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SHORT COMMUNICATION OPEN ACCESS

Preferential Phosphorus Placement Improves the Productivity and Competitiveness of Tropical Pasture Legumes

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Received: 1 February 2024 | **Revised:** 17 April 2024 | **Accepted:** 17 April 2024

Funding: This work was funded by Meat and Livestock Australia and the Australian Government through the Meat Donor Company, through an investment in 'Phosphorus management and requirements of tropical legume pasture swards' Project P.PSH.1050.

Keywords: ^{32}P-radioisotope tracer | *Centrosema pubescens* | *Chloris gayana* | *Desmanthus pernambucanus* | *Digitaria eriantha* | fertiliser recovery

ABSTRACT

Extensive grazing systems often receive minimal fertiliser due to the risk associated with using relatively expensive inputs. Nevertheless, nutrient applications are known to improve pasture productivity, and the benefit of applying fertiliser is being more widely accepted. Two tropical pasture mixes (Digit/Desmanthus and Rhodes/Centro) were established in plastic boxes containing phosphorus (P) responsive soil to investigate shoot yield and P fertiliser recovery. The grasses and legumes were planted in separate rows, and three P treatments were applied along with the seed ('BOTH low-P' had 2 kg P ha^{-1} banded below both components, 'BOTH high-P' had 12 kg P ha^{-1} banded below both components and 'LEGUME superhigh-P' had 12 kg P ha^{-1} banded below the legume only). The P applied below the legumes was labelled with ^{32}P-radioisotope tracer. When P fertiliser was applied below both components, the grasses consistently out-yielded the legumes (avg. legume content = 29%). Preferential fertiliser application below the legumes increased the average legume content of the two pasture mixes to 66%. Legume tissue P derived from applied P fertiliser increased from 20% to 77% as the P application rate was increased. However, total recovery of applied P by the legumes was relatively low in each of the treatments (≤ 7% of applied P). These collective results demonstrate that a preferential application of P fertiliser can benefit legume productivity, with applied P being a significant proportion of plant tissue P. Although only a small proportion of applied P was recovered within the seven-week growth period, it is expected that this fertiliser application at planting will remain beneficial for a large proportion of the growing season following pasture establishment.

1 | Introduction

The extensive grazing systems of northern Australia are based on C_4 grasses such as *Cenchrus ciliaris* L., *Chloris gayana* Kunth, *Digitaria eriantha* Steud and *Panicum* spp. (Boschma et al. 2021; Haling et al. 2013; Robertson, Myers, and Saffigna 1997). These grasses are often highly productive when first established, but the continual run-down of available nutrients such as nitrogen (N) reduces pasture productivity (Clem and Hall 1994; Graham, Webb, and Waring 1981; Jones, McDonald, and Silvey 1995). The addition of tropical legumes, including those of the genera *Centrosema* and *Desmanthus*, are expected to improve pasture productivity by facilitating atmospheric N-fixation and increasing pasture quality (Clem and Hall 1994; Jones and Rees 1997). However, legume growth is often highly variable, and persistence can be poor. This may be because tropical pasture legumes are more palatable than constituent pasture grasses, so they are preferentially grazed when there is limited grazing management (McCaskill et al. 2019; Muir et al. 2014). Alternatively, legumes may have a higher requirement for nutrients such as phosphorus (P) than grasses, meaning they would be less competitive in the nutrient-deficient soils of northern Australia.

Fertiliser applications have historically been limited in the extensive grazing systems of northern Australia (McIvor, Guppy, and Probert 2011). This is likely due to the risk associated with using relatively expensive inputs in conjunction with the extensiveness of these grazing systems. Nevertheless, fertiliser applications are known to improve pasture productivity, particularly as many soils in northern Australia are nutrient deficient, and the benefit of applying fertiliser at pasture establishment is being more widely accepted in these systems. This change in practice opens new opportunities for the management of pasture nutrition in extensive grazing systems, which could improve the success of pasture establishment and long-term legume persistence. However, limited information is available about the benefit of P fertiliser at planting in mixed tropical pasture swards. Phosphorus fertiliser recovery has also not been quantified for tropical pasture species. A better understanding of how the application of P fertiliser impacts legume productivity, particularly at pasture establishment, would help producers make more informed decisions about nutrient management. The objective of this experiment was to determine the benefit of a localised application of P fertiliser below the grass and/or legume components of mixed tropical pasture swards. It was expected that preferential fertiliser application below the legume would improve the productivity and competitiveness of this component.

2 | Materials and Methods

Four tropical pasture species were grown in two mixed swards to investigate shoot yield and P 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. In particular, mixes of Digit and Desmanthus are common in moderate rainfall areas (e.g., southern-central Queensland) while mixes of Rhodes and Centro are common in higher rainfall areas (e.g., tropical Queensland).

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, Sale, and Gourley 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. Thirty kilograms (oven-dry basis) of the crushed soil was then placed into individual large plastic boxes (360-mm width, 530-mm length, 150-mm soil height) (Figure 1). This resulted in a bulk density of ~1.05 g cm^{-3}. 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% and 100% field capacity (based on water content of the soil after excess water has drained) for the duration of the experiment. Water could be added to the reservoir by hose without wetting the soil surface, which mimicked the moisture profile of soil in the field.

FIGURE 1 | Large plastic boxes were filled with a phosphorus responsive soil to investigate the shoot yield and phosphorus fertiliser recovery of two tropical grass/legume combinations. The grasses (right-hand side) and legumes (left-hand side) were planted in separate rows, and phosphorus fertiliser was applied below the seed. The phosphorus 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 1 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 row^{-1} banded below the grass and legume ('BOTH low-P'), 95.4 mg P row^{-1} banded below the grass and legume ('BOTH high-P') and 190.7 mg P row^{-1} banded below the legume only ('LEGUME superhigh-P'). These P treatments were equivalent to 2, 12 and 12 kg P ha^{-1}, respectively, based on the surface area of the boxes. The 12 kg P ha^{-1} application rate was selected based on typical industry practice where starter P fertiliser is applied at pasture establishment. The same application rate of 12 kg P ha^{-1} was used to preferentially fertilise the legume component, which meant that the concentration of P fertiliser in the legume row was twice as high. The 2 kg P ha^{-1} application rate was selected to be sufficiently low so that the soil remained P responsive, but high enough to allow fertiliser recovery to be calculated accurately. The P fertiliser solutions applied below the legume component were labelled with ^{32}P-radioisotope, whereas the solutions applied below the grass component were unlabelled.

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 emerged seedlings were thinned to achieve plant populations of 24 grass plants row^{-1} (right-hand side of the plastic boxes) and 24 legume plants row^{-1} (left-hand side of the plastic boxes) (Figure 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 to ensure that most of the soil profile was between 80% and 100% field capacity.

Plants were harvested after 7 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 predigested 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:

$$\textit{Specific activity} = \frac{\textit{radioactivity in shoots}}{\textit{total P in shoots}} \tag{1}$$

$$\textit{Plant P derived from applied P} = \frac{\textit{specific activity of shoots}}{\textit{specific activity of applied P}} \times 100 \tag{2}$$

$$\textit{Recovery of applied P fertiliser} = \frac{\textit{applied P in shoots}}{\textit{total applied P}} \times 100 \tag{3}$$

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

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 mix' 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, Bates, DebRoy, Sarkar, and Team 2020). The models included 'species mix', '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. Main effects were calculated from the fitted linear models (R package: emmeans) (Lenth 2020) and were compared using Tukey's honest significant differences (HSD). Normal quantile–quantile plots and Shapiro–Wilk 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.

3 | Results and Discussion

When equal amounts of P fertiliser were applied below both components of the mixed swards (i.e., the 'BOTH low-P' and 'BOTH high-P' treatments), the two grasses (Digit and Rhodes) consistently out-yielded the two legumes (Desmanthus and Centro) with which they were grown ($p < 0.001$; Figure 2a). In these two P treatments, the legume content of the mixed swards averaged 29%. This legume content is adequate for N-fixation in temperate and tropical pastures (Thomas 1992) but is relatively low considering there was an equal number of grass and legume plants in each box. When P fertiliser was applied below the legumes only, grass shoot yields were lower (which demonstrated that both grasses were responsive to P in the soil that was used in the present experiment) whereas legume shoot yields were, on average, higher ($p = 0.001$). These relative changes in shoot yield resulted in the average legume content increasing to 66%, compared to when both components received a banded application of P fertiliser. This legume content is higher than the desired level for optimal pasture production. However, strong legume growth following pasture establishment is desired to maximise

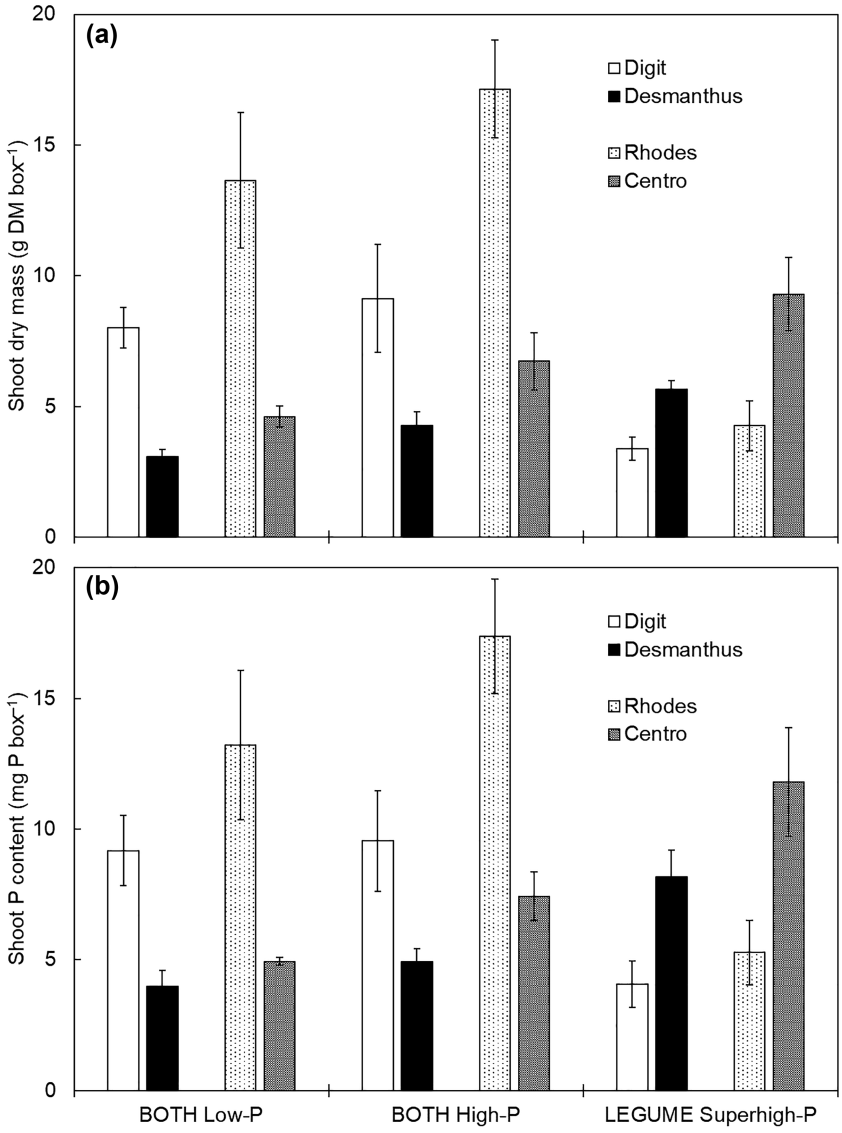

FIGURE 2 | Shoot dry mass (a) and shoot phosphorus content (b) of two tropical pasture mixes (Digit/Desmanthus and Rhodes/Centro) grown in response to three soil phosphorus treatments. The phosphorus fertiliser was applied as a band, either below both the grass and legume (BOTH) or below the legume only (LEGUME). Values show the mean ± standard error ($n = 4$).

the opportunity for seed-set and recruitment in subsequent years, in which grasses are likely to become progressively more competitive. When both components of the mixed swards received the same amount of P fertiliser, the average productivity of the species was as follows: Rhodes > Digit > Centro > Desmanthus ($p < 0.001$). These differences in productivity show that it is important to consider differences in early growth when pairing tropical grasses and legumes in mixed swards, so that both components are represented in appropriate proportions after the swards have established. Large differences in early growth between grasses and legumes will likely lead to poor legume establishment and persistence, particularly if grazing cannot be carefully managed to control pasture biomass.

Shoot P concentrations were, on average, higher for the two legumes (avg. 1.2 mg P g^{-1} DM) compared to the two grasses (avg. 1.1 mg P g^{-1} DM) across the different treatment combinations ($p = 0.002$; data not shown). There was no difference in shoot P concentration between Digit and Rhodes or between Desmanthus and Centro ($p = 0.078$). The shoot P concentrations of the grasses and legumes were both relatively low, which suggests that P supplementation to grazing animals may still be required in legume-enriched pasture swards of northern Australia due to poor soil fertility. For example, the soil used in the present experiment had a Colwell P concentration of 3 mg kg^{-1}, which is expected to constrain both pasture and animal production. Nevertheless, the low shoot P concentrations indicate that tropical grasses and legumes produce dry matter efficiently, even when a large amount of P fertiliser is applied at planting. This is consistent with previous experimentation, which demonstrated that Desmanthus cultivar JCU 9 (also used in the present experiment) utilised acquired P more efficiently than other Desmanthus varieties (McLachlan, Guppy, and Flavel 2021). The differences in shoot yield and shoot P concentration meant that shoot P content (an indicator of plant P uptake) differed according to species mix ($p < 0.001$) and component ($p < 0.001$) and, to a lesser extent, P treatment ($p = 0.012$) (Figure 2b).

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 was 20% in the 'BOTH low-P' treatment and increased to 77% in the 'LEGUME superhigh-P' treatment as the rate of P fertiliser below that component was increased (Figure 3a). Although shoot yield did not increase between the banded low-P and high-P treatments, there was a substantial increase in P derived from fertiliser. This demonstrates that the applied P fertiliser was an important source of P for plant growth, as has been seen in previous work (McLachlan et al. 2020). Even though the grasses were not fertilised directly with the labelled P fertiliser, there was a significant amount of plant P derived from applied P in both species (4%–27% across the treatments). This indicates that the grasses effectively foraged the soil profile for available P, acquiring some of the P fertiliser that had been 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.

The recovery of labelled P fertiliser that had been applied below the legumes was relatively low across the three P treatments for each of the species. It ranged between 0.6% and 5.6% for the grasses, which had intercepted some of the labelled P fertiliser applied below the legumes, and between 2.6% and 7.1% for the legumes, which had been planted directly above the labelled P fertiliser (Figure 3b). Other experiments have shown that P fertiliser recoveries can be much 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, in the present experiment, plants were only grown for 7 weeks, 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

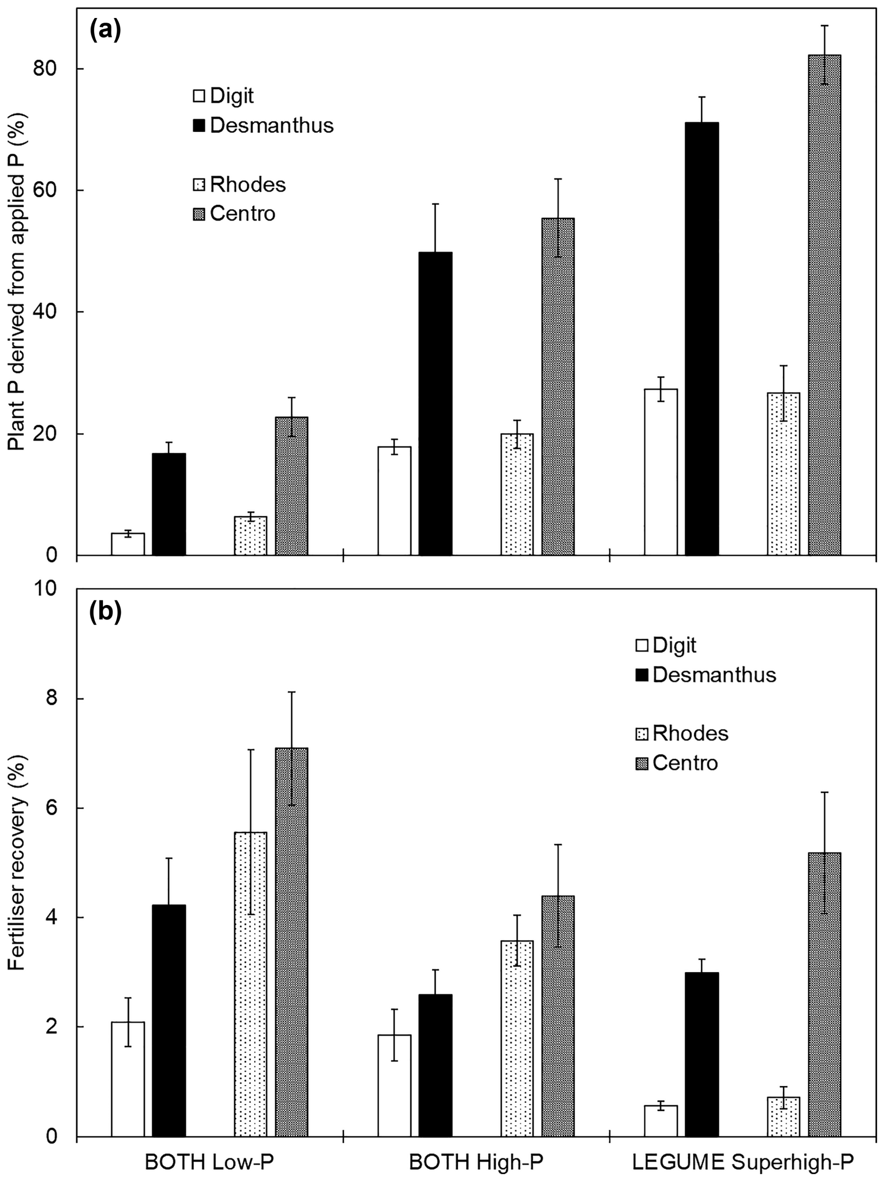

FIGURE 3 | Plant phosphorus derived from applied phosphorus (a) and phosphorus fertiliser recovery (b) of two tropical pasture mixes (Digit/Desmanthus and Rhodes/Centro) grown in response to three soil phosphorus treatments. The phosphorus fertiliser was applied as a band, either below both the grass and legume (BOTH) or below the legume only (LEGUME). Values show the mean ± standard error ($n = 4$).

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.

4 | Conclusions

The collective results demonstrate that banded applications of P fertiliser are appropriate for tropical grasses and legumes. However, if both components are fertilised, the grass component is likely to be highly competitive which could reduce legume productivity and persistence over time. The preferential placement of P fertiliser below only the legume component 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 P fertiliser was only applied below the legume component, the grasses still acquired labelled fertiliser. This suggests that, over time, the grass will also benefit from the applied fertiliser. Future research should focus on quantifying the benefit of preferentially placed P fertiliser for the legume component of a mixed pasture sward grown under field conditions, because the interaction between fertiliser placement and water availability is likely to influence the success of this application strategy.

Author Contributions

Jonathan W. McLachlan: Conceptualization; Formal analysis; Investigation; Methodology; Writing – original draft; Writing – review and editing. **Richard J. Flavel:** Conceptualization; Investigation; Methodology; Writing – review and editing. **Chris N. Guppy:** Conceptualization; Investigation; Methodology; Writing – review and editing.

Acknowledgements

This work was funded by Meat and Livestock Australia and the Australian Government through the Meat Donor Company, through an investment in 'Phosphorus management and requirements of tropical legume pasture swards' Project P.PSH.1050. Emma Flavel is thanked for technical assistance. Chris Gardiner and Nick Kempe are thanked for providing the soil used in the experiment. Open access publishing facilitated by University of New England, as part of the Wiley - University of New England agreement via the Council of Australian University Librarians.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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