Impact of P fertilizer and arbuscular mycorrhizal fungi on forage legume growth, chlorophyll content and productivity (#102558)

First revision

Guidance from your Editor

Please submit by **17 Oct 2024** for the benefit of the authors .

Structure and Criteria
Please read the 'Structure and Criteria' page for guidance.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files

Download and review all files from the materials page.

1 Tracked changes manuscript(s)
3 Rebuttal letter(s)
2 Figure file(s)
4 Table file(s)
1 Raw data file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- **Impact and novelty is not assessed.** Meaningful replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

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

Sanele Mpongwana [1] , **Alen Manyevere** [2] , **Confrence Thando Mpendulo** [1] , **Johnfischer Mupangwa** [3] , **Wandile Mashece** [Corresp., 4] , **Mthunzi Mndela** [Corresp. 4]

[1] Livestock and Pasture, University of Fort Hare, Alice, South Africa

[2] University of Fort Hare, Agronomy, Alice, South Africa

[3] Agribusiness & Economics, University of Namibia, Neudamm, Windhoek, Namibia

[4] Livestock and pasture, University of Fort Hare, Alice, Eastern Cape, South Africa

Corresponding Authors: Wandile Mashece, Mthunzi Mndela
Email address: wandilemashece@gmail.com, mmndela@ufh.ac.za

Soil phosphorous is the most limiting nutrient globally, minimizing forage plant performance and productivity. Although inorganic P fertilizers are used, about 75-90% of P becomes unavailable for plant uptake, hence, the strategies to enhance P uptake e.g., arbuscular mycorrhizal fungi (AMF) inoculation are crucial. A pot study was conducted in a controlled environment where three legume species (*Vigna unguiculata*, *Lablab purpereus* and *Mucuna pruriens*) were grown in the pots for 90 days 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, and leaf and stem yield were affected significantly ($P < 0.001$) by the interaction of P fertilizer levels, AMF inoculation and legume species. Inoculated plants were significantly taller (94.2 to 159.0 cm) than uninoculated plants (61.1 to 117.0 cm), with their stem diameters being 2-fold bigger than the latter under 40 kg P/ha than other P fertilizer levels at day 90 for all legume species. Likewise, the chlorophyll content of inoculated plants (78.1-90.7 SPAD) was significantly higher than uninoculated plants (56.9-69.1 SPAD) at 40 kg P/ha compared to 0, 20 and 80 kg P/ha. Moreover, inoculated plants attained relatively higher leaf (123.3-144.0 g/pot) and stem yield (75.2-121.8 g/pot) than uninoculated plants at 40 kg P/ha compared to 0, 20 and 80 kg P/ha. Overall, AMF inoculation improved the 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. The findings of this study provide insights into how AMF inoculation and P fertilizer can be incorporated into soil nutrient management plans to boost the performance and productivity of legume pastures.

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

Sanele Mpongwana[1], Alen Manyevere[2], Thando C Mpendulo[1], Johnfischer Mupangwa[3], Wandile Mashece[1], Mthunzi Mndela[1]*

[1]Department of Livestock and Pasture Science, University of Fort Hare, Private Bag X 1314, Alice 5700, South Africa

[2]Department of Agronomy, University of Fort Hare, Private Bag X 1314, Alice 5700, South Africa

[3]Department of Animal Production, Agribusiness & Economics, University of Namibia, Neudamm Campus, Private Bag, Windhoek 13188, Namibia

Correspondence: wandilemashece@gmail.com

Abstract

Soil phosphorous is the most limiting nutrient globally, minimizing forage plant performance and productivity. Although inorganic P fertilizers are used, about 75-90% of P becomes unavailable for plant uptake, hence, the strategies to enhance P uptake e.g., arbuscular mycorrhizal fungi (AMF) inoculation are crucial. A pot study was conducted in a controlled environment where three legume species (*Vigna unguiculata*, *Lablab purpereus* and *Mucuna pruriens*) were grown in ~~the pots~~ for 90 days 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, and leaf and stem yield were affected significantly ($P < 0.001$) by the interaction of P fertilizer levels, AMF inoculation and legume species. Inoculated plants were significantly taller (94.2 to 159.0 cm) than uninoculated plants (61.1 to 117.0 cm), with their stem diameters being 2-fold bigger than the latter under 40 kg P/ha than other P fertilizer levels at day 90 for all legume species. Likewise, the chlorophyll content of inoculated plants (78.1-90.7 SPAD) was significantly higher than uninoculated plants (56.9-69.1 SPAD) at 40 kg P/ha compared to 0, 20 and 80 kg P/ha. Moreover, inoculated plants attained relatively higher leaf (123.3-144.0 g/pot) and stem yield (75.2-121.8 g/pot) than uninoculated plants at 40 kg P/ha compared to 0, 20 and 80 kg P/ha. Overall, AMF inoculation improved the 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. The findings of this study provide insights into how AMF inoculation and P fertilizer can be incorporated into soil nutrient management plans to boost the performance and productivity of legume pastures.

Keywords: AMF inoculation, Forage plant performance and productivity, Legume species, Inorganic P fertilizer, Optimum fertilizer rate

Introduction

Low forage production is the main constraint limiting livestock production in developing countries ~~globally~~, owing amongst other drivers to rangeland degradation, and climate change (Mpongwana et al. 2023ab). Thus, the reliance on rangelands alone for livestock feeding is inadequate, given the increase in human population that demands more animal products. Legume pastures have the potential to complement rangelands as a source of highly nutritious and digestible forage (Aucamp 2008). However, poor soil fertility, largely phosphorus (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).

AMF plays a vital role in tripartite mutualistic interactions with rhizobia and the host plant, thereby enhancing the efficiency of N_2 fixation, water uptake and disease resistance and reducing the effects of environmental stresses e.g., drought and heavy metal stress (Hack et al. 2019; Murrel et al. 2020). These together with increased P uptake promote photosynthesis and enhance plant health, thereby increasing LAI and overall forage productivity of legumes (Puschel et al. 2017). The AMF-mediated benefits are more important given that P and N are the main limiting factors for plant growth and productivity (Liu et al. 2021; Mpongwana et al. 2024). Thus, the inclusion of AMF inoculation in soil nutrient management and planting programs may help reduce the costs of fertilizer application for financially disadvantaged farmers.

A collaborative initiative on legume pasture establishment was launched in 2006 by the Eastern Cape government of South Africa and the Australian government to improve livestock production in communal areas (Davis et al. 2008). However, little has been done to ascertain factors that limit legume pasture productivity including low availability of soil P (Chen et al. 2023; Hack et al. 2019). This further limits the derivation of soil nutrient management and strategies to enhance nutrient use efficiency for sustainable pasture production. Previous studies demonstrate that the interactions of AMF and Rhizobia to influence legume productivity depend on the amount of P in the soil, with low and super high P reducing the benefits of AMF (Puschel et al. 2017). While there is plenty of evidence indicating that AMF enhances P availability and uptake to increase productivity (Unger et al. 2021), a knowledge gap exists regarding the optimal rate of P fertilizer at which AMF maximizes legume growth and productivity. This information is crucial to designing an 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? 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

A greenhouse pot experiment was conducted at the University of Fort Hare (32º 46' S 26º 50' E) greenhouse from 01 January 2017 to 30 March 2017, with the conditions in the greenhouse set to mimic conditions of subtropical climate with a 12-hour daylight. Before the experiment commences the baseline soil data was collected to analyze the nutrient composition (Table 1).The experimental design was a split-split plot design (SSPD) with a 3 x 2 x 5 factorial arrangement. The SSPD comprised 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), including as the main factor, subfactor, and sub-sub factor respectively. Each legume was planted in 40 pots, with 20 pots planted with inoculated seeds and the other 20 pots planted with uninoculated seeds. For both the pots of inoculated and uninoculated seeds, each of the P fertilizer rates was applied in four replicate pots. Thus, splitting 40 pots per legume species (main factor) into 2 levels of AMF inoculation (sub-factor) and splitting these AMF levels into 5 levels of P fertilizer levels (sub-sub factor) qualified the experiment to be regarded as a split-split plot design. 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 ^{o}C for 30 minutes (Ortas 2012). Before planting, seeds were inoculated with *Rhizobium* inoculum (*Bradyrhizobium* strain). A commercial AMF product (Mycoroot™ Supreme) was purchased from Rhodes University Microbiological Lab. A mixture of 1 ml of Mycoroot™ Supreme per seed was applied, followed by the application of a single superphosphate fertilizer (P_2O_5) of 20, 40, 60 or 80 kg/ha in a 15 kg soil per pot. The AMF comprised various isolates including *Claroideoglomus etunicatum, Funneliformis mosseae*, *Gigaspora gigantean*, *Paraglomus occulum* and *Rhizophagus clarus* (Mpongwana et al. 2023). The AMF was applied to the soil as the powder mixture during the sowing of forage legume seeds in planting holes. Calculation of the amount of P fertilizer levels was based on downscaling the amount from per hectare basis to the surface area of a pot. The pot diameter was 30 cm and the soil depth in the pot was 30 cm (15 kg soil mass). ~~The nutrient composition of the soils used for the experiment is presented in **Table 1.**~~ The forages were not supplied with any fertilizer except the P fertilizer and a standard *Rhizobium* inoculation for all the forage legumes as a standard control.

Three seeds per pot were planted at a 4-6 cm depth of soil and thinned to two after the seedling emergency. All the pots were randomly placed in a greenhouse with temperatures of 27 ^{o}C with natural light. The cooling of the greenhouse was achieved by regulating the air condition. Irrigation 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 manual weeding of any emerging weed.

Data collection

The legume plant height was measured from the plant base to the tip of a primary shoot in all plants at 30, 60 and 90 days after sowing using a measuring tape. Stem diameter (mm) was measured in each plant at 10 cm above the soil surface using the Vernier Calliper (Mitutoyo, 150 mm Vernier Caliper 0.02 mm, Metric, Zhejiang, China) at 30, 60 and 90 days after sowing. The leaf chlorophyll content was measured using a SPAD meter (SPAD-502 Plus, Minolta Camera Cooperative, Japan) (Rodriguez 2000) at 15-day intervals starting from day 30 of sowing by randomly selecting three leaves on each plant per pot.

The forage legumes were cut at a stubble height of 10 cm above the ground, 90 days after sowing. The forage samples were bagged in brown paper bags and transported to the lab where they were separated into leaf and stem, after which the fresh weight of each forage component was determined. Thereafter, forage samples were oven-dried at 65 ºC for 48 hours and weighed to determine dry matter production. The leaf-to-stem ratio weight was used to calculate the leaf-to-stem ratio.

3.1 Statistical analysis

Normality and homoscedasticity of the data were assessed using Kolmogorov-Smirnof and Levenne's tests, respectively and all the data met these assumptions. Repeated measures analysis of variance (RMANOVA) using mixed effects models was conducted using SAS version 9.1.3 (SAS 2003), with time since sowing entered as within-subject factor, whereas legume species (n = 2), inoculation (n = 2) and P fertilizer levels (n = 5) were added as between-subject factors. When interactions were significant at α = 5%, the means were separated using Tukey's test. A Pearson correlation matrix was conducted between plant agronomic traits and P fertilizer levels using Jamovi 2.5.6 software.

Results

Shoot height and stem diameter.

The interactions between P fertilizer, AMF inoculation and legume species on plant height and stem diameter over time after emergence 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 (30.77-34.15 cm), more so under 40 P kg/ha fertilizer level compared to uninoculated plants (17.75-23.07 cm) 30 days post-sowing. However, when inoculated plants were compared alone, *V. unguiculata* plants were relatively taller under 40 kg P/ha (30.77-86.72 cm) and 60 kg P/ha (28.17-74.15 cm) 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 a twofold larger stem diameter than the latter, more significantly ($P < 0.05$) at 40 and 60 kg P/ha after days 60 and 90 post-sowing.

Chlorophyll content

The interactions between P fertilizer, AMF inoculation and legume species on chlorophyll content over time 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-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 kg P/ha (80.9-87.1 SPAD) and 60 kg P/ha (74.2-80.2 SPAD) compared to other P fertilizer levels from day 30 to 45post-emergence. For all P fertilizer levels, the AMF-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 kg P/ha (79.3-84.4 SPAD) and 60 kg P/ha (73.1-80.5 SPAD), with plants grown at 80 kg P/ha (48.5-56.5 SPAD) exhibiting lower chlorophyll content than plants grown in other P fertilizer levels until 90 days post-sowing. However, the results showed, that the chlorophyll content of the AMF-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-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-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 ($P < 0.05$) higher at 40 kg P/ha (142.5 vs 87.4 g/pot) and 60 kg P/ha (126.25 vs 84.17 g/pot) 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 remarkably higher leaf: stem ratio.

The AMF-AMF-inoculated *L. purpereus* plants attained a significantly higher leaf and stem dry matter of 123.25 and 81.30 g/pot at 40 kg P/ha and 106.25 and 79.55 g/pot at 60 kg P/ha, respectively compared to other P fertilizer levels. At a 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 of 142.5 and 126.25 g/pot at 40 and 60 kg P/ha, respectively, which were significantly higher than that of uninoculated plants in all P fertilizer levels. However, the stem dry matter for AMF-AMF-inoculated plants was significantly higher at 40 kg P/ha (120.00 g/pot) 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.

The correlations between the agronomic traits of forage legume species

The leaf and stem yield were correlated significantly ($P < 0.001$) for uninoculated ($r = 0.95$) and inoculated plants ($r = 0.93$; Figures 1 and 2). Chlorophyll content also increased significantly ($P < 0.001$) with stem and leaf yield ($r = 0.64$-0.76) for uninoculated plants (Figure 1). Similarly, inoculated plants exhibited positive correlations between chlorophyll content and leaf and stem yield (Figure 2). There were negative correlations between plant height and leaf yield ($r = -0.51$), stem yield ($r = -0.64$) and chlorophyll content ($r = -0.77$). P level was unrelated to any agronomic trait for both inoculated and uninoculated plants (Figures 1 and 2).

Discussion

The interactions between legume species, arbuscular mycorrhizal fungi (AMF) and P fertilizer level suggest that legume growth and chlorophyll 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 chlorophyll 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 stunted growth (Table 2), indicating that growth stimulation by AMF is limited in soils with excess P. Similarly, Liu et al. (2020) and Xia et al. (2023) found similar responses in which chlorophyll content and productivity of Alfalfa increased with an increased P rate to some degree and declined at a relatively high P level. As indicated previously by Nwaga et al. (2003); Khan et al. (2008); Nishita and Joshi (2010); and Tobisa and Uchida (2017), the relatively high soil P reduces the symbiotic association between legumes and AMF, with AMF tending to be parasitic to the host plant at higher P levels. 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 e.g., Yaseen et al. (2011), Nazir et al. (2011), Singh and Yadav (2008), Nouri et al. (2014), and Chen et al. (2023), that AMF compensates for low soil P via enhancing acquisition of other nutrients. It can therefore be, deduced that the enhancement of productivity and growth of legumes by AMF depends on the optimal rate of P fertilizer supply, which in this study appears to be 40 kg P/ha. In congruence with our findings, Dillon and Vig (1996) also found that 40 kg P/ha in a P_2O_5 form optimized forage yield of a leguminous species (*Vigna radiata*). Although not examined in this study, AMF colonization rate and diversity are constrained at excessive P application, thereby negating the responses of biomass and growth of legumes (Xia et al. 2023). Hence, a higher P level (80 kg P/ha) dramatically reduced the growth, productivity and chlorophyll content of almost all legumes studied here. The systematic review of Mitran et al. (2028), however, indicates that the optimal rate of P fertilizer is species- and area-specific, depending largely on the P status of the soil. Thus, in soils highly deficient in P, the optimal rate of P application may be higher than in soils rich in P (Mitran et al. 2018). The significant three-way interactions also highlighted that growth responses of legumes do not only vary by AMF inoculation and P fertilizer level but also interspecifically. Of the tested inoculated legume species, *Mucuna pruriens* was generally more responsive to 40 kg P/ha relative to *Vigna unguiculata* and *Lablab purpereus* (Table 3), suggesting that the former utilizes P more effectively than the latter two species. These findings form the basis for species selection for legume pasture establishment and production. Generally, AMF inoculation enhances P use efficiency in plants, thereby enhancing growth (Ibrahim et al. 2022). Because P is not readily available to plants, AMF via producing enzymes that solubilize P, induces higher uptake of P in inoculated plants. Apart from enhancing P uptake, AMF enhances the uptake of other essential nutrients (e.g., N, K and Fe) and reduces the uptake of salt ions, thereby stimulating vigorous plant growth and photosynthesis (Begum et al. 2019). We found, however, that for inoculated *Vigna unguiculata* and *Lablab pupereus* plants, the differences in chlorophyll content from 0-60 kg P/ha disappeared with time since sowing (Table 3). This could be ascribed to the fact that as plants matured at high P levels (40-60 kg P/ha), the pot size possibly limited further root growth and biomass production, thereby downregulating photosynthesis. This was further depicted by a higher leaf-to-stem ratio for inoculated plants at these low P fertilizer levels (Table 3), suggesting that the little available P was invested in leafiness rather than stem production. Generally, as legume plants grow, especially AMF-inoculated plants with their tap root system, have better access to nutrients in the deeper zones of the soil profile. For all legume species, however, the chlorophyll content remained different only at 80 kg P/ha compared to other P fertilizer levels, implying that excessive P does not only stunt legume growth, but also inhibits photosynthesis.

Both the leaf and stem biomass were higher in inoculated than uninoculated plants, with these responses being remarkably 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 in leaf production but also in 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 a 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.

Study limitations and future research prospects

This study was conducted in the glasshouse using a pot experiment. While AMF inoculation enhances root growth and high development of hyphae ~~that enhance~~ uptake of P and other essential nutrients (Chen et al. 2023), rooting depth and mycelial production in pots are likely to be restricted by pot size (Chalk et al. 2006). This has serious implications for the above-ground responses of legumes, as the inhibition of below-ground productivity may limit shoot growth and productivity. As a result, it is not surprising that chlorophyll content did not differ from day 75 to day 90 post-sowing (Table 3). This could be due to the downregulation of photosynthesis because of restricted vertical growth of tap roots and low root biomass production due to the limited carrying capacity of pots (Qin et al. 2022; Mndela et al. 2022). Apart from the pot size, glasshouse conditions do not mimic a wide range of climate scenarios, thus we caution that the applicability of these results is restricted to areas in which climate conditions resemble those set at the glasshouse in this study. Due to financial constraints, the root biomass and AMF colonization rate were not determined along the P fertilizer gradient studied here, instead, these parameters were investigated only at optimal P level (40 kg P/ha) under field conditions (Mpongwana et al. 2023a). This, therefore, limits the understanding of how the interaction of AMF and P fertilizer influences below-ground processes across a range of P fertilizer levels. Thus, to bridge this gap, future research should be directed towards understanding how AMF interacts with P fertilizer to influence below-ground responses and how these responses feedback to above-ground shoot responses under field conditions. This research may include an assessment of the effect of AMF on root growth, rooting depth and productivity and mycelial biomass. This research may further investigate how belowground productivity including root and mycelial productivity influences P use efficiency and nutrient uptake of AMF inoculated vs uninoculated plants.

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, chlorophyll 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 regard to pasture establishment in communal arable lands of South Africa. Therefore, the findings suggest that the incorporation of AMF inoculation in pasture establishment enhances plant growth and productivity, particularly under the optimal rate of P fertilizer application (40 kg P/ha). The results also showed that *Mucuna pruriens* was the highly responsive legume to AMF and P fertilizer in terms of growth and productivity, highlighting the significance of this species in pasture establishment.

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.

Funding statement

This was supported by National Research Foundation (T353) of South Africa and the Govan Mbeki Research and Development Centre (C203) (South Africa) for financial support.

Conflict of interest

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

References

Antunes PM, Lehmann A, Hart MM, Baumecker M, Rillig MC. 2012. Long-term effects of soil nutrient deficiency on arbuscular mycorrhizal communities. Functional Ecology. 26: 532-540.

Aucamp AJ. 2008. Linking cultivated pastures with rangelands. Grassroots: Newsletter of the Grassland Society of Southern Africa. 8: 19-25.

Bastida F, Siles JA, Garcia C, Garcia-Diaz C, Moreno JL. 2023. Shifting the paradigm for phosphorus fertilization in the advent of the fertilizer crisis. *Journal of Sustainable Agriculture and Environment*. 1-4. https://doi.org/10.1002/sae2.12040.

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L. 2019. Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. *Frontiers in Plant Science*. 10: 1068. doi:10.3389/fpls.2019.01068.

Davis JK, Ainslie A, Finca A. 2008. Coming to grips with 'abandoned arable' land in efforts to enhance communal grazing systems in the Eastern Cape province, South Africa. *African Journal of Range and Forage Science*. 25: 55-61. doi: 10.2989/ AJRFS.2008.25.2.3.482.

Helfenstein J, Tamburini F, von Sperber C, Massey MS, Pistocchi C, Chadwick OA, Frossard E. 2018. Combining spectroscopic and isotopic techniques gives a dynamic view of phosphorus cycling in soil. *Nature Communications*. 9: 312–32.

Ibrahim M, Iqbal M, Tang YT, Khan S, Guan DX, Li G. 2022. Phosphorus Mobilization in Plant–Soil Environments and Inspired Strategies for Managing Phosphorus: A Review. Agronomy. 12: 2539. https://doi.org/10.3390/agronomy12102539.

Mganga KZ, Ndathi AJN, Wambua SM, Bosma L, Kaindi EM, Amollo KO, Kaindi EM, Kioko T, Kadenyi N, Musyoki GK, van Steenbergen F, Musimba NKR. 2021. Forage value of vegetative leaf and stem biomass fractions of selected grasses indigenous to African rangelands. *Animal Production Science*. 61: 1476–1483. https://doi.org/10.1071/AN19597.

Mitran T, Meena RS, Lal R, Layek J, Kumar S, Datta R. 2018. Role of Soil Phosphorus on Legume Production. In: Legumes for Soil Health and Sustainable Management. 15-510. https://doi.org/10.1007/978-981-13-0253-4_15.

Mpongwana S, Manyevere A, Mupangwa J, Mpendulo CT, Mashamaite CV. 2023a. Foliar nutrient content responses to bio-inoculation of Arbuscular Mycorrhizal Fungi and Rhizobium on three herbaceous forage legumes. Front. Sustain. Food Syst. Sec. 7, https://doi.org/10.3389/fsufs.2023.1256717.

Mpongwana S, Manyevere A, Mupangwa J, Mpendulo CT, Mashamaite CV. 2023b. Optimizing biomass yield of three herbaceous forage legumes through dual inoculation of Arbuscular Mycorrhizal Fungi and Rhizobia. *South African Journal of Botany*. 159: 61-71.

Nouri E, Breuillin-Sessoms F, Feller U, Reinhardt D. 2014. Phosphorus and Nitrogen Regulate Arbuscular Mycorrhizal Symbiosis in Petunia hybrida. *PLoS ONE*. 9: e90841. doi:10.1371/journal.pone.0090841.

Ortas I. 2012. The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. *Field Crop Research*. 125: 35–48.

Tobisa M, Uchida Y. 2017. Effect of Phosphorus Application and Arbuscular Mycorrhizal Fungi Inoculation on the Growth of American Jointvetch and Greenleaf Desmodium. *American Journal of Agricultural and Biological Sciences*. 12: 85-94. doi:10.3844/ajabssp.2017.85.94.

Unger S, Habermann FM, Schenke K, Jongen M. 2021. Arbuscular Mycorrhizal Fungi and Nutrition Determine the Outcome of Competition Between *Lolium multiflorum* and *Trifolium subterraneum*. *Frontiers in Plant Science*. 12: 778861. doi: 10.3389/fpls.2021.778861.

Figure 1

The correlation between the agronomic traits of uninoculated forage legumes grown under varying levels of Phosphorous

List of figures

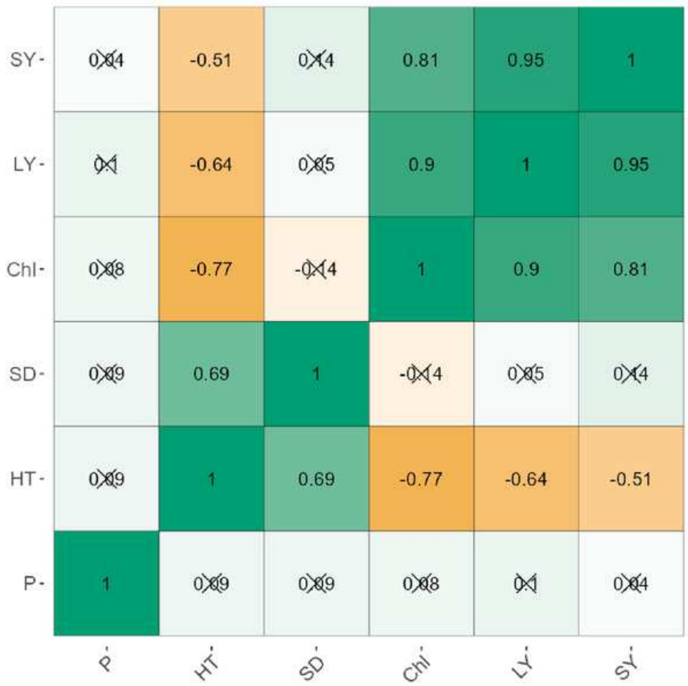

Fig1

Figure 2

The correlation between the agronomic traits of inoculated forage legumes grown under varying levels of Phosphorous

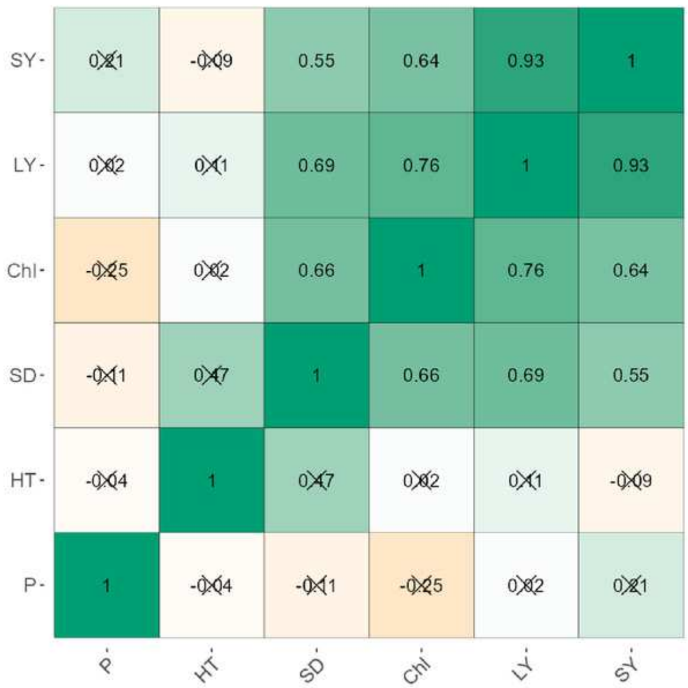
SY
LY
Chl
SD
HT
P
0.21
-0.09
0.55
0.64
0.93
1
0.02
0.11
0.69
0.76
1
0.93
-0.25
0.02
0.66
1
0.76
0.64
-0.11
0.47
1
0.66
0.69
0.55
-0.04
1
0.47
0.02
0.11
-0.09
1
-0.04
-0.11
-0.25
0.02
0.21
P
HT
SD
Chl
LY
SY

Fig 2

Table 1(on next page)

The nutrient composition of the soils used for the experiment

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 2 (on next page)

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

Table 2: 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(on next page)

Interactions between legume species, AM fungi inoculation and P fertilizer level on <!--[if !supportLists]-->20 <!--[endif]-->chlorophyll content over time since sowing

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 $P \leq 0.05$. *= $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$. L = legume, AMF = Arbuscular mycorrhizal fungi, and P = Phosphorous

Table 4(on next page)

102558Table4StaffHC.docx

1 **Table 4**. Interactions between legume species, AM fungi inoculation and P fertilizer level on dry
2 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 $P \leq 0.05$. * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$. L = legume, AMF = Arbuscular mycorrhizal fungi, and P = Phosphorous