Does intercropping of legumes with oilseeds modify mycorrhizal colonisation?

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Research Article

Keywords: Lentil, chickpea, canola, linseed, mycorrhizal symbiosis

Posted Date: September 27th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2070874/v1

Abstract

Background and Aims Legume-oilseed intercrops are increasingly grown in mechanised agricultural systems for their improved nutrient use efficiency. However, the mechanisms that underpin this advantage are not well known. This study aimed to investigate the effect of intercropping and species mixture on the arbuscular mycorrhizal fungi (AMF) colonisation of oilseed and legume crops, and subsequent effects on crop phosphorus nutrition.

Methods We sampled legume-oilseed intercrops in field experiments and measured the level of AMF root colonisation and shoot phosphorus. Additionally, we grew legume-oilseed intercrops in the glasshouse using AMF-inoculated (Rhizophagus irregularis) and mock-inoculated treatments. Measurements included mycorrhizal colonisation, root and shoot biomass, and shoot phosphorus.

Results Mycorrhizal colonisation and the subsequent effect on phosphorus nutrition was host plant dependent. Lentil was the most mycorrhizal plant, followed by linseed, chickpea, and then canola. Only in lentil in the glasshouse was there a correlation between mycorrhizal colonisation and shoot phosphorus ($R = 0.79$, $p<0.001$). Intercropping reduced mycorrhizal colonisation of lentil in the glasshouse but not in the field; intercropping did not affect AMF colonisation in any other species. The interaction between intercropping and AMF had a limited effect on crop growth and shoot phosphorus, while intercropping alone increased canola shoot phosphorus.

Conclusion The role of AMF in the growth and phosphorus nutrition of legume-oilseed intercropping systems appears host specific, and lacks a “one size fits all” solution. Research should be directed towards host plant-AMF specificity, and field studies using diverse soil P profiles.

Introduction

In the face of rising production costs (USDA, 2022) and a changing climate (Crane et al., 2011; Leriorato & Nakamura, 2019), farmers are seeking alternatives to the current high input systems that improve resource use efficiency and reduce costs without sacrificing yield (Fletcher et al, 2020; Khanal et al., 2021). One approach, that embraces principles of agroecology and conservation agriculture, focuses on the sustainable intensification of agriculture through the utilisation of naturally occurring interspecies interactions and ecosystem services (Andres & Bhullar, 2016; Rillig et al., 2016; Duchene et al., 2017). The practice of intercropping, wherein multiple crop species are grown together in the same area for a sustained period of time, is central to this approach (Betencourt et al., 2012; Dowling et al., 2021). Intercropping increases species diversity, and is thought to improve the overall nutrient use efficiency of the system through improved utilisation of soil phosphorus (Latati et al., 2016; Zhang et al., 2019) and nitrogen (Andersen et al., 2004; Cadoux et al., 2015; Génard et al., 2017).

The agroecological and conservation approach also focuses on the symbiosis between plants and arbuscular mycorrhizal fungi (AMF) to increase plant phosphorus supply (Hontoria et al. 2019; Guzman et al., 2021). Symbiosis with mycorrhizas is correlated with increased early phosphorus nutrition (Miller et

al., 1995; Gavito & Miller, 1998) and increased plant growth and yield (Thingstrup et al., 1998; Smith & Smith, 2011; Zhang et al., 2016). Few studies have investigated the intersection of intercropping and AMF, and the interaction with crop yield and nutrient efficiency (Guzman et al., 2021; Rezaei-Chiyaneh et al., 2021). While the extensive work on monocrops can be extrapolated to intercropping systems, there are many interspecies interactions that may generate an outcome different to the sum of their parts.

Some studies suggest that AMF are important in intercropping systems, providing both a phosphorus mining function and a network where resources can flow between species (Bethlenfalvay et al., 1991; Eason et al, 1991; He et al., 2003, 2009; Simard et al., 2012; Walder et al., 2012; Nie et al., 2016), but more research is needed, particularly in the context of legume-oilseed intercrops. Further, canola is often used as the oilseed component of legume-oilseed intercrops (e.g. field pea-canola, Madsen et al., 2022; faba bean-canola, Suraweera et al., 2022; lentil-canola, Roberts et al., 2019) due to its high market relevance, yet it is a non-mycorrhizal crop (Fester & Sawers, 2011; French, 2017; Floc'h et al., 2022). Does the canola component of a legume-canola intercrop affect the extent or quality of the AMF symbiosis with the mycorrhizal legume, and how does this impact phosphorus dynamics within the system? Crop rotation studies have shown reduced mycorrhizal colonisation and yield reductions in mycorrhizal crops planted in rotation following canola (Grant et al., 2009; McGonigle et al., 2011; Bakhshandeh et al., 2017; Higo et al., 2017), but little is known about its effect in an intercrop.

This study aimed to investigate the effect of intercropping and species mixture on the AM colonisation of legume-oilseed intercrops in the field and in the glasshouse, and the subsequent effect on plant phosphorus nutrition. We hypothesised i) that mycorrhizal colonisation of roots would improve phosphorus acquisition, ii) that intercropping would affect mycorrhizal colonisation and subsequent phosphorus acquisition, and iii) that the direction of this effect would be dependent on companion species.

Materials And Methods

2.1 Study location and trial design

Field

Multiple legume-oilseed intercropping field trials located at Hart, South Australia (33°45'34.1"S 138°24'49.7"E), were sampled in 2020 and 2021. The experiment sampled in 2020 is referred to as H2020, and the experiment sampled in 2021 is referred to as H2021 (Table 1).

Table 1
Environmental variables at the two experimental sites.

	H2020	H2021
Pre-season rainfall (mm)	211	54.8
Sowing to sampling rainfall (mm)	56.8	157.2
Background soil nitrate 0-10cm (mg kg^{-1})	10	11
Background soil Colwell phosphorus 0-10cm (mg kg^{-1})	35	10
Soil pH (H2O) 0 to 10cm	8.5	8.2
Soil organic C 0 to 10cm (%)	1.37	0.97
EC 0 to 10cm (dS m^{-1})	0.179	0.177

Chickpea (*Cicer arietinum* L., cv. 'Genesis 090') and lentil (*Lens culinaris* L. cv. 'Hurricane') were the legume species used in the study, with mycorrhizal linseed (*Linum usitatissimum* L. cv. 'Croxton') and non-mycorrhizal canola (*Brassica napus* L., cv. 'Thumper') being the oilseed species used. Intercrop combinations comprised chickpea-linseed, chickpea-canola, and lentil-canola, with sole crop iterations of all four species. At H2020, sole chickpea, sole linseed, and sole canola, as well as chickpea-linseed and chickpea-canola plots were sampled. At H2021, the sole chickpea, sole lentil, sole linseed and sole canola plots, as well as the chickpea-linseed, chickpea-canola, and lentil-canola intercrop plots were sampled. There were three replicates of each plot, totalling 15 plots sampled at H2020 and 40 plots sampled at H2021. At H2020, the intercrop plots had a mixed sowing arrangement, meaning legume and oilseed seeds were sown together in the same row, while at H2021 the intercrop plots had a double skip sowing arrangement, meaning that oilseed and legume species were sown separately in a 2:2 alternate row arrangement.

Glasshouse

Plants were grown in a greenhouse at the Waite campus of the University of Adelaide from late June to August 2021. Over the course of the experiment, the glasshouse had an average maximum temperature of 27.6°C and an average minimum of 18.3°C, with supplemental lighting in a 9:15 day:night photoperiod and average of 3551.4 lux.

The soil used was a mixture of 85% sterilized sand (2mm) and 15% field soil (as used by Tosti & Thorup-Kristensen, 2010), collected from the Kingsford Field Research site, Kingsford, South Australia. The field soil is a hard-setting red-brown clay loam with a H_2O pH of 7.3, and KCl extractable concentrations of nitrate and ammonium N of 21mg kg^{-1} and 3.1mg kg^{-1}, respectively. The field soil had a plant available (Colwell) P concentration of 63.5mg P kg^{-1} and a plant available (Colwell) K concentration of 783mg K kg^{-1}. The soil contained 2% organic carbon. The soil was sieved to 2mm to remove any debris,

autoclaved and oven dried at 60°C before being mixed with the sand. The final sand:soil mixture contained 14.5 mg P kg^{-1} of plant-available (Colwell) P.

All pots were filled with 900mL of the sand:soil mixture. To half the pots (28 pots) an AMF inoculum (*Rhizophagus irregularis* WFVAM10) was added at 10% total pot volume (100mL). The inoculum was made up of dried soil, hyphae and a small amount of root material from colonised Marigold (*Tagetes patula*) trap. To the other half of the pots (28 pots), a mock inoculum was added, composed of soil and root material from non-colonised Marigold plants (Watts-Williams & Gilbert, 2020).

Species and cultivars used were the same as in the field section of the study. Seeds were germinated in the soil:sand mixture, with staggered sowing date to ensure all plants were at the same growth stage for transplanting. When all plants had germinated and were showing two true leaves, seedlings were transplanted into pots in a sole crop 4:0 ratio and an intercrop 2:2 ratio (total four plants per pot). The pots were arranged randomly on the glasshouse bench and were rearranged weekly. Plants were watered three times a week to 10% of field capacity.

2.2 Plant harvest and sample analysis

Field

In both years the same methods were followed. At chickpea growth stage ~ V5 (GRDC GrowNotes, 2017) 10 plants per species per plot (i.e. 10 plants per sole crop plot and 20 plants per intercrop plot) were randomly selected and carefully extracted, keeping roots as intact as possible. Plants were harvested at this stage because of the importance of phosphorus uptake at the vegetative stage in determining overall plant yield. Shoots were cut from the roots at the soil level. Shoot biomass (dry weight, g) was determined after oven drying at 60°C for 120 hours. A subsample of finely ground shoot was then digested in a 4:1 (v/v) mix of nitric acid and hydrogen peroxide (Miller, 1998), and total P concentration determined using inductively-coupled plasma atomic emission spectroscopy (ICP-AES). After legume plants had been given a nodule score, roots were cut into ~ 10mm pieces and placed in 50% ethanol for storage. The fresh root samples fixed in ethanol were then rinsed using RO water, and cleared in 10% KOH at room temperature for 7 days. Cleared roots were rinsed and then stained in 5% Sheaffer Black Ink in vinegar (Modified from Vierheilig et al., 1998) at 60°C for 10 minutes, before being destained in acidified water for 12 hours. Roots were then washed and moved to RO water for storage. Mycorrhizal colonisation was determined on stained root samples according to the gridline intersect method (Giovannetti & Mosse, 1980).

Glasshouse

Counting the day of transplant as day 0 (0 DAT), plant heights were measured weekly for five weeks. At 39 DAT (when ~ 15% of plants were in flower), all plants were destructively harvested as follows. Plants were removed from the pot. Plant shoots were cut at soil level and the roots gently washed in water. Root and shoot fresh weight (g) were then taken. A subsample of fresh root (~ 0.25–0.8 g) was placed into 50% ethanol for storage. The DW of the remaining root biomass and total shoot biomass was determined

after oven drying at 60°C for 120 hours. Shoot biomass was ground finely, and the total P concentration was determined using the same method as for the field sample shoots and the rhizosphere soil. The subsamples of fresh root fixed in ethanol were rinsed using RO water, and the subject to the same methods as the field roots (above) to determine mycorrhizal colonization (%).

2.3 Statistical analysis and calculations

Prior to analysis, all data were tested for normality via the Shapiro-Wilk test, and were log-transformed if required.

Field

Data were initially analysed using ASReml-R in the statistical program R (Rstudio Team, 2020). Each site by year was considered a separate environment, 'site-year', and data were combined across sites for multiple environment trial (MET) analysis. A separate linear mixed model was built for the mycorrhizal root colonisation and shoot phosphorus. The model specified site-year · treatment (intercropping · legume species · oilseed species) as the fixed effects and replicate · site-year as the random effects. Additional site-year-specific extraneous fixed and random terms were included as needed. The residual errors for each site were modelled using spatial methods. The correlation between intercropping, intercropping companion, shoot phosphorus, and root mycorrhizal colonisation was explored through Pearson correlation using the corrplot package (Wei et al., 2018) in R.

Glasshouse

Data were initially analysed using ASReml-R in the statistical program R (Rstudio Team, 2020). A separate linear mixed model was built for each mycorrhizal root colonisation, shoot and root dry weight, and shoot phosphorus. The model specified intercropping · legume species · oilseed species as the fixed effects and replicate as the random effects. The correlation between intercropping, intercropping companion, shoot phosphorus, root mycorrhizal colonisation and root dry weight was explored through Pearson correlation using the corrplot package (Wei et al., 2018) in R.

Results

3.1 Mycorrhizal colonisation

Field

In the field experiments, mycorrhizal colonisation of both legume and oilseed roots did not differ between sole and intercrop treatments. There was no difference in root colonization between the legume species (Fig. 1a), whilst linseed had greater colonisation than the canola ($p < 0.001$; Fig. 1b). Site-year did not affect mycorrhizal root colonisation in any of the crops.

Glasshouse

All four species were colonized by *R. irregularis* in the inoculated treatment (Fig. 2). For lentil and linseed, mycorrhizal root colonisation was greater in the inoculated treatment than in the mock treatment ($p < 0.05$), while there was no inoculation effect on the chickpea or canola (Fig. 2). In the inoculated treatment, lentil had the greatest mycorrhizal colonisation, followed by linseed, chickpea and then canola (Fig. 2). Lentil had greater mycorrhizal colonisation in the sole crop than the intercrop ($p < 0.05$), while intercropping had no effect on chickpea mycorrhizal colonisation (Fig. 2a). Intercropping did not affect mycorrhizal colonisation in either of the oilseed species (Fig. 2b).

It is important to note that the small amount of AM colonisation in the canola (< 5%) is superficial, occurring only on the epidermis and outer cells of the root, and is not sufficient to sustain a functional mycorrhizal symbiosis (Floc'h et al., 2022).

3.2 Biomass

Glasshouse

Inoculation alone did not affect root or shoot dry weight of either the legumes or the oilseeds ($p > 0.05$; Fig. 3). Dry weight of all crop species was greater in the sole crop compared with the intercrop ($p < 0.001$; Fig. 3). The inoculation · intercropping interaction decreased lentil shoot biomass ($p < 0.05$, Fig. 3b), but increased linseed biomass ($p < 0.05$, Fig. 3d). Generally, there was greater difference between treatments in shoot dry weight compared with root dry weight (Fig. 3). For example, while intercropped lentil had a smaller shoot weight than sole lentil, root dry weight between treatments was similar (Fig. 3b). Similarly, canola intercropped with lentil had less shoot biomass than sole canola, but a similar root biomass (Fig. 3c).

3.3 Shoot phosphorus

Field

There was no difference in shoot phosphorus between intercropped and sole cropped legumes (Fig. 4a). Similarly, canola shoot phosphorus did not vary between intercropping treatments. Intercropped linseed, however, had greater shoot phosphorus than the sole crop at H2021 ($p < 0.05$), but at H2020 there was no difference between treatments (Fig. 4b). Averaged across the site-years, however, intercropped and sole cropped linseed had similar shoot phosphorus amounts (3.58 and 4.13 g kg^{-1}, respectively). Shoot phosphorus was different between species of the same crop type ($p < 0.001$), with greater phosphorus in the lentil than in the chickpea, and greater phosphorus in the canola than in the linseed.

Glasshouse

Shoot phosphorus was lower in the glasshouse than in the field. In the glasshouse, legume shoot phosphorus was affected by the inoculation · species interaction ($p < 0.001$). Inoculation increased shoot phosphorus in both the sole and intercropped lentil but not in the chickpea (Fig. 5a). The inoculation · intercropping interaction also affected legume species differently ($p < 0.01$). While there was no difference between sole and intercropped lentil in the mock treatment, sole lentil had more shoot

phosphorus than the intercrop in the inoculated treatment ($p < 0.05$; Fig. 5a). Conversely, chickpea was not affected and had the same level of shoot phosphorus across intercropping and inoculation treatments (Fig. 5a). Oilseed species shoot phosphorus was not affected by inoculation alone, but was affected by the inoculation · intercropping · species interaction ($p < 0.01$). Canola shoot phosphorus was similar across species combinations in the inoculated treatment, but in the mock treatment canola intercropped with chickpea had greater shoot phosphorus ($p < 0.05$, Fig. 5b). Linseed shoot phosphorus was affected by neither inoculation nor intercropping (Fig. 5b).

Canola phosphorus was highly positively correlated with chickpea intercropping ($R = 0.71$, $p < 0.001$), but was unaffected by intercropping with lentil ($R = -0.12$, $p > 0.05$). Shoot phosphorus in the linseed and legume species was unaffected by intercropping ($p > 0.05$).

3.4 Mycorrhizal colonisation and shoot phosphorus

In the field experiment, there was no correlation between shoot phosphorus and mycorrhizal root colonization for either the legumes or the oilseeds (Fig. 6a-d).

In the glasshouse, a correlation between mycorrhizal root colonisation and shoot phosphorus was only observed in lentil ($p < 0.001$); as root colonisation increased, so too did shoot phosphorus (g kg^{-1}) (Fig. 6f). While there was a weak positive correlation between the root colonisation and shoot phosphorus in the linseed, the relationship was not significant due to scatter around the regression line (Fig. 6h).

Discussion

The integration of agroecological principles into conventional agriculture is increasingly being championed as a means to sustainably increase agricultural production (Rillig et al., 2016; French, 2017; Begum et al., 2019). In the present study, we investigated the effect of intercropping on mycorrhizal root colonisation and plant shoot phosphorus.

Comparison of root colonisation in the field and glasshouse experiments suggests some level of host plant preference for specific AMF species, and vice versa. Chickpea and lentil had

similarly high levels of colonisation in the field (~ 60%), but in the glasshouse colonisation of chickpea roots was between 15–30%, less than both the glasshouse lentil and field chickpea and lentil. While plant-AM specificity was previously thought to be low (Mosse, 1975; Brundrett, 2009), a meta-analysis by Van Geel et al. (2016) found that symbiosis with different AM species produces different growth and nutrition responses in a given plant. Similarly, Xavier & Germida (2002) report that plant response to inoculation with AMF varies significantly depending on the AMF species. In our study, the field soil would have harboured a number of different mycorrhiza species (Vályi et al., 2016; Guzman et al., 2021) while the inoculum in the glasshouse experiment contained only *Rhizophagus irregularis* spores. The low colonisation of chickpea roots in the glasshouse but high colonisation in the field suggests that chickpea prefers to associate with AMF species other than *R. irregularis*, while lentil freely associates with *R.*

irregularis and potentially other species. The literature reports colonisation of chickpea roots of ~ 60% by AMF species *Funneliformis mosseae* and *Glomus intraradices* (Tavasolee et al., 2011; Li et al., 2022), a similar level of colonisation to the chickpea in the field, supporting the notion of host-AMF species specificity. This suggests that farmers should potentially focus on cultivating diverse communities of mycorrhiza in the soil to ensure improved growth of multiple crops (Guzman et al., 2021).

Root mycorrhizal colonisation was only correlated with shoot phosphorus in the lentil in the glasshouse experiment, contrary to our first hypothesis. While a lack of correlation between mycorrhizal root colonisation and shoot phosphorus is to be expected for non-mycorrhizal canola (Fester & Sawers, 2011; French, 2017), it is especially surprising for linseed, which is known to be highly mycorrhizal (Thingstrup et al., 1998; McGonigle et al., 2011; Rahimzadeh & Pirzad, 2019). As in the literature, we also found high levels of mycorrhizal root colonisation in linseed in both the field and glasshouse experiments.

The lack of correlation between mycorrhizal colonisation and shoot phosphorus in the field experiment at H2020 could be explained by the adequate supply of background soil phosphorus (Table 1). The most significant benefits from AMF have been reported under phosphorus limitation, with adequate N, light, and water supply (Thingstrup et al., 1998; Smith & Smith; 2011; Ryan & Graham, 2018; Tran et al., 2019). The background Colwell-P value of the soil at H2020 was well above the critical range for most oilseeds (16–19 mg kg^{-1}) and legumes (20–29 mg kg^{-1}) in low to mid rainfall environments (Bell et al., 2013). Given ample phosphorus supply, plants would not have had to rely heavily on the AMF symbiosis to fulfil their growth requirements. At H2021 and in the glasshouse, however, the soil did not have sufficient Colwell P (10 mg kg^{-1} and 14.5 mg kg^{-1}, respectively). In these scenarios, the lack of correlation between shoot and colonisation could be a function of the short duration of the experiment. Perhaps P limitation at later growth stages would have necessitated greater reliance on the AMF symbiosis to provide phosphorus as plant phosphorus demands change over time (Veneklaas et al., 2012). Field studies of longer duration and on P-limited soils are needed to investigate the role of AMF in intercrop phosphorus nutrition.

In contrast to mycorrhizal colonisation, intercropping did affect shoot phosphorus. This is in line with the literature, which suggests that intercrop component interactions alter P availability in the rhizosphere (Costa et al., 2014; Nie et al., 2016). In the glasshouse experiment, canola shoot phosphorus was highly positively correlated with chickpea intercropping, and was greater in the intercrop with chickpea than in either the sole crop or when intercropped with lentil. This suggests a phosphorus benefit of intercropping with some species combinations and not others. Enhanced resource acquisition in an intercrop can be explained by positive interspecific interactions such as resource partitioning or facilitation (Hinsinger et al., 2011; Li et al., 2018), or by competitive dominance wherein one crop component increases resource acquisition at the expense of the other (Loreau & Hector, 2001; Li et al., 2018). In the case of the chickpea-canola intercrop, the increased canola shoot phosphorus but similar chickpea shoot phosphorus relative to their respective sole crops suggests that the chickpea facilitates increased uptake in the canola, rather than the canola outcompeting the chickpea. Chickpea, along with field pea and faba bean, has been shown to have a superior ability to mobilise soil phosphorus compared with other legumes (Miheguli et

al., 2018). It exudes large amounts of acid phosphates that hydrolyses organic phosphorus into plant available inorganic forms (Li et al., 2003; Liao et al., 2020). More research is needed to test compatible legume-oilseed intercrop combinations with regards to phosphorus acquisition. Research should focus on investigating the mechanisms behind the intercrop benefit, with selection for complementary and facilitative interactions that enhance the nutrient dynamics of the whole system, over competitive dominance, which enhances one crop at the expense of the other.

Contrary to our second hypothesis, mycorrhizal colonisation of plant roots was not affected by intercropping. The one exception to this was the lentil in the glasshouse experiment, which had decreased colonisation in the intercrop compared with the sole crop. Most surprisingly, being intercropped with the non-mycorrhizal canola did not affect lentil or chickpea root colonisation (excepting lentil in the glasshouse) relative to the sole crop or being intercropped with the highly mycorrhizal linseed. This is unexpected, as canola is known to release glucosinolates into the soil (Gimsing & Kirkegaard, 2009; Couëdel et al., 2019), that are toxic to fungi and are thought to contribute to the plant's non-mycorrhizal status (Floc'h et al., 2022). Indeed, mycorrhizal plants, such as maize and linseed, had reduced mycorrhizal colonisation and yield in years where they follow canola in crop rotation, compared with another mycorrhizal crop (McGonigle et al., 2011; Higo et al., 2017).

In explanation of the nil canola effect, Trenbath (1993) and Boudreau (2013) suggest that sowing brassica species with a companion legume may negate any negative effects on soil biota, and it is possible this was the case with the mycorrhiza in our lentil-canola and chickpea-canola intercrops. Alternatively, it is possible that we harvested the plants before the canola could exude sufficient amounts of glucosinolates to affect the mycorrhizal colonisation of its companion, and that it would have released potentially more toxic levels when more established. Glucosinolate content in canola is reported to increase up until budding (~ 100 DAS, growth stage 2.2; Berkenkamp, 1973), with the majority of total glucosinolate content at this time in the plant roots (40–86%) (Clossais-Besnard & Larher, 1991; Sarwar & Kirkegaard, 1998). Further research involving whole-season experiments, with sampling at multiple growth stages (such as early flowering, podding and maturity) is needed to establish the longer-terms effects of intercropping with canola. An important aspect of this is break crop rotation research. If the yield of mycorrhizal cereal crops such as wheat is worse following canola, as much of the literature suggests (Owen et al., 2010; Bakhshandeh et al., 2017), could legume-canola intercrops take the place of sole canola? This would maintain the break crop and market relevance benefits, whilst also providing continuation of the mycorrhizal community for the following cereal crop.

Our study found lentil to be highly mycorrhizal, which is consistent with many previous studies (Khan et al., 1988; Amirnia et al., 2019). As discussion of sustainable farming practices gains momentum, farmers are increasingly focussing more attention on enhancing and maintaining abundant communities of mycorrhiza in the soil (Ryan & Graham, 2018; Kirkegaard & Condon, 2019). Although linseed is a highly mycorrhizal crop (Grant et al., 2009), it has a relatively small global market (FAOSTAT, 2022). By using lentil as their 'mycorrhizal' crop, farmers could ensure the maintenance of soil mycorrhizal populations with a species that has high market relevance. In Australia, for example, lentil has the third largest planted

area of the pulse crops ('000 ha; ABARES, 2022), and is already included as break crops in cereal dominant rotations (GRDC GrowNotes, 2018).

Conclusion

In line with much of the literature on AMF in monocultures, our study found that root colonisation by the AMF species *Rhizophagus irregularis*, and the subsequent effect on plant phosphorus nutrition in the early stages of growth, was largely host plant species dependent. The interaction between intercropping and inoculation with AMF had limited effect on crop growth and shoot phosphorus, and intercropping with non-mycorrhizal canola did not reduce legume colonisation. Outside of the interaction with AMF, intercropping increased canola shoot phosphorus, but did not affect shoot phosphorus of the other three species. Interestingly, lentil had the highest mycorrhizal colonisation of the four study species, and was the only species to have a positive correlation between colonisation and shoot phosphorus. This offers lentil as a potential mycorrhizal community enhancer, either in intercrop with an oilseed, or as a sole crop as part of a crop rotation. The intersection of intercropping and AMF poses a complex and multi-faceted situation that lacks a "one-size fits all" solution (Rillig et al., 2016). More research into host plant-AMF specificity is needed, as are intercropping field studies of longer duration and with various soil P profiles, with information gathered at different plant growth stages up until harvest.

Abbreviations

AMF
Arbuscular Mycorrhizal Fungi
AM
Arbuscular Mycorrhiza
DAT
Days After Transplant

Declarations

Acknowledgements

This work would not have been possible without the financial support of the Australian Government Research Training Program scholarship, the Tim Healy Memorial Trust Scholarship, the South Australian Research and Development Institute (SARDI), and the Grains Research and Development Corporation through research funding DAV00150. We acknowledge the support of the technical and research teams at SARDI Clare and Hart Field Site. We extend our deepest gratitude to Dr Judith Rathjen for reviewing the manuscript prior to submission.

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Figures

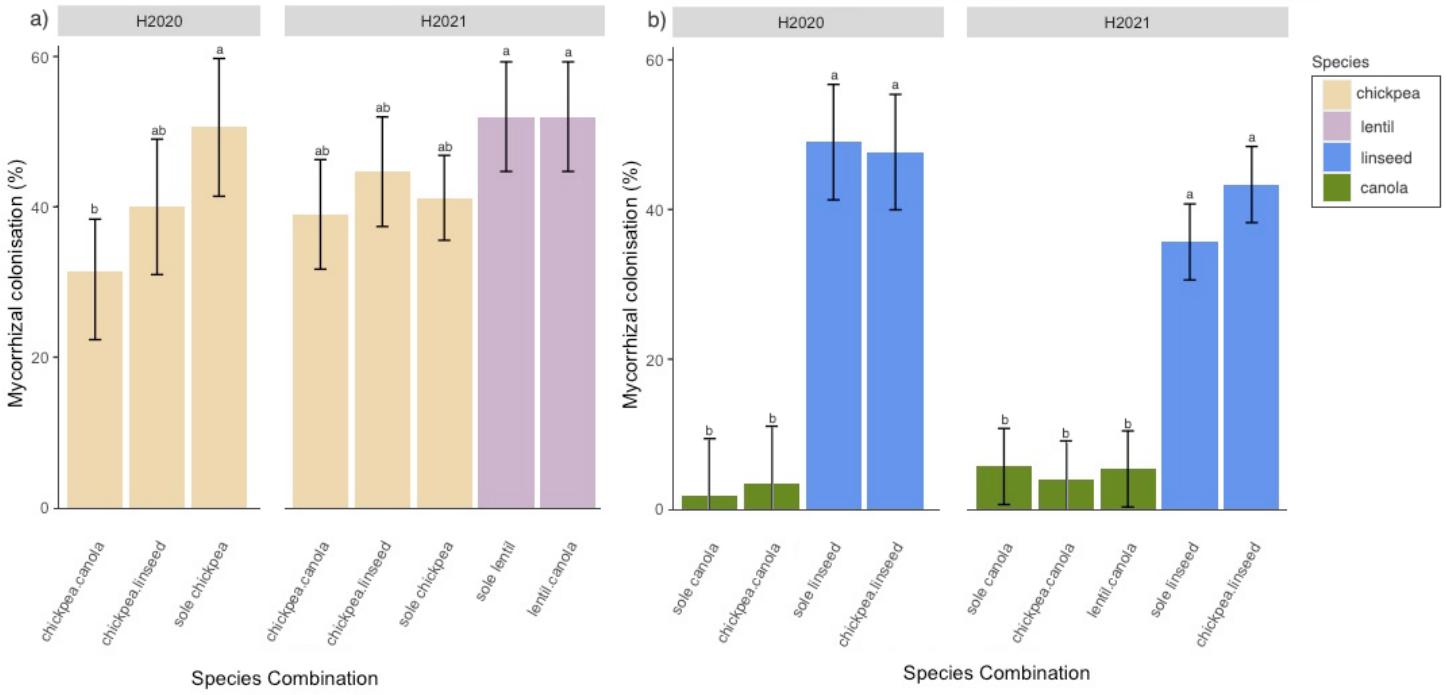

Figure 1

a) Chickpea (beige) and lentil (purple) and b) canola (green) and linseed (blue) root mycorrhizal colonisation (%) in intercrop and as sole crops at H2020 and H2021. Error bars indicate ± 95% confidence interval. Different letters indicate significant difference at p<0.05.

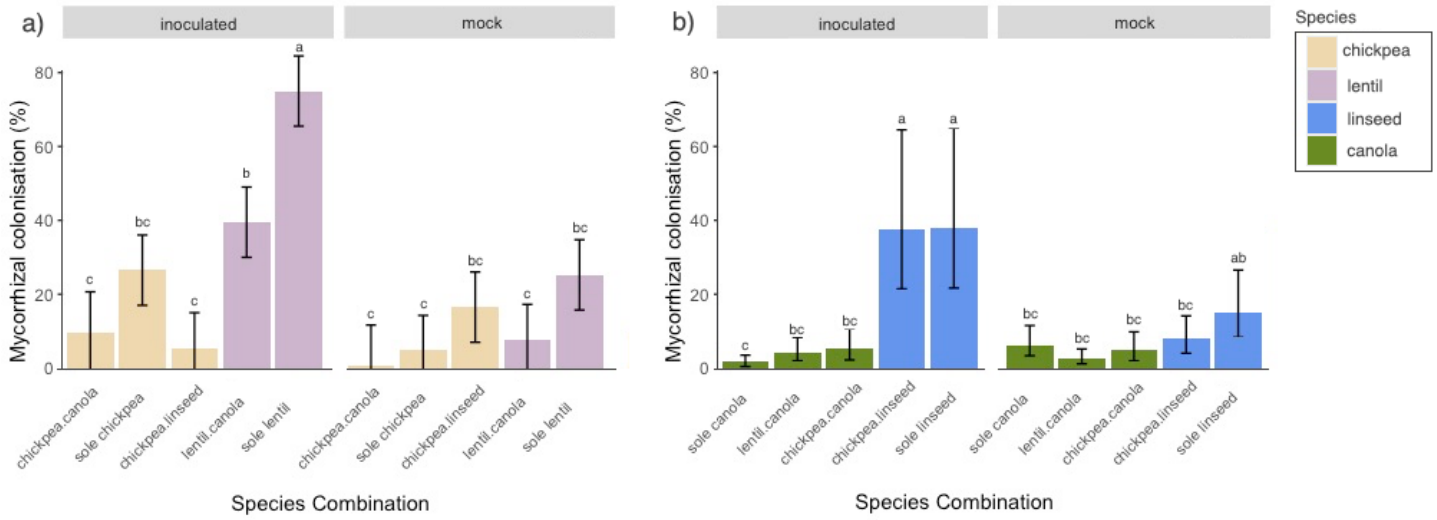

Figure 2

a) Chickpea (beige) and lentil (purple), and b) canola (green) and linseed (blue) root mycorrhizal colonisation (%) in intercrop and as sole crops in the inoculated and mock treatments. Error bars indicate

± 95% confidence interval. Different letters indicate significant difference at p<0.05.

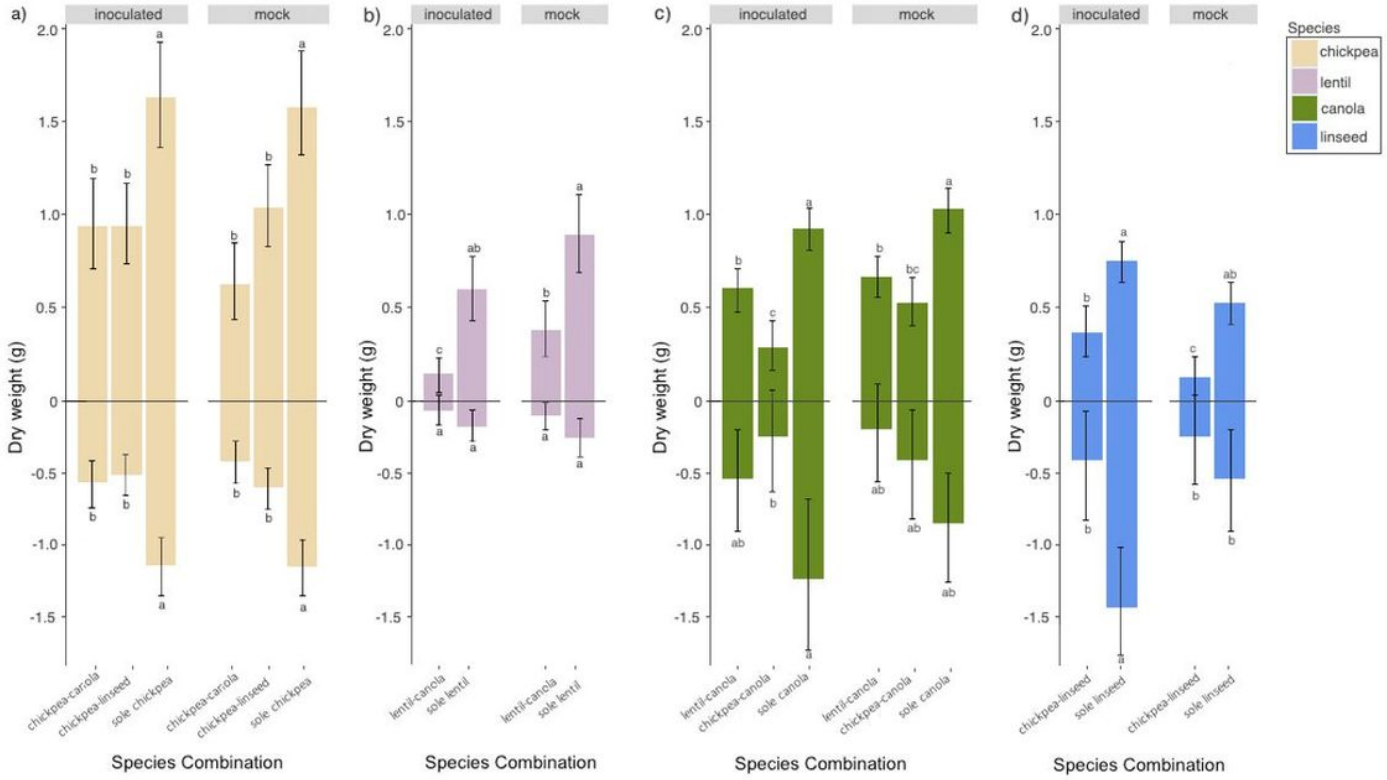

Figure 3

Mean dry weight (DW, g) at harvest of shoot (above x-axis) and root (below x-axis) of a) chickpea, b) lentil, c) canola, and d) linseed in intercrop and as sole crops in the inoculated and mock treatments. Error bars indicate ± 95% confidence interval. Different letters indicate significant difference at p<0.05.

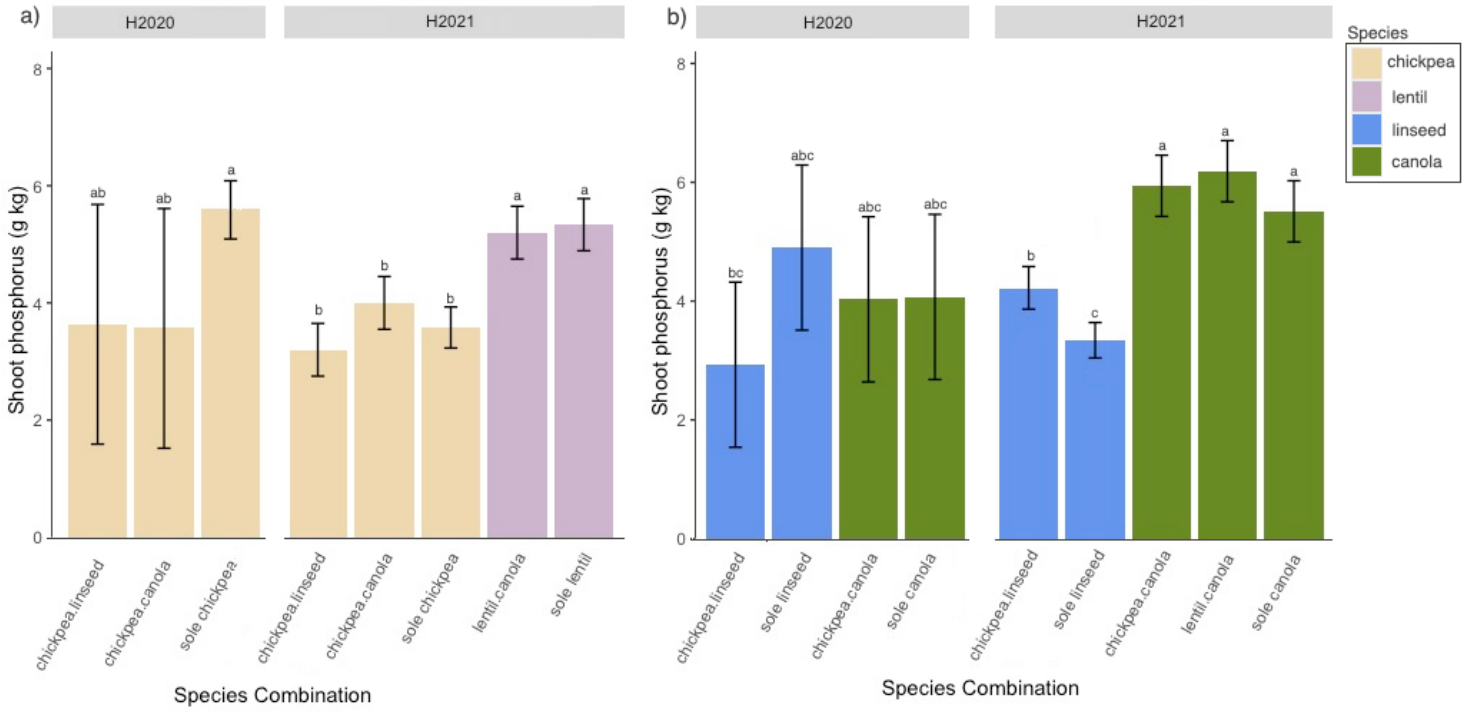

Figure 4

Shoot phosphorus (g kg-1) of a) chickpea and lentil and b) canola and linseed in intercrop and as sole crops at H2020 and H2021. Error bars indicate ± 95% confidence interval. Different letters indicate significant difference at p<0.05.

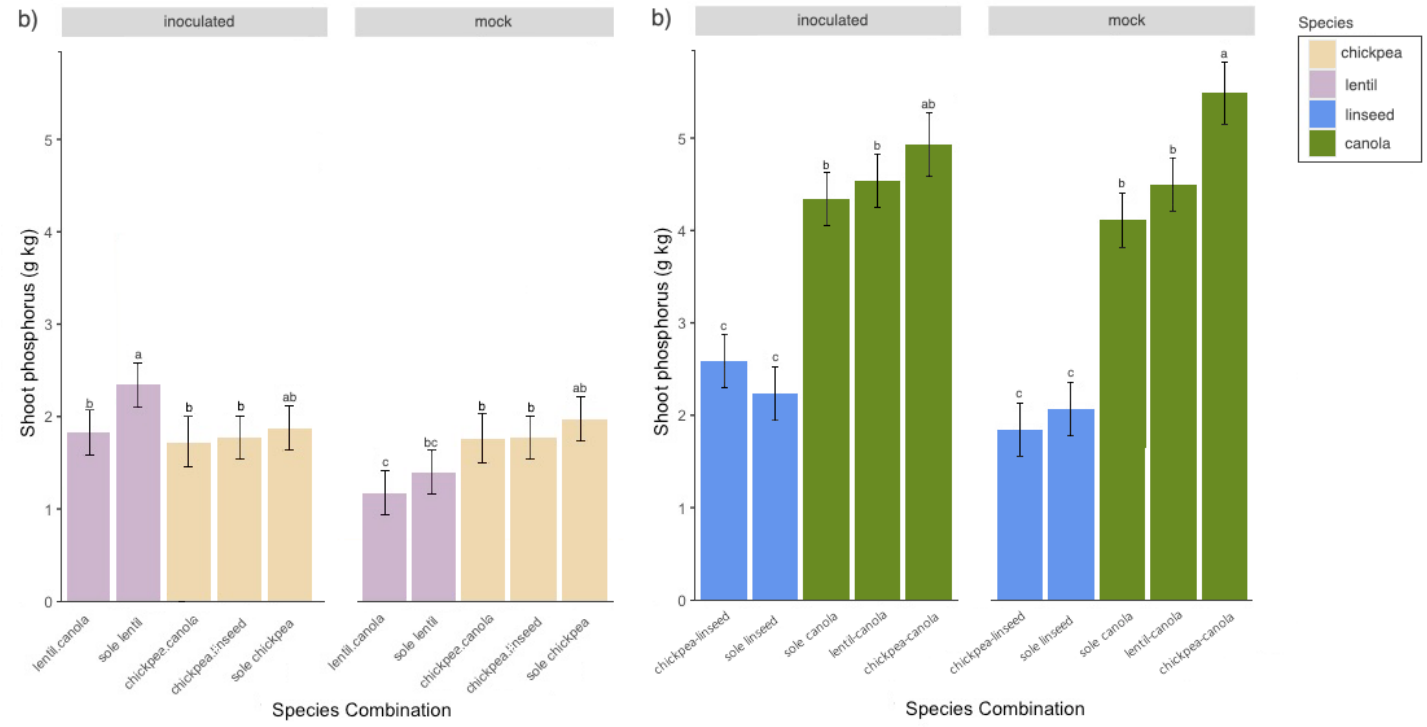

Figure 5

Shoot phosphorus (g kg-1) of a) chickpea and lentil and b) canola and linseed in intercrop and as sole crops in the inoculated and mock treatments. Error bars indicate ± 95% confidence interval. Different letters indicate significant difference at p<0.05.

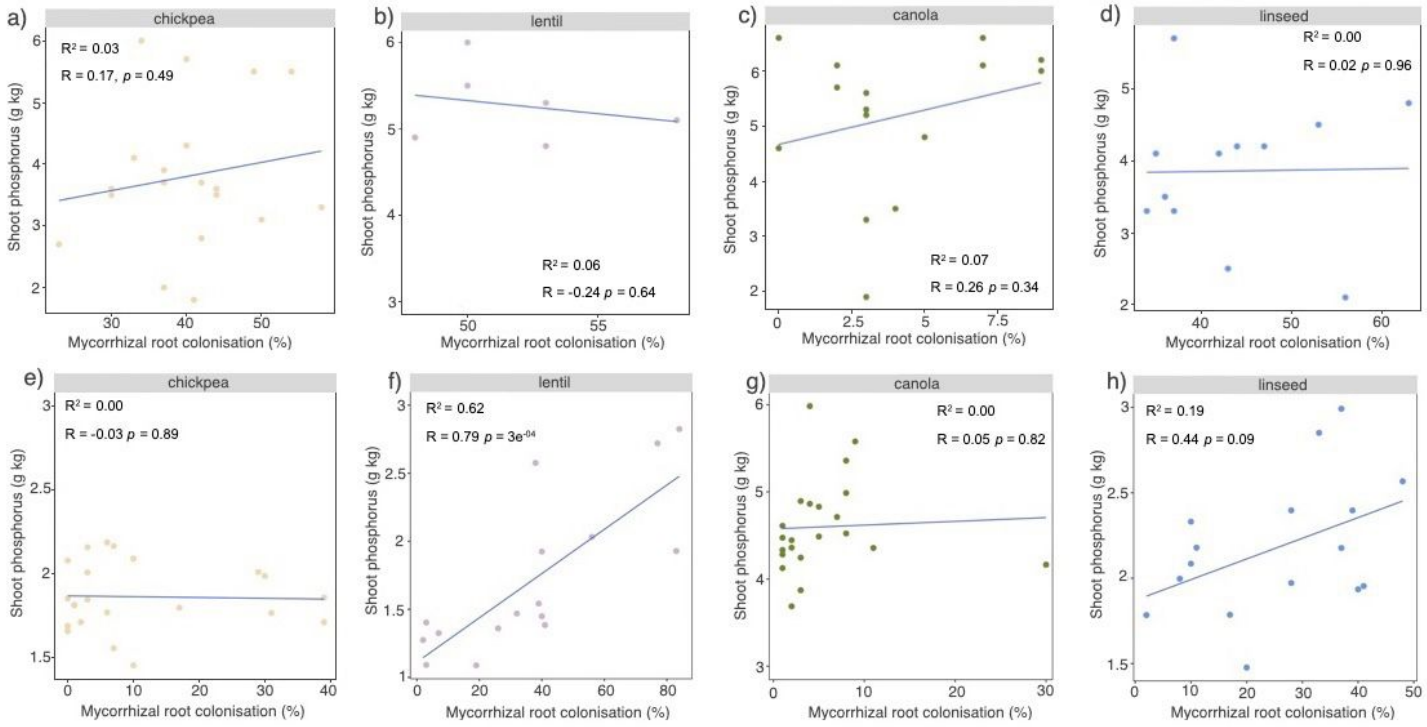

Figure 6

Regression of shoot phosphorus (g kg-1) by mycorrhizal root colonisation (%) of both sole crops and intercrops in the field experiment (a-d) and glasshouse experiment (e-h). Correlation between the two variables is shown by R, fit of the line by R2.