Impact of Phosphorus fertilizer and arbuscular mycorrhizal fungi on forage legume growth, chlorophyll content and productivity

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Abstract

Phosphorous deficiency limits forage productivity in South African soils. Although inorganic P fertilizers are used, about 75-90% P becomes unavailable for plant uptake, hence, the strategies to enhance P uptake e.g., arbuscular mycorrhizal fungi (AMF) inoculation are crucial. Three legume species (*Vigna unguiculata*, *Lablab purpereus* and *Mucuna pruriens*) were grown under five P fertilizer levels (0, 20, 40, 60 and 80 kg P/ha) with or without AMF inoculation in the pots, resulting in 30 treatment combinations, each replicated 4 times. Agronomic responses to P fertilization and AMF inoculation were assessed. Plant height, stem diameter, chlorophyll content, leaf and stem yield were affected by the interaction between P fertilizer levels, AMF inoculation and legume species ($P < 0.001$). The remarkable increases in plant height, stem diameter, chlorophyll content and yield were noticeable in AMF inoculated than uninoculated plants for all legume species, more so at 40 kg P/ha compared to other P fertilizer levels. Uninoculated plants particularly, were less responsive to 0, 20 and 80 kg P/ha fertilizer levels. Overall, AMF inoculation improved growth and productivity of forage legumes, but its effects depended on the P fertilizer level, with 40 kg P/ha being the potential optimal fertilizer rate for soil nutrition of legume pastures.

Keywords: AMF inoculation, Plant height and stem diameter, Legume species, P fertilizer rate, Plant yield

Introduction

Low forage production is the main constraint limiting livestock production in smallholder farming in South Africa, owing amongst other drivers, to rangeland degradation, and climate change (Mpongwana et al. 2023a and b). Thus, the reliance on rangelands alone for livestock feeding is not adequate, given the increase in human population that demands more animal products. Legume pastures have a potential to complement rangelands as a source of highly nutritious and digestible forage (Aucamp 2008). A collaborative initiative by the Eastern Cape government of South Africa and the Australian government was launched in 2006 to improve livestock production in selected communal areas through planting legume pastures (Davies et al. 2008). To the best of our knowledge, this was the first empirical attempt to establish legume pastures in communal arable lands of South Africa. Hence, little has been done to ascertain factors that limit legume establishment and productivity. Generally, poor soil fertility, largely phosphorous (P) deficiency remains a central constraint to sustainable legume pasture establishment and productivity (Mitran et al. 2018; Mpongwana et al. 2023b).

For forage legumes particularly, soil P deficiency limits nodulation, and rhizobium establishment, thereby reducing legume growth and forage productivity (Mitran et al. 2018). The deficiency of soil P has led to the reliance on inorganic P fertilizers (Bastida et al. 2023), with P accessed as orthophosphate anions ($H_2PO_4^-$ and HPO_4^{2-}), P forms restricted to soil pH of 6.0 to 6.5 (Ibrahim et al. 2022). Furthermore, only a small proportion (10-20%) of P applied as an inorganic fertilizer is available for uptake by plants (Helfenstein et al. 2018). P immobilization and insolubility are

the main factors limiting P uptake, as P tends to be adsorbed by Al, and Fe together with clay minerals (Bastida et al. 2023; Mpongwana et al. 2023).

This has stimulated more research interest in finding strategies to maximize P availability, uptake, and efficient use by forage plants (Bastida et al. 2023). Of these strategies, plant inoculation with arbuscular mycorrhizal fungi (AMF) holds great promise through its mutualistic relationship with plants via carbon-for-nutrient trade (Ibrahim et al. 2022). The AMF acquires C from plants in exchange for P and to some degree N (Antunes et al. 2012; Nouri et al. 2014). The enzyme phosphatase produced by AMF solubilizes immobile P, thereby increasing P availability and uptake by plants (Begum et al. 2019) and the extraradical mycelia formed by the fungi on plant roots grow beyond plant rooting depth to acquire soil nutrients (Nouri et al. 2014; Ibrahim et al. 2022).

While there is plenty evidence indicating that AMF enhances P availability and uptake (Unger et al. 2021), knowledge gap exists regarding the rate of P fertilizer at which AMF maximizes legume growth and productivity. This deprives us an opportunity to design appropriate soil nutrition management program for sustainable legume pasture establishment and production. This study, therefore, answers the following questions: 1) does the influence of P fertilizer application on legume growth, chlorophyll content and productivity depend on the AMF inoculation? 2) what is the optimal rate of P fertilizer at which AMF maximizes forage legume growth, chlorophyll content and productivity? and 3) do the growth and productivity responses of forage legumes to AMF and P fertilizer vary with legume species?

Materials and methods

Study site and experimental design

The study was conducted at the University of Fort Hare (32º 46' S 26º 50' E) greenhouse during January 2017. The experimental design was a split-split plot design (SSPD) with 3 x 2 x 5 factorial arrangement. The SSPD comprised of three legume species (*L. purpureus, M. purpureus* and *V. inguculata*), two arbuscular mycorrhiza fungi levels (inoculated or uninoculated) and five P fertilizer rates (0, 20, 40, 60 and 80 kg P/ha), included as the main plot, subplot, and sub-sub plot, respectively. This resulted in 30 treatment combinations (3 species × 2 arbuscular mycorrhiza fungi levels × 5 fertilizer levels), with each combination replicated four times, giving a total of 120 pots. The potting soil was collected at a depth of 20 cm from arable fields of the University of Fort Hare Crop Research Farm. The potting soil was sterilized to kill potential arbuscular mycorrhiza fungi (AMF) that might be present in the soil through oven drying at 82 - 92 ºC for 30 minutes (Ortas 2012). Before planting, seeds were inoculated with *Rhizobium* inoculum (*Bradyrhizobium* strain). Commercial AMF product (Mycoroot^{TM} Supreme) was purchased from Rhodes University Microbiological lab. A mixture of 1 ml of Mycoroot^{TM} Supreme per seed was applied, followed by application of single superphosphate fertilizer (P_2O_5) of 20, 40, 60 or 80 kg/ha in a 15 kg soil per pot. The diameter of the pot was 30 cm and the soil depth in the pot was 8 cm.

Three seeds per pot were planted at 4-6 cm depth of soil and thinned to two after seed germination. All the pots were randomly placed in a greenhouse with temperatures of 27 ºC with natural light. The cooling of the greenhouse was achieved by regulating the air condition. Watering was done once a day in the morning to maintain moisture at 50% field capacity to avoid leaching. This was achieved by measuring soil moisture content using calibrated soil moisture probes Delta T device (SM150T Soil Moisture Sensor, United Kingdom). The pots were kept weed-free through hand ~~removal of any emerging weed~~.

Data collection

105 The legume plant height was measured from the base to the tip of a primary shoot in all plants at
106 30, 60 and 90 days after sowing using a measuring tape. Stem diameter (mm) was measured in
107 each plant at 10 cm above the soil surface using the Vernier Calliper (Mitutoyo, 150 mm Vernier
108 Caliper 0.02 mm, Metric, Zhejiang, China) at 30, 60 and 90 days after sowing. The leaf chlorophyll
109 content was measured using a SPAD meter (SPAD-502 Plus, Minolta Camera Cooperative, Japan)
110 (Rodriguez 2000) on 15-day intervals starting from day 30 of sowing by randomly selecting three
111 leaves on each plant per pot.
112 The forage legumes were cut at a stubble height of 10 cm above the ground, 90 days after sowing.
113 The forage samples were bagged and transported to the lab where they were separated into leaf
114 and stem, after which the fresh weight of each forage component was determined. Thereafter,
115 forage samples were oven-dried at 65 °C for 48 hours and weighed to determine dry matter
116 production. The leaf and stem weight were used to calculate the leaf to stem ratio.

117 **3.1 Statistical analysis**

118 Firstly, data normality and homoscedasticity were assessed using Kolmogorov-Smirnof and
119 Levenne's tests, respectively and all the data met these assumptions. Repeated measures analysis
120 of variance (RMANOVA) using mixed effects models was conducted using SAS version 9.1.3
121 (SAS 2003), with time since sowing entered as within-subject factor, whereas legume species (n
122 = 2), inoculation (n = 2) and P fertilizer levels (n = 5) were added as between-subject factors. When
123 interactions were significant at $\alpha = 5\%$, the means were separated using Tukey's test.

124 **Results**

125 **Shoot height and stem diameter**

The interactions between P fertilizer, AMF inoculation and legume species on plant height and stem diameter overtime since sowing are presented in **Table 2**. The interaction between legume species, P fertilizer levels and AMF had a significant ($P < 0.05$) effect on the plant height. For all legume species, inoculated plants were significantly ($P < 0.05$) taller, more so under 40 P kg/ha fertilizer level compared to uninoculated plants 30 days post-sowing. However, when inoculated plants were compared alone, *V. unguiculata* plants were relatively taller under 40 and 60 kg P/ha after 30 and 60 days of sowing, after which the differences disappeared between 0, 20, 40 and 60 kg P/ha on day 90 post-sowing. For *L. purpereus* and *M. pruriens*, inoculated plants had similar height at 40 and 60 kg P/ha, but the plants under these P fertilizer levels were consistently taller than at 0, 20 and 80 kg P/ha throughout the study period.

There were significant interactions of legume species, P fertilizer and AMF on stem diameter (SD), with stem diameter increasing with P fertilization, peaking at 40 kg P/ha, above which it declined. There were obvious differences between inoculated and uninoculated plants for all legume species. The former exhibited larger stem diameters than the latter, more significantly ($P < 0.05$) at 40 and 60 kg P/ha after day 60 and 90 post-sowing.

Chlorophyll content

The interactions between P fertilizer, AMF inoculation and legume species on chlorophyll content overtime since sowing are presented in **Table 3**. The three-way interactions of legume species, P fertilizer level and AMF inoculation were again significant ($P < 0.05$) for chlorophyll content. The AMF inoculated plants showed interspecific responses to P fertilizer over time. AMF inoculated plants of *V. unguiculata* attained significantly ($P < 0.05$) higher chlorophyll content at 40 and 60 kg P/ha compared to other P fertilizer levels from day 30 to 45 post-sowing. For all P fertilizer

levels, the AMF inoculated *L. purpereus* plants had higher chlorophyll content than uninoculated plants until 90 days post-sowing. The chlorophyll content for the AMF inoculated *L. purpereus* was highest at 40 and 60 kg P/ha, with plants grown at 80 kg P/ha exhibiting low chlorophyll content than plants grown in other P fertilizer levels until 90 days post-sowing. The results showed however, that the chlorophyll content of the AMF inoculated *L. purpereus* plants grown at 20 kg P/ha was comparable ($P > 0.05$) to that of plants grown at 40 and 60 kg P/ha on day 90 post-sowing. The remarkable responses of AMF inoculated compared to uninoculated *M. pruriens* plants were evident 45 days post-sowing, more so at 40 and 60 kg P/ha compared to 0, 20 and 80 kg P/ha.

Leaf and stem yield

The interactions between P fertilizer, AMF inoculation and legume species on leaf and stem yield and leaf to stem ratio are presented in **Table 4**. The three-way interaction between legume species, P fertilizer and AMF on leaf and stem dry matter production and leaf:stem ratio was significant ($P < 0.05$). For both AMF inoculated and uninoculated *M. pruriens* plants, the leaf dry matter was significantly higher at 40 and 60 kg P/ha compared to other P fertilizer levels, but the former attained significantly ($P < 0.05$) higher leaf dry matter than the latter in these P fertilizer rates. Nonetheless, the leaf:stem ratio for AMF inoculated *M. pruriens* was similar ($P > 0.05$) across the P fertilizer levels, with uninoculated plants grown at 0 and 80 kg P/ha attaining remarkable higher leaf:stem ratio.

The AMF inoculated *L. purpereus* plants attained a significantly higher leaf and stem dry matter from 0-60 kg P/ha compared to uninoculated plants, more so at 40 and 60 kg P/ha than other P fertilizer levels. At high P fertilizer level (80 kg P/ha), the leaf dry matter of AMF inoculated plants

was not different from uninoculated plants. The leaf:stem ratio was highest in all P fertilizer levels for uninoculated plants relative to AMF inoculated ones at 40 and 60 kg P/ha. For *M. pruriens* also, the AMF inoculated plants attained similar leaf dry matter at 40 and 60 kg P/ha, which was significantly higher than uninoculated plants in all P fertilizer levels. However, the stem dry matter for AMF inoculated plants was significantly higher at 40 kg P/ha than all P fertilizer levels. However, the leaf:stem ratio was relatively low in AMF inoculated *M. pruriens* grown at 40 and 60 kg P/ha compared to uninoculated plants.

Discussion

The interactions between legume species, arbuscular mycorrhizal fungi (AMF) and P fertilizer level suggest that legume growth and chlorophyl content responses are determined by synergistic effects of P fertilizer and AMF depending on the type of legume species. This highlights that the crucial role of AMF for efficient utilization of P by legumes also depends on the amount of P in the soil. This was justified by high legume growth and chlorophyl content at 40 kg P/ha, above which the stimulatory effect of AMF was negated in all legume species, regardless of the time elapsed since sowing (Table 2). Indeed, at 80 kg P/ha, inoculated legumes exhibited a stunted growth (Table 2), indicating that growth stimulation by AMF is limited in soils with excess P. Generally, relatively high soil P reduces the symbiotic association between legumes and AMF, with AMF tending to be parasitic to the host plant (Tobisa & Uchida 2017). Even at 0-20 kg P/ha, growth enhancement by AMF inoculation was minimal probably due to P deficiency. This finding disagrees with several previous studies that suggest that AMF compensates for low soil P via enhancing acquisition of nutrients (e.g., Nouri et al. 2014). We, therefore, deduce that the enhancement of the uptake and efficient use of P by AMF depends on the optimal rate of P fertilizer

192 supply, which in this study appears to be 40 kg P/ha. The significant three-way interactions also
193 highlighted that growth responses of legumes do not only vary by AMF inoculation and P fertilizer
194 level, but also interspecifically. Of the tested inoculated legume species, *Mucuna pruriens* was
195 generally more responsive to 40 kg P/ha relative to *Vigna unguiculata* and *Lablab purpereus*
196 (Table 3), suggesting that the former utilizes P more efficient than the latter two species. These
197 findings form basis for species selection for legume pasture establishment and production.
198 Generally, AMF inoculation enhances P use efficiency in plants, thereby enhancing growth
199 (Ibrahim et al. 2022). Because P is not readily available to plants, AMF via producing enzymes
200 that solubilize P, induces higher uptake of P in inoculated plants. Apart from enhancing P uptake,
201 AMF enhances uptake of other essential nutrients (e.g., N, K and Fe) and reduces the uptake of
202 salt ions, thereby stimulating vigorous plant growth and photosynthesis (Begum et al. 2019). We
203 found, however, that for inoculated *Vigna unguiculata* and *Lablab pupereus* plants, the differences
204 on chlorophyl content from 0-60 kg P/ha disappeared with time since sowing (Table 3). This could
205 imply that despite low P content at 0 and 20 kg P/ha, as plants grew, they were able to acquire
206 more P and efficiently channel it to photosynthetic rather than non-photosynthetic apparatus. This
207 was further depicted by higher leaf to stem ratio for inoculated plants at these low P fertilizer levels
208 (Table 3), suggesting that the little available P was invested in leafiness rather than stem
209 production. Generally, as legume plants grow, especially AMF inoculated plants with their tap
210 root system, have a better access to nutrients on the deeper zones of the soil profile. For all legume
211 species, however, the chlorophyl content remained different only at 80 kg P/ha compared to other
212 P fertilizer levels, implying that excessive P does not only stunt legume growth, but also inhibits
213 photosynthesis.

Both the leaf and stem biomass were highest in inoculated than uninoculated plants, with these responses being remarkable at 40 kg P/ha (Table 4). This is not surprising, given that the plant growth was higher in AMF inoculated compared to uninoculated plants at these P fertilizer levels. This could be ascribed to the fact that P was optimal to permit high stimulation of legume productivity by AMF via increased photosynthesis and growth at 40 kg P/ha relative to uninoculated plants. However, the leaf:stem ratio was highest for uninoculated relative inoculated plants (Table 4), suggesting that the latter invested more P not only to leaf production but also to stem production. This has significant implications for animal nutrition, as the higher stem production translates to low forage quality due to low crude protein and high fiber content in stems relative to leaves (Mganga et al. 2021). The forage material with higher stem component has a longer retention time in the rumen due to low digestibility (Mganga et al. 2021). It should be noted, however, that despite low leaf:stem ratios for inoculated plants, they still exhibited more leafiness than uninoculated plants. Thus, a well-timed grazing management will be needed to ensure that these legumes are utilized while they are still nutritious before their stems become more fibrous and ligneous.

Conclusion

This study provides evidence that AMF inoculation is key in enhancing legume growth, and productivity. Our results show, however, that the influence of AMF depends largely on its interaction with P fertilizer and legume species. For instance, remarkable responses of forage legumes in terms of growth, chlorophyl content and dry matter production to AMF inoculation were noticeable at 40 kg P/ha, implying that this P fertilizer level is optimal for legume growth and productivity. However, we noted that responses to AMF inoculation and P fertilizer were

interspecific, emphasizing the importance of appropriate species selection for pasture establishment. These findings are a basis for soil nutrition management of forage legume pastures and may play a crucial role in policy making with regards to pasture establishment in communal arable lands of South Africa.

Author contributions

Sanele Mpongwana: Conceptualization; data curation; formal analysis; investigation; methodology; Writing. Allen Manyevere: Funding project administration; resources; software; supervision; validation; visualization; writing—review and editing. Johnfischer Mupangwa: Funding project administration; resources; software; supervision; validation; visualization; writing—review and editing. Thando C Mpendulo: Funding project administration; resources; supervision; validation; visualization; writing—review and editing. Wandile Mashece: Investigation—original draft; writing—review and editing. Mthunzi Mndela: Investigation—original draft; writing—review and editing.

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Conflict of interest

There is no known conflict of interest associated with this study.

Data availability

Data will be made available on request from the main author.

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List of tables

Table 1: The nutrient composition of the soils used for the experiment

Soil nutrient	**Amount**
pH (H_20)	6.9
Organic carbon (%)	0.65
Organic matter (%)	0.92
Total nitrogen (%)	0.066
P (mg/kg)	2.34
K (mg/kg)	44.7
Ca (mg/kg)	546.5
Mg (mg/kg)	183.5
Fe (mg/kg)	17.7
Na (mg/kg)	1.87
Zn (mg/kg)	34
Mn (mg/kg)	45
Cu (mg/kg)	40

Table 1: Interactions between legume species, AM fungi inoculation and P fertilizer level on plant height and stem diameter over time since sowing.

Independent variables			Plant height (cm)			Stem diameter (mm)		
			Time elapsed since sowing (days)					
Species	**AMF**	**Fertilizer (kg P/ha)**	**30**	**60**	**90**	**30**	**60**	**90**
V. anguiculata	Uninoculated	0	16.00^{d}	48.77^{e}	61.12^{f}	0.47^{de}	0.85^{b}	1.32^{d}
		20	17.85^{d}	48.70^{e}	67.62^{ef}	0.60^{de}	0.95^{b}	1.80^{d}
		40	23.07^{bc}	49.52^{e}	61.12^{f}	0.52^{de}	0.95^{b}	2.25^{cd}
		60	26.40^{b}	44.67^{e}	62.37^{f}	0.57^{de}	0.97^{b}	1.82^{d}
		80	15.75^{d}	41.32^{e}	66.47^{f}	0.55^{de}	0.97^{b}	1.60^{d}
	Inoculated	0	25.65^{b}	63.77^{d}	73.75^{ef}	1.22^{b}	1.67^{b}	2.42^{cd}
		20	26.57^{b}	69.60^{c}	76.25^{ef}	1.27^{b}	1.67^{b}	2.62^{cd}
		40	30.77^{a}	86.72^{b}	94.20^{e}	1.62^{a}	3.72^{a}	6.02^{a}
		60	28.17^{ab}	74.15^{bc}	85.82^{e}	1.47^{ab}	2.55^{a}	4.12^{bc}
		80	29.72^{a}	45.27^{e}	61.12^{f}	0.70^{d}	1.02^{b}	2.40^{cd}
L. purpereus	Uninoculated	0	9.85^{e}	22.25^{g}	88.77^{de}	0.77^{cd}	1.20^{b}	1.67^{d}
		20	12.05^{de}	26.45^{fg}	99.42^{de}	1.15^{b}	1.32^{b}	1.80^{d}
		40	17.75^{d}	55.05^{d}	112.50^{cd}	1.25^{b}	1.52^{b}	2.32^{cd}
		60	15.67^{d}	47.95^{e}	109.50^{cd}	1.10^{c}	1.52^{b}	2.10^{cd}
		80	11.55^{e}	49.72^{e}	102.07^{d}	0.90^{c}	1.50^{b}	1.97^{d}
	Inoculated	0	19.42^{bc}	74.95^{c}	115.75^{cd}	1.55^{a}	1.87^{b}	3.40^{bc}
		20	20.85^{bc}	80.72^{c}	121.50^{c}	1.57^{a}	1.77^{b}	3.70^{bc}
		40	34.15^{a}	97.37^{b}	143.00^{a}	1.77^{a}	2.35^{a}	4.50^{ab}
		60	26.75^{bc}	84.65^{b}	127.32^{ab}	1.15^{b}	2.10^{ab}	4.10^{b}
		80	13.25^{de}	42.07^{e}	112.82^{cd}	0.80^{c}	1.57^{b}	2.72^{cd}
M. pruriens	Uninoculated	0	10.60^{e}	63.10^{d}	103.00^{d}	0.35^{e}	1.10^{b}	2.22^{c}
		20	16.25^{d}	74.25^{c}	112.25^{cd}	0.37^{e}	0.80^{b}	2.70^{cd}
		40	22.47^{c}	88.45^{b}	117.00^{cd}	0.50^{de}	1.20^{b}	2.90^{cd}
		60	22.07^{c}	87.87^{b}	109.50^{cd}	0.47^{de}	0.87^{b}	2.57^{cd}
		80	21.80^{c}	83.50^{b}	103.75^{d}	0.47^{de}	0.67^{b}	1.95^{d}
	Inoculation	0	25.12^{bc}	91.65^{b}	121.75^{c}	0.80^{c}	1.50^{b}	3.45^{bc}
		20	27.60^{bc}	71.05^{c}	133.00^{b}	0.65^{de}	1.77^{b}	3.80^{bc}
		40	32.70^{a}	121.70^{a}	159.25^{a}	1.17^{b}	2.27^{a}	4.82^{ab}
		60	27.97^{bc}	110.60^{a}	152.62^{a}	1.12^{bc}	2.05^{ab}	4.17^{b}
		80	12.57^{de}	68.07^{d}	106.50^{cd}	0.52^{de}	1.12^{b}	1.82^{d}
Significance level		L×AMF	***	***	***	***	***	***
		L×P	**	***	***	**	***	***
		AMF×P	**	***	***	***	**	***
		L×AMF×P	***	***	***	***	***	***

^{a,b,c,d}Means with different superscripts in the same column differ significantly at P≤ 0.05. *= P≤ 0.05, **=P≤ 0.01***=P≤ 0.001. L = legume, AMF = Arbuscular mycorrhizal fungi, and P = Phosphorous

Table 3: Interactions between legume species, AM fungi inoculation and P fertilizer level on chlorophyl content over time since sowing

Independent variables			Chlorophyl content (°Spad)				
			Time elapsed since sowing (days)				
Species	**AMF**	**Fertilizer (kg P/ha)**	**30**	**45**	**60**	**75**	**90**
V. anguiculata	Uninoculated	0	45.1^{d}	53.1^{de}	63.2^{de}	67.8^{cd}	72.4^{c}
		20	47.8^{d}	54.8^{de}	66.0^{d}	70.1^{c}	74.2^{c}
		40	55.9^{cd}	58.7^{d}	69.1^{d}	73.2^{c}	77.9^{b}
		60	48.5^{cd}	51.8^{de}	68.6^{d}	71.6^{c}	75.5^{b}
		80	47.7^{cd}	50.4^{e}	63.4^{d}	67.5^{cd}	73.1^{c}
	Inoculated	0	57.2^{cd}	60.8^{d}	80.2^{b}	83.1^{bc}	86.4^{ab}
		20	60.4^{c}	75.4^{bc}	82.7^{ab}	85.2^{ab}	88.3^{ab}
		40	80.9^{a}	87.1^{a}	90.7^{a}	96.2^{a}	98.2^{a}
		60	74.2^{ab}	80.2^{ab}	86.7^{a}	91.2^{a}	95.4^{a}
		80	48.0^{d}	52.9^{de}	63.2^{d}	68.3^{cd}	74.4^{c}
L. purpereus	Uninoculated	0	40.6^{d}	45.1^{f}	50.4^{f}	54.2^{e}	58.6^{d}
		20	41.8^{d}	47.7^{f}	53.4^{ef}	56.2^{de}	61.3^{d}
		40	47.4^{d}	50.9^{ef}	56.9^{e}	60.2^{de}	63.5^{d}
		60	44.6^{d}	49.1^{f}	54.9^{e}	57.2^{de}	62.4^{d}
		80	42.0^{d}	43.8^{f}	49.2^{f}	53.2^{e}	60.3^{d}
	Inoculated	0	66.9^{ab}	70.7^{c}	75.9^{b}	80.2^{bc}	84.3^{ab}
		20	70.0^{ab}	72.5^{c}	77.8^{b}	82.2^{bc}	87.2^{ab}
		40	79.3^{ab}	82.7^{ab}	84.4^{a}	87.1^{a}	93.3^{a}
		60	73.1^{ab}	76.6^{bc}	80.5^{b}	84.8^{b}	90.3^{ab}
		80	48.5^{cd}	54.7^{de}	56.5^{e}	59.9^{de}	58.6^{d}
M. pruriens	Uninoculated	0	44.1^{d}	49.4^{f}	53.9^{ef}	58.2^{de}	62.4^{d}
		20	46.7^{cd}	50.4^{ef}	55.9^{e}	60.2^{de}	64.3^{d}
		40	55.0^{c}	58.5^{de}	60.0^{e}	64.3^{de}	68.4^{d}
		60	46.6^{cd}	52.4^{de}	57.8^{e}	62.4^{de}	66.6^{d}
		80	41.2^{d}	48.4^{f}	53.9^{e}	57.4^{de}	62.5^{d}
	Inoculated	0	49.4^{c}	56.2^{d}	61.4^{de}	66.4^{de}	70.4^{c}
		20	50.0^{c}	58.5^{d}	64.3^{c}	68.3^{cd}	72.4^{c}
		40	64.4^{bc}	74.0^{bc}	78.1^{b}	82.4^{bc}	86.4^{ab}
		60	57.3^{c}	70.2^{c}	73.3^{bc}	79.8^{bc}	88.4^{ab}
		80	42.2^{d}	50.4^{e}	49.8^{f}	58.8^{de}	64.5^{d}
Significance level		L×AMF	***	***	***	***	***
		L×P	**	***	***	**	***
		AMF×P	***	**	**	***	***
		L×AMF×P	***	***	***	***	***

[a,b,c,d]Means with different superscripts in the same column differ significantly at P≤ 0.05. *= P≤ 0.05, ** = P≤ 0.01, *** = P≤ 0.001. L = legume, AMF = Arbuscular mycorrhizal fungi, and P = Phosphorous

361 **Table 4**. Interactions between legume species, AM fungi inoculation and P fertilizer level on dry
362 matter production over time since sowing

Species	AMF	Fertilizer (kg P/ha)	Leaf DM (g/pot)	Stem DM (g/pot)	Leaf to stem ratio
V. anguiculata	Uninoculated	0	83.32^{e}	43.47^{gh}	1.94^{a}
		20	103.25^{d}	71.77^{de}	1.44^{bc}
		40	121.75^{bc}	73.42^{de}	1.65^{ab}
		60	114.50^{bc}	68.67^{de}	1.66^{ab}
		80	82.40^{ef}	37.25^{g}	2.24^{a}
	Inoculated	0	104.00^{cd}	77.22^{de}	1.34^{cd}
		20	122.75^{bc}	91.32^{cd}	1.34^{cd}
		40	144.00^{a}	114.62^{a}	1.26^{cd}
		60	130.75^{a}	110.00^{ab}	1.19^{d}
		80	102.50^{d}	95.17^{bc}	1.08^{d}
L. purpereus	Uninoculated	0	64.20^{fg}	30.32^{h}	2.12^{a}
		20	70.47^{f}	37.40^{gh}	1.88^{ab}
		40	75.22^{f}	35.7^{gh}	2.10^{a}
		60	73.60^{f}	36.10^{gh}	2.04^{a}
		80	69.15^{f}	33.20^{gh}	2.09^{a}
	Inoculated	0	86.30^{de}	44.25^{gh}	1.95^{ab}
		20	87.60^{de}	47.27^{fg}	1.86^{ab}
		40	123.25^{bc}	81.30^{cde}	1.52^{bc}
		60	106.25^{cd}	79.55^{de}	1.34^{cd}
		80	71.60^{ef}	48.77^{fg}	1.46^{bc}
M. pruriens	Uninoculated	0	75.62^{ef}	38.00^{gh}	1.99^{a}
		20	79.62^{ef}	44.62^{g}	1.78^{ab}
		40	87.40^{de}	51.45^{f}	1.71^{ab}
		60	84.17^{de}	53.80^{f}	1.57^{ab}
		80	78.75^{ef}	45.45^{fg}	1.73^{ab}
	Inoculated	0	95.12^{de}	53.60^{f}	1.77^{ab}
		20	98.82^{de}	59.15^{ef}	1.67^{ab}

		40	142.50[a]	120.00[a]	1.18[d]
		60	126.25[ab]	92.32[c]	1.36[cd]
		80	87.75[de]	46.35[f]	1.89[ab]
Significance level		L×AMF	**	**	*
		L×P	**	**	**
		AMF×P	***	***	**
		L×AMF×P	***	***	**

[a,b,c,d]Means with different superscripts in the same column differ significantly at P≤ 0.05. * = P≤ 0.05, ** = P≤ 0.01, *** = P≤ 0.001. L = legume, AMF = Arbuscular mycorrhizal fungi, and P = Phosphorous