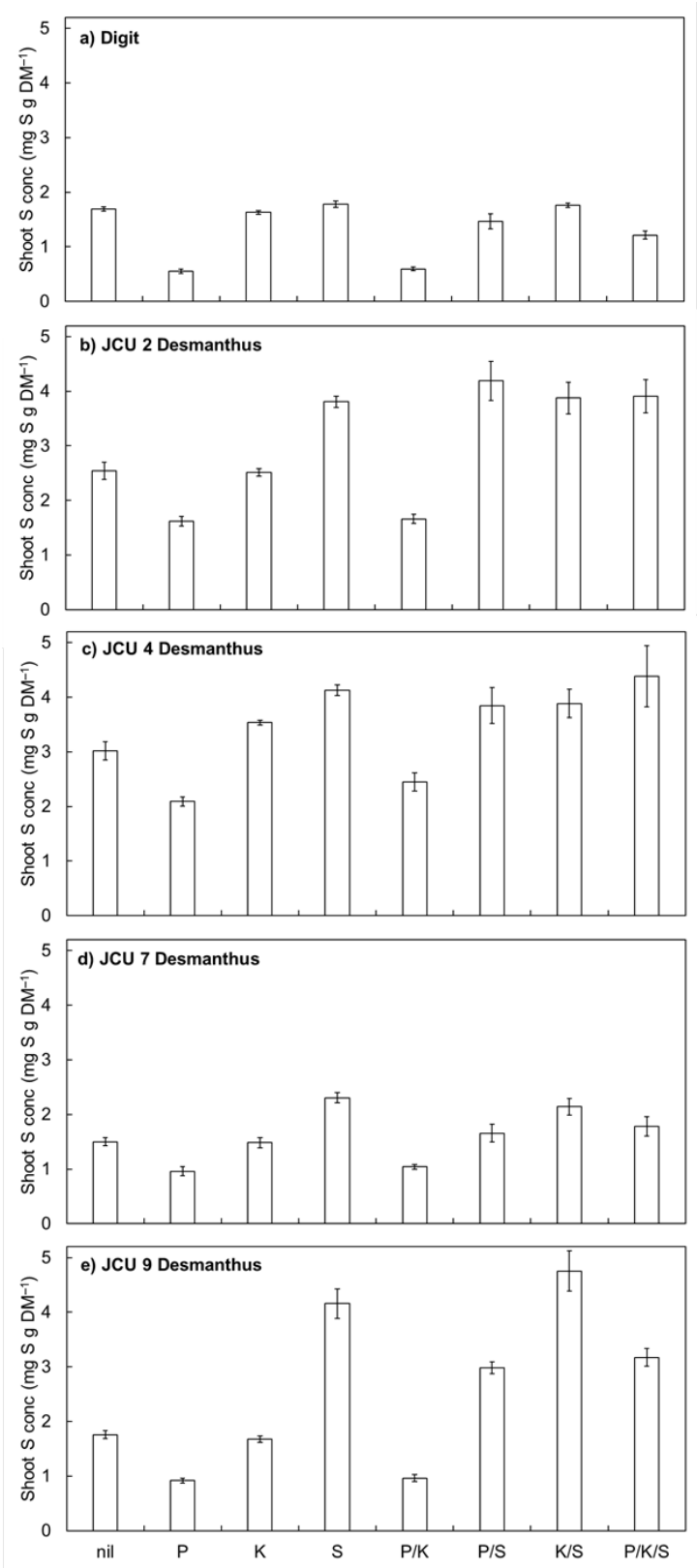

Figure 66. Shoot S concentrations of five tropical pasture species grown in response to eight nutrient treatments that were combinations of P, K and S. Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, nutrient treatment P < 0.001, species × nutrient treatment interaction P < 0.001.

Practical implications

The collective results demonstrate that, in this particular soil, the addition of P was the most important nutrient for increasing the shoot yield of both Digit and Desmanthus. This is unsurprising given the Colwell extractable P concentration of the soil was only 3 mg P kg^{-1}, which is expected to be below the critical P requirements of the species as determined in previous research. The addition of P also had a beneficial effect on the shoot P concentrations of the pasture species. The application of P fertiliser is therefore likely to improve the productivity and quality of tropical pastures that are grown in the nutrient-deficient soils of northern Australia. Nevertheless, the addition of P generally had a negative effect on shoot S concentrations, even though this did not lead to a yield penalty. This result suggests that fertiliser containing both P and S (e.g. single superphosphate) would be appropriate in nutrient-deficient soils for maintaining biomass quality, particularly for some *Desmanthus* spp. genotypes that tend to accumulate S in their tissue.

8.17 Appendix 17 – Sulfur response curves for Premier Digit and two *Desmanthus* spp. genotypes

Materials and Methods

Plant growth conditions

Three tropical pasture species were grown to investigate shoot yield and tissue nutrient concentrations in response to P and S. The pasture species were JCU 7 Desmanthus (*D. leptophyllus* cv. JCU 7), JCU 9 Desmanthus (*D. pernambucanus* cv. JCU 9) and Digit (*Digitaria eriantha* cv. Premier). A clay loam soil was collected from Gregory Downs Station, Gregory, QLD, Australia. The soil had a Colwell extractable P concentration of 3 mg P kg^{-1} and a KCl-40 concentration of 3 mg S kg^{-1}. The soil was dried, crushed by hand and homogenised. The soil was then amended with a basal nutrient solution that included 30 mg N kg^{-1} (as CH4N2O) and 1 mg kg^{-1} Librel BMX (which contains boron, copper, iron, manganese, molybdenum and zinc). Two P treatments were prepared by adding either 10 or 40 mg P kg^{-1} (as KH_2PO_4) to the soil, with the K in the P10 treatment balanced using KCl. Cylindrical PVC pots (87 mm internal diameter, 200 mm height) were filled with 1.3 kg (oven-dry basis) of the amended soils. The total depth of soil was ~190 mm. Eight S treatments were then prepared by adding $CaSO_4$. $2H_2O$ to the soil surface at the following rates: 0, 2, 4, 6, 8, 12, 16 and 20 mg S kg^{-1} soil (hereafter referred to as S0, S2, S4, S6, S8, S12, S16 and S20, respectively).

Micro-swards of the pasture species were established by sowing seed to achieve a target density of 5 plants pot^{-1}. Two replicate pots of each species were prepared for each P and S treatment combination. After planting, the pots were moved to a glasshouse (natural daylight; 30/25ºC, day/night) in Armidale, NSW, Australia. Plants were grown between March–May 2022. Pots were arranged in a randomised complete block design (blocks comprised the different replicates). Soil moisture was maintained at 80% field capacity by watering daily to a predetermined weight. An additional application of 30 mg N kg^{-1} was applied after five weeks' growth due to signs of N deficiency in the Digit grass.

Harvest and analysis

Plants were harvested after eight weeks' growth. Shoots were cut at the soil surface and were oven-dried at 70ºC for 72 h and weighed. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were digested using a Milestone UltraWAVE 640. The S concentration of the digested samples was determined using ICP-OES. Shoot S content was calculated by multiplying shoot S concentration and shoot dry mass. Measured parameters were analysed using R (R Core Team, 2020). Critical external S requirements were calculated as the amount of S required to achieve 90% of maximum yield based on a Weibull growth function, with the 95% confidence intervals determined by bootstrapping the residuals. Critical internal S requirements were calculated as the shoot P concentrations that corresponded with the critical external S requirements.

Results and Discussion

Shoot yield and critical external S requirements

Shoot dry mass increased in response to the application of S for Digit and the two *Desmanthus* spp. genotypes, but only between the S0 and S4 treatments (Fig. 67). Digit was more productive than both of the Desmanthus spp. genotypes. There was no shoot yield response to P application rate. Critical external S requirements ranged between 1.5–1.8 mg S kg^{-1} soil for Digit, and between 2.4–3.7 mg S kg^{-1} soil for the two Desmanthus spp. genotypes (Table 19).

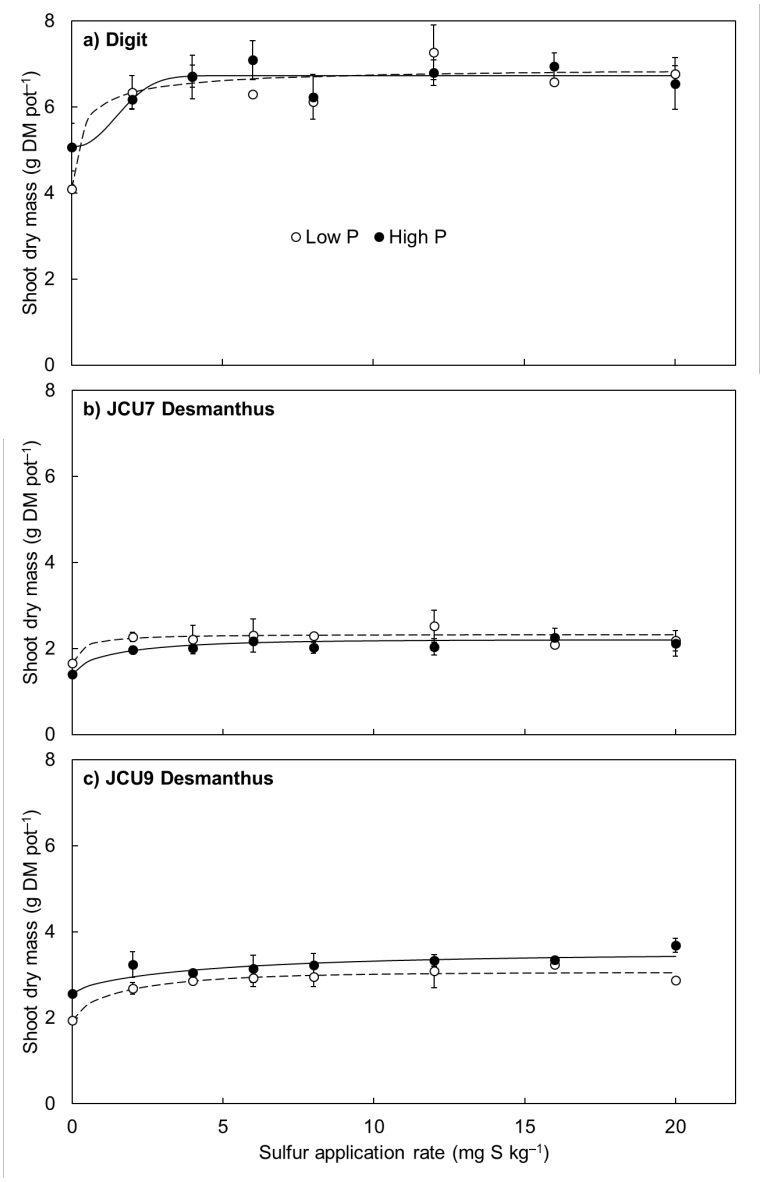

Figure 67. Shoot dry mass of Digit, JCU 7 Desmanthus and JCU 9 Desmanthus at two levels of P (10 or 40 mg P kg^{-1}) when grown in response to eight rates of S (0–8 mg S kg^{-1}) . Values show the mean ± s.e. (n = 2). The curves that were fitted to the shoot yield data show Weibull growth functions (Crawley, 2013).

Table 19. The critical external and internal S requirements of Digit, JCU 7 Desmanthus and JCU 9 Desmanthus in low and high-P soil. Critical external S requirements were calculated as the amount of S applied to achieve 90% maximum yield, with 95% confidence intervals shown in parentheses. Critical internal S requirements were the shoot S concentrations that corresponded with the critical external S requirements. * shows the values that could not be calculated.

Species Low/High P	**Critical external S requirement (mg S kg^{-1} soil)**	**Critical internal S requirement (mg S g^{-1} DM)**
Digit		
Low-P	**1.5** (*–2.7)	**0.73**
High-P	**1.8** (*–2.9)	**0.79**
JCU7 Desmanthus		
Low-P	*	*
High-P	**2.4** (*–4.1)	**1.95**
JCU9 Desmanthus		
Low-P	**2.7** (1.4–4.7)	**1.60**
High-P	**3.7** (2.2–5.4)	**1.82**

Shoot S concentration and critical internal S requirements

Shoot S concentrations increased on response to the application of S for Digit and the two Desmanthus spp. genotypes (Fig. 68). The two Desmanthus spp. genotypes accumulated more shoot S than Digit, which was reflected by the higher critical internal S requirements of the legume compared to the grass (Table 19).

Practical implications

Even though the species only responded to S in the lowest S treatments, there was still a significant difference in critical external and internal S requirements between Digit and Desmanthus. This indicates that S should be considered when fertilising tropical pastures that contain Desmanthus.

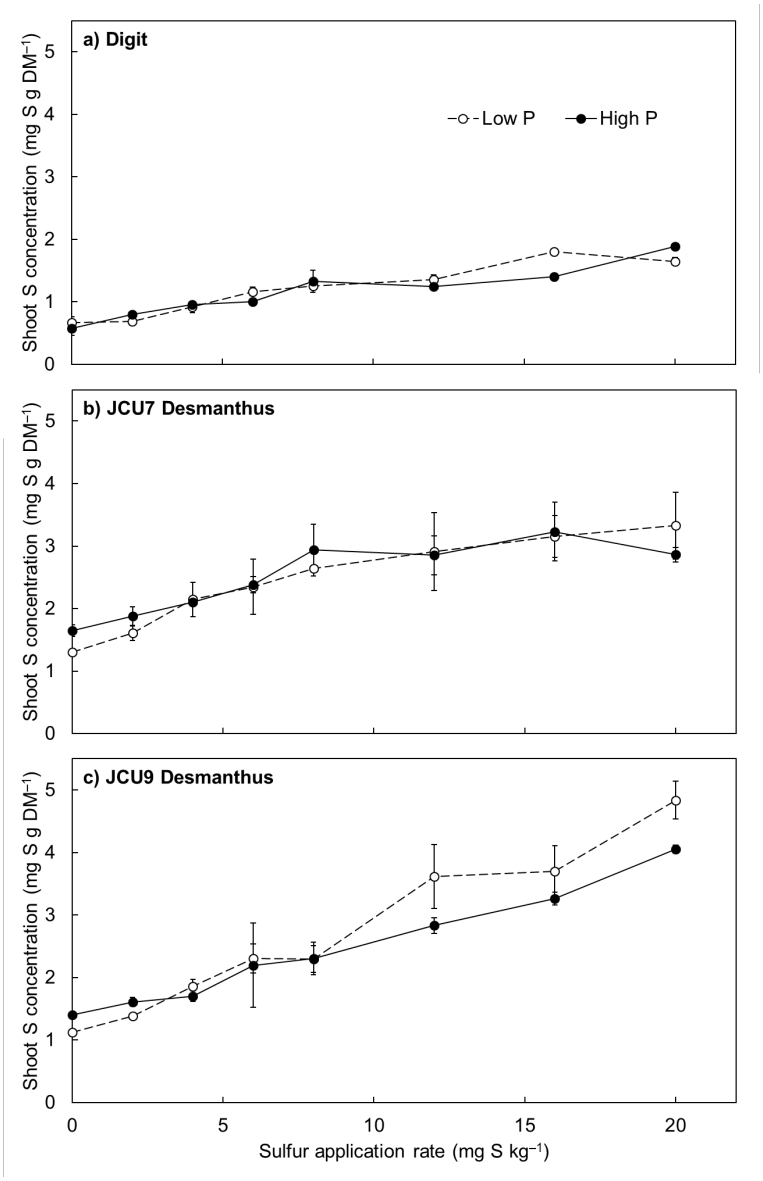

Figure 68. Shoot S concentration of Digit, JCU 7 Desmanthus and JCU 9 Desmanthus at two levels of P (10 or 40 mg P kg^{-1}) when grown in response to eight rates of S (0–8 mg S kg^{-1}) . Values show the mean ± s.e. (n = 2).

8.18 Appendix 18 – Phosphorus acquisition by tropical pasture species in response to watering method

Materials and Methods

Plant material

Four tropical pasture species were grown to determine the effect of watering method on shoot yield and tissue P when plants were grown in four soil-P distribution treatments. The pasture species were Digit (*Digitaria eriantha* cv. Premier), Rhodes (*Chloris gayana* cv. Katambora), Desmanthus (*Desmanthus pernambucanus* cv. JCU 9) and Centro (*Centrosema pubescens* cv. Cardillo). These species are commonly grown in northern Australia.

Soil and nutrient treatments

A sandy loam soil (Brown Chromosol; Isbell 1996) was collected from the upper 2–15 cm soil layer of a field at Kirby SMART Farm, Armidale, NSW, Australia (30°25'35.0"S 151°39'08.4"E). The soil had a Colwell extractable P concentration of 7 mg P kg^{-1} (as measured by the method of Colwell (1963)), a Phosphorus Buffering Index (PBI) of 51 (as measured by the modified method of Burkitt et al. (2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of ~4.6. The soil was passed through a 5 mm sieve and lime (0.5 g $CaCO_3$ kg^{-1}) was mixed through the soil to raise the pH to ~4.8. While mixing the soil, basal nutrients were applied as a solution including 34 mg kg^{-1} soil $MgSO_4.7H_2O$, 36 mg kg^{-1} $CaSO_4.2H_2O$, 141 mg kg^{-1} KNO_3, 23 mg kg^{-1} $(NH_4)_2SO_4$, 14 mg kg^{-1} NH_4NO_3, 99 µg kg^{-1} H_3BO_3, 635 µg kg^{-1} $MnCl_2.4H_2O$, 301 µg kg^{-1} $ZnSO_4.7H_2O$, 28 µg kg^{-1} $CuSO_4.5H_2O$, 60 µg kg^{-1} $(NH_4)_2MoO_4$, 17 µg kg^{-1} $CoCl_2{\cdot}6H_2O$ and 1283 µg kg^{-1} FeNa-EDTA. Four P-amended soils (0, 35, 135 and 300 mg P kg^{-1}) were prepared by adding KH_2PO_4 to the nutrient solution before the soil was amended. KCl was also applied to the 0 mg P kg^{-1} treatment to balance the K to be equivalent to that of the 35 mg P kg^{-1} treatment. After the addition of lime and nutrients, the Colwell extractable P concentrations of the soils were: 7, 33, 113 and 275 mg Colwell P kg^{-1}.

The four amended soils were used to prepare four contrasting soil-P distribution treatments (uniform low-P, uniform high-P, surface high-P and banded high-P), as shown in Figure 69. The three high-P treatments were each intended to achieve a comparable average Colwell extractable P concentration throughout the soil volume, based on the proportion of each amended soil used in the three soil layers. The calculated average Colwell extractable P concentrations were: 33, 33 and 35 mg Colwell P kg^{-1} for the uniform high-P, surface high-P and banded high-P treatments, respectively. The surface high-P treatment provided a stratified soil profile with P concentrated in the topsoil layer, while the banded high-P treatment provided a concentrated source of P that mimicked a shallow band of P fertiliser below the soil surface. The uniform high-P treatment provided a positive control whereas the uniform low-P treatment provided a negative control. These soil-P distribution treatments were prepared in cylindrical PVC pots (87 mm internal diameter; 200 mm height) using three layers of soil; a 'subsoil' layer (840 g oven-dry soil; 123 mm soil height), a 'band' layer (140 g oven-dry soil; 20 mm soil height) and a 'topsoil' layer (320 g oven-dry soil; 47 mm soil height). Alkathene beads were placed around the interior edge of these pots to mark the interface between each of the soil layers.

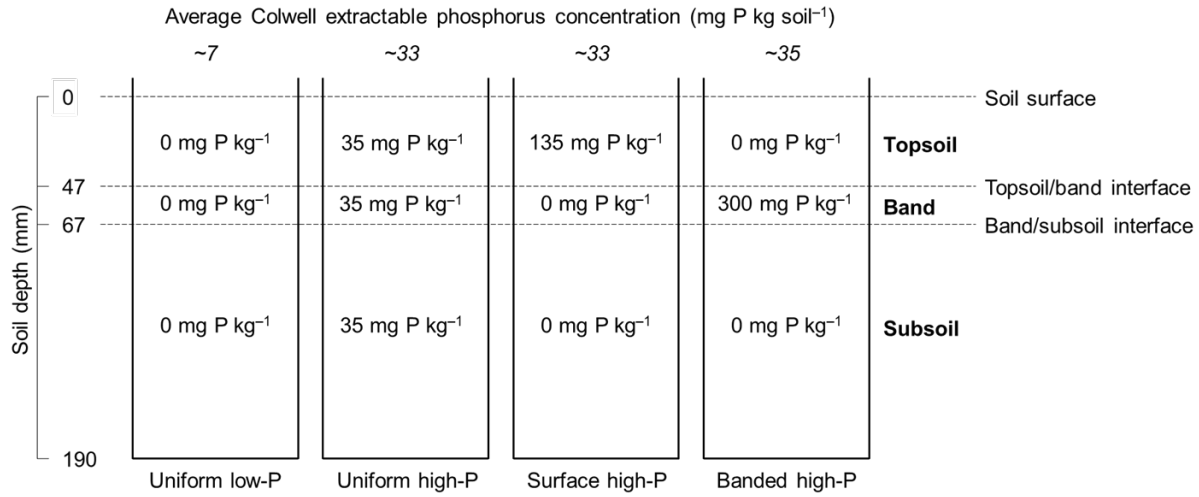

Figure 69. Diagrammatic illustration of the four soil-P distribution treatments used to investigate the interaction between P and water among four tropical pasture species. Thick lines represent the pot confine and dashed lines represent the soil surface and soil layer interfaces. The 0, 35, 135 and 300 mg P kg^{-1} labels depict the P applied to each soil layer (i.e. topsoil, band and subsoil). These four P application rates resulted in Colwell extractable P concentrations of: 7, 33, 113 and 275 mg Colwell P kg^{-1}, respectively. The average Colwell extractable P concentration throughout the soil volume of each treatment (i.e. uniform low-P, uniform high-P, topsoil high-P and banded high-P) was calculated based on the proportion of amended soil and the corresponding Colwell extractable P concentration applied to each soil layer.

Plant growth conditions

Micro-swards of each species were established by sowing seed (~5 mm depth) to achieve a density of 4 plants pot^{-1}. Prior to sowing, the Desmanthus seeds were heat-treated to break dormancy and increase germination rates. Four replicate pots of each species in each P treatment and watering treatment (as described below) were prepared. After planting, the pots were watered and moved to a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25ºC, day/night) in Armidale, NSW, Australia. Humidifiers were used to increase the humidity within the glasshouse to ~50%. Plants were grown between May–July 2020. Pots were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006). Soil moisture was initially maintained at 80–100% field capacity by watering daily to a predetermined weight on the soil surface. After three weeks, the plants were watered daily to 80% field capacity by applying water either to the soil surface or to the base of the pots. After a further two weeks, the watering rate was increased to 90% field capacity, with water applied to either the soil surface or to the base of the pots. An additional 50 mg N kg soil^{-1} as CH_4N_2O was applied to the surface of each pot six weeks after planting due to signs of N deficiency.

Harvest and measurements

Plants were harvested after eight weeks of growth. Shoots were cut at the soil surface and rinsed to remove any soil. Shoots were then oven-dried at 70°C for 72 h and weighed. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass.

Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with 'species', 'P treatment' and 'water treatment' as predictor variables. When appropriate, the effect of 'rep' was included in the most parsimonious model. Means were compared using Tukey's honest significant differences (HSD). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. The linear models that were fitted to shoot dry mass and shoot P content were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield

Shoot dry mass was influenced by soil-P supply because the largest shoot yields were achieved in the three high-P treatments, regardless of whether P fertiliser was uniformly distributed throughout the soil profile or applied in a localised manner (i.e. the surface high-P and banded high-P treatments) ($P < 0.001$; Fig. 70). Shoot dry mass was also influenced by watering treatment because the pasture species generally produced larger shoot yields when water was applied to the surface of the pots ($P < 0.001$; Fig. 70). Regardless of these treatment effects, there were significant varietal differences in shoot yield ($P < 0.001$; Fig. 70). On average, the grasses produced larger shoot yields than the legumes (Rhodes > Digit > Centro > Desmanthus).

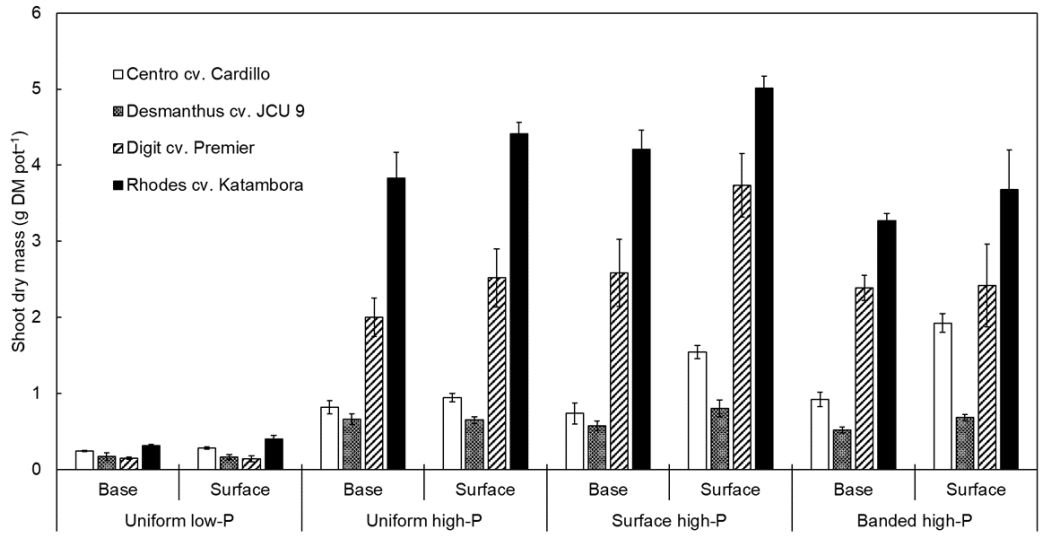

Figure 70. The shoot dry mass of four tropical pasture species grown in response to four soil-P distribution treatments and two watering treatments. Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, water treatment P < 0.001, species × P treatment interaction P < 0.001, species × water treatment interaction P = 0.034, P treatment × water treatment interaction P = 0.033. The 3-way interaction was not significant (P = 0.342).

Shoot P

Shoot P concentration was influenced by P treatment, with the largest concentrations achieved in the uniform high-P and banded high-P treatments, followed by the surface high-P treatment then the uniform low-P treatment ($P < 0.001$; Fig. 71). Shoot P concentration was also influenced by watering treatment because the pasture species generally produced larger concentrations when water was applied to the surface of the pots ($P < 0.001$; Fig. 71). On average, the largest shoot P concentrations were achieved by Desmanthus and Digit, and the smallest was achieved by Rhodes, with the shoot P concentration of Centro being intermediate ($P < 0.001$; Fig. 71).

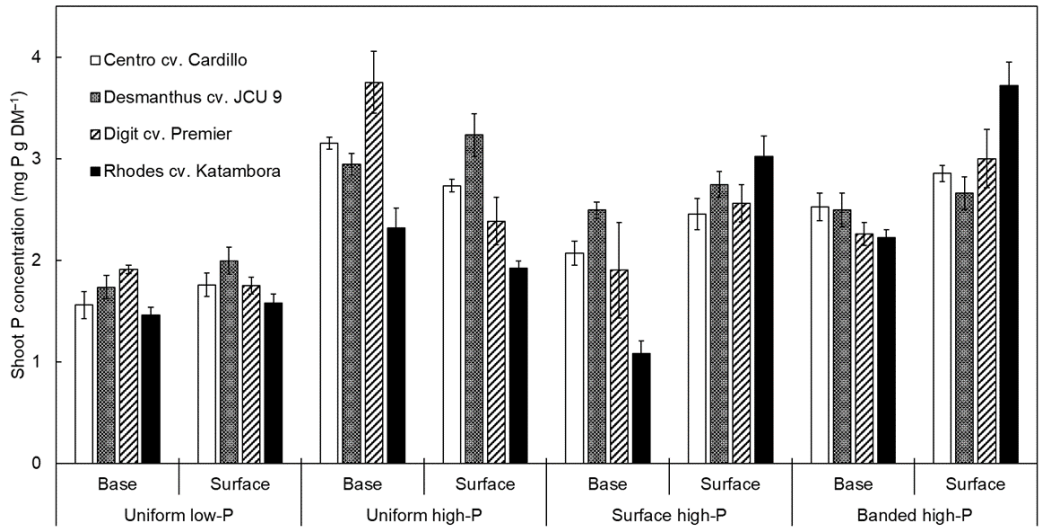

Figure 71. The shoot P concentration of four tropical pasture species grown in response to four soil-P distribution treatments and two watering treatments. Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, water treatment P < 0.001. The 2-way and 3-way interactions were also significant (P < 0.001).

Shoot P content responded to the P treatments and watering treatments in a similar manner to shoot dry mass (Fig. 72); the largest shoot P contents were achieved in the high-P treatments ($P < 0.001$) and when water was applied to the surface of the pots ($P < 0.001$). However, there was a significant P treatment × water treatment interaction because the difference in shoot P content between watering treatments was most apparent in the localised P treatments (i.e. the surface high-P and banded high-P treatments) ($P < 0.001$; Fig. 72). On average, the grasses produced larger shoot P contents than the legumes (Rhodes > Digit > Centro > Desmanthus).

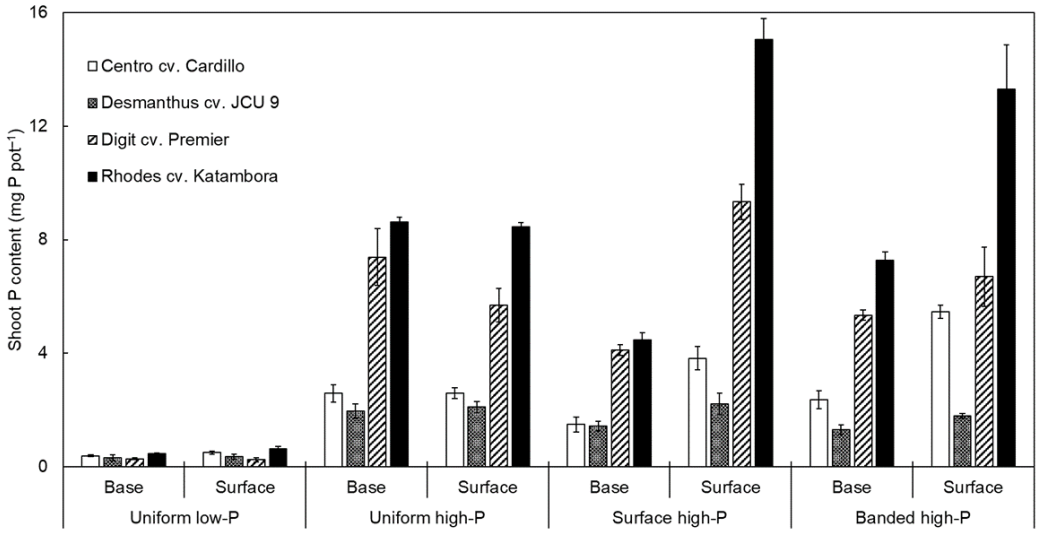

Figure 72. The shoot P content of four tropical pasture species grown in response to four soil-P distribution treatments and two watering treatments. Values show the mean ± s.e. (n = 4). Analysis of variance results were: species P < 0.001, P treatment P < 0.001, water treatment P < 0.001, species × P treatment interaction P < 0.001, species × water treatment interaction P = 0.001, P treatment × water treatment interaction P < 0.001. The 3-way interaction was not significant (P = 0.420).

Practical implications

Watering method generally did not influence the P acquisition and shoot yield of the four pastures when grown in the uniform low-P and high-P treatments. The species were generally more productive when watered from the surface in the surface high-P treatment, and Centro and Rhodes were more productive when watered from the surface in the banded high-P treatment. It was expected that watering from the base would have improved the P acquisition and shoot yield of the species in the banded high-P treatment, due to the higher interaction between available P and water. However, the species may have produced enough roots within the relatively small pots to negate this benefit. Nevertheless, it is expected that the interaction between P and water is likely to be important in the soils of northern Australia, where broadcast applications of P fertiliser may be constrained by regular moisture deficit in the soil surface.

8.19 Appendix 19 – Preferential water drawdown in the vicinity of banded fertiliser reduces legume productivity

Materials and Methods

Plant material

Two tropical pasture species were grown to determine the effect of preferential water drawdown within the vicinity of banded P fertiliser on shoot yield and P acquisition. The pasture species were Digit (*Digitaria eriantha* cv. Premier) and Desmanthus (*Desmanthus pernambucanus* cv. JCU9).

Soil and nutrient treatments

A clay soil (Red Ferrosol; Isbell 1996) was collected from a field at Kingaroy, QLD, Australia. The soil had a Colwell extractable P concentration of 12, a Phosphorus Buffering Index (PBI) of 385, and a pH (1:5 w/v; 0.01 M $CaCl_2$) of 4.6. The soil was passed through a 5 mm sieve and lime (1 g $CaCO_3$ kg^{-1}) was mixed through the soil to raise the pH. While mixing the soil, basal nutrients were applied as a solution including 34 mg kg^{-1} soil $MgSO_4.7H_2O$, 36 mg kg^{-1} $CaSO_4.2H_2O$, 141 mg kg^{-1} KNO_3, 23 mg kg^{-1} $(NH_4)_2SO_4$, 14 mg kg^{-1} NH_4NO_3, 99 µg kg^{-1} H_3BO_3, 635 µg kg^{-1} $MnCl_2.4H_2O$, 301 µg kg^{-1} $ZnSO_4.7H_2O$, 28 µg kg^{-1} $CuSO_4.5H_2O$, 60 µg kg^{-1} $(NH_4)_2MoO_4$, 17 µg kg^{-1} $CoCl_2{\cdot}6H_2O$ and 1283 µg kg^{-1} FeNa-EDTA. Two P-amended soils (26.8 and 142.5 mg P kg^{-1} soil) were prepared by adding KH_2PO_4 to the nutrient solution before the soil was amended.

The soils were used to prepare two contrasting soil-P distribution treatments. The uniform P treatment included three layers of 26.8 mg P kg^{-1} soil while the banded P treatment included a layer each of 26.8, 142.5 and 26.8 mg P kg^{-1} soil. These soil-P distribution treatments were prepared in cylindrical PVC pots (150 mm internal diameter; 290 mm height); a 'subsoil' layer (2850 g oven-dry soil; 150 mm soil height), a 'band' layer (950 g oven-dry soil; 50 mm soil height) and a 'topsoil' layer (1330 g oven-dry soil; 70 mm soil height). Small PVC pipe (30 mm internal diameter) that had numerous holes was placed down the centre of the pot and filled with coarse sand, to facilitate water movement down the soil profile when watering. Half of the pots had a 10 mm layer of coarse sand placed at the interface of the soil layers, to act as a capillary break and stop water movement between the soil layers. The other half of the pots did not have any coarse sand between the soil layers, but the sand was placed at the bottom of the soil profile to keep soil volume consistent.

Plant growth conditions

Micro-swards of Digit and Desmanthus were established by sowing seed (~5 mm depth) to achieve a density of 16 plants pot^{-1}. Four replicate pots of each species in each P treatment both with and without the coarse sand capillary break were prepared. After planting, the pots were watered and moved to a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25ºC, day/night) in Armidale, NSW, Australia. Pots were arranged in a randomised complete block design (blocks comprised the different replicates) which was generated using DiGGer (Coombes, 2006). Soil moisture was initially maintained at 80–100% field capacity by watering daily to a predetermined weight on the soil surface. Following this, the soil profile was watered to 100% field capacity and then watering ceased to allow for water drawdown within the soil profile. The soil profiles were dried until the first plants showed signs of wilting, before the pots were watered to 100% field capacity and then the process of dried started again. This process was repeated four times during the experiment.

Harvest and measurements

The Digit plants were harvested after 8 weeks of growth and the Desmanthus plants were harvested after 9 weeks of growth (the Desmanthus plants grew much slower than the Digit). Shoots were cut at the soil surface and rinsed to remove any soil. Shoots were then oven-dried at 75°C for 72 h and weighed. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 4 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. . Digested shoot samples were also analysed for ^{32}P- and ^{33}P-radioisotope activity using a PerkinElmer Tri-Carb 2810TR (PerkinElmer, Waltham, United States of America). A scintillation cocktail was prepared by mixing 3 mL of sample with 17 mL of scintillant (PerkinElmer UltimaGold AB). All samples were analysed by liquid scintillation counting (LSC) for 5 min and were corrected for radioactive decay and dilution. The ^{32}P- and ^{33}P-radioisotope counts were used to calculate the specific activity of the shoot material, the amount of plant P derived from applied P, and the recovery of applied P as follows:

$$Specific\ activity = \frac{radioactivity\ in\ shoots}{total\ P\ in\ shoots} \quad (1)$$

$$Plant\ P\ derived\ from\ applied\ P = \frac{specific\ activity\ of\ shoots}{specific\ activity\ of\ applied\ P} \times 100 \quad (2)$$

$$Recovery\ of\ applied\ P\ fertiliser = \frac{applied\ P\ in\ shoots}{total\ applied\ P} \times 100 \quad (3)$$

Pots were stored in a cool room until the radioisotope had decayed, at which point roots were washed from the soil over 2 mm sieves. Roots were then oven-dried at 70°C for 72 hr and weighed.

Statistical analyses

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with ‘species’ and ‘P treatment’ as predictor variables. When appropriate, the effect of ‘rep’ was included in the most parsimonious model. Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. The linear models that were fitted to shoot dry mass and shoot P content were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield

Digit out-yielded Desmanthus in each of the treatments (Fig. 73), even though both species were planted at the same density and the Desmanthus plants were grown for an extra week. On average, the shoot yields of Digit and Desmanthus were higher in the banded-P treatments compared to the uniform-P treatments. The capillary break did not influence the growth of Digit, but it did influence the growth of Desmanthus. The legume was more productive without the coarse sand capillary break.

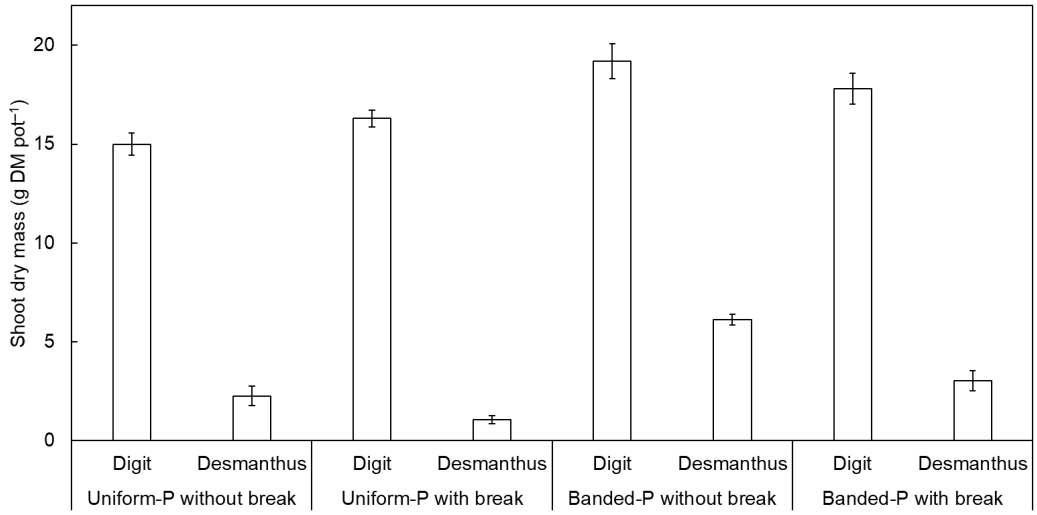

Figure 73. Shoot dry mass of Digit and Desmanthus grown in response to uniformly distributed or banded P fertiliser, both with and without a coarse sand capillary break that restricted water movement into the band layer. Values show the mean ± s.e. (n = 4).

Shoot P

The shoot P concentrations of Desmanthus were higher than that of Digit in each of the soil treatments (Fig. 74). There were no clear trends in the accumulation of shoot P across the different soil treatments.

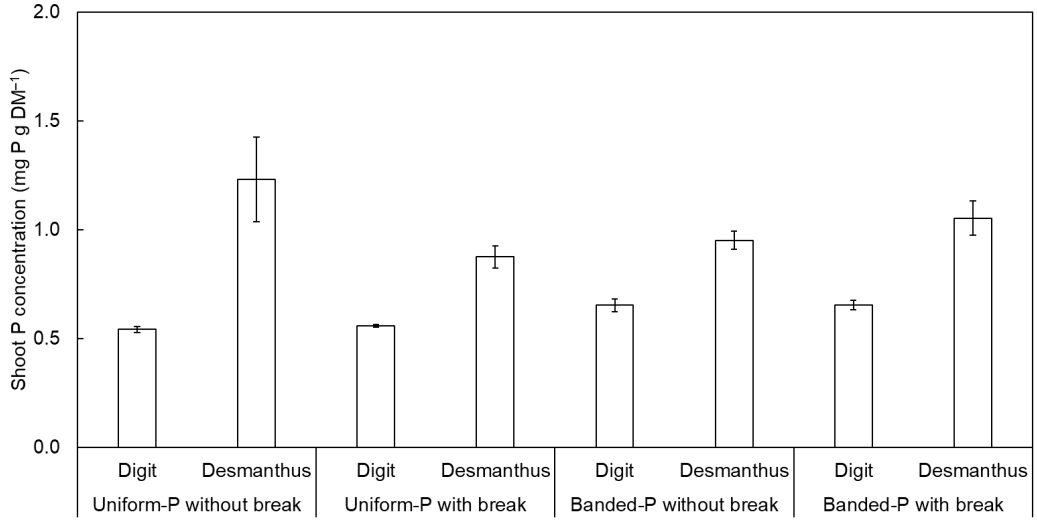

Figure 74. Shoot P concentration of Digit and Desmanthus grown in response to uniformly distributed or banded P fertiliser, both with and without a coarse sand capillary break that restricted water movement into the band layer. Values show the mean ± s.e. (n = 4).

Root traits

Digit produced more roots than Desmanthus, as indicated by the larger root mass density (i.e. root mass per unit soil volume) of the grass (Fig. 75). Considering the roots of Digit are often longer and thinner than that of Desmanthus, this difference in root mass density is likely to mean a substantial difference in the development of root length for P acquisition. In general, both species did not proliferate root length in response to the banded-P treatments.

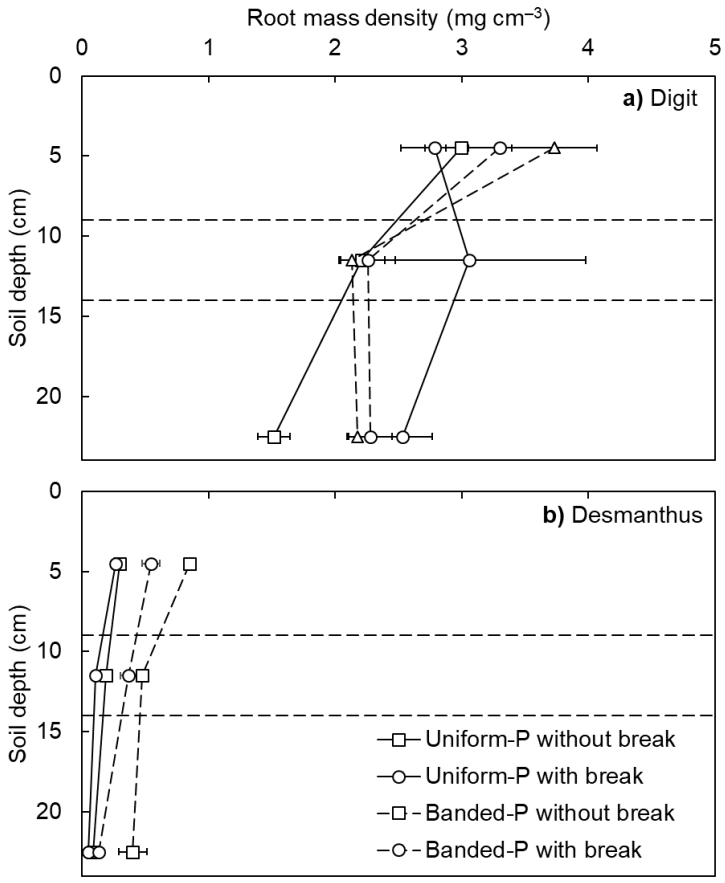

Figure 75. Root mass density in the topsoil, band and subsoil layers of Digit and Desmanthus grown in response to uniformly distributed or banded P fertiliser, both with and without a coarse sand capillary break that restricted water movement into the band layer. Soil depth values correspond to the depth below the soil surface of the mid-point of each soil layer (i.e. topsoil = 4.5 cm, band = 11.5 cm and subsoil = 22.5 cm) and the horizontal lines depict the interfaces between soil layers. Values show the mean ± s.e. (n = 4). Data points of the uniformly distributed treatments are joined by solid lines whereas data points of the banded treatment are joined by dashed lines.

Phosphorus acquisition

Shoot P derived from P applied to the band was highest in the banded-P treatments (Fig. 76), when P fertiliser was highly concentrated in the relatively thin band layer. In these treatments, Digit had higher levels of shoot P derived from P applied to the band than Desmanthus. In the uniform-P treatments, the shoot P derived from P applied to the band of Desmanthus was comparable or higher than that of Digit.

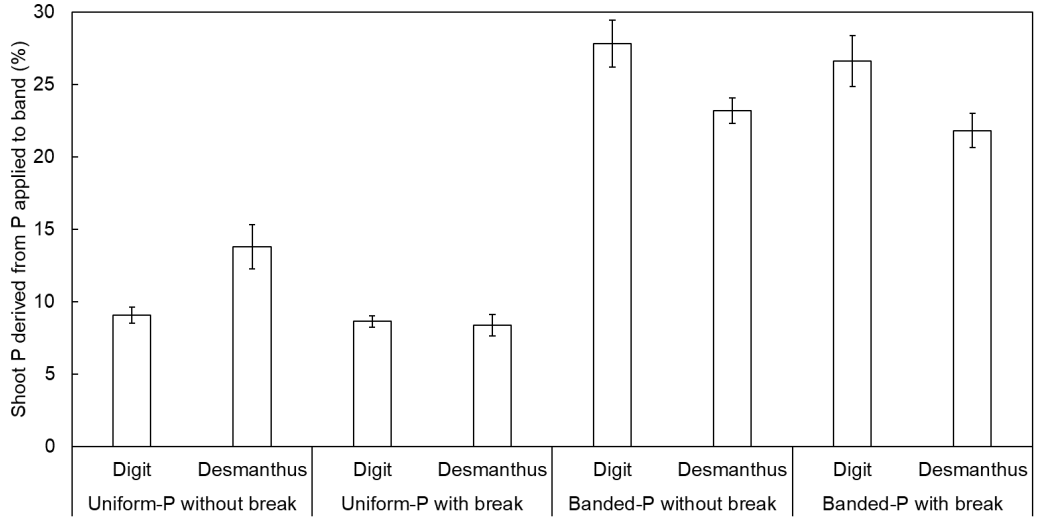

Figure 76. Shoot P derived from P applied to the band layer of Digit and Desmanthus grown in response to uniformly distributed or banded P fertiliser, both with and without a coarse sand capillary break that restricted water movement into the band layer. Values show the mean ± s.e. (n = 4).

Digit recovered more of the P that was applied to the band than Desmanthus (Fig. 77).

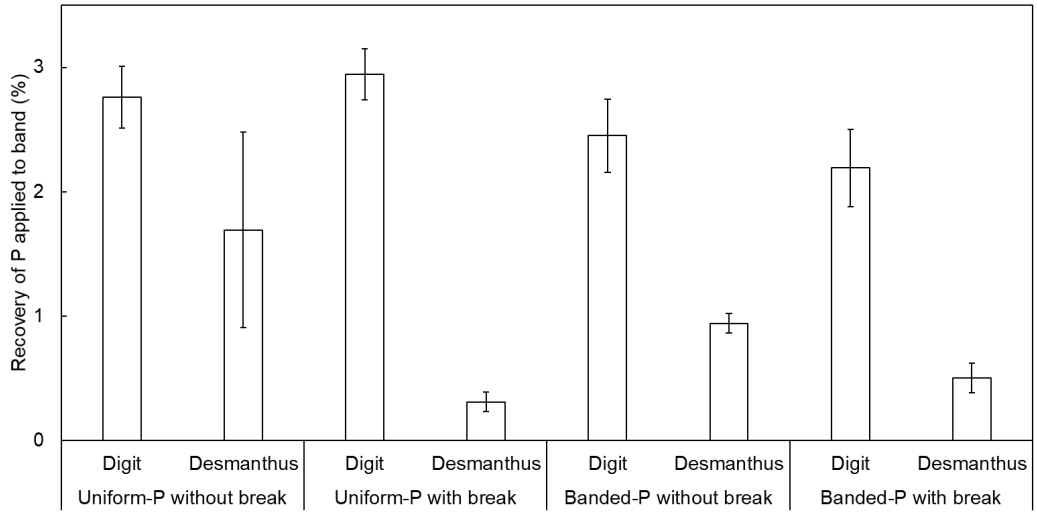

Figure 77. Recovery P derived from P applied to the band layer of Digit and Desmanthus grown in response to uniformly distributed or banded P fertiliser, both with and without a coarse sand capillary break that restricted water movement into the band layer. Values show the mean ± s.e. (n = 4).

Practical implications

Water drawdown reduced the shoot yield of Desmanthus in both the uniform-P and banded-P treatments that had the coarse sand capillary break. This indicates that preferential placement of fertiliser within the vicinity of legume roots may lead to a large enough proliferation response that leads to drying of the root zone. Under these conditions, it is expected that drying will lead to a temporary reduction in P acquisition that will be overcome when the soil is re-wet. Even though Digit would have produced more roots within the band and was expected to dry the soil profile quicker, it is likely that the extensive root development by the grass means that any drying within the vicinity of the band is overcome by roots that are growing elsewhere in the soil profile. This suggests that tropical pasture grasses may be highly competitive for P, even during periods of lower soil moisture, due to the amount of the soil profile that is explored.

8.20 Appendix 20 – Soil temperature influences the root growth and phosphorus acquisition of tropical pasture species

Materials and Methods

Plant material

Digit (*Digitaria eriantha* cv. Premier) and Desmanthus (*Desmanthus pernambucanus* cv. JCU 9) were grown together to investigate shoot yield, root traits and P acquisition under different soil temperature profiles. These pasture species are commonly grown together in the extensive grazing systems of northern Australia.

Soil preparation

A sandy soil (Grey Tenosol; Isbell 1996) was collected from the upper 2–15 cm soil layer of a field at Newholme SMART Farm, Armidale, NSW, Australia (30°26'21.4"S 151°39'55.5"E). The soil had a Colwell extractable P concentration of 3 mg P kg^{-1} (as measured by the method of (Colwell, 1963)), a Phosphorus Buffering Index (PBI) of 29 (as measured by the modified method of (Burkitt et al., 2008)), and a pH (1:5 w/v; 0.01 M $CaCl_2$) of ~5.3. The soil was passed through a 5 mm sieve and a basal nutrient solution was applied that included 34 mg kg^{-1} soil $MgSO_4.7H_2O$, 36 mg kg^{-1} $CaSO_4.2H_2O$, 141 mg kg^{-1} KNO_3, 23 mg kg^{-1} $(NH_4)_2SO_4$, 14 mg kg^{-1} NH_4NO_3, 99 µg kg^{-1} H_3BO_3, 635 µg kg^{-1} $MnCl_2.4H_2O$, 301 µg kg^{-1} $ZnSO_4.7H_2O$, 28 µg kg^{-1} $CuSO_4.5H_2O$, 60 µg kg^{-1} $(NH_4)_2MoO_4$, 17 µg kg^{-1} $CoCl_2 \cdot 6H_2O$ and 1283 µg kg^{-1} FeNa-EDTA.

A4 document scanners (Canon CanoScan LiDE 300 Flatbed Scanners) were modified so that plant roots could be scanned regularly. A hard-plastic spacer and backboard were attached to each document scanner which could contain a thin layer of soil (270 mm height, 180 mm width, 10 mm depth). 660 g (oven-dry equivalent) of the amended soil was packed into to each rhizoscanner. Following this, the backboards were removed to expose the packed soil profile. Phosphorus solutions were then applied to the soil as follows; 40 mg P kg $soil^{-1}$ as KH_2PO_4 labelled with ^{33}P-radioisotope to the top 90 mm (topsoil), 40 mg P kg $soil^{-1}$ as KH_2PO_4 labelled with ^{32}P-radioisotope to the middle 90 mm (band), and 40 mg P kg $soil^{-1}$ as unlabelled KH_2PO_4 to the bottom 90 mm (subsoil). The labelled P solutions enabled P acquisition from the different soil layers to be traced.

Plant growth conditions and experimental design

Micro-swards of Digit and Desmanthus were established by sowing seed (~5 mm depth) to achieve a population of two grass and two legume plants pot^{-1}. There were four replicates of each treatment. The rhizoscanners were located under shade cloth, to reduce surface temperatures, in a glasshouse (natural daylight, ~1800 µmol m^{-2} s^{-1} peak intensity; 30/25°C, day/night) in Armidale, NSW, Australia. Plants were grown between December–January 2023. Soil moisture was maintained at 80–100% field capacity by watering daily. The rhizoscanners were fitted with thermoelectric (Peltier) cells to control soil temperature which enabled five contrasting soil temperature profiles to be maintained. For the duration of the experiment, the temperature profiles were; uniform cool (20/20/20°C), uniform warm (34/34/34°C), warm band (20/34/20°C), cool band (34/20/34°C), and gradient (34/27/20°C). The rhizoscanners were also programmed to scan plant roots every 2 h, from plant emergence until the plants were harvested. Root length and average root diameter were determined using WinRHIZO™ software (Regent Instruments Inc., Quebec, Canada) (Bouma et al., 2000).

Harvest and measurements

Plants were harvested five weeks after planting. Shoots were cut at the soil surface, oven-dried at 70°C for 72 h and weighed. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 12 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Shoot P content was calculated by multiplying shoot P concentration and shoot dry mass. Digested shoot samples were also analysed for ^{32}P- and ^{33}P-radioisotope activity using a PerkinElmer Tri-Carb 2810TR (PerkinElmer, Waltham, United States of America). A scintillation cocktail was prepared by mixing 3 mL of sample with 17 mL of scintillant (PerkinElmer UltimaGold AB). All samples were analysed by liquid scintillation counting (LSC) for 5 min and were corrected for radioactive decay and dilution. The ^{32}P- and ^{33}P-radioisotope counts were used to calculate the specific activity of the shoot material, the amount of plant P derived from applied P, and the recovery of applied P as follows:

$$Specific\ activity = \frac{radioactivity\ in\ shoots}{total\ P\ in\ shoots} \quad (1)$$

$$Plant\ P\ derived\ from\ applied\ P = \frac{specific\ activity\ of\ shoots}{specific\ activity\ of\ applied\ P} \times 100 \quad (2)$$

$$Recovery\ of\ applied\ P\ fertiliser = \frac{applied\ P\ in\ shoots}{total\ applied\ P} \times 100 \quad (3)$$

Statistical analysis

Measured parameters were analysed in R Version 4.0.2 (R Core Team, 2020) by fitting linear models and using an analysis of variance with ‘soil temperature’ as the predictor variable. Linear mixed effects models were fitted to measured variables where the two components (i.e. grass and legume) were compared (R package: nlme) (Pinheiro et al., 2020). The models included ‘soil temperature’ and ‘component’ as fixed effects, and ‘rhizoscanner’ as a random effect. When appropriate, the effect of ‘replicate’ was included in the most parsimonious model. Means and standard errors were calculated from the fitted linear models (R package: emmeans) (Lenth, 2020) and means were compared using Tukey’s honest significant differences (HSD). Normal quantile-quantile plots and Shapiro-Wilkes tests were used to test the normality of the residuals for all fitted models. When required, these models were log-transformed to meet the assumptions of normality. A 5% level of significance was applied for all statistical tests.

Results and Discussion

Shoot yield

Digit and Desmanthus were most productive in the uniform cool soil (Fig. 78). It was expected that the species would have also been equally productive in the warmer soils. However, the constant high temperatures that were imposed on the plants may have negatively impacted growth. For example, relatively high temperatures overnight may have increased the rate of respiration thus reducing plant productivity. Alternatively, water use may have been higher which would be problematic because the soil profile was only ~10 mm thick. Root traits and radioactivity were not determined due to the relatively poor growth within the experiment.

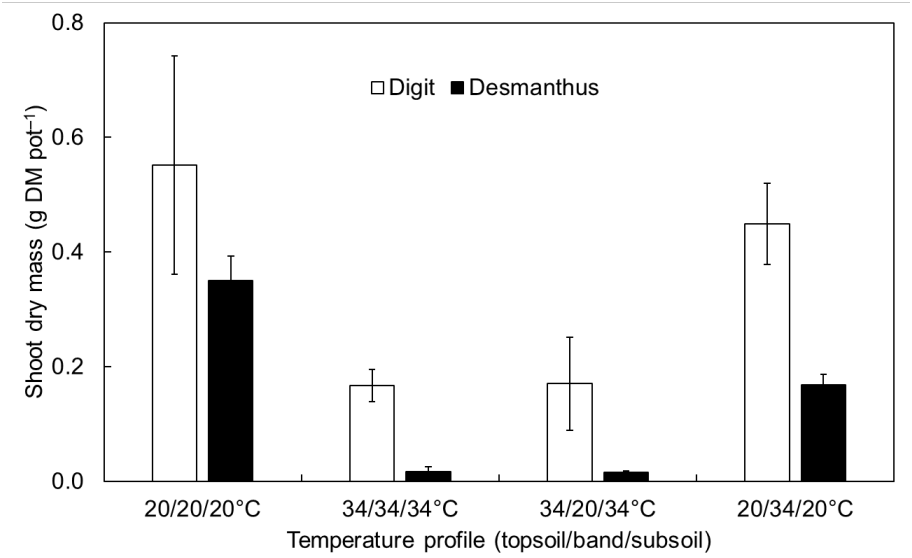

Figure 78. Shoot dry mass of Digit and Desmanthus grown in response to four soil temperature profiles. Values show the mean ± s.e. (n = 4).

Practical implications

The results of the experiment are not conclusive, nevertheless they suggest that Digit and Desmanthus could both be grown in the relatively cooler soils of northern NSW. In previous experiments that used the rhizoscanners to investigate the shoot yield and root traits of Digit and Desmanthus, it was apparent that Digit was much more productive than Desmanthus. Yet in the present experiment, Desmanthus was still productive and was a significant component within the mixed sward. Further research is warranted to investigate the effects of soil temperature on Digit and Desmanthus.

8.21 Appendix 21 – Translating between controlled-environment studies and field-relevant recommendations for tropical pasture legumes

Materials and Methods

Paired plant (to determine shoot P concentration) and soil (to determine Colwell extractable P concentration) samples were collected from five sites across the south-eastern Queensland Brigalow belt bio-region. The sites were selected based on differences in native soil fertility and a history of growing tropical pasture legumes. Plant samples were collected by harvesting all of the above-ground biomass, while soil samples were collected by bulking five 10 cm deep cores into one sample. Following collection, plant samples were dried at 60°C and soil samples were dried at 40°C for seven days. Shoot samples were finely cut before a ~50 mg subsample was pre-digested in a glass tube with 1 mL deionised water and 4 mL 70% (v/v) nitric acid for at least 12 h. Samples were then digested using a Milestone UltraWAVE 640 (Milestone Srl, Sorisole, Italy). The P concentration of the digested samples was determined colorimetrically at 630 nm using the malachite green method (Irving and McLaughlin, 1990). Soil samples were crushed and weighed to 0.4 g, which was then tumbled for 16 hr in 40 mL of 0.5 M NaHCO3 and analysed using malachite green reagent at 630 nm (Colwell, 1963; Motomizu, Wakimoto, & Toei, 1983).

Results and Discussion

Shoot P concentrations in the field

The location of the sampling site significantly predicted the observed shoot P concentrations for both Desmanthus and Stylosanthes ($P < 0.001$; Fig. 79), whereas the collocated Colwell extractable P concentrations and historic P application rates did not. In the context of extensive grazing systems that receive limited annual P input, it is unsurprising that historic P applications have little influence on the shoot P concentrations of the collected samples. It is expected that P nutrition in these systems will be dominated by spikes of mineralised P which occur following rainfall events and, with established perennial root systems, it is expected that plants will efficiently capture P in luxury amounts when soil P is mineralised. These factors contribute to the findings whereby, under severe P constraint (i.e. Colwell P of less than 10 mg P kg^{-1}), there was a cluster of P stressed plants with a mean shoot P concentration of 2.1 mg P g^{-1} DM for both Desmanthus and Stylosanthes (standard deviations were 0.6 and 0.4, respectively). In soil that had Colwell extractable P concentrations of 10-15 mg P kg^{-1}, and similar increments beyond, the mean shoot P concentrations rapidly increased to 2.9 mg P g^{-1} DM (standard deviation = 1.2) and 2.6 mg P g^{-1} DM (standard deviation = 0.6) for Desmanthus and Stylosanthes, respectively. The increase in mean shoot P concentration and approximate doubling of the standard deviation for both genera indicates that these legumes are highly responsive to the variation observed in site, with clustering of location values being a dominant effect (sites are indicated by coincident point colouring; Desmanthus in Figure 79a and Stylosanthes in Figure 79b).

Practical implications

The paired plant and soil samples collected from the field suggest that the data produced in the controlled-environment experiments is comparable to expected field responses. We have found that critical internal P requirements have generally ranged from 1.5–2.5 mg P g^{-1} DM, which means that plants grown in soils with a Colwell P of less than 10 mg P kg^{-1} are likely to be responsive to P

fertiliser. Soils with Colwell P values about this level are less likely to be responsive. Indeed, numerous of our controlled environment experiments demonstrated a yield response with soils that had Colwell P values of 3 mg P kg^{-1} but not in soils with a Colwell P of 13 mg P kg^{-1}.

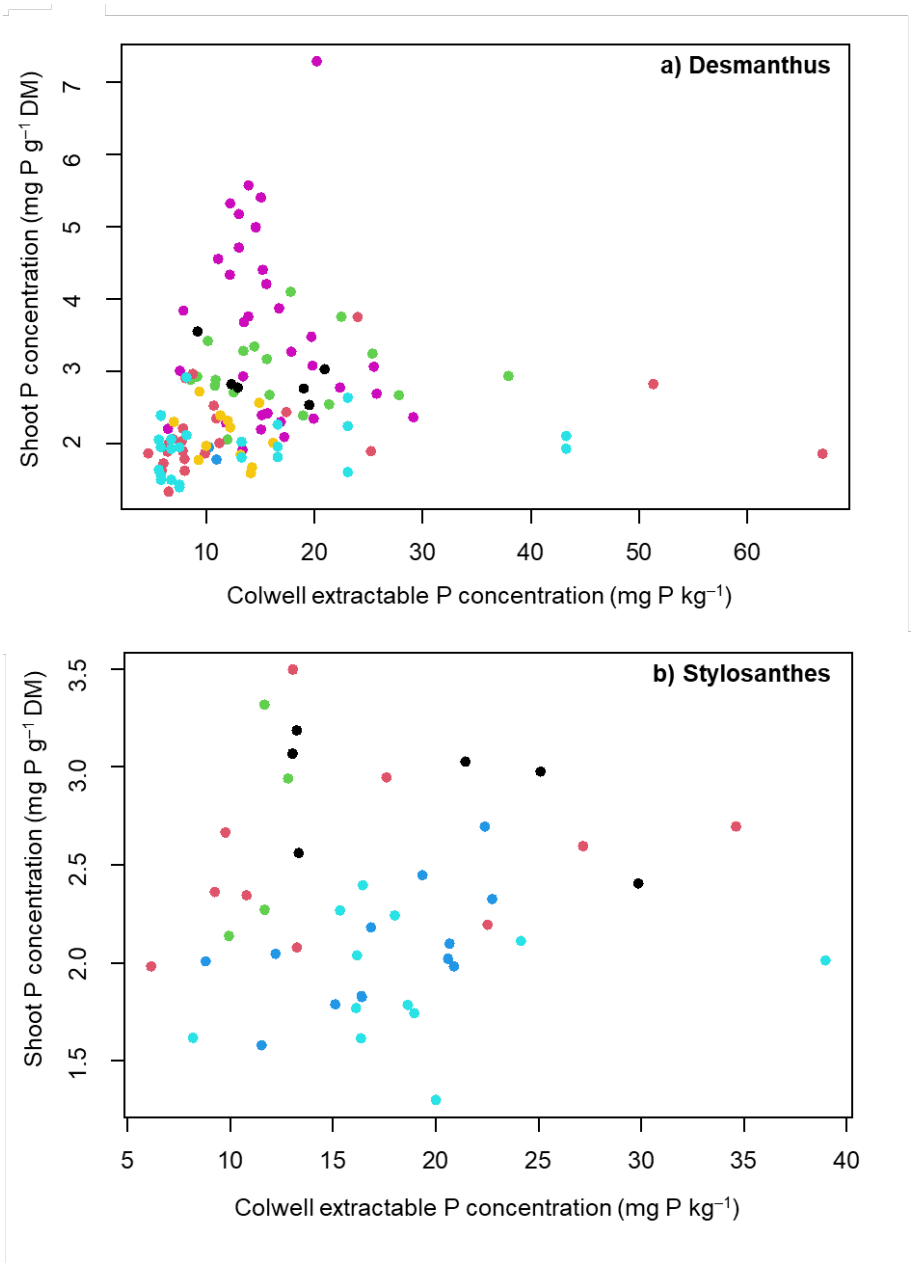

Figure 79. Field sampling of coincident Desmanthus (a) and Stylosanthes (b) plants and the soil, and the relationship between shoot P concentrations and Colwell extractable P concentration across five sites from the south-eastern Queensland Brigalow belt bio-region. Points from the same sites are denoted by colour.