THE INTERACTION BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI, RHIZOBIA AND ROOT-LESION NEMATODES (*PRATYLENCHUS THORNEI*) IN MUNG BEAN (*VIGNA RADIATA*)

A Thesis submitted by

Elaine Gough, BSc (Hons)

For the award of

Doctor of Philosophy

2021

ABSTRACT

There are a limited number of reports on interactions between the beneficial microsymbionts arbuscular mycorrhizal fungi (AMF) and rhizobia which co-occur with the root-lesion nematode *Pratylenchus* sp. within the roots of legumes. Mung bean (*Vigna radiata*) is an important summer legume in the sub-tropical grain region of eastern Australia. It is a host of AMF, *Pratylenchus thornei* and nitrogen (N) fixing *Bradyrhizobium* bacteria. These microorganisms are dependent on mung bean for photosynthates and their interactions influence host production and nutrition. Nodulation failure in mung bean reduces plant production, nutrition and N budgets in soils and could be explained by a lack of mycorrhizal inoculum in the soil and/or by infestation with *P. thornei.* Furthermore, AMF colonisation of the roots may alter the population densities of *P. thornei* in mung bean.

Initially, in this thesis, a systematic review was carried out to clarify the effect of interactions between AMF and *Pratylenchus* spp., which showed that their interactions depended on the taxonomic order and genus of AMF, along with host plant functional groupings. With this specificity in mind, the interaction of AMF, rhizobia and *P. thornei* was investigated for mung bean cv. Jade-AU grown in a vertisol with a full factorial of these biological treatments in glasshouse experiments.

In the first study, AMF and rhizobia acted synergistically, increasing nodulation, biological N fixation, nutrition, growth and seed yield. These positive effects were complicated by *P. thornei*. Nodulation was reduced by *P. thornei* infestation which negatively impacted N fixation efficiency. However, mycorrhizal colonisation conferred tolerance to *P. thornei* which was indicated by maintained plant biomass. Unexpectedly, the population density of *P. thornei* increased in mycorrhizal mung bean, and the population density was positively correlated with concentrations of phosphorus (P), zinc (Zn) and copper (Cu) in the mung bean shoot.

Therefore, investigations were undertaken to elucidate the role of nutrients behind (i) improved nodulation and biological nitrogen fixation when mung bean was co-inoculated with AMF and rhizobia and (ii) increased population densities of *P. thornei* in mycorrhizal mung bean. A full factorial experiment included treatments of AMF, rhizobia, and *P. thornei*, with N, P and Zn fertilisers. It was shown that AMF increased (i) nodulation and N fixation to a level equal to or

greater than the application of fertiliser P, (ii) concentrations of P and Zn in the shoot, greater than the application of fertiliser alone and, (iii) the concentration of Cu in the shoot. Rhizobia and/or AMF conferred improvements greater than the addition of fertiliser N, including increased nodulation, shoot N concentration, biomass and yield greater than when rhizobia alone was added; and increased biomass and yield when AMF and rhizobia were both added. Increased population densities of *P. thornei* in mycorrhizal mung bean was again demonstrated but the application of fertilisers N, P and Zn decreased *P. thornei.* This result suggested the role of other mechanisms of increased susceptibility, such as AMF may decrease concentrations of defensive compounds against *P. thornei* in the roots or that AMF colonised roots may provide organic compounds that nutritionally stimulate *P. thornei* reproduction.

The research presented in this thesis contributes to understanding the complex multipartite interactions that occur between microorganisms in the roots mung bean and their impacts on N fixation, nutrition, biomass and yield. The conservation of AMF within farming systems is strongly advocated to promote and protect their valuable role in increasing biological nodulation and N fixation efficiency by rhizobia, and in improved crop nutrition and yield, while reducing fertiliser inputs. However, it is also crucial to understand that AMF may increase population densities of *P. thornei.* Agronomic practices and plant breeding to promote the synergism between AMF and rhizobia for mung bean yield, while limiting population densities of *P. thornei* will benefit mung bean production and subsequent crops in long-term sustainable farming systems.

CERTIFICATION OF THESIS

This Thesis is the work of Elaine Gough except where otherwise acknowledged, with the majority of the authorship of the papers presented as a Thesis by Publication undertaken by the Student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal supervisor: Dr Kirsty Owen

Associate supervisor: Prof John Thompson

Associate supervisor: Dr Rebecca Zwart

Associate supervisor: Dr Alla Marchuk

Student and supervisors signatures of endorsement are held at the University.

STATEMENT OF CONTRIBUTION

The following detail is the agreed share of contribution for candidate and co-authors in the presented publications in this thesis:

Article 1. Gough, E. C., Owen, K. J., Zwart, R. S., Thompson, J. P. (2020) A systematic review of the effects of arbuscular mycorrhizal fungi on root-lesion nematodes, *Pratylenchus* spp. Frontiers in Plant Science 11:923. (Q1; Impact Factor: 4.407) https://doi.org/10.3389/fpls.2020.00923 **(Chapter 2).**

EG contributed 65% of the conception and design, database search, collation of data, statistical analyses, writing and drafting. JT contributed 15% to the conception and design, statistical analyses, interpretation of data, writing and drafting. KO contributed 10% to the conception and design, integrating information on tables, writing and drafting. RZ contributed 10% to integrating information on tables, writing and drafting.

Article 2. Gough, E. C., Owen, K.J., Zwart, R.S., Thompson, J. P. (2021) Arbuscular mycorrhizal fungi acted synergistically with *Bradyrhizobium* sp. to improve nodulation, nitrogen fixation, plant growth and seed yield of mung bean (*Vigna radiata*) but increased the population density of the root-lesion nematode *Pratylenchus thornei*. Plant and Soil 1-22, (Q1; Impact Factor: 5.753) https://doi.org/10.1007/s11104-021-05007-7 **(Chapter 3).**

EG contributed 65% of the conception and design, data collection, statistical analysis and interpretation, writing and drafting. JT contributed 15% to the conception and design, statistical analyses, interpretation of data, writing and drafting. KO contributed 10% to the conception and design, interpretation of data, writing and drafting. RZ contributed 10% to writing and drafting.

Article 3. Gough, E. C., Owen, K.J., Zwart, R.S., Thompson, J. P. (2021) The role of nutrients underlying interactions among root-nodule bacteria (*Bradyrhizobium* sp.), arbuscular mycorrhizal fungi (*Funneliformis mosseae*) and root-lesion nematodes (*Pratylenchus thornei*) in nitrogen fixation and growth of mung bean (*Vigna radiata*) (Prepared for submission to Plant and Soil, Impact Factor: 4.192) **(Chapter 4).**

EG contributed 65% of the conception and design, data collection, statistical analysis and interpretation, writing and drafting. JT contributed 15% to the conception and design, statistical analyses, interpretation of data, writing and drafting. KO contributed 10% to the conception and design, interpretation of data, writing and drafting. RZ contributed 10% to interpretation of data, writing and drafting.

ACKNOWLEDGEMENTS

"Twenty years from now you will be more disappointed by the things you didn't do,

than by the ones you did do."

I heard this quote leaving school at 18 years old, and I stand, almost 20 years on, at the end of a very rewarding academic journey of which I could not have succeeded without the help of some integral people I have had the pleasure of knowing along the way.

First and foremost, I am indebted to the guidance and instruction received by my supervisors– Dr Kirsty Owen, Dr Rebecca Zwart, Dr Alla Marchuk and Prof John Thompson. This really was a collaborative work strengthened by a network of mentors that I have learned so much from in these four years. My research has followed on from the excellent research in soil biology carried out in the sub-tropical grain region of eastern Australia, and without these strong foundations, and aided by my supervisors' depth of knowledge and wisdom, I could not have finished this journey. Standing on the shoulders of giants indeed!

A special thanks to the CCH Crop Nematology team; Tim Clewett, Ros Reen, Hannah Rostad, Jason Sheedy, Neil Robinson, Mei Wang, Jing Lin, Martin Fiske, Michelle Thompson, Motiur Rahaman, Sonal Channale and Iman Elmor. Thanks to my colleagues and all the PhD students at the Centre for Crop Health. Thanks to Dr. Nikki Seymour and staff at the Queensland Department of Agriculture and Fisheries, Toowoomba. Thanks to Paul McIntosh (Pulse Australia) for promoting the research on the ground and getting growers excited about AMF. Thanks to Daniel Burrell (USQ) and Clayton Forknall (Statistics for the Australian Grain Industry) for statistical help. Thanks to Prof Gavin Ash (Executive Director at Institute of Life Sciences and the Environment, USQ), and Prof Levente Kiss (Director at Centre for Crop Health, USQ) for their support. Thanks to Juanita Ryan and all the members of the Graduate Research Office, for the help with research and University Guidelines. Thanks to all of the members of Action Learning Group. The invaluable constructive criticism and positive feedback improved each manuscript and contributed to improving my writing skills tremendously.

This research has been supported by an Australian Government Research Training Program Scholarship. I would like to thank the Dean of Graduate Research School Prof Peter Terry and the University of Southern Queensland Research Training Program Scholarship. Thanks also to the Grains Research and Development Corporation (GRDC) for awarding a research scholarship through Project USQ1912-003RSX which has supported my research and invited me to present my studies to a wider audience. Thanks also to the Australasian Association of Nematologists for a bursary to attend the "Stay Connected for Plant Health" Australasian Plant Pathology Society Conference 2021.

Raising two little boys in the midst of a PhD and a global pandemic is not easy and I couldn't have done it without the help of my family and friends, both here in Australia and over in Ireland. Thanks to Dr Bree Wilson for academic mentorship and friendship, Hannah Rostad for being the funniest nematologist I've ever come across, and Dom Carr, a fountain of patience and understanding. Thanks to my wonderful family–my mother and father and all those marvellous Goughs of Carrigarea who raised me well and believed unwaveringly that I could do it.

For Tom and Rafa, the reason I get up in the morning. Kids you'll move mountains.

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ABBREVIATIONS

% Ndfa	% nitrogen derived from the atmosphere
δ15N	Nitrogen isotope natural abundance
AMF	Arbuscular mycorrhizal fungi
BNF	Biological nitrogen fixation
CTR	Copper transporter family
JA	Jasmonic acid
LFD	Long Fallow Disorder
MAMP/DAMP	Microbe/Pathogen associated molecular pattern molecules
MTI	MAMP triggered immunity
MIR	Mycorrhiza induced resistance
NVT	National Variety Trial
Pi	Inorganic phosphate
Pi	Initial population
Pf	Final population
PAW	Plant available water
PCA	Principal components analysis
PGPR	Plant growth-promoting rhizobacteria
PPA	Pre-penetration apparatus
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSB	Phosphate solubilizing bacteria
RF	Reproduction factor
RLK	Receptor like kinase
RLP	Receptor like protein
SA	Salicylic acid
ZIP	Zinc-regulated, Iron-regulated transporter-like Protein family

CHAPTER 1

REVIEW OF LITERATURE

This review of literature covers a general discussion of the literature relevant to the PhD topic "The interaction between arbuscular mycorrhizal fungi, rhizobia and root-lesion nematodes (*Pratylenchus thornei*) in mung bean (*Vigna radiata*)". Research questions have been postulated after an extensive review of the literature and critical discussions and analysis of this topic. The chapter has been formatted according to the journal Australasian Plant Pathology.

1. Agriculture in the sub-tropical grain region of eastern Australia

The sub-tropical grain region of eastern Australia is an area of great agricultural productivity extending from Dubbo in Central NSW (32.23° S, 148.63° E) to Clermont (22.83° S, 147.63° E) in Central Queensland. The area is characterised by summer dominant rainfall, with an annual precipitation of 550–880 mm (Webb, 1997). Soils of the region are mainly heavy textured soils with high water storage capacity, classified into the soil Orders of Vertosols, Chromosols and Sodosols (Webb, 1997; Isbell, 1996). Australian Vertosols are generally derived from alluvium and sedimentary rocks formed of basic igneous rocks particularly basalt and they contain >35% montmorillonitic clay with a high cation exchange capacity (Isbell, 1996). In the course of this thesis, the term "Vertosol" as classified by Isbell (1996) is used in when discussing the soil order in relation to Australian agriculture, while the term "vertisol" is used in reference to the general soil type.

Historically, vertisols have had high levels of fertility. However, after years of intensive cropping, the soils have become deficient in soil organic matter and nutrients particularly nitrogen (N), phosphorus (P), potassium (K), zinc (Zn) and copper (Cu) (Dalal et al., 1991; Bell et al., 2010). Best agricultural land management incorporates conservation agriculture practices to improve soil health and reduce soil degradation. These include soil testing to assess the nutritive status of the soils and to guide fertiliser rates and depths of application, encouragement of zero to minimal tillage practices, maintenance of stubble cover to reduce soil erosion and improve water infiltration, and promotion of crop rotations with N-fixing grain and pasture legumes (Dalal et al., 1991; Chen et al., 2008).

Economically important crops grown in this region include wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.) in winter, and sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), cotton (*Gossypium hirsutum* L.) and mung bean (*Vigna radiata* (L.) R Wilczek) in summer. These crops are predominantly grown using a

combination of moisture accumulated in the soil profile during fallow periods and incident rainfall.

1.1 Mung bean production in the sub-tropical grain region of eastern Australia

Mung bean is a high-value short-season summer pulse crop. Originally domesticated in India, it is now cultivated on >7 million ha globally including Asia, particularly the Indian subcontinent and, South East Asia and in Australia (Fuller et al., 2007; Nair et al., 2020). As mung bean is a nutritious legume, high in starch, rich in essential amino acids and with a protein content of between 20 to 24% (Tang et al., 2014), it is an important plant-based protein source for many consumers globally, with the additional benefit of being high in micronutrients including iron and zinc (Nair et al., 2015). In Australia, mung bean is cultivated as a spring (September to November) or summer (December to February) crop predominantly in the sub-tropical grain region. The 5-year average production in Australia is 90,000 tonnes, with an export value of AUD$118 million (pulseaus.com.au). In 2016–2017, the production area of mung bean in Australia increased to 129,000 ha from 35,000 ha in 2014–2015 (ABARES 2017) due to increased price. Ninety percent of Australian production of mung bean is exported to the Indian sub-continent, South East Asia and China (https://www.pulseaus.com.au).

Mung bean is advantageous as it fits well in rotations with cereals where minimal or no tillage farming is practised and cereal stubble retained. Mung bean assist breaking weed and disease cycles, for example, by reducing the build-up of cereal pathogens such as crown rot caused by the fungus *Fusarium pseudograminearum* (Chauhan and Williams 2018; Gentry 2010). Mung bean also benefit cropping systems due to its ability to fix nitrogen through a beneficial symbiosis with rhizobia in root nodules, reducing the reliance on mineral N in the soil for the mung bean crop, increasing yield and protein content of subsequent cereal crops in the sequence and improving overall revenue for growers in the region (Dalal et al., 1998; Hochman et al., 2020). Furthermore, as mung bean require only 70–80 days from sowing to harvest it can be

double cropped after winter cereals, or a short fallow after summer crops when soil moisture levels are sufficient.

1.2 Abiotic and biotic factors affecting mung bean production

Mung bean has the capacity to produce 2.5 to 3 t/ha, however the average global productivity is much lower at 0.5 t/ha due to biotic and abiotic stressors (Nair et al., 2019). Abiotic factors that limit production both in Australia and globally include drought, susceptibility to heat stress at flowering and pod fill, salinity stress and waterlogging (Nair et al., 2019, Singh & Singh, 2011).

In Australia, biotic factors leading to reductions in yield potential of mung bean include bacterial pathogens causing tan spot (*Curtobacterium flaccumfaciens*) (Osdaghi et al., 2020) and halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) (Noble et al., 2019), fungal pathogens such as powdery mildew (*Podosphaera xanthii*) (Kelly et al., 2021), insect damage caused by mirids (*Creontiades* sp.) and cotton boll worm (*Helicoverpa armigera*) (Gentry, 2010), the soil-borne root-lesion nematode (*Pratylenchus thornei*) (Owen et al., 2014) and nodulation failure by rhizobia (Herridge et al., 2005) (Fig. 1). The constraints of the root-lesion nematode *P. thornei* and nodulation failure by rhizobia and the impacts of these on mung bean production are addressed in this thesis.

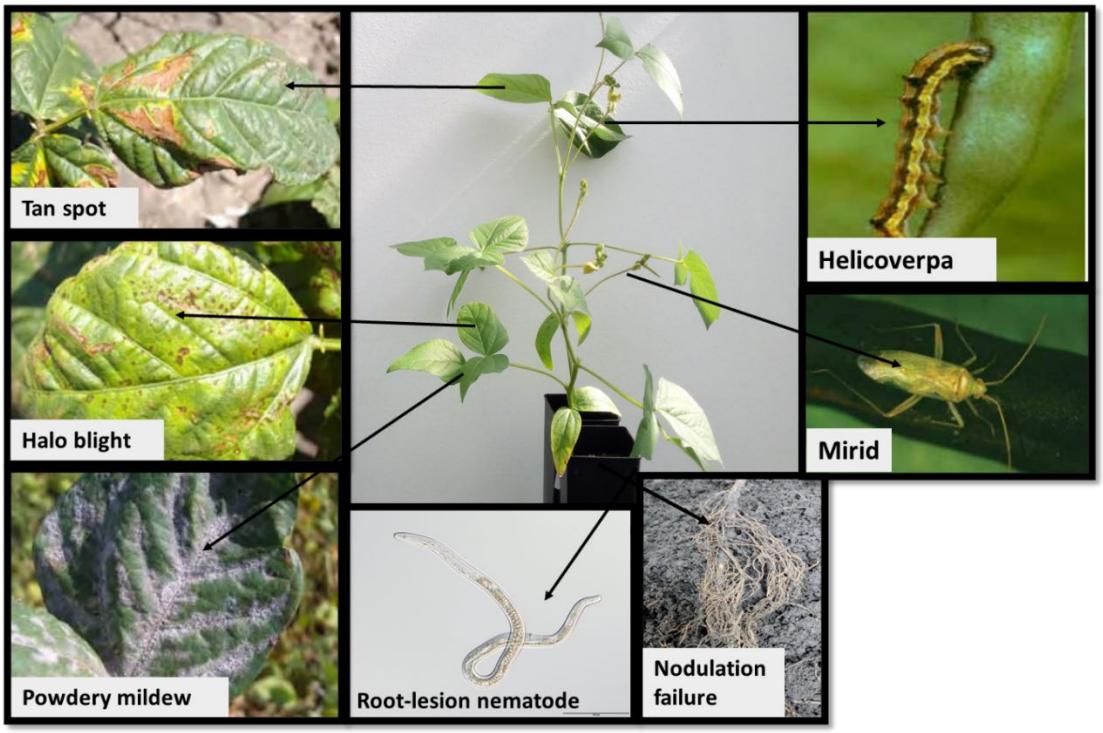

Figure 1 Biotic stressors of mung bean. Left to Right: tan spot (*Curtobacterium flaccumfaciens*), halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*), powdery mildew (*Podosphaera xanthii*), root-lesion nematode (*Pratylenchus thornei*), nodulation failure, mirids (*Creontiades* sp.), cotton boll worm (*Helicoverpa armigera*). Figure source: Adapted with permission from Mung Bean Management Guide (2nd Edition), by J. Gentry, 2010, the State of Queensland, Department of Employment, Economic Development and Innovation. Copyright 2010 by the State of Queensland, Department of Employment, Economic Development and Innovation

2. Plant-parasitic nematodes

Nematodes are thread-like unsegmented animals in the Phylum Nematoda. They represent the most abundant group of animals on earth encompassing 80% of all animals on land with an estimated 60 billion nematodes for each person, and are distributed ubiquitously in every habitat worldwide from sub-Arctic regions, to temperate and tropical regions (van der Hoogen et al., 2019). While the majority of nematodes are beneficial to ecosystem functioning, soil nutrient cycling and geochemical processes (Ingham et al., 1985; Bardgett et al., 1999; Hunt & Wall, 2002), some are parasitic to animals and plants.

There are over 4,100 species of plant-parasitic nematodes identified (Perry & Moens, 2006) and new species are continually being described. The impact of these plant-parasites on agriculture is significant with estimates of valuated losses of > US$100 billion worldwide (Nicol et al., 2011). However, damage from these below-ground plant-parasites is not always as obvious compared to many other soil-borne pathogens, for example damage by *Fusarium* or *Phytophthora* species. Some plant-parasites are biotrophic—feeding on living cellular plant tissue, therefore the level of damage to plant roots may not be as destructive as those pathogens or parasites that adopt a necrotrophic mechanism for survival.

The symptoms produced by nematode infestation including yellowing and wilting can be ambiguous and are often attributed to nutritional or water deficiencies. This often results in incorrect diagnoses and a misplacement of resources to treat the symptoms, not the cause of the disorder. Nematodes can remain undetected in the soil, where they build-up to damaging levels, and analyses of soil and plant roots are required to diagnose their presence.

Plant-parasitic nematodes can be grouped according to their feeding location in the host plant. Ecto-parasites feed externally from the tissues, while endo-parasites feed from within their host plant. Plant-parasitic nematodes can be further classified into

sedentary or migratory according to their pattern of movement once parasitism commences (Nicol et al., 2011).

The most economically important nematodes reducing agricultural production worldwide include the root knot nematodes (*Meloidogyne* spp.), the cyst nematodes (*Heterodera* and *Globodera* spp.) and the root-lesion nematodes (*Pratylenchus* spp.) (Jones et al., 2013). These nematode species affect the staple food crops of the majority of the world's population reducing crop productivity and causing substantial yield losses (Nicol et al., 2011). The root-lesion nematodes (*Pratylenchus* spp.) are the third most important group of plant-parasitic nematodes responsible for substantial crop losses worldwide including yield losses in the sub-tropical grain region of eastern Australia. Mung bean are a susceptible host to the root-lesion nematode *Pratylenchus thornei*, therefore *Pratylenchus* spp. are discussed in further detail in this section.

2.1 Introduction to Pratylenchus

Pratylenchus species are migratory, intracellular endo-parasites of the root cortex approximately 0.35 to 0.9 mm in length (Jones and Fosu-Nyarko, 2014). There are over 60 species of *Pratylenchus* distributed in diverse habitats throughout the world (Castillo and Vovlas, 2007). They are polyphagous with one of the broadest host ranges of all genera of plant-parasitic nematodes affecting many important crop species worldwide such as cereals (rice, maize, wheat), legumes, sugarcane, coffee, banana, potatoes, vegetables, and fruit trees (Castillo and Volvas, 2007). The species with the greatest economic impact on crop production include *P. thornei* Sher and Allen 1953*, P. neglectus* Rensch 1924, *P. penetrans* Cobb 1917*, P. crenatus* Loof 1960, *P. zeae* Graham 1951*, P. vulnus* Allen and Jensen 1951 and *P. coffeae* Goodey 1951 (Jones et al., 2013).

2.2 Feeding and migration of Pratylenchus *spp.*

Pratylenchus spp. are migratory in the root cortex and generally do not penetrate or feed on the endodermis or the stele (Perry & Moens, 2006). Extensive observations have been carried out on feeding and migration patterns of *P. penetrans* by Zunke (1990a, 1990b) using a range of microscopy techniques, including transmission electron microscopy and high-resolution video enhanced microscopy. The research demonstrated that *P. penetrans* are attracted to the zone of root hair development or to the zone of root elongation. Subsequent to a period of probing epidermal root cells with the lips, the root cell is perforated by the nematode using its characteristic hollow stylet, generally 15–22 µm long, which is thrust repeatedly into the epidermis. Once the epidermis has been punctured, the nematode pauses for a period of salivation and subsequently ingests the cytoplasm before moving through the epidermal cells. On puncturing the epidermal cell, the area then becomes attractive to other nematodes. They aggregate around the damaged area and can enter the cell via the feeding site. A brief feeding period by *Pratylenchus* spp. does not induce cell death. However, the cell is destroyed when the nematode passes through intracellularly on its migratory path. After feeding, *Pratylenchus* spp. undertake a resting phase of several hours before returning to feed and migrate to fresh cortical tissue.

2.3 Reproduction of Pratylenchus *spp.*

The adult females deposit eggs inside the cortical tissue, near the root surface or in the soil (Jones & Fosu-Nyarko, 2014). There are four juvenile stages in the life cycle of *Pratylenchus* spp. generally completed in 3 to 8 weeks under favourable conditions, depending on nematode species and host susceptibility (Jones & Fosu-Nyarko, 2014; Linsell et al., 2014). *Pratylenchus* spp. develop within the egg to the first stage juvenile (J1). The J1 moults to the second stage juvenile (J2), which hatches from the egg within 14 days. The juvenile then moults to the third stage juvenile (J3) and then to the fourth stage juvenile (J4). The J4 resembles the adult but without the sexually

mature reproductive organs (Fig. 2). An adult female may lay 1–2 eggs per day and this reproduction may be sexual or parthenogenic (Jones & Fosu-Nyarko, 2014). In the absence of a host, *Pratylenchus* spp. can survive for long periods in all stages of development (Glazer & Orion, 1983).

Figure 1 Pratylenchus spp biology

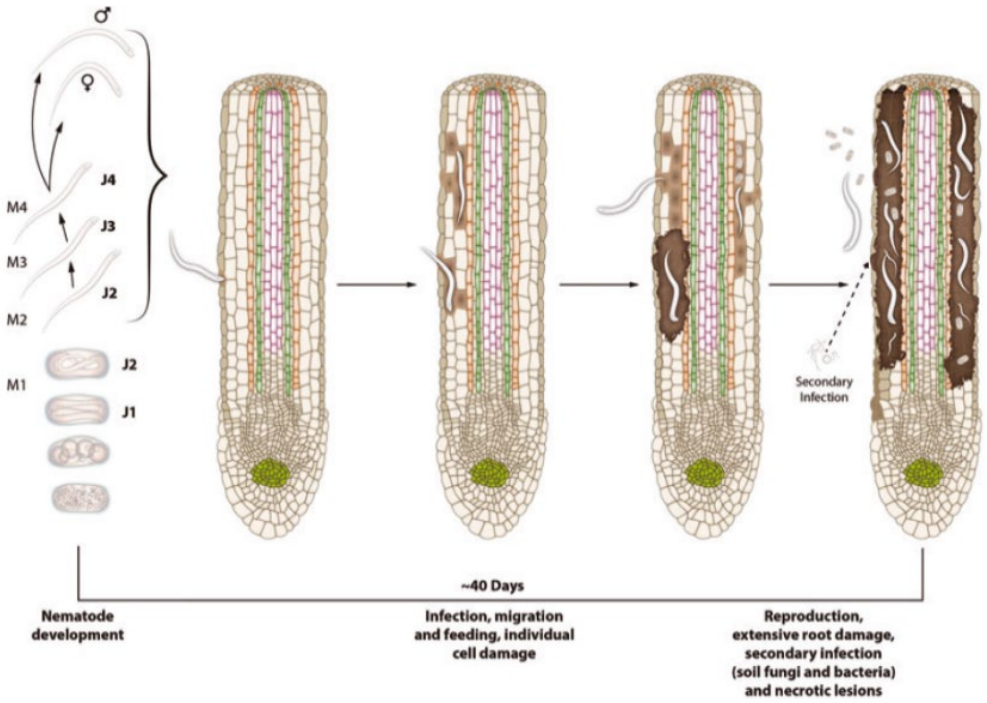

Figure 2 *Pratylenchus* spp. biology. Reproduced with permission from "Molecular biology of root lesion nematodes (*Pratylenchus* spp.) and their interaction with host plants: Molecular biology of root lesion nematodes" by M. G. K. Jones and J. Fosu-Nyarko, 2014, *Annals of applied Biology*, 164(2), 163-181. Copyright 2014 by Association of Applied Biologists.

2.4 Symptoms of Pratylenchus *spp. infection*

Pratylenchus spp. secrete cellular degrading enzymes and effector proteins which aid the enzymatic degradation of the cell wall during feeding and salivation and facilitate migration through the cells (Fosu-Nyarko & Jones, 2016). On migration through root

tissue, they destroy cortical cells resulting in reddish to brown necrotic lesions in the roots of the host plant (Perrine-Walker, 2019). Lesions expand by coalescence between lesions or by increasing in size over time and in some species such as in *P. penetrans*, the females induce larger lesions and in greater quantities than the males (Saikai & MacGuidwan, 2020). This destruction of root cortical tissue results in a reduction in root mass and loss of root function. As a result, the intolerant plant has increased susceptibility to water stress and a reduced water extraction rate of the damaged roots resulting in stunting, yellowing and yield loss (Davis & MacGuidwan, 2000; Pinochet et al., 1996; Robinson et al., 2019; Thompson et al., 2012; Whish et al., 2014). These lesions may also act as a pathway for pathogens to enter the root increasing plant susceptibility to secondary infection by pathogenic bacteria and fungi (Fig. 2) (Jones & Fosu-Nyarko, 2014). The combination of *Pratylenchus* infestation with secondary infections caused by pathogens can lead to synergistic interactions, for example in potato (*Solanum tuberosum*), *P. penetrans* and Verticillium wilt fungi interact and result in "potato early dying syndrome" with symptoms more severe when combined than on infection with either alone (Davis & MacGuidwin, 2000).

As a consequence of the destruction of cortical tissue, infestation by *Pratylenchus* spp. also results in symptoms of nutrient deficiencies. Wheat infested by *P. thornei* exhibited symptoms of N deficiencies including increased chlorosis and reduced tillering resulting in a subsequent yield reduction in field trials (Thompson et al., 1995). The concentration and uptake of both P and N were reduced with increasing *P. thornei* population densities in wheat at stem elongation and anthesis in field trials (Thompson et al., 2021). Inoculation with *P. vulnus* resulted in deficiencies of N and P in pear rootstock (*Pyrus communis*) (Lopez et al., 1997), and reduced concentrations of the nutrients N, P, K, Ca, Mg, Mn and Cu in plum rootstock (*Prunus cerasifera* X *P. munsoniana*) when grown in low P soils (Pinochet et al., 1998). Conversely, plants grown under conditions of sufficient soil moisture and soil nutrition may exhibit tolerance to infestation with *Pratylenchus*, as optimal plant growth conditions may increase the efficacy of root function and therefore compensate for the damage inflicted by the nematode (Calvet et al., 1995; Melakeberhan et al., 1997).

Interactions may also occur between nutrient applications and alterations to *Pratylenchus* population densities. Walker (1971) found that populations of *P.*

penetrans decreased after adding nitrogenous amendments to cultures predominantly due to ammonification. In field trials on wheat, P application of 20–30 kg/ha reduced *P. neglectus* population densities by 30% (Vanstone et al., 2002). Potassium fertilisation led to increased reproduction of *Pratylenchus* spp. in cotton, though the plants became tolerant to the infestation (Oteifa & Diab, 1961), while in sour cherry trees (*Prunus cerasus*), K deficient trees hosted the highest population of *P. penetrans* (Kirkpatrick et al., 1964). The effects of nutrition on population dynamics of *Pratylenchus* spp. are likely to be quite specific.

2.5 Pratylenchus thornei *in the sub-tropical grain region of eastern Australia*

During the 1960s, conservation agriculture and the benefits of reduced tillage, no till and retaining crop residues led to a major transformation in Australian rain-fed agriculture (Thomas et al., 2007). This reduction of tillage and retaining crop residues resulted in benefits including improving soil structure, water retention, soil moisture conservation, reducing soil erosion, increasing soil organic content and increasing beneficial soil-borne micro-organisms (Bowles et al., 2017; Gupta et al., 2019). The broad-acre cropping systems in the sub-tropical grain region allows the cultivation of both summer and winter crops and is dependent on water stored in the heavy textured soils from summer dominant rains. Soil moisture is conserved through reduced tillage and also by the use of weed free fallows which vary in length from <6 months termed 'short fallow' and >12 months termed 'long fallow' (Page et al., 2013). Surveys carried out in the 1960's in paddocks of the region cultivated with wheat identified the presence of the root-lesion nematodes *P. thornei* and *P. neglectus* (Colbran and McCulloch, 1964). However, the continuous cultivation of susceptible wheat varieties had a knock-on effect of allowing the proliferation of plant-parasitic nematodes including *P. thornei* in the soil (Fig. 3) (Thompson et al., 1995; Thompson et al., 2021).

Surveys on the distribution of root-lesion nematodes carried out in eastern Australia showed that in the sub-tropical grain region, *P. thornei* was found in 67% and *P. neglectus* in 32% of 795 wheat fields sampled, with population densities for damage

of 2000 *P. thornei*/kg soil exceeded in 31% of the area sampled (Thompson et al., 2008; Thompson et al., 2010). In the southern grain region *P. neglectus* predominates over *P. thornei*, while in the western grain region *P. neglectus* predominates over *P. quasitereoides* and *P. penetrans* (Hodda et al., 2014; Vanstone et al., 2008).

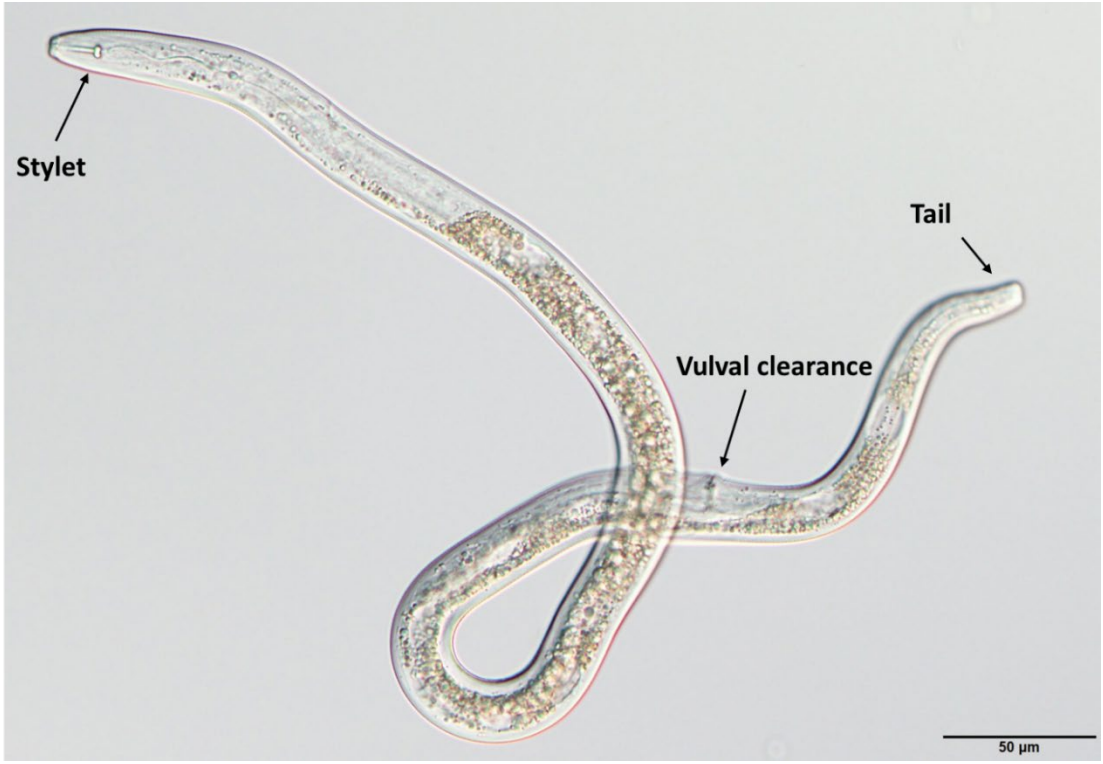

Figure 3 *Pratylenchus thornei* adult female. Source: Modified with permission by Kirsty Owen.

Mung bean is a susceptible host to *P. thornei* and the level of nematode multiplication is dependent on cultivar. Increases in multiplication of *P. thornei* in mung bean and other susceptible hosts can have detrimental effects on the yield of a subsequent intolerant wheat cultivar (Owen et al., 2014). Regression analyses of crop rotation experiments in the region demonstrated a negative linear relationship between wheat biomass and yield and increasing population density of *P. thornei* (Owen et al., 2014). Some chickpea and wheat genotypes can lose up to 25% and 65% of crop yields respectively in fields containing high population densities of *P. thornei* (Reen et al., 2014; Thompson et al., 1999). Under optimal conditions, *P. thornei* can complete its life cycle in ~ 6 weeks (Larson, 1959). Therefore, when a susceptible wheat with an 18-week cropping cycle is cultivated into soil with *P. thornei*, under ideal conditions for reproduction, the population increase of *P. thornei* can exceed the damage

threshold of 2000 *P. thornei*/kg soil (Thompson et al., 2015). It is imperative therefore, to reduce the population densities of *P. thornei* in soils of the region to a level below the economic damage threshold to maintain yields of economically important crops.

2.6 *Management of* Pratylenchus thornei

Management of *P. thornei* in the sub-tropical grain region has followed a multi-faceted approach utilising farm hygiene, development of resistant and tolerant cultivars, soil amendments and crop rotations. Murray and Brennan (2009) demonstrated that these current strategies reduced the potential loss of wheat by *P. thornei* infestation in the sub-tropical grain region of Australia from $104 million to $38 million.

The reaction of a plant to *P. thornei* infestation can be categorised as tolerant or resistant. Tolerance of a plant to nematode infestation indicates the ability of the plant to maintain biomass and yield despite multiplication of the nematode within the roots, while resistance of a plant indicates the inability of the parasite to reproduce within the roots of the plant (Roberts, 2002; Thompson et al., 2008; Wallace, 1971). Resistance and tolerance to nematodes are usually controlled by separate genetic traits (Roberts, 2002). Screening for resistance and tolerance to *P. thornei* infestation is undertaken in both glasshouse and field trials. Assessments of the difference between the final *P. thornei* population (P*f*) versus the initial *P. thornei* population (P*i*) in the roots of the plant over the growing cycle indicates the level of tolerance or resistance of the host plant. Varieties with higher levels of resistance are currently being developed by breeders by conducting analyses of accessions with greater genetic diversity from the centres of origin of both wheat and chickpea to manage *P. thornei* infestation (Reen et al., 2019; Sheedy & Thompson, 2009). Introgressing these polygenic traits for resistance have increased yields of wheat compared to tolerant varieties in the sub-tropical grain region (Thompson et al., 2001). However, to date only moderately resistant varieties of wheat and chickpea are available for use by growers. Notwithstanding this, research is currently being undertaken to incorporate higher levels of tolerance and resistance in research breeding programmes in wheat

and chickpea (Reen et al., 2019; Thompson, 2008; Thompson et al., 2021; Zwart et al., 2019).

Application of nematicides and/or soil amendments have been used in experimental research in the region to control *P. thornei*. Nematicides are not effective in the deep levels in the soil profile where *P. thornei* can survive (0.9 m) (Reen et al., 2014; Thompson et al., 2012; Thompson et al., 2021). They are environmentally destructive and none are registered for use in broadacre crop production in this grain region. Soil amendments including green manures and biochars can reduce nematode population densities such as *Pratylenchus* (Akhtar & Malik, 2000; George et al., 2016; Zhang et al., 2013), but these are not economical in the large farm sizes of Australia and are not likely to reach the depths in the soil profile where *P. thornei* can survive (Owen et al., 2014; Reen et al., 2014).

Crop rotations by growing sequences of non-host crops consecutively such as sorghum, cotton, linseed (*Linus usitatissimum* L.), canary seed (*Phalaris canariensis* L.) or canola (*Brassica napus* L.) currently remains the most important cultural technique to reduce the levels of *P. thornei* in the soils of the sub-tropical grain region (Owen et al., 2014; Reen et al., 2014). Strategic crop rotations can be utilised to manipulate and reduce the populations of detrimental *P. thornei,* while improving beneficial soil-borne micro-organisms such as arbuscular mycorrhizal fungi (AMF) to increase yields for subsequent crops in the rotation (Owen et al., 2014). Research to reduce *P. thornei* while increasing beneficial micro-organisms is further discussed in Section 4.6. Cultivars of wheat, barley, maize and soybean selected in the region for their economic advantages may have a range of responses to *P. thornei* infestation ranging from resistant/moderately resistant to susceptible. The selection and growth of cultivars that are evaluated as tolerant or susceptible may facilitate the multiplication of *P. thornei* population densities throughout the growing season. The resistance and tolerant rating systems to *P. thornei* are assessed using glasshouse and field trials and the ranking of cultivars is available on the National Variety Trial (NVT) database (www.nvt.grdc.com.au). As of 2021, mung bean is not screened in the NVT trials for any trait related to yield or disease ratings including *P. thornei* resistance ratings.

3 Mycorrhizal fungi

Mycorrhizal fungi form a symbiotic relationship with the roots of many terrestrial plants worldwide. They can be divided into ectomycorrhizal fungi and endomycorrhizal fungi, differentiated on how the fungal hyphae colonise plant root cells. In ectomycorrhizal fungi, the hyphae do not enter root cells, but form a mycelial mantle around the root and grow in the space between epidermal and cortical cells in a complex of intracellular hyphae termed the Hartig net (Figure 4A) (Peterson & Bonfante, 1994). Ectomycorrhizal fungi form associations with the roots of approximately 80–90% of temperate and boreal forest trees (Stuart & Plett, 2020). In endomycorrhizal fungi, which include arbuscular mycorrhizae, ericoid mycorrhizae, orchid mycorrhizae and monotropoid mycorrhizae, the hyphae penetrate plant root cells by invagination of the cell membrane (Figure 4B) (Allen, 1991). As the focus of this thesis research is on arbuscular mycorrhizal fungi, the subsequent section discusses arbuscular mycorrhizal fungi alone.

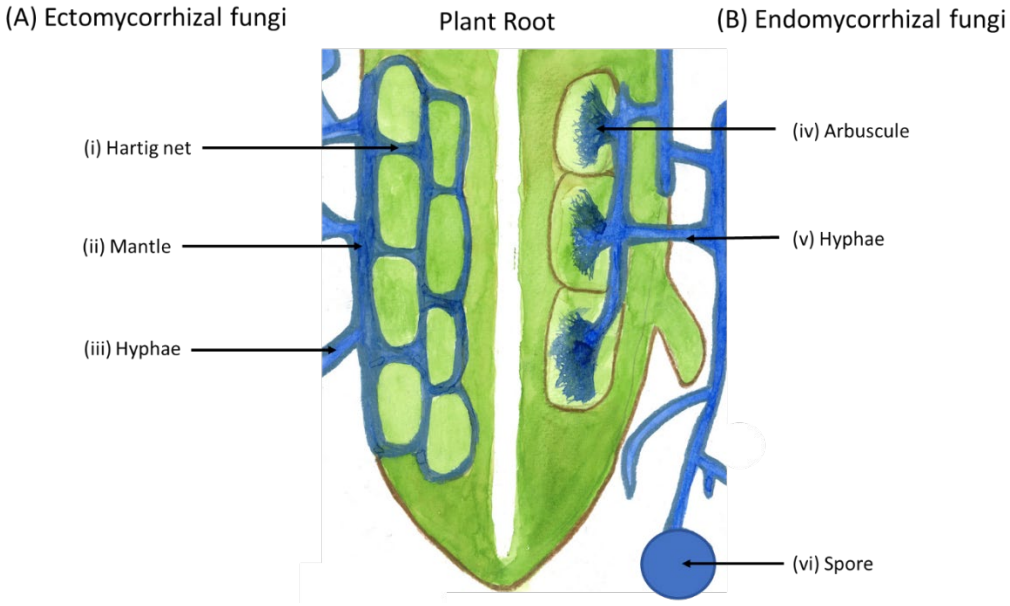

Figure 4 Schematic of mycorrhizal fungi (A) ectomycorrhizal fungi illustrating (i) Hartig net, (ii) mantle, (iii) hyphae and, (B) endomycorrhizal fungi illustrating the (iv) arbuscule, (v) hyphae, and (vi) mycorrhizal spore. Figure source: Elaine Gough

3.1 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi are obligate biotrophs from the phylum *Glomeromycota*, which evolved > 450 million years ago and are thought to have been crucial to the colonisation of soil by ancestral land plants (Redecker et al., 2000). Arbuscular mycorrhizal fungi have existed relatively unchanged for millions of years due to their important mutually beneficial association with the roots of an estimated 80% of terrestrial plants, improving water and nutrient supply to the plant in exchange for photosynthetic carbon from the plant (Smith & Read, 2008). The phylum *Glomeromycota* can be further divided into the orders Glomerales, Diversisporales, Paraglomerales and Archaeosporales. These orders are based on phylogenetic molecular taxonomy from the analyses of the small subunit rRNA gene, the large subunit rRNA gene and the internal transcribed region (Schüßler & Walker, 2010). There are approximately 288 described species of AMF and this relatively low level of diversity may be related to their asexual manner of reproduction, though recent advances in molecular sequencing, indicates that diversity of species may be higher, with approximately 1700 putative species proposed by Öpik and Davison (2016). Soils are likely to contain multiple species of AMF and agricultural systems may influence the community structures of AMF. Increased phylogenetic diversity is observed under systems of reduced tillage and reduced fertiliser N application (Jansa et al., 2002; Williams et al., 2017).

Along with taxonomic diversity, functional diversity is also used to aid classification of mycorrhizal fungi. Functional diversity is determined by the variability in plant responses to mycorrhizal colonisation including growth responses, P uptake and tolerance to biotic and abiotic stressors (Smith and Read, 2008) and variations in these traits can occur both intra-specifically and inter-specifically (Munkvold et al., 2004). This functional diversity has been proposed to be more useful indicator to predict the outcome of the host/AMF response to plant growth, nutrition and stressors compared to predictions of outcomes based on species alone (Van der Heijden et al., 2004). Van der Heijden and Scheublin (2007) have identified 13 different functional traits of AMF including P uptake, extra-matrical hyphal length that could be used to predict the effect mycorrhizal fungi have on plant growth. It has been suggested that functional diversity

may be more conserved in higher taxonomic clades, for example via family compared to by species (Maherali & Klironomos, 2007). For example, in a meta-analysis by Yang et al. (2017), the Glomeraceae family improved P uptake and fungal pathogen protection to a greater level than Gigasporaceae, which in turn, improved plant response against heavy metal stressors.

Phosphorus is an essential nutrient for plant growth, energy transfer, photosynthesis and other key biological processes and is often the most limiting nutrient for plant growth after nitrogen (Schachtman et al., 1998). Plants take up P from the soil in the form of $H_2PO_4^-$ and HPO_4^{2-} and this P is moved by diffusion in the bulk soil. However, this is a slow process (10^{-12} to 10^{-15} m^2 s^{-1}) and due to the high requirement for P by the plant, the rhizosphere is often depleted of phytoavailable P (Schachtman et al., 1998). A plant may increase the uptake of P by the use of root hairs in the plant mediated pathway via high affinity inorganic P transporters (PiTs) and also by the use of mycorrhizal fungi.

Plants that form associations with mycorrhizal fungi vastly increase the surface area available to them to forage for P. The absorptive extra-matrical hyphal network composed of fine hyphae (2–10 μm) (Fig. 5) and there can be hundreds of metres of fungal hyphae per cm^3 soil (Miller et al., 1995). Furthermore, mycorrhizal fungi absorb P from the soil and using fungal PiTs, deposit P as polyphosphate directly into the cortical cells of the plant (Smith et al., 2011). Consequently, AMF can improve the acquisition of inorganic, poorly mobile nutrients from the soil in particular P, but also including NH_4, Zn, S and Cu, and can access nutrients from the bulk soil outside the nutrient interception zone of the roots (Cavagnaro et al., 2015; Jakobsen et al., 1992; Lehmann et al., 2014; Miller et al., 1995; Smith et al., 2011). The fine fungal hyphae also facilitate access to moisture and nutrients from soil micropores, which would otherwise be inaccessible to the coarser roots, improving water extraction efficiency along with nutrient uptake from the soil (Allen, 2011; Bitterlich et al., 2018). The efficiency of AMF depends on the level of P in the soil (Koide and Li, 1990). In soils with very high levels of P available to the roots via direct uptake and diffusion, the percentage mycorrhization is reduced due to an increase in root biomass, a reduction in arbuscle development and a reduction in fungal biomass per plant (Smith & Read, 2008).

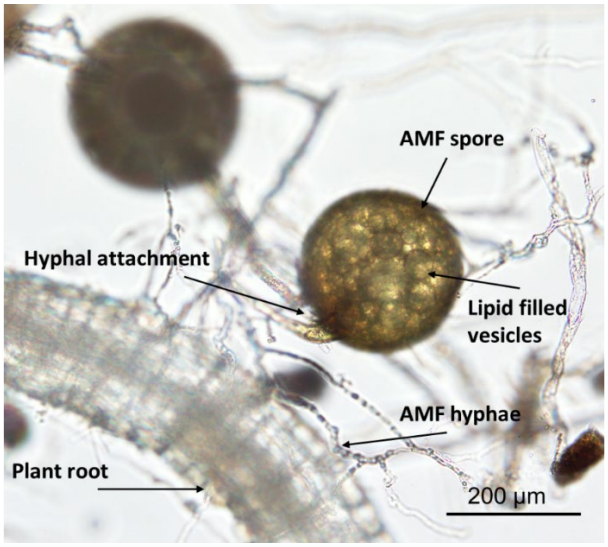

Figure 5 Micrograph of mycorrhizal fungi. Spore of arbuscular mycorrhizal species *Funneliformis mosseae* near root of maize showing fine AMF hyphae, hyphal attachment to the spore and lipid filled vesicles within the spore. Scale bar 200 µm. Figure source: Elaine Gough

3.2 Infection of AMF in the plant root

Mycorrhizal fungi exhibit relatively low host specificity and will colonise a broad range of plant species (Smith and Read 2008). Individual roots may also be colonised with multiple species of AMF and the interaction between AMF and host may be a functionally-dependent relationship, as opposed to a species-dependent relationship (Öpik & Davison 2016). Initiation of mycorrhizal symbiosis with AMF begins with the host plant exuding a chemical signal from the roots in the form of the phytohormone strigolactone, which induces mycorrhizal spore germination and hyphal branching (Figure 6) (Besserer et al., 2006; Akiyama et al., 2005). This begins a series of cross communication between the plant and the fungus. Mycorrhiza factors (Myc factors) are produced in the fungus which induce calcium oscillations in the plant host cells, initiating the symbiosis signalling pathway (Kosuta et al., 2008). Appresoria, called hyphopodium, are developed by the fungus and the plant cells produce a tunnel like structure called the pre-penetration apparatus (PPA) in anticipation of fungal penetration. This PPA extends across the vacuole of the

epidermal cell of the host allowing the fungal hyphae to penetrate the cortical cells (Genre et al., 2005). Additional secondary PPA are produced within cortical cells in which the fungus produces the tree like structures known as arbuscules (Figure 7). These act as functional sites of exchange, transferring sugars and fatty acids as a fixed carbon source from the plant in exchange for nutrients especially P and ammonium (NH_4^+) to the plant, via a shared permeable peri-arbuscular membrane (Luginbuehl & Oldroyd, 2017). The fungus also produces lipid filled vesicles within the roots (Figure 7), believed to function as storage organs for the fungus and these vesicles are high in nutrients including P, Ca, K, S, Zn and Si (Olsson et al., 2011). Vesicles, along with spores, may also function as infective propagules in certain AMF species (Biermann & Linderman, 1983; Müller et al., 2017).

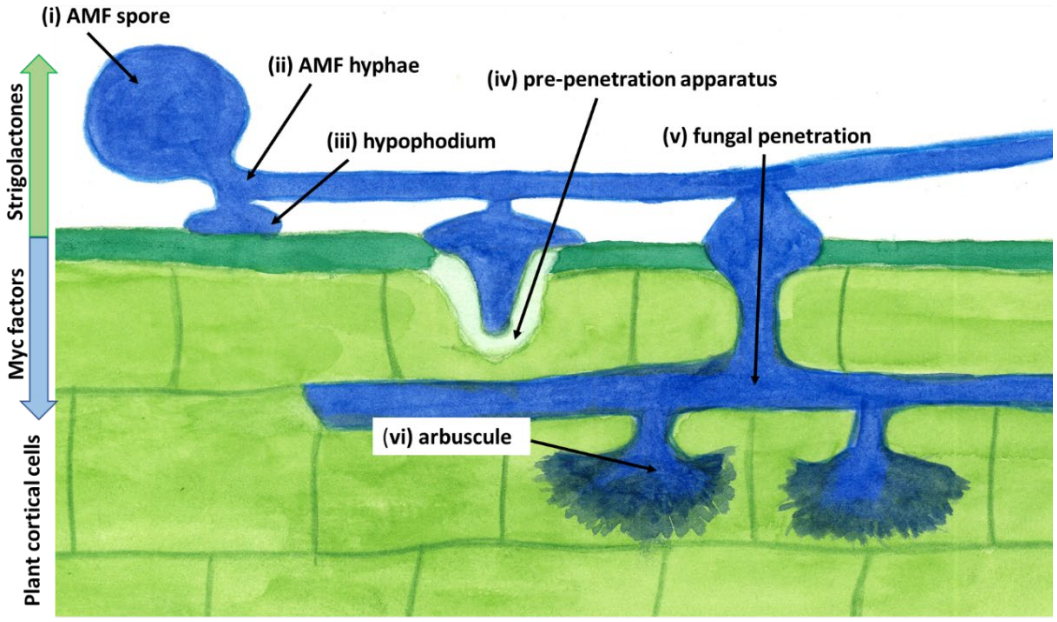

Figure 6 Schematic of steps in arbuscular mycorrhizal development. The (i) mycorrhizal spore germinates and (ii) hyphae extends in the presence of strigolactones exuded by a suitable plant host. Myc factors are released by AMF initiating the cross talk between plant and fungus and the (iii) hyphopodium extends and grows into the plant cell in the form of a (iv) pre-penetration apparatus. (v) Fungal penetration into the cortical cells results in (vi) arbuscules formed from the pre-penetration apparatus and the mature symbiosis results in the exchange of nutrients between plant and fungus. Figure source: Elaine Gough

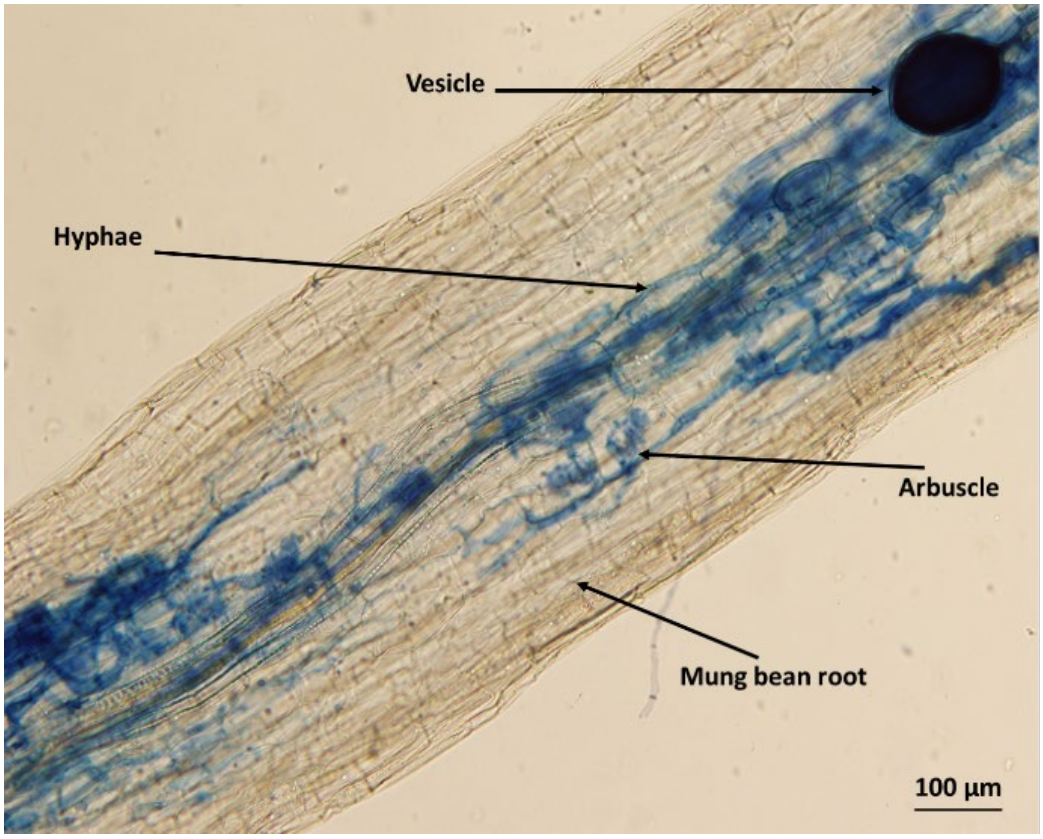

Figure 7 Mycorrhizal colonisation of mung bean root. Roots of mung bean cv. Jade-AU colonised with arbuscular mycorrhizal fungi showing the stained arbuscules, vesicles and fungal hyphae. Root stained with Trypan blue. Scale bar 100 µm. Figure source: Elaine Gough

3.3 AMF and abiotic stressors

Many crop species grown in the sub-tropical grain region of eastern Australia including sorghum, maize, sunflower, cotton, faba bean, chickpea and mung bean have a high level of mycorrhizal dependency (Thompson, 1987). Some crop families have no association with mycorrhizae such as the *Brassicaceae*, which include agriculturally important crops such as canola and lupins (Lambers and Teste 2013).

Mycorrhizal dependency has been defined as the level of dependence of a plant on mycorrhizae to produce maximum growth or yield at a given level of soil fertility (Gerdemann, 1975). According to the "resource economics hypothesis" root

morphological characteristics depend on the trade-off between the acquisition of a resource (for example inorganic phosphate (Pi)), and the conservation of a resource (for example photosynthates required to gain extra Pi). Plants adopt a number of strategies to acquire resources such as Pi efficiently with some plants increasing the production of fine secondary and tertiary roots to acquire Pi, while others have an increased dependence on the mycorrhizal association to increase Pi uptake (Wen et al., 2020). Baylis (1975) hypothesized that coarser rooted plants would have a greater mycorrhizal growth response than plants with fine root systems, though this hypothesis was challenged by Maherali (2014) who found that having coarse roots did not necessarily influence the mycorrhizal growth response.

It is estimated that up to 20% of the photosynthetic carbon of plants is relegated to maintaining the association with AMF (Smith & Read, 2008) and this high cost in resources is generally outweighed by the many benefits of the fungi to the plant. Arbuscular mycorrhizal fungi have been promoted as a tool to maintain and promote sustainable agriculture due to their role as natural biofertilizers, increasing the levels of N, P and Zn in the crop (Baum et al., 2015; Berruti et al., 2016; Parniske 2008; Smith et al., 2011). Phosphorus and Zn are poorly mobile in the soil through sorption to colloids and limit crop production in the vertisols of the sub-tropical grain region of eastern Australia. Crops grown in vertisols of the region that have low levels of AMF, exhibit symptoms of P or Zn deficiency and fail to thrive even when there is adequate soil moisture in the profile. This is known as Long Fallow Disorder (LFD) (Thompson, 1987) and is caused by a deficiency of mycorrhizal inoculum in the soil after periods of long fallow (>12 months), for example after a period of extended drought, or from changes in cropping sequences from winter to summer crops. Other factors that result in a reduction in AMF inoculum include tillage practices, which destroy the fungal hyphal network and reduce mycorrhizal species diversity, and stubble burning which destroy the mycorrhizal spores that are predominantly located in the top 10–15 cm of the soil profile (Kabir, 2005). The symptoms of LFD can be ameliorated by the application of high rates of Zn and P often to levels that may be uneconomical or the application of mycorrhizal spores (Seymour et al., 2019; Thompson, 1990; Thompson, 1996; Thompson et al., 2013; Wellings et al., 1991). In short-season crops with high mycorrhizal dependency such as mung bean, there may not be sufficient time to treat any observed nutrient deficiencies in the plant before

harvest. Soil tests have been developed by the South Australian Research and Development Institute (SARDI) Molecular Diagnostic Centre to quantitatively determine mycorrhizal DNA levels in the soil and these tests may indicate a potential risk for LFD, though the technology is in its early stages of development (Owen et al., 2018).

In addition to nutrient and water acquisition, AMF have been linked to improvements of abiotic traits such as improved: (i) soil structure and soil aggregation via exudation of the glycoprotein glomalin from the hyphae (Rillig & Mummey, 2006), (ii) tolerance to salt (Chandrasekaran et al., 2014), (iii) tolerance to heavy metal stress (Audet & Charest, 2007), (iv) drought tolerance (Augé et al., 2015) and, (v) soil carbon sequestration (Wilson et al., 2009).

3.4 AMF and plant-parasitic nematodes as biotic stressors

Alongside its role in improvement of abiotic stress, AMF also interact with a number of other micro-organisms in the roots and soil of plant hosts. Inoculation of plants with AMF can play an important role in biocontrol against biotic stressors such as fungal soil-borne diseases, bacterial pathogens and nematodes resulting in reductions of the symptoms of infection, reductions of disease incidence and decreased inoculum in the soil (Pozo & Azcón-Aguilar, 2007; Veresoglou & Rillig, 2012; Whipps, 2004; Yang et al., 2014).

Plant-parasitic nematodes and AMF occupy a very similar ecological niche in the root cortex of the host plant. *Pratylenchus* spp. at various life stages occupy the same root cortical tissue as the mycelium, arbuscules and vesicles of AMF (Pinochet et al., 1995). Many analyses have been carried out on the effects of AMF colonisation with respect to plant-parasitic nematodes, some with contradictory results (Borowicz, 2001; Hol & Cook, 2005; Veresoglou & Rillig, 2012; Yang et al., 2014). These analyses document the generally suppressive effect that AMF have on plant-parasitic nematodes, although some studies showed no effect or even an increase in nematode numbers on inoculation with AMF (Hol & Cook, 2005; Pinochet et al., 1996). A meta-

analysis undertaken by Veresoglou and Rillig (2012) demonstrated that inoculation with AMF reduced plant-parasitic nematode infestations by 44–57%. There was a reciprocal inhibition between AMF and plant-parasitic nematodes, and an AM species mix was more effective than single isolates in reducing nematode population densities. The only exception was in experiments with AMF isolates from the family *Acaulosporaceae* which stimulated nematode effects. The analysis demonstrated that there was no difference with respect to the lifestyle or mode of feeding of the nematode pathogens (sedentary versus migratory plant-parasitic nematodes). This was corroborated by another meta-analysis of 144 papers carried out by Yang et al. (2014) where there was reciprocal inhibition between AMF and nematode growth performance measured as reduction in galls, eggs or nematode population density/volume of soil. However, these analyses contrast to the findings of the meta-analyses by Hol and Cook (2005) and Borowicz (2000) who argued that the mode of feeding of the nematode was significant to AMF interactions and ectoparasites damaged AMF plants more than endoparasites. Hol and Cook (2005) found that experiments on migratory nematodes were characterised by lower levels of damage to plants inoculated with AMF compared to plants without AMF even though the populations of nematodes increased. These conflicting results may be due to differences in environmental and nutritional factors, AMF species and genera, nematode species and/or crop hosts.

Fungal species composition (multiple vs single species), fungal species diversity, source of inoculum (native indigenous vs commercial inoculant) and host interactions can all lead to varying reports of the effects of AMF on nematode suppression and on plant performance (Ji et al., 2010; Pinochet et al., 1998; Varga, 2015). There are also differences in how AMF aid tolerance to nematodes based on plant family with positive effects of AMF demonstrated on *Fabaceae* (Yang et al., 2014). In the meta-analysis undertaken by Veresoglou and Rillig (2012), there was a trend for nitrogen fixing dicotyledons to be better protected by AMF against nematode damage than non-nitrogen fixing dicotyledons. Due to the controversy surrounding these analyses, and to disentangle the conflicting results from previous papers, a systematic analysis on the interactions on AMF and *Pratylenchus* spp. alone was carried out as a part of this thesis. The analysis and subsequent paper are presented in Chapter 2.

3.5 AMF and P. thornei *interactions in the sub-tropical grain region of eastern Australia*

Both arbuscular mycorrhizal fungi and *P. thornei* are found in the vertisols of the sub-tropical grain region of eastern Australia and both organisms have an important effect on the growth and yield of crops cultivated in the region (Thompson, 1994). Summer crops that are partially resistant or poor hosts of *P. thornei* include sunflower, maize, panicum (*Setaria italica* L.), millets (*Panicum miliaceum* L. and *Echinochloa* spp.) and pigeon pea (*Cajanus cajan* L.), all of which are hosts to the beneficial AMF (Thompson et al., 1997; Owen et al., 2014). In crop rotation research, cultivation of canary seed, a non-host of *P. thornei*, produced levels of *P. thornei* to 88% below the levels of susceptible wheat and increased the levels of AMF in the soil. This in turn, resulted in an increase of 25% in yield of a subsequent crop of chickpea that followed at the site (Reen et al., 2014). Canola has a role as a break-crop in cropping rotations to reduce the population of *P. thornei* and other soil-borne diseases. However, work by Owen et al. (2010) demonstrated that canola reduced levels of AMF which led to a subsequent reduction in wheat yields. Research by Thompson (1994) also demonstrated a link between low nematode numbers, increased AMF populations in the soil and increased biomass of wheat. Rotations of crops to increase AMF spore densities and reduce *P. thornei* populations are therefore desirable in cropping systems in the sub-tropical grain region.

There has been no research into the interaction between the migratory endoparasite *P. thornei* and AMF in the N-fixing legume mung bean. As discussed above, both of these organisms infect the roots of crops grown in the sub-tropical grains region and both have effects on plant growth, nutrition and crop yield. Chapter 3 and Chapter 4 of this thesis will investigate the interaction between *P. thornei* and AMF, and their effects on nodulation by *Bradyrhizobium* and subsequent effects on mung bean production and nutrition.

3.6 Putative mechanisms in the interaction between AMF and P. thornei

The putative mechanisms involved in the interaction between *P. thornei* and AMF may include: (i) enhanced plant tolerance to nematodes as a result of increased nutrient uptake and altered root morphology, (ii) effects through mycorrhizal induced resistance, (iii) a direct competition between organisms for resources and space or, (iv) altered rhizosphere interactions (Pozo & Azcón-Aguilar, 2007; Schouteden et al., 2015). How AMF and plant-parasitic nematodes may interact within the rhizosphere is discussed below.

3.6.1 Enhanced plant tolerance

An increase in nutrient uptake by AMF has been proposed as a mechanism for pathogen biocontrol (Linderman, 1994; Pinochet et al., 1996). Inoculation with AMF is well known to result in increased yield and improved plant nutrition in many agriculturally important crop species (Berruti et al., 2016). Mung bean colonized by AMF had increased biomass, grain yield, enhanced root nodulation by rhizobia, acid phosphatase levels and nutrient uptake (namely N, P, Cu, Zn) in field trials (Manjunath & Bagyaraj, 1986; Tarafdar & Rao, 1997; Xiao et al., 2010). As discussed in Section 3.6. tolerance of a plant indicates that the plant can perform well, maintaining biomass and yield even under constraints of the nematode infestation. Increased growth and yield in the plant as a result of colonisation with AMF may induce a tolerance effect in plants infested with plant-parasitic nematodes and increased nutrition may be as a result of direct nutrient uptake by the mycorrhizal hyphae, or increased root branching in the mycorrhizal plant. However, the nematode may still multiply in the infested roots resulting in a build-up of the plant-parasite in the roots and soil.

On fungal spore germination, AMF exude short chain chito-oligosaccharides which initiate the AMF symbiosis and may also stimulate plant root growth and branching (Atkinson et al., 1994; Maillet et al., 2011). The effects on root branching in the plant may be dependent on nutrition, genotype and be related to the mycorrhizal dependency

of the plant. For example, in *Musa* spp. (banana and plantain), genotypes with high secondary and tertiary root biomass had low mycorrhizal dependency and inoculation with AMF had no influence on the number of root branches. However, genotypes with a high proportion of primary roots had both a higher mycorrhizal dependency and a higher induction of root branching in response to AM inoculation (Elsen et al., 2003a). In contrast, *Pratylenchus* spp., by the very nature of their pattern of feeding and migration through the cortical cells of intolerant plants, destroy root functionality, reduce root biomass and length of root branches, resulting in reduced nutrient and water uptake (as discussed in section 3.4).

Therefore, a complex interplay may occur between the two organisms. Inoculation with AMF should increase nutrient uptake and maintain root system functionality, resulting in more vigorous plants. This improved 'fitness' may then play a role in damage compensation and inducing a tolerance to the nematode, counteracting the reduction in root quality and root efficiency by nematode infestation. The increased nutrient status of AMF-inoculated plants has not, however, conclusively been shown to be the cause of increased tolerance to nematodes (Pozo & Azcón-Aguilar, 2007; Schouteden et al., 2015). On the other hand, increased nutrient uptake may render the plant more palatable for the plant-parasite as a nutrient rich food source. In this thesis it was aimed to answer if increased nutrition in the plant or increased root biomass may play a role in affecting population densities of *P. thornei* in the roots of mycorrhizal mung bean.

3.6.2 Mycorrhiza induced resistance

On initial infection of plant roots by the mycorrhizal hyphae, the plant may perceive the fungus as a potential pathogen on recognition of the molecular signature highly conserved by many microbes known as microbe (or pathogen) associated molecular pattern molecules (MAMPs (or PAMPs)). These MAMPs are recognised by the plant recognition receptors (PRR) located on the plant cell wall which consist of plasma membrane localised receptor kinases (RLKs) or receptor like proteins (RLPs). The recognition of the microbe triggers a cascade of signalling events resulting in an

upregulation of the plant defence systems including release of reactive oxygen species, modifications of the cell wall, production of antimicrobial compounds and pathogenesis related proteins. This cascade of events is termed the MAMP-triggered immunity response or MTI response (Newman et al., 2013). The MTI response leads to transcriptional and hormonal changes in the host plant and involves induction of the jasmonate biosynthesis pathway (Pozo and Azcón Aguilar 2007). The phytohormone jasmonic acid (JA) is generally induced in the plant in response to wounding, for example by chewing insects, and is also involved in plant resistance against necrotrophs. On the other hand, salicylic acid (SA) is induced as a defence mechanism in response to phloem feeding insects and to biotrophic pathogens. On infection with AMF, the plant induces the JA-dependent response and, despite initially increasing the levels of SA, once mycorrhizal colonisation is established there is a subsequent reduction in the SA defence mechanism. These modifications to phytohormone levels, which can prime the plants defence system is known as Mycorrhiza Induced Resistance or MIR. It is via MIR that AMF can improve plant resistance or tolerance to pathogens (Jung et al., 2012). Induction of MIR in the plant can modify the expression of defence related genes resulting in increases in phenolics such as ferulic acid, which may be involved in reducing nematode population densities and infection levels of the burrowing nematode *Radopholus similis* (Li et al., 2015). A transcriptomic study found that mycorrhizal tomato (*Lycopersicon esculentum*) had an increased expression of defence related plant genes, signal transduction genes and changes in the expression of proteins, which reduced the infection of the root-knot nematode *Meloidogyne incognita* in the plant roots (Vos et al., 2013). While research on MIR for pathogens and other plant-parasitic nematodes is available in the literature, reports on MIR and the subsequent effects on *Pratylenchus* spp. is lacking. The suppression of nematodes in AM inoculated plants may be via local or systemic mechanisms. Systemic effects of MIR have been demonstrated in AM plants inoculated with various fungal and bacterial pathogens (Jung et al., 2012; Liu et al., 2007), though there are fewer reports on systemic reductions in migratory nematodes. Systemic resistance effects inducing bio-control have been observed against *Pratylenchus* spp. in experiments with banana and tomato using split root systems (Elsen et al., 2008; Vos et al., 2012a). However, this is in contrast to work by De la Peña et al. (2006) who, when using a split root system in the dune grass *Ammophilia arenaria*, demonstrated a local mechanism for *P. penetrans* suppression.

3.6.3 Competition for resources

As both AMF and root-lesion nematodes inhabit the cortex, competition between them for resources such as feeding sites or photosynthates may occur. This competition may affect the beneficial mycorrhizal symbiosis by alteration of the level and efficacy of mycorrhizal colonisation. On the other hand, the competition might affect the population densities of nematodes in the roots. Experiments have found that the level of mycorrhizal root colonisation may influence the protective effects against other root pathogens (Khaosaad et al., 2007; Slezack et al., 2000). In crops including banana (*Musa* sp.) and pineapple (*Ananas comosus*), *Pratylenchus* infestation reduced the level of mycorrhizal colonisation but not the development of active arbuscules with no effects of AMF on reducing *Pratylenchus* population densities (Elsen et al., 2003b; Guillemin et al., 1994). The intraradical mycorrhizal structures including arbuscules, hyphae and vesicles may also represent a sink for sugars with storage of carbon resources as lipids in the vesicles and spores (Rich et al., 2017). Notwithstanding the high lipid content of vesicles, they also contain high levels of other nutrients. For example, particle induced X-ray emission in vesicles of *Glomus intraradices* showed that P totalled 0.5% of the dry weight of the vesicles, with additional nutrients such as Ca, K, Zn and Si also present (Olsson et al., 2011). These vesicles may be an additional nutrient dense source of food for the nematodes.

The timing of inoculation of AMF and the biotic stressor may affect tolerance levels in the plant. Azcón-Aguilar and Barea (1997) hypothesized that the maturity of AMF infection, as gauged by the presence of arbuscules, may influence the bio-protective effects. On early inoculation of mung bean with *Glomus coronatum,* the mycorrhizal fungi reduced the incidence of disease severity of binucleate *Rhizoctonia* sp. and *R. solani*, while co-inoculation at the same time resulted in a reduction in root colonisation by the pathogen of binucleate *Rhizoctonia* sp only (Kasiamdari et al., 2002). The timing of inoculation of both organisms may affect the tolerance levels to nematodes. Inoculation with *P. coffeae* four months after inoculation with either *Glomus clarum* or *Acaulospora mellea* improved the tolerance of coffee (*Coffea*

arabica) to *P. coffeae* infestation, as assessed by leaf area and root length, and also resulted in fewer, more localised lesions on the roots compared to inoculation of AMF and *P. coffeae* at the same time (Vaast et al., 1997). Investigation on the timing of inoculation of AMF and the subsequent effects on *Pratylenchus* population densities and plant nutrition has its merits in arboriculture and horticulture where transplantation from nursery to field sites is common. However, in the broad-acre cropping systems, both organisms are present concurrently in the soil at planting.

3.6.4 Changes to exudation in the mycorrhizosphere

Plant roots release between 5–21% of their photosynthates into the soil and this in turn alters microbial populations in the zone around the root (Marschner, 1995). The mycorrhizosphere is the soil zone around the roots which is influenced by both the root and the mycorrhiza. This zone is differentiated from the bulk soil by altered biological and chemical properties due to the release of enzymes, protons and carbohydrates from the mycorrhized root and extra radical mycelium (Marschner & Timonen, 2006). Alterations in exudations in the mycorrhizosphere change the abundance of many microorganisms including bacteria, fungi, protozoa and nematodes (Linderman, 1988; Wang and Feng, 2021).

These exudations may also play a role in influencing population changes in plant-parasitic nematodes. Changes in root exudation may alter the level of chemotaxic attractants used by nematodes in migration towards roots and thereby reduce nematode reproduction (Siddiqui & Mahmood, 1995). In tomato, pre-inoculation with *Glomus fasciculatum* significantly reduced populations of the root-knot nematode *M. javanica* as a result of increased lignin and phenolic compounds in the mycorrhizal root (Singh et al., 1990). Furthermore, exudations from tomato inoculated with *F. mosseae* also reduced penetration of *M. incognita in vitro* and resulted in paralysis of J2 juveniles (Vos et al., 2012b).

4 Rhizobia

Rhizobia are motile gram-negative bacteria that in symbiosis with legumes, fix nitrogen gas from the air into ammonium using the enzyme nitrogenase in nodules on the plant. Genera include *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium* and *Sinorhizobium* (Shamseldin et al., 2017). In this mutually beneficial symbiosis, the plant supplies carbohydrates and oxygen buffered by leghaemoglobin for respiration to the bacteria in exchange for N fixation. Globally, pasture and forage legumes fix up to 40 million tonnes of N annually (Herridge et al., 2008). It has been estimated that 10 tonnes of CO_2 are emitted in the production of each tonne of N fertiliser used in agricultural systems. Therefore, biological N fixation represents a major contribution to sustainable agricultural systems, through reduction of exogenous N fertiliser application and carbon emissions (Abberton et al., 2010; Stagnari et al., 2019)

4.1 Mechanism for N fixation by rhizobia

In the soil, flavonoids are exuded from the roots of a suitable host and initiate the production of Nod factors in rhizobia. These Nod factors are perceived by the plant host and initiate root hair curling around the rhizobial cells. The bacteria begin to multiply and a pre-infection thread is then produced within the plant root, allowing the bacteria to enter the root epidermal cells. This pre-infection thread is believed to have evolved from the pre-penetration apparatus of AMF (Parniske, 2008). Once inside the root cells, nodule organogenesis is undertaken and the rhizobia are converted into bacteroids inside symbiosomes. These symbiosomes are specialised organelles where ammonium is converted to amino acids such as glutamine and asparagine by the rhizobia bacteroids using nitrogenase before being exported to the plant (Geurts & Bisseling 2002). The fixation of N_2 by nitrogenase is an energy intensive process and it has been estimated that a minimum of 16 ATP molecules are required to reduce each

molecule of N_2 (Simpson and Burris, 1984). Nitrogenase activity decreases when plants are grown in P deficient conditions (Høgh-Jensen et al., 2002; Schulze et al., 2011).

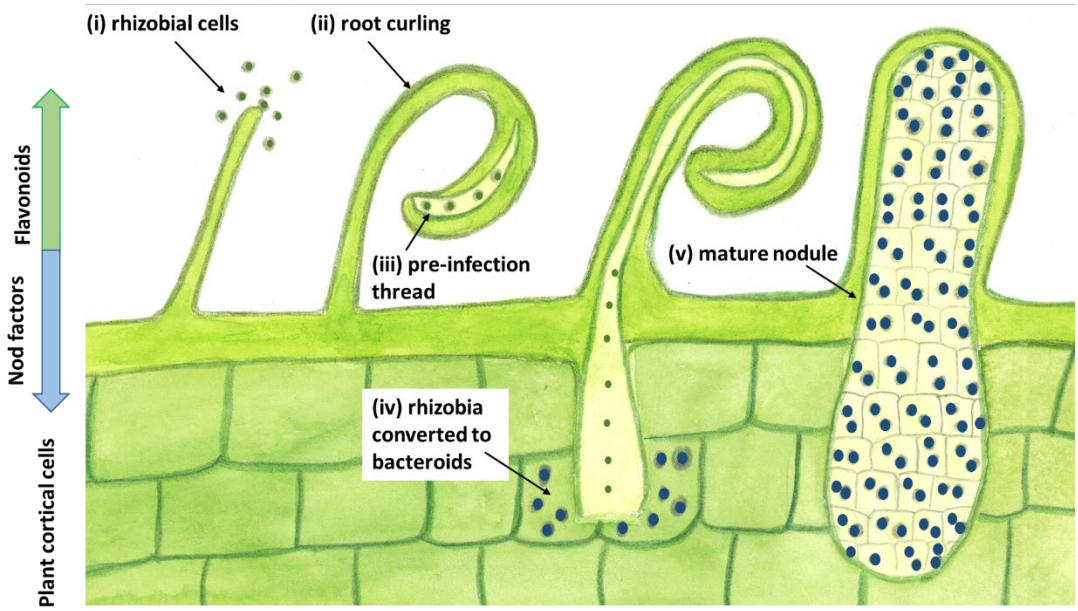

Figure 8 Schematic of steps in nodule development. Flavonoids are exuded from host roots and nod factors (i) produced in rhizobia cells, which are perceived by the plants and (ii) initiate root curling. The bacteria multiply and (iii) a pre-infection thread is produced to facilitate the bacterial entry into the root epidermal cells. In the root cells (iv) the rhizobia are converted into bacteroids in the (v) mature nodule. Figure source: Elaine Gough

The amount of N fixed by the rhizobial nodulated plant is dependent on the host species, soil environmental conditions of heat and moisture, soil pH, soil nitrate, soil P and other macro and micronutrients including K, S, Fe, Mo, Cu, Zn (O'Hara, 2001; Peoples et al., 2009). There are various methods to quantify nitrogen fixation in the crop and their respective advantages and limitations have been reviewed by Herridge et al. (2008).

4.2 Inoculation of mung bean with Bradyrhizobium *sp.*

The role of rhizobia in N fixation is well studied and inoculation of legumes with rhizobia is currently practised agronomically worldwide. In Australia for mung bean, the commercially available *Bradyrhizobium* strain is CB 1015 from inoculant group I, originally obtained from Haringhata, West Bengal, India. This is also effective for nodulating other species of *Vigna* including *V. angularis, V. mungo* and *V. parkeri* (Eagles & Date, 1999). This strain has been provisionally identified by MALDI-TOF as *Bradyrhizobium* cf. *diazoefficiens* (Ndungu, 2017), but will be referred to as *Bradyrhizobium* sp. in this thesis. While other strains of *Bradyrhizobium* indigenous to the soils of Burdekin region in northern Queensland also are effective at nodulating and fixing N in mung bean to a level comparable to the commercially available strain (Christopher et al., 2018), so to date only CB 1015 is used in commercial inoculum.

Inoculation of mung bean seed at sowing with *Bradyrhizobium* to improve N fixation for the growing crop and to prevent further expenditure from existing soil N reserves is a common agricultural practice in the sub-tropical grain region. It is highly advocated where soil nitrate levels are low and where paddocks have not previously had mung bean under cultivation (Gentry, 2010). Surveys carried out in the region found that nodulation failure in mung bean is widespread even after inoculation with the correct strain of rhizobium. Nodulation failure can result in low biological N fixation (mean value of 35% Ndfa when soil nitrate is <50 kg N/ha), low biomass, low yields and a high extraction of N from the soils to support mung bean growth (Herridge et al., 2005; Herridge et al., 2008).

Inoculation failure and subsequent low N fixation rates evident on cultivation of mung bean may be as a result of various abiotic factors. These include (i) incorrect inoculum storage, handling and application, (ii) incorrect strain being used, (iii) extremes of temperature or water stress on sowing, (iv) incorrect pH level (optimal range is 5.0–7.5), (v) high soil nitrate levels—the energy required to fix N biologically is greater than assimilating a readily available source in the soil and thus fewer nodules are produced, (vi) fertilisers applied at sowing, such as those containing Zn, can be toxic to rhizobia.

Other hypotheses to explain nodulation failure that merit consideration have their foundation in biology. Could nodulation failure in the region be caused by a lack of mycorrhizal spores in the soil, or the presence of plant-parasitic nematodes such as *Pratylenchus* spp.? These hypotheses are investigated in Chapter 3 of this thesis.

4.3 Interaction between rhizobia and AMF

The rhizobial symbiosis is believed to have evolved 65 million years ago from a similar pathway to the AMF endosymbiosis. A common symbiotic pathway is shared by both the AM and rhizobial symbiosis with elements of the Nod signalling pathway also integral to the establishment of the mycorrhizal symbiosis (Oldroyd et al., 2017). At least seven plant genes have been described in legumes that are required for AM and root nodule symbiosis (Markmann et al., 2008; Parniske, 2008). Interestingly, similar to the initial infection in the plant by AMF, the suppression of the SA pathway is also involved in the rhizobium symbiosis (Stacey et al., 2006).

Rhizobia and AMF can interact in a synergistic or additive manner to improve plant growth, nodulation, mineral nutrition and fix N in leguminous plants such as chickpea, soybean and common bean (Artursson et al., 2017; Barea et al., 2002; Chalk et al., 2006). Synergism between the symbionts can be due to their functional complementary—rhizobia increase the uptake of N to the plant while AMF increases the uptake of P. However, other mechanisms are also likely to aid in this synergism. Recent transcriptomic studies in soybean demonstrated that genes were specifically upregulated on co-inoculation with the AMF species *Gigaspora rosea* and *Bradyrhizobium diazoefficiens*, including nodulin genes and genes involved in sugar transport (SWEET transporters) which the authors attribute to the increased nodulation, biomass and yield observed (Sakamoto et al., 2019).

Co-inoculation with *Rhizophagus intraradices* and two strains of *Bradyrhizobium japonicum* resulted in increased yield and improved nutrient content of mung bean in an additive manner compared to inoculation with either inoculant type alone (Yasmeen et al., 2012). However, as yet there is no information on the interaction

between AMF and the commercial *Bradyrhizobium* strain CB 1015 on N fixation levels, nodulation, plant growth and plant nutrition. It is likely that these organisms will interact and as both beneficial symbionts are present in the soils of the sub-tropical grain region, research is required to assess the magnitude of the interaction and the contribution of the symbionts to mung bean production systems. The interaction between these symbionts and their impact on biomass, yield and plant nutrition will be investigated in Chapter 3 and 4.

4.4 Rhizobia and Pratylenchus *interactions*

Rhizobium nodulation levels can be affected by root-knot nematodes (*M. incognita, M. javanica*), *P. thornei* or *Rotylenchus reniformis* resulting in a decrease in functionality in chickpea (Castillo et al., 2008). Hussey and Barker (1976) found *P. penetrans* stimulated nodule formation on soybean, but inhibited nitrogen fixing capacity. *Pratylenchus penetrans* also readily entered nodules of the pea (*Pisum sativum*) cv. Wandoo, though remained in the cortex of soybean and peanut (*Arachis hypogaea*) nodules (Barker & Hussey, 1976). Elhady et al. (2020) found effects on nodule numbers and N fixation in soybean were density dependent with increases in populations of *P. penetrans* reducing nodule number, nodule weight and N fixation. However, when repeated in a split root experiment, inoculation with the nematode increased nodule counts, though the nodule weights and numbers of bacteroids were reduced, indicating the nematode disrupted the symbiosis and resulted in more non-functional, immature nodules.

The interaction between host, plant-parasitic nematode, and beneficial symbionts is likely to be quite specific. So far, there has been no research into this multipartite interaction in the vertisols of the sub-tropical grain region of Australia and these interactions are the focus of this thesis.

5. Objectives of the study and thesis outline

5.1 Aims and objectives of the study

In the course of this thesis, it was aimed to determine if there was an interaction between AMF, *P. thornei* and rhizobia with respect to nodulation, plant nutrition, biomass and seed yield in mung bean. The knowledge gaps determined from an extensive literature review and from experimental work carried out in this research are outlined below.

Research Question 1: From reports in the literature, what are the interactions between root-lesion nematodes (*Pratylenchus* spp.) and arbuscular mycorrhizal fungi in different plant species?

Previous reviews of the literature reported conflicting conclusions of the effects AMF have on population densities of plant-parasitic nematodes. These reviews classified plant-parasitic nematodes broadly into their mode of feeding including ecto-parasitic, sedentary endo-parasitic and migratory endo-parasitic nematodes.

In Chapter 2, a systematic analysis of the literature was undertaken using PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. I hypothesized that using broad groupings of nematodes may have contributed to contradictory findings on how these plant-parasitic nematodes interact with AMF. *Pratylenchus* represents a genus of economically important plant-parasitic nematode, therefore it was proposed that investigations should be made exclusively between these nematodes and AMF. Additionally, a traditional nomenclature based on spore morphology of AMF species may also have contributed to the conflicting conclusions of these reviews. Therefore, I also hypothesized that specific families or genera of AMF may have different effects on population densities of *Pratylenchus* and biomass of hosts, due to mycorrhizal functional diversity. As part of the systematic review, parameters of changes in population density of *Pratylenchus* spp., plant biomass and mycorrhization were analysed under the revised nomenclature of the phylum

Glomeromycota by Schüßler and Walker (2010), and by plant host functional group to clarify the interactions between these organisms.

Research Question 2: What are the interactions between the root-lesion nematode *P. thornei* and arbuscular mycorrhizal fungi in mung bean?

Mung bean is an import summer legume in the sub-tropical grain region of eastern Australia and is a host for both AMF and *P. thornei*. However, it is unknown what the interactions between these organisms are and what the impact of these interactions may be on plant biomass and nutrition in mung bean. The systematic review undertaken in Chapter 2, highlighted the very limited reports in the literature on interactions between AMF and *Pratylenchus* spp. in legumes, with only one reported on navy bean (*Phaseolus vulgaris*) (Elliott et al., 1984). In Chapter 3, I hypothesized that changes in population densities of *P. thornei* may be influenced by AMF inoculation, and these changes could be as a result of improved biomass and nutrition of mycorrhizal mung bean. Furthermore, I hypothesized that *P. thornei* infestation may reduce mycorrhizal colonisation, with subsequent impacts on plant nutrition in mycorrhizal mung bean.

Research Question 3. What are the interactions between the symbionts AMF and rhizobia in mung bean, and are these interactions affected by *P. thornei* infestation?

In the literature, AMF and rhizobia can interact improving biomass and plant nutrition in other leguminous crops. It was hypothesized that the beneficial symbionts can interact positively, which benefit mung bean biomass, yield and nutrition. In Chapter 3, it was also determined if these beneficial symbionts interact in an additive or synergistic manner. It was hypothesized that *P. thornei* negatively affected the outcome of the interaction between AMF and rhizobia, potentially by reducing root biomass and nutrient uptake. The reported research explored the effect of the interactions between these organisms on mung bean biomass, yield and nutrition and determined the magnitude of the relationship between the beneficial symbionts in mung bean.

Research Question 4: Is nodulation failure in mung bean due to a lack of AMF inoculum in the soil and/or due to infestation with *P. thornei*?

Nodulation by rhizobia can be reduced by infestation by plant-parasitic nematodes, and can be improved by co-inoculating with AMF in other legumes. Investigations reported in Chapter 3 hypothesized that inoculation with both AMF and *P. thornei* alone or combined affected nodulation and biological nitrogen fixation in mung bean. This research contributes to understanding a cause of nodulation failure in mung bean.

In Chapter 3, a full factorial designed experiment between AMF, *P. thornei* and rhizobia was undertaken to investigate the hypotheses as outlined in the Research Questions 2, 3 and 4.

Research Question 5. What are the relative contributions of the symbionts AMF and rhizobia compared to the application of fertiliser N, P and Zn on improving mung bean biomass, yield, nodulation, N fixation, and nutrition?

In Chapter 4, it was hypothesized that rhizobia can improve the concentration of N in the plant to a level comparable to application of fertiliser N, and AMF can improve the concentration of P and Zn in the plant to a level comparable to application of fertiliser P or Zn, even at high rates of fertiliser application. Furthermore, it was hypothesized that AMF can improve biological N fixation by rhizobia to a level comparable to the application of fertiliser P. In Chapter 4, investigations on the interactions between the biological symbionts and the application of fertilisers N, P and Zn on biomass, yield, N fixation and plant nutrition are reported. This research explores the valuable contribution that AMF and rhizobia can make to sustainable mung bean production.

Research Question 6. What are the interactions between the application of fertilisers N, P and Zn and *P. thornei* in mung bean?

It was hypothesized that increased plant nutrition can alter population densities of *P. thornei.* This research further investigated if *P. thornei* can alter the nutrient status of plants under N, P and Zn fertilisation, in the presence and absence of AMF and rhizobia. This research will contribute towards understanding the interaction between *P. thornei* and nutrition in mung bean.

In Chapter 4, a full factorial designed experiment between AMF, *P. thornei*, rhizobia, N, P and Zn was undertaken to investigate the hypotheses as outlined in the Research Questions 5 and 6.

5.2 Thesis outline

This thesis is structured as a PhD Thesis by Publication in which three papers are presented (two published and one submitted for review) in Chapters 2, 3 and 4. Chapter 5 provides a discussion and conclusions regarding the outcomes of the research on interactions between AMF, *P. thornei* and rhizobia and how these organisms affect biomass, yield, nodulation, nitrogen fixation and nutrition in mung bean. Implications for mung bean production and prospects for future investigations are discussed.

CHAPTER 2

A SYSTEMATIC REVIEW OF THE EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI ON ROOT-LESION NEMATODES, *PRATYLENCHUS* SPP.

The paper presented in this chapter is published in the international Q1 journal *Frontiers in Plant Science* (MDPI, Switzerland), July 2020

Gough EC, Owen KJ, Zwart RS and Thompson JP (2020) A systematic review of the effects of arbuscular mycorrhizal fungi on root-lesion nematodes, *Pratylenchus* spp. *Frontiers in Plant Science* 11, 923. (Q1; Impact Factor: 4.41) doi: 10.3389/fpls.2020.00923

This study describes a synthesis and evaluation of the effects of arbuscular mycorrhizal fungi on *Pratylenchus* spp. distinct from other nematode genera. In the literature, previous reports investigating interactions between AMF and plant-parasitic nematodes have combined nematode species into categories of their pattern of feeding and migration. These broad categorisations have resulted in contradictory reports on how AMF and plant-parasitic nematodes interact.

In this study, a systematic review was carried out using the PRISMA set of guidelines. This review investigated the interactions between AMF species using a revised taxonomy as proposed by Schüßler and Walker (2010) and *Pratylenchus* species. The results of the systematic review demonstrated that AMF improve plant tolerance to *Pratylenchus* spp. infestation as indicated by maintenance of plant biomass. Effects on *Pratylenchus* spp. population densities were found to be dependent on order and genus of AMF and on host functional group. This research contributes towards clarification of the response of root-lesion nematode *Pratylenchus* spp. to AMF inoculation.

[Supplementary material associated with this Chapter is attached in Appendix A.]

frontiers in Plant Science

SYSTEMATIC REVIEW
published: 14 July 2020
doi: 10.3389/fpls.2020.00923

A Systematic Review of the Effects of Arbuscular Mycorrhizal Fungi on Root-Lesion Nematodes, *Pratylenchus* spp.

Elaine C. Gough, Kirsty J. Owen, Rebecca S. Zwart and John P. Thompson*

Centre for Crop Health, Institute for Life Sciences and the Environment, University of Southern Queensland, Toowoomba, QLD, Australia

Root-lesion nematodes (*Pratylenchus* spp.) and arbuscular mycorrhizal fungi (AMF) occupy the same ecological niche in the phytobiome of many agriculturally important crops. Arbuscular mycorrhizal fungi can enhance the resistance or tolerance of a plant to *Pratylenchus* and previous studies have been undertaken to investigate the relationship between these organisms. A restructuring of the AMF phylum Glomeromycota has reallocated the species into genera according to molecular analysis. A systematic review of the literature was synthesized to assess the interaction between *Pratylenchus* spp. and AMF using the revised classification. Plants inoculated with AMF generally exhibited greater tolerance as demonstrated by increased biomass under *Pratylenchus* pressure. Species of AMF from the order Diversisporales tended to increase *Pratylenchus* population densities compared to those from the order Glomerales. Species from the genera *Funneliformis* and *Glomus* had a reductive effect on *Pratylenchus* population densities. The interaction between AMF and *Pratylenchus* spp. showed variation in responses as a result of cultivar, crop species, and AMF species. Putative mechanisms involved in these interactions are discussed.

Keywords: arbuscular mycorrhizal fungi, *Pratylenchus*, root-lesion nematodes, phytobiome interactions, Glomeromycota, systematic review

OPEN ACCESS

Edited by:
Johannes Hallmann, Julius Kühn-Institut - Braunschweig, Germany

Reviewed by:
Martina Janouskova, Institute of Botany (ASCR), Czechia
Raffaella Balestrini, Italian National Research Council, Italy

****Correspondence:***
Elaine C. Gough
Elaine.Gough@usq.edu.au

Specialty section:
This article was submitted to Plant Microbe Interactions, a section of the journal Frontiers in Plant Science

Received: *27 February 2020*
Accepted: *05 June 2020*
Published: *14 July 2020*

Citation:
Gough EC, Owen KJ, Zwart RS and Thompson JP (2020) A Systematic Review of the Effects of Arbuscular Mycorrhizal Fungi on Root-Lesion Nematodes, Pratylenchus spp. Front. Plant Sci. 11:923. doi: 10.3389/fpls.2020.00923

INTRODUCTION

Pratylenchus spp. or root-lesion nematodes, are migratory endoparasites (Singh et al., 2013). They feed and move through the root cortex, penetrating parenchyma cells with their stylet, excreting cell degrading enzymes, ingesting the cellular contents, and destroying cortical tissue. This results in necrotic lesions, loss of root function and consequently, reductions in plant vigor, and yield of economic products (Jones et al., 2013).

Root-lesion nematodes are polyphagous and have the broadest host range of all plant-parasitic nematodes. They are responsible for substantial yield losses of many important crop species including cereals, legumes, sugarcane, coffee, banana, potato, vegetables and fruit trees (Castillo and Vovlas, 2007). There are over 68 recognized species of *Pratylenchus* associated with the phytobiome and they are distributed in diverse habitats worldwide (Castillo and Vovlas, 2007). Historically, *Pratylenchus* spp. were distinguished on the basis of their morphometric characteristics. With the advent of molecular techniques, differences in the sequences of ribosomal DNA can distinguish

between species despite high levels of intraspecific variation in some *Pratylenchus* spp. High levels of intraspecific variability occur within some *Pratylenchus* spp. such as *P. coffeae* and *P. penetrans* while other species exhibit less intraspecific internal transcribed spacer (ITS) variation, for example, *P. goodeyi* and *P. vulnus* (de Luca et al., 2011; Jones et al., 2013).

Arbuscular mycorrhizal fungi (AMF), from the phylum Glomeromycota are a ubiquitous group of soil microorganisms associated with the phytobiome. Arbuscular mycorrhizal fungi form a complex symbiosis with land plants which originated in the Ordovician period 400 million years ago (Parniske, 2008). They have remained morphologically unchanged since then, forming an intrinsic part of ecosystem functionality (Powell and Rillig, 2018). These obligate biotrophs form beneficial mutualistic associations with the roots of an estimated 80% of land plants including many agriculturally important crop species with the notable exception of most species in the families *Brassicaceae* and *Chenopodiaceae* (Lambers and Teste, 2013). Their characteristic arbuscules (microscopic tree-like structures) within the root cortical cells of compatible plants enable the photosynthetically derived organic compounds supplied by the plant to be exchanged for inorganic nutrients and water supplied by the fungus from the soil. The fungus also aids in the stabilization of soil aggregates through hyphal binding and exudation of glomalin (Smith and Read, 2008; Leifheit et al., 2014). It is estimated that up to 20% of the photosynthetic carbon of plants is allocated to maintaining the fungal association (Smith and Read, 2007). This carbon cost to the plant is outweighed by the many benefits conferred by the fungi, foremost of which are improved acquisition by the fungal hyphae of immobile nutrients from the soil such as phosphorus (P) and zinc (Zn) (Parniske, 2008).

Arbuscular mycorrhizal fungi have been promoted as a natural tool to maintain and promote sustainable agriculture due to their role as natural biofertilizers; increasing the levels of nitrogen (N), P and Zn in the crop (Thompson, 1993; Parniske, 2008; Smith et al., 2011; Baum et al., 2015; Berruti et al., 2016). They also play a role in drought tolerance (Zhao et al., 2015) and as bio-protectants against fungal, bacterial, and nematode pathogens (Whipps, 2004; Pozo and Azcón-Aguilar, 2007; Veresoglou and Rillig, 2012; Yang et al., 2014).

Early classifications defined species within the order Glomerales of the phylum Glomeromycota on the basis of spore morphology (Morton and Benny, 1990). Schüßler and Walker (2010) restructured the phylum Glomeromycota according to molecular phylogenies based on the small subunit (SSU) rRNA gene, the large subunit (LSU) rRNA gene, β-tubulin sequence data and the ITS region. Consequently, the current classification of the order Glomerales consists of two families — the *Glomeraceae* and the *Claroidoglomeraceae*. A number of *Glomus* species have been transferred to the genera *Funneliformis* and *Rhizophagus*. **Table 1** shows the phylum Glomeromycota and the subdivisions into the orders Glomerales, Diversisporales, Archaeosporales, and Paraglomerales (Redecker et al., 2013).

Plant-parasitic nematodes are classified according to their feeding strategies. These include (i) ecto-parasitic nematodes which feed externally on root cells and remain in the rhizosphere such as *Tylenchorhynchus* spp., (ii) migratory endo-parasitic nematodes which enter the plant root, feed, and move through the root tissues destroying cells as they migrate such as *Pratylenchus* spp., and, (iii) sedentary endo-parasitic nematodes which convert vascular cells into specialized feeding cells where they remain, such as the root-knot nematodes (*Meloidogyne* spp.) and the cyst nematodes (*Heterodera* and *Globodera* spp.) (Decraemer and Hunt, 2013).

TABLE 1 | Classification of the phylum Glomeromycota according to Redecker et al. (2013).

Order	Family	Genus*
Diversisporales	Diversisporaceae	*Tricispora*
		Otospora
		Diversispora
		Corymbiglomus
		Redeckera
	Acaulosporaceae	***Acaulospora***
	Sacculosporaceae	*Sacculospora*
	Pacisporaceae	*Pacispora*
	Gigasporaceae	***Scutellospora***
		Gigaspora
		Intraornatospora
		Paradentiscutata
		Dentiscutata
		Centraspora
		Racocetra
Glomerales	Claroideoglomeraceae	***Claroideoglomus***
	Glomeraceae	***Glomus***
		Funneliformis
		Septoglomus
		Rhizophagus
		Sclerocystis
Archaeosporales	Ambisporaceae	*Ambispora*
	Geosiphonaceae	*Geosiphon*
	Archaeosporaceae	*Archaeospora*
Paraglomerales	Paraglomeraceae	*Paraglomus*

**Genera in bold were considered in this review.*

The coexistence of AMF and nematodes in the phytobiome has prompted a number of investigations into their interactive effects on plants (reviews: Pinochet et al., 1996; meta-analyses: Borowicz, 2001; Hol and Cook, 2005; Veresoglou and Rillig, 2012; Yang et al., 2014). Published meta-analyses describe the generally suppressive effect that AMF have on nematodes (Veresoglou and Rillig, 2012; Yang et al., 2014). These analyses included nematodes belonging to different genera and they grouped plant-parasitic nematodes into their feeding modes (sedentary or migratory). AMF reduced the numbers of the sedentary endo-parasitic nematodes (*Meloidogyne, Heterodera*, and *Globodera* spp.) and the ectoparasitic nematodes (*Tylenchorhynchus* spp.). However, some analyses showed an increase in migratory

endo-parasitic nematode numbers on inoculation with AMF (Borowicz, 2001; Hol and Cook, 2005). Grouping the nematodes into their broad feeding modes has the effect of obscuring the data on interactions of AMF with *Pratylenchus* spp. and those with other migratory endo-parasites including *Radopholus* spp. and *Hirschmanniella* spp.

Due to the ubiquitous distribution and the great economic importance of *Pratylenchus* spp. to agricultural crops worldwide, this systematic review examines the relationship exclusively between *Pratylenchus* spp. and AMF taking into account the current classification of AMF genera. All life stages of *Pratylenchus* spp., adults, juveniles, and eggs occupy the same root cortex tissue as the AMF structures of hyphae, arbuscules, and vesicles (Pinochet et al., 1996) and co-occur with AMF extraradical hyphae and spores in the rhizosphere soil.

The aims of this review are to determine (a) the responses in *Pratylenchus* population densities to AMF, (b) the effects of AMF on the growth of plants infested with *Pratylenchus* and, (c) the effects of degree of AMF colonization on *Pratylenchus* population density. The outcomes of the systematic review are discussed in relation to putative mechanisms involved in the interaction between *Pratylenchus* spp. and AMF. These mechanisms may include: (a) enhanced plant tolerance to *Pratylenchus* as a result of increased nutrient uptake and altered root morphology, (b) direct competition between *Pratylenchus* and AMF for resources and space, (c) effects on *Pratylenchus* through plant defense mechanisms such as induced systemic resistance in the plant from AMF colonization, and (d) altered rhizosphere interactions (Pozo and Azcón-Aguilar, 2007; Schouteden et al., 2015).

METHODS

Selection of Studies

A systematic review of the literature was performed according to PRISMA systematic review guidelines (Moher et al., 2009). Studies investigating interactions between *Pratylenchus* spp. and AMF were obtained from the databases,—Web of Science (www.webofknowledge.com), SCOPUS (https://www.scopus.com) and Google Scholar (https://scholar.google.com/).

The search parameters included the following terms, "*Pratylenchus*," "arbuscular mycorrhizal fungi" AND "root-lesion nematode." The papers were further screened to select original research with quantitatively measured data of the following response variables: (a) effects of AMF on *Pratylenchus* population densities, (b) effects of *Pratylenchus* spp. on degree of AMF colonization in the roots (mycorrhization), and (c) effects of both organisms on plant biomass. Other pre-requisites for eligibility for inclusion in the review were (a) studies with one or more AMF species, but not mixed treatments with other beneficial organisms, (b) studies with *Pratylenchus* species alone not mixed with other plant-parasitic nematodes, and (c) studies with a non-inoculated control. Reviews, meta-analyses and book chapters were excluded from the analyses, but the original research papers cited within were cross referenced and assessed for suitability for inclusion.

Analyses of Response Variables

The "nematode response" was calculated using the following formula:

$$nematode\ response = \frac{(Pratylenchus - Pratylenchus\ plus\ AMF)}{Pratylenchus} * 100 \quad (1)$$

where "*Pratylenchus*" is the final population density of *Pratylenchus* in nematode only treatments and "*Pratylenchus plus AMF*" is the population density of *Pratylenchus* in co-inoculated AMF and nematode treatments.

The "biomass response" was calculated using the following formula:

$$biomass\ response = \frac{(Pratylenchus\ biomass - Pratylenchus\ plus\ AMF\ biomass)}{Pratylenchus\ biomass} * 100 \quad (2)$$

Where "*Pratylenchus biomass*" is the plant biomass in nematode only inoculated treatments and "*Pratylenchus plus AMF biomass*" is the plant biomass in co-inoculated AMF and nematode treatments. Biomass data were expressed as shoot, root and total biomass where available.

The "AMF response" was calculated using the following formula:

$$AMF\ response = AMF\ \%\ colonisation - AMF\ \%\ colonisation\ plus\ Pratylenchus \quad (3)$$

where "*AMF % colonization*" is the percentage of mycorrhization of plants with AMF alone and "*AMF % colonization plus Pratylenchus*" is the percentage of mycorrhization of plants co-inoculated with AMF and nematodes.

The effect of inoculation with AMF on the *Pratylenchus* population density was categorized as decrease, no effect, or increase based on statistical significance ($P<0.05$) of studies in the original publications. A chi-squared test for independence was performed to assess the relationship between order of AMF (Glomerales and Diversisporales) and effect on *Pratylenchus* population densities. Chi-squared values were calculated from two-way contingency tables (Steel and Torrie, 1960) of AMF order by *Pratylenchus* density effect for the 56 studies using the following function:

$$\chi^2 = \Sigma\{(\text{observed number} - \text{expected number})^2 / \text{expected number}\} \quad (4)$$

The percentage AMF colonization of the roots of the plants in these three categories of AMF effects on *Pratylenchus* population densities for the studies with relevant data was subjected to one-way analysis of variance (ANOVA) using GenStat (VSN International, 2014).

The data were examined under other independent groupings such as (a) restructured AMF genera according to the current classification by Schüßuler and Walker (Schüßler and Walker, 2010) and (b) host plant functional group (grasses, trees, herbs, shrubs).

RESULTS

The initial search conducted on all available literature in the three databases provided 519 potential papers for inclusion. Further screening by removing duplicates and ineligible papers resulted in 22 full text articles selected for the systematic review (**Table 2**). Experiments within papers were treated as separate studies when; (a) two or more AMF species were studied independently, (b) more than one plant cultivar was included, and (c) more than one time of inoculation was used. If there were various times of assessment for plant biomass over multiple years, the most recent data set was used. In total, 60 studies were analyzed (**Supplementary Table 1**).

Table 3 shows the response of *Pratylenchus* sp., arbuscular mycorrhizal fungi (AMF) and plants to co-inoculation of AMF and *Pratylenchus* sp. compared to *Pratylenchus* sp. alone in glasshouse and microplot experiments. The data is statistically significant as stated in the original papers. The majority of the crops assessed were agriculturally or horticulturally important with the exception of dune grass (*Ammophilia arenaria*). In general, the experiments were undertaken in glasshouses with some transplanting of pre-inoculated AMF colonized plants to field microplots. There were 14 individual species of AMF used

TABLE 2 | PRISMA Flow Diagram for eligible articles to include in the qualitative review.

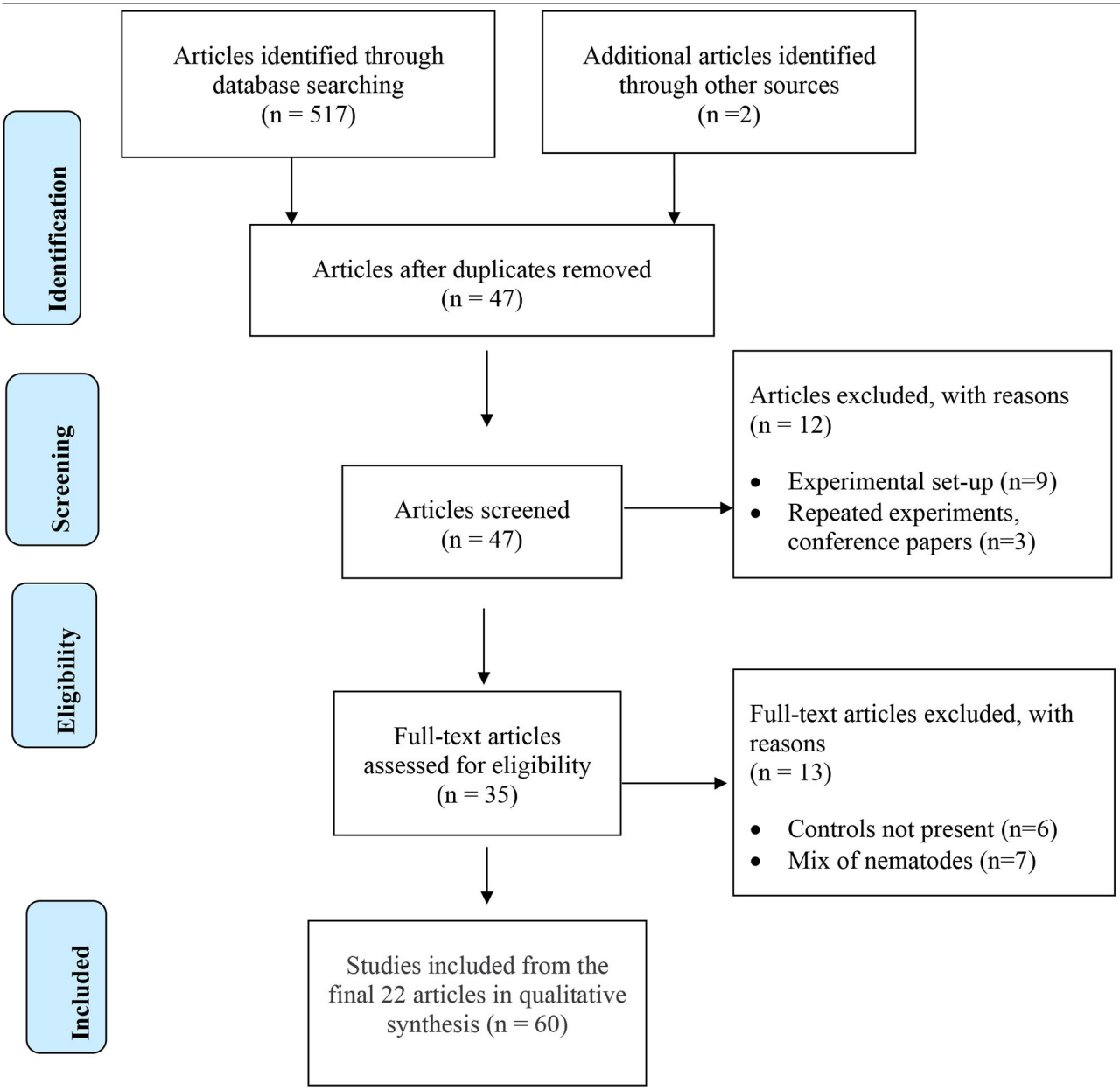

TABLE 3 | Response of *Pratylenchus* spp., arbuscular mycorrhizal fungi (AMF) and plants to co-inoculation of AMF and *Pratylenchus* spp. compared to *Pratylenchus sp.* alone in glasshouse and microplot experiments.

Plant species (common name)		*Pratylenchus* species	Response to AMF-*Pratylenchus* interaction (%)					
	AMF species		Nematode	AMF	Biomass	Shoot wt	Root wt	Reference
GRASS								
Triticum aestivum (wheat)	Mix: *Claroideoglomus etunicatum, F. coronatum, Rhizophagus irregularis, F. mosseae*	*P. neglectus*	47 to 117[1]	ns	↓30 to ↓40[1]	ND	↓31 to ↓44[1]	Frew et al., 2018
Zea mays (maize)	*R. clarus*	*P. brachyurus*	990	ND	ND	ns	ND	Brito et al., 2018
	Dentiscutata heterogama		353	ND	ND	ns	ND	
	Gigaspora rosea		447	ND	ND	ns	ND	
	C. etunicatum		441	ND	ND	ns	ND	
	G. margarita		353	ND	ND	ns	ND	
	S. calospora		900	ND	ND	ns	ND	
Ammophila arenaria (dune grass)	*Glomus* sp.	*P. dunensis*	↓38[2]	ns	↓44[2]	ND	ND	Rodríguez-Echeverría et al., 2009
	Glomus sp.	*P. penetrans*	↓67[2]	ns	ns	ND	ND	
	Mix: *Glomus* spp., *S. castanea*	*P. penetrans*	↓47 to ↓86[3]	ns	ns	ND	ns	de La Peña et al., 2006
Tree								
Cydonia oblonga (quince)	*R. intraradices*	*P. vulnus*	ns	↓26	ND	65	51	Calvet et al., 1995
Malus domestica (apple)	*C. claroideum*	*P. penetrans*	ns	ND	ND	ND	ns	Ceustermans et al., 2018
	Acaulospora longula		ns	ND	ND	ND	ns	
	C. claroideum, A. longula		ns	ND	ND	ND	165	
	R. intraradices		ns	ND	ND	ND	ns	
	AMF species mix (13)		↓97	ND	ND	ND	ns	
	C. etunicatum		ns	ns	ns	8	ns	Forge et al., 2001*
	R. aggregatus		ns	ns	ns	ns	ns	
	R. clarus		ns	ns	ns	ns	ns	
	F. mosseae		ns	ns	19 to 45[1]	9 to 54[1]	1 to 32[1]	
	R. intraradices		ns	ns	19 to 43[1]	12 to 49[1]	5 to 37[1]	
	G. versiforme		ns	ns	ns	47	ns	
Malus silvestris (crab apple)	*F. mosseae*	*P. vulnus*	↓51	ns	ND	201	142	Pinochet et al., 1993
Pyrus communis (pear)	*R. intraradices*		↓57	ns	ND	403	209	Lopez et al., 1997
	F. mosseae		↓63	ns	ND	341	202	
Prunus mahaleb (cherry)	*R. intraradices*		ns	ns	ND	89	78	Pinochet et al., 1995a
Prunus persica (peach)	*F. mosseae*		↓42	ns	ND	ns	ns	Pinochet et al., 1995b
Prunus cerasifera X *P. munsoniana* (Prunus rootstock)	*R. intraradices*		ns	↓14	ND	ns	28	Pinochet et al., 1998
	F. mosseae		ns	↓14	ND	ns	ns	
Prunus cerasifera (cherry plum)	*F. mosseae*		ns	↓34[1]	ND	ns	86[1]	Camprubi et al., 1993
Herb								
Musa sp. (banana)	*F. mosseae*	*P. coffeae*	↓76[1]	ns	ND	175 to 433[1]	192 to 310[1]	Elsen et al., 2003a
	F. mosseae		↓79 to ↓80[1]	↓17 to ↓24[1]	ND	ns	ns	Elsen et al., 2003b
	F. mosseae	*P. goodeyi*	ns	ND	ND	16	ns	Jaizme-Vega and Pinochet , 1997
	R. aggregatus		ns	ND	ND	14	ns	
	R. intraradices		ns	ND	ND	8	ns	
Phaseolus vulgaris (common bean)	*R. fasciculatus*	*P. penetrans*	ns	ND	ND	ND	ND	Elliott et al., 1984

(Continued)

TABLE 3 | Continued

Plant species (common name)	AMF species	*Pratylenchus* species	Response to AMF-*Pratylenchus* interaction (%)					Reference
			Nematode	AMF	Biomass	Shoot wt	Root wt	
Daucus carota (carrot)	*F. mosseae*	*P. penetrans*	↓48	ns	207	ND	ND	Talavera et al., 2001
Lycopersicon esculentum (tomato)	*F. mosseae*		↓87	ns	ND	ns	ns	Vos et al., 2012
Ananas comosus (pineapple)	*Glomus* sp.	*P. brachyurus*	↓24 to ↓74[4]	↓9 to ↓32[4]	ND	105 to 359[4]	50 to 269[4]	Guillemin et al., 1994
Shrub								
Gossypium hirsutum (cotton)	*Gigaspora margarita*	*P. brachyurus*	↓66	ND	ND	556	544	Hussey and Roncadori, 1978
Coffea arabica (coffee)	*A. mellea*		1049[3]	↓32[3]	946[3]	ND	ND	Vaast et al., 1997
	R. clarus		432[3]	↓26[3]	504[3]	ND	ND	

*[1]Cultivar dependent; [2]AMF, country of origin dependant; [3]Time of inoculation dependent; [4]Cultivar and time of inoculation dependent; ns, non-significant result; ND, not determined. Nematode response, difference between Pratylenchus alone and co-inoculated with AMF; AMF response, difference between percentage mycorrhization of AMF alone and co-inoculated with Pratylenchus; Biomass response; difference between Pratylenchus alone and co-inoculated with AMF; ↓ indicates negative effect of AMF x Pratylenchus interaction; *Glasshouse data only.*

in 43 studies, one undetermined species in ten studies, and a mix of AMF species in seven studies. These species came from both the order Glomerales which included the genera *Rhizophagus, Glomus, Funneliformis, Claroideoglomus,* and the order Diversisporales, which included the genera *Acaulospora, Dentiscutata, Gigaspora*, and *Scutellospora.*

The studies involved seven *Pratylenchus* spp. namely *P. penetrans, P. vulnus, P. neglectus, P. coffeae, P. goodeyi, P. brachyurus* and *P. dunensis*. These species reviewed are many of the species of *Pratylenchus* causing the most economic damage worldwide (Jones and Fosu-Nyarko, 2014).

Responses in *Pratylenchus* Population Densities to AMF

The effects of AMF inoculation on *Pratylenchus* population densities varied from a decrease in population densities ($n = 22$), no effect on *Pratylenchus* population densities ($n = 28$), to an increase in *Pratylenchus* population densities ($n = 10$).

The taxonomic order of AMF species used had an effect on *Pratylenchus* densities, whereby inoculation with species from the order Glomerales tended to decrease *Pratylenchus* population densities compared with species from the order Diversisporales which tended to increase *Pratylenchus* population densities (**Table 4**). Although there were fewer studies with comparisons for Diversisporales than for Glomerales, the differences in response between these groupings were highly significant (**Table 4**). Within the Glomerales, inoculation with the genera *Glomus* and *Funnelifomis* had a neutral to reductive effect on *Pratylenchus* population densities.

Increases in *Pratylenchus* population densities due to AMF inoculation in studies subdivided in relation to the host plant functional group were predominantly found in the grasses (increases in 8 out of 15 studies). No increase in *Pratylenchus* population densities were found in trees (0 increases in 24 studies), or herbs (0 increases in 16 studies).

Effects of AMF on the Growth of Plants Infested With *Pratylenchus*

Plant shoot biomass increased when AMF were co-inoculated with *Pratylenchus* compared with infection with *Pratylenchus* alone. From the 34 studies with data providing comparisons on shoot biomass, 24 showed an increase in shoot biomass while 10 had no effect. No studies showed a reduction in shoot biomass. Most studies calculated shoot biomass ($n = 35$) and root biomass independently ($n = 41$), with fewer reporting results on total biomass ($n = 28$). From these 28 studies, eight showed an increase in total plant biomass, and three studies a decrease in total plant biomass with 17 having no significant effect.

The change in root biomass between plants inoculated with *Pratylenchus* and the plants co-inoculated with AMF and *Pratylenchus* is shown in **Table 3**. The majority of the studies showed an increase in root biomass when inoculated with AMF ($n = 22$) in the presence of *Pratylenchus* with the exception of two studies by Frew et al. (2018).

Effects of Degree of AMF Colonization on *Pratylenchus* Population Density

There were 58 studies with data on the degree of AMF colonization of the roots. In most studies there was a decrease ($n = 21$) or no effect ($n = 27$) on *Pratylenchus* population densities, which were associated with relatively high percentage AMF colonization of the roots (43.9 and 42.2% respectively), compared to an increase in *Pratylenchus* population densities ($n = 10$), which were associated with a significantly lower percentage AMF colonization (20.1%) (**Table 5**).

DISCUSSION

This review is the first to examine the effects of specific genera and order of AMF acting on *Pratylenchus* population densities and demonstrates that the taxonomic order of AMF

TABLE 4 | Number of studies investigating AMF-*Pratylenchus* interaction included in the systematic review and the effect of AMF order on *Pratylenchus* populations.

Order	Genus	Effect on *Pratylenchus* populations			Total studies
		Increase	No effect	Decrease	
Glomerales	*Rhizophagus*	2	12	1	15
	Glomus	0	2	8	10
	Funneliformis	0	8	8	16
	Claroideoglomus	1	2	0	3
	AMF mix (*Claroideoglomus*, *Rhizophagus*, *Funneliformis*)	2	0	1	3
	Total	5	24	18	47
Diversisporales	*Acaulospora*	1	2	0	3
	Dentiscutata	0	1	0	1
	Gigaspora	2	0	1	3
	Scutellospora	2	0	0	2
	Total	5	2	2	9
		$\chi 2 = 10.43$ with 2 d.f. $P < 0.01$			

TABLE 5 | Effects of AMF inoculation on change in *Pratylenchus* population densities in relation to degree of AMF colonization in the roots.

Change in *Pratylenchus* population density	Number of comparisons	AMF % colonization in presence of *Pratylenchus*		
		$\log_e$	SE[a]	BTM (%)[b]
Decrease	21	3.7818	0.1419	43.9
No effect	27	3.7421	1.1252	42.2
Increase	10	2.9994	0.2057	20.1

Fprobability, 0.006 from ANOVA of the transformed data.
[a]*SE, standard error.*
[b]*BTM, back-transformed mean.*

has a significant influence on *Pratylenchus* population densities. Previous reviews and meta-analyses showed a varied response of AMF on migratory endo-parasites ranging from a suppressive (Veresoglou and Rillig, 2012; Yang et al., 2014) to a stimulatory effect (Borowicz, 2001; Hol and Cook, 2005).

Variation in functionalities between AMF families has been reported (Smith et al., 2004). Members of the Glomeraceae are typically fast colonizers, concentrating their hyphae within the plant roots and can increase P uptake and promote plant growth under pathogen attack and drought stress (Klironomos, 2000; Hart and Reader, 2002; Maherali and Klironomos, 2007; Yang et al., 2015; Seymour et al., 2019). Members of the Diversisporales are typically slower to colonize roots, concentrating hyphae externally to the plant root in the soil and are effective at enhancing plant phosphorus uptake (Klironomos, 2000; Hart and Reader, 2002; Maherali and Klironomos, 2007). However, from the studies in this review, there was lack of data on the percentage of AMF colonization of the controls in the order Diversisporales ($n = 2$) therefore it remains unclear if Diversisporales are slower to colonize from these studies.

From our review, species from the genera *Glomus* or *Funneliformis*, in the order Glomerales decreased or had no significant effect on the *Pratylenchus* population densities compared with *Rhizophagus* and *Claroideoglomus*. The difference in effects that AMF genera have on *Pratylenchus* population densities could be due to differences in the secondary metabolites produced under the symbiotic relationship. For example, in tomato, although the metabolic pathways altered by the AMF symbiosis were similar, different metabolites were produced, depending on inoculation with *F. mosseae* or *R. irregularis* (Pozo et al., 2002). An increase in the accumulation of bioactive forms of jasmonic acid was found in roots colonized by *F. mosseae* (Rivero et al., 2015). Jasmonic acid and its derivative methyl jasmonate play a role in plant defense against herbivores and they can reduce susceptibility of plants to infestation by *Pratylenchus* (Soriano et al., 2004). Root metabolites may influence populations of plant parasitic nematodes by acting as attractants, repellents or affecting hatch rates of nematodes (Sidker and Vestergård, 2019). Mycorrhizal colonization can increase phenolics such as ferulic acid and gallic acid in the host plants (López-Ráez et al., 2010; Li et al., 2015). Ferulic acid inhibits mobility and is toxic to the burrowing nematode *R. similis* but is ineffective against *Pratylenchus penetrans* (Wuyts et al., 2006). Gallic acid acts as a nematicide to the root-knot nematode *M. incognita* (Seo et al., 2013). High constitutive total phenol contents were found in synthetic hexaploid wheat genotypes resistant to *P. thornei* combined with high levels of induced phenol oxidases (Rahaman et al., 2020). These studies indicate that the biochemical responses of host plants to both inoculation with AMF and infestation by plant-parasitic nematodes are highly complex.

Even within populations of a single species of AMF, there is a high genetic variability which may affect the host/fungal relationship (Koch et al., 2006, 2017). Variations in the effects that a single species of AMF have on *Pratylenchus* population

densities were observed in the studies by Elsen et al. (2003b) and Jaizme-Vega and Pinochet (1997). Both studies used the same cultivar of banana and the same species of AMF, but obtained different results depending on the *Pratylenchus* sp. tested. Elsen et al. (2003b) stated that it was difficult to explain the contrary results, however, the AMF strain and the environmental conditions differed between experiments. As a different isolate of *F. mosseae* was used as inoculum, it is important to emphasize the traceability of isolates that are used in experiments. A similar observation was made in dune grass whereby *Pratylenchus* sp. were only reduced in the interaction with a community of AMF isolated from Wales and not from an AMF community isolated from Belgium (Rodríguez-Echeverría et al., 2009). This highlights the need to study interactions between specific crops, cultivars and AMF species or communities.

Plant functional group influenced *Pratylenchus* population densities in grasses but not in herbs and trees. Interestingly, response to AMF can be attributed to plant functional groups in which non-nitrogen fixing forbs and woody plants, and C4 grasses benefit more in plant growth by the fungal association, compared to nitrogen fixing plants and C3 grasses (Hoeksema et al., 2010). However, Yang et al. (2016) concluded that nitrogen fixing plants had a greater mycorrhizal growth response only when the host plant was a forb and not woody. A practical application to improve tolerance, or plant growth, when *Pratylenchus* is present may therefore be to pre-inoculate tree species with AMF prior to transplanting into orchards, taking into account the interaction between cultivars, their mycorrhizal dependency and AMF species used as inoculum sources (Pinochet et al., 1996). The potential of AMF inoculum conferring benefits to crop production in high economic value vegetable crops has been reviewed by Baum et al. (2015). These include advantages such as increases in yield, increases in commercial quality of the crop, protection against nematodes and other pathogens, tolerance to drought and other abiotic stressors and nutrient uptake. As the interaction between host, AMF inoculum and environment can be very specific, future research is needed to optimize the inoculation protocols to target specific crop production limitations.

The outcomes of the present systematic review, in relation to putative mechanisms involved in the interaction between *Pratylenchus* spp. and AMF, are discussed below.

Enhanced Plant Tolerance

Plant shoot biomass increased when AMF were co-inoculated with *Pratylenchus* compared with infection with *Pratylenchus* alone. A number of studies investigated tolerance to *Pratylenchus* spp. as a reflection of increasing vegetative plant nutrition. AMF can increase the uptake of P and other nutrients such as Zn from the soil (Parniske, 2008; Seymour et al., 2019). This increase in nutrition can lead to a greater plant biomass response conferring a compensatory effect against the damage done by nematodes. Previous studies have shown that AMF confers tolerance to *Pratylenchus* spp. by compensating for root damage caused by *Pratylenchus* spp. through increasing the uptake of P and other micronutrients, such as Fe, Mn, Zn, and Cu (Calvet et al., 1995; Pinochet et al., 1998). However, improvement in the nutritional status of the plant is not believed to be wholly responsible for the biocontrol effect of AMF (Bødker et al., 1998; Jung et al., 2012).

Tolerance conferred by AMF to a crop under *Pratylenchus* pressure has been described in the majority of the reviewed papers ($n = 41$) with the exception of the following; peach, *Musa* sp., maize, tomato, dune grass and wheat (Pinochet et al., 1995b; Elsen et al., 2003a; Rodríguez-Echeverría et al., 2009; Vos et al., 2012; Brito et al., 2018; Frew et al., 2018). This may be a reflection of the mycorrhizal dependency of the cultivars assessed as some tomato and wheat cultivars have a low mycorrhizal dependency (Smith et al., 2009) while cultivars of maize, *Musa* sp. and peach generally have higher mycorrhizal dependency (Pinochet et al., 1995b; Kaeppler et al., 2000; Elsen et al., 2003a). A study by Martín-Robles et al. (2018) found that domesticated crops benefit more from the symbiosis with AMF under P limiting conditions. It is worthwhile to note that most of the studies analyzed in this review were undertaken in low P experimental conditions where AMF function most efficiently (**Supplementary Table 1**).

The studies assembled in **Table 3** demonstrate the predominantly beneficial effects AMF have on crop species, alleviating the damage to the root and shoot biomass caused by *Pratylenchus*. There were only three studies where AMF decreased total biomass and root weight when co-inoculated with *Pratylenchus*. These studies were on wheat and dune grass, both C3 crops (Rodríguez-Echeverría et al., 2009; Frew et al., 2018). Variations in root morphology between C3 and C4 grasses determine their dependency on the mycorrhizal symbiosis (Hetrick et al., 1991), which may help explain the reduction in biomass. Wheat has a low to intermediate dependency on mycorrhiza depending on genotype (Lehnert et al., 2017) and modern plant breeding may contribute to a reduction in dependency on the mycorrhizal symbiosis by screening and selecting new varieties in high phosphate or highly fertile soils (Hetrick et al., 1993). However, a modern wheat cultivar Batavia was found to have high dependency on AMF colonization under drought conditions on a field site infested with *P. thornei* (Owen et al., 2010). Dune grass forms an association with AMF promoting plant growth (Tadych and Blaszkowski, 1999). de La Peña et al. (2006) suggested that evidence of biomass reduction in dune grass was related to a species-specific interaction between a geographically unique community of AMF from Wales and the species of *Pratylenchus* (*P. dunensis*) studied. Biomass reduction was not significant in another study of the interaction between AMF and *P. penetrans* on dune grass (de La Peña et al., 2006).

Previous reviews have also demonstrated this positive effect that AMF have on increasing plant growth under attack by migratory nematodes (Hol and Cook, 2005; Yang et al., 2014). This is contrary to the study by Borowicz (2001) that concluded AMF increased the negative effects of nematodes on plant biomass, indicating a reduced nematode tolerance.

The majority of studies showed an increase in root biomass in the presence of *Pratylenchus* when inoculated with AMF. *Pratylenchus* infestation negatively impacts root biomass, resulting in a reduction in the quantity and length of root branches (Fosu-Nyarko and Jones, 2016). Colonization by AMF can also result in alterations to root morphology, causing either an increase or decrease in root branching (Hooker et al., 1992;

Sikes, 2010). A study on morphological changes within the root system in *Musa* sp. under *Pratylenchus* pressure showed that AMF increased root branching counteracting the negative consequences of *Pratylenchus* infection (Elsen et al., 2003a). Berta et al. (1995) also demonstrated in cherry plum (*Prunus cerasifera)* that AMF increased the branching of all root orders. However, there were variable effects on root diameter depending on which genera of AMF were used.

Baylis (1975) hypothesized that plants with extensive fine root systems with long dense root hairs were less reliant on the mycorrhizal symbiosis in comparison to coarsely rooted plants. However, recent evidence suggests that coarse roots are not necessarily a good predictor of crop dependency on the AMF symbiosis (Maherali, 2014). A meta-analysis by Yang et al. (2016) found that although plants with fibrous roots responded less to mycorrhizal colonization than tap rooted plant species, this was only evident for C3 and not C4 grass species. Notwithstanding this, plants that have a highly branched root system may still benefit from the AMF association via other ecosystem functions such as pathogen protection (Newsham et al., 1995).

Competition for Space Between *Pratylenchus* and AMF

Degree of AMF colonization had an effect on the population densities of *Pratylenchus*. Inoculation with AMF that resulted in low levels of AMF colonization was associated with increases in *Pratylenchus* population densities compared with other cases with high levels of AMF colonization that were associated with decreases or no effects on *Pratylenchus* population densities. The nematode population density could also affect the rate of colonization by AMF indicating a competition between species. Both AMF and *Pratylenchus* occupy the same ecological niche within the root cortical cells as described in various crop species, for example, quince, cherry, peach, pear, banana, plum, and coffee (Calvet et al., 1995; Pinochet et al., 1995a,b, 1998; Lopez et al., 1997; Vaast et al., 1997; Elsen et al., 2003b). *Pratylenchus* sp. and AMF were considered to have competed for space within the cortical cells in quince, coffee, banana and dune grass (Calvet et al., 1995; Vaast et al., 1997; Elsen et al., 2003b; de La Peña et al., 2006).

Arbuscules are the metabolically active sites of exchange between the plant and the fungus and a mature mycorrhizal colonization of the plant, as evidenced by the production of arbuscules, has been thought to be the prerequisite for a biocontrol effect (Khaosaad et al., 2007). It has been hypothesized that a greater colonization of AMF in plant roots would lead to a greater biocontrol effect on nematodes.

Pratylenchus can affect the quantity and morphology of AMF within the root cortical cells. For example, in quince, AMF increased the production of arbuscules reflecting a metabolically active state under *Pratylenchus* infestation, compared to an increase in the production of vesicles in the absence of infestation (Calvet et al., 1995). In banana, nematodes reduced the frequency of colonization but not the intensity (Elsen et al., 2003b). In pineapple, although nematodes reduced the frequency of arbuscules when applied at a later time point during transplanting, they did not affect the efficiency of the symbiosis (Guillemin et al., 1994).

The time of inoculation was not a factor in how the nematode population densities responded to AMF inoculation. AMF was applied to the plants prior to nematode inoculation in the majority of studies ($n = 42$), which gave the symbiosis a chance to establish before being challenged with *Pratylenchus*. However, this established symbiosis was not reflected in a decrease in nematode population density, but may have aided the plant in tolerance to nematode infestation through increased vegetative growth as previously discussed.

Plant Defense and Induced Systemic Resistance

Mycorrhiza-induced resistance that can operate systemically can be effective against plant-parasitic nematodes and may contribute toward the biocontrol effect of AMF (Jung et al., 2012). Induced systemic resistance has no association with pathogenesis related proteins or salicylic acid but is regulated by jasmonic acids and ethylene (Pieterse et al., 1998).

There is little available research on induced systemic resistance by AMF against *Pratylenchus* as compared to other plant pathogens. However, using split root experiments, the systemic biocontrol effects of the AMF species *F. mosseae* and *R. irregularis* on *Pratylenchus* were demonstrated in banana and tomato. *Rhizophagus irregularis* induced a systemic suppression of *P. coffeae* and *R. similis* in banana, though the pathways involved in this suppression were not determined (Elsen et al., 2008). In tomato, inoculation with *F. mosseae* reduced the number of females of *P. penetrans* through a localized mechanism and the number of juveniles through a systemic mechanism (Vos et al., 2012). Contrary to this, only a localized suppression of *Pratylenchus* population densities was observed in dune grass (de La Peña et al., 2006).

Investigations into the metabolomics of AMF showed that AMF colonization increased the production of AMF plant signaling compounds and anti-herbivory defenses (Hill et al., 2018). There is still very little research available on the interactions between *Pratylenchus* and AMF on effects on the metabolome. Frew et al. (2018) reported that AMF reduced plant defense metabolites, specifically benzoxazinoids, which accounted for an increase in *P. neglectus* population densities in wheat. Studies involving root organ cultures of carrot showed significant suppressive effects of AMF on *P. coffeae* female population densities believed to be a result of biochemical changes in the mycorrhized root (Elsen et al., 2003c). Exudates from AMF can reduce the motility and penetration of sedentary nematodes (Vos et al., 2012) but little research has been done on their effects on migratory endo-parasites. An *in-vitro* chemotaxic assay on the migratory endo-parasite *R. similis* demonstrated that the exudation of a water-soluble compound, produced by mycorrhizal roots, reduced attraction at a pre-infection stage (Vos et al., 2012), but there is little information on how exudates affect *Pratylenchus* spp. Further research is needed to assess the mechanisms of AMF in influencing *Pratylenchus* population densities.

Alterations in the Rhizosphere

Alterations in chemical compounds in the rhizosphere as a result of interactions between plant-parasitic nematodes and AMF have been reviewed (Schouteden et al., 2015). These involve changes in exudation of sugars, organic acids, amino acids, phenolic compounds, flavonoids and strigolactones in AMF colonized plants as compared to non-AMF plants. AMF exudations into the rhizosphere promote beneficial microorganisms such as plant-growth promoting rhizobacteria (PGPR) (Jung et al., 2012; Javaid, 2017) and resultant changes can be induced systemically, influencing the bacterial community structure (Marschner and Baumann, 2003). This enhanced microbial activity around plant roots has been termed the mycorrhizosphere effect (Linderman, 1988). Plant growth promoting rhizobacteria have been implicated in nitrogen fixation, phosphate solubilization, modulating phytohormone levels and the production of antibiotics and lytic enzymes (Glick, 2012). Cameron et al. (2013) proposed that AMF and PGPR act together to increase plant defenses against biotic stressors in mycorrhiza-induced resistance. Studies on multipartite interactions between *Pratylenchus*, AMF, PGPR and crop hosts are lacking in the literature.

Species of PGPR in the genera *Pseudomonas*, *Bacillus*, *Streptomyces* and *Lysobacter* have been implicated in reducing *Pratylenchus* population densities (Walker et al., 1966; Stirling, 2014; Castillo et al., 2017), and some research has been conducted on the interaction between AMF and these PGPR. In strawberry, *Pseudomonas chlororaphis* suppressed populations of *P. penetrans* (Hackenberg et al., 2000) while extracts from the AMF species *R. irregularis* stimulated the growth of *Pseudomonas chlororaphis in vitro* (Filion et al., 1999). *Streptomyces* spp. can reduce *Pratylenchus* population densities (Meyer and Linderman, 1986; Samac and Kinkel, 2001) and they can also stimulate spore germination in *F. mosseae* and *Gigaspora margarita* (Tylka et al., 1991). This indicates a link between the three types of phytobiome organisms, though further research is needed to assess AMF and PGPR combined effects on *Pratylenchus* population densities.

LIMITATIONS OF THE REVIEW AND FUTURE RESEARCH

The crops assessed in this review were agriculturally or horticulturally important with the exception of dune grass (*Ammophilia arenaria*). Most studies looked at a single species of AMF alone and not in combination with species from different orders and genera of AMF, or other beneficial microbes such as PGPR. The taxonomic orders of AMF used in the studies reviewed were limited to the Glomerales and Diversisporales. Other orders such as the Archaeosporales and the Paraglomerales are also present in soils, though they are under-represented in experimental work. A study by Gosling et al. (2014), found a wide distribution of the Paraglomerales in agricultural soils in the UK. AMF species such as *F. mosseae* and *R. irregularis* have a tendency to be over represented in this type of experimental work due to their ease of multiplication in trap cultures. The studies in this review were undertaken in low P soils, predominantly in glasshouses, with some transplantations to microplots. Arbuscular mycorrhizal fungi function most efficiently under low to moderately high P conditions, and therefore the benefit of AMF in improving plant nutrition and plant biomass under *Pratylenchus* pressure could be overstated for agricultural systems receiving continued high rates of P fertilizers. Better matching of P fertilizer inputs to crop removal is required in some agricultural systems to avoid excessive levels of available P in soils for better harnessing of AMF functions, stewardship of global P supplies and environmental quality (Gianinazzi et al., 2010).

The number of studies in this highly specific review of the interaction between *Pratylenchus* spp. and AMF was limited to only 60 studies suitable for inclusion. Further research needs to be undertaken in the area, using a broad range of crop cultivars and AMF species from diverse orders to further increase our understanding of the relationship between these organisms in the rhizosphere.

Further research needs to be done in assessing the mechanisms involved in the effect of AMF on *Pratylenchus* population densities through investigations into induced systemic resistance and changes in the metabolome. As research is lacking on the effects of AMF, *Pratylenchus* and beneficial bacteria in the rhizosphere, more studies need to be undertaken on multipartite interactions between these organisms in crop hosts.

CONCLUSION

The interactions between *Pratylenchus* and AMF reveal some unique effects as influenced by crop species, crop cultivar, AMF order and AMF genus. Our review showed increased *Pratylenchus* densities in plants inoculated with species from the order Diversisporales. Inoculation with the AMF genera *Glomus* and *Funneliformis* from the order Glomerales, reduced or had no effect on *Pratylenchus* densities in host roots. AMF aids the tolerance of plants to *Pratylenchus* through increased vegetative growth. The biocontrol effect of AMF is likely to be a combination of increasing host tolerance, competition between organisms, and systemic resistance, though further research is needed to identify the mechanisms involved. Further studies will need to take into account the specific interactions between crop, cultivar and AMF species in both glasshouse and field trials.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

JT and EG conceptualized the paper. EG performed the database search, collated the data, and drafted the manuscript. JT and EG conducted the statistical analyses. EG, KO, and RZ integrated information on tables. All authors contributed to revising

the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

EG acknowledges support from the University of Southern Queensland Research Training Program Scholarship and Grains Research and Development Corporation (GRDC) Research Scholarship Project USQ1912-003RSX. RZ acknowledges support from USQ. KO acknowledges co-funding by the GRDC through Project DJP1907-002RMX. KO and JT acknowledge support from the Queensland Department of Agriculture and Fisheries (QDAF) through the Broadacre Cropping Initiative with USQ.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00923/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CHAPTER 3

ARBUSCULAR MYCORRHIZAL FUNGI ACTED SYNERGISTICALLY WITH *BRADYRHIZOBIUM* SP. TO IMPROVE NODULATION, NITROGEN FIXATION, PLANT GROWTH AND SEED YIELD OF MUNG BEAN (*VIGNA RADIATA*) BUT INCREASED THE POPULATION DENSITY OF THE ROOT-LESION NEMATODE *PRATYLENCHUS THORNEI*

The paper presented in this chapter is published in the international Q1 journal *Plant and Soil* (Springer Nature, The Netherlands), 09 June 2021. Reproduced with permission from Springer Nature.

Gough EC, Owen KJ, Zwart RS and Thompson JP (2021) Arbuscular mycorrhizal fungi acted synergistically with *Bradyrhizobium* sp. to improve nodulation, nitrogen fixation, plant growth and seed yield of mung bean (*Vigna radiata*) but increased the population density of the root-lesion nematode *Pratylenchus thornei*. *Plant and Soil*, (Q1; Impact Factor: 3.299) https://doi.org/10.1007/s11104-021-05007-7

Mung bean is the most important summer pulse crop grown on vertisols in the subtropical grain region of eastern Australia, where it can suffer nodulation problems even though inoculated with *Bradyrhizobium*, resulting in poor biomass and yield. In this study, it was demonstrated for the first time that dual inoculation with the beneficial symbionts arbuscular mycorrhizal fungi (AMF) and *Bradyrhizobium*, resulted in a marked synergistic response causing increased nodulation, nitrogen fixation, mineral nutrition, growth and seed yield of mung bean. The population density of *P. thornei* increased in the roots of plants inoculated with AMF and was positively correlated with increased concentration of P, Zn and Cu in the plant shoot. This knowledge indicates that inoculation with *Bradyrhizobium* sp. combined with management practices to promote natural population densities of AMF propagules, but reduce those of *P. thornei,* will increase yield, nutrition, and improve N fixation in mung bean.

[Supplementary material associated with this Chapter is attached in Appendix B.]

Plant Soil
https://doi.org/10.1007/s11104-021-05007-7

REGULAR ARTICLE

Arbuscular mycorrhizal fungi acted synergistically with *Bradyrhizobium* sp. to improve nodulation, nitrogen fixation, plant growth and seed yield of mung bean (*Vigna radiata*) but increased the population density of the root-lesion nematode *Pratylenchus thornei*

Elaine C. Gough · Kirsty J. Owen · Rebecca S. Zwart · John P. Thompson

Received: 10 January 2021 / Accepted: 10 May 2021

Abstract

Purpose Mung bean is a host of the root-lesion nematode *Pratylenchus thornei* (Sher & Allen) and the beneficial symbionts arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing *Bradyrhizobium* bacteria. The purpose of this research was to investigate interactions among these organisms affecting their reproduction and functional impact on mung bean nodulation, nutrition, biological nitrogen fixation, growth and seed yield.

Methods A glasshouse experiment was conducted with mung bean in pots of a pasteurised vertisol using a factorial design of treatments to investigate the interactive effects of AMF, *Bradyrhizobium* and *P. thornei*. The plants were assessed at 6 and 12 weeks after sowing for variables of shoot biomass, seed yield, nodulation, *P. thornei* population density, AMF colonisation of the roots, and nutrients in the plant shoot, including nitrogen isotope natural abundance ($\delta^{15}N$) to quantify fixed nitrogen.

Results Arbuscular mycorrhizal fungi and rhizobia acted synergistically to substantially increase nodulation, nitrogen fixation, nutrition, seed yield and biomass of the plants. The population density of *P. thornei* in roots of the mung bean at 12 weeks increased in plants inoculated with AMF and was positively correlated with plant nutrition namely increased phosphorus, zinc and copper concentrations in the plant shoot.

Conclusion Understanding these interactions should inform changes in agronomic practices, to promote the synergism between mycorrhiza and rhizobia for mung bean yield, while managing to limit *P. thornei* population densities to benefit mung bean itself and subsequent crops in the farming system.

Keywords Arbuscular mycorrhizal fungi · *Bradyrhizobium* · *Pratylenchus thornei* · Microbiota plant interactions · *Vigna radiata* · Synergy

Responsible Editor: Jan Jansa.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-021-05007-7.

E. C. Gough (✉) · K. J. Owen · R. S. Zwart · J. P. Thompson
Centre for Crop Health, Institute for Life Sciences and the Environment, University of Southern Queensland, Toowoomba, QLD, Australia
e-mail: elaine.gough@usq.edu.au

Introduction

Mung bean (*Vigna radiata* (L.) R. Wilczek) is a high protein, short season legume, with a global production area of 7.3 million ha, predominantly in South Asia (Nair and Schreinemachers 2020). In the subtropical grain region of eastern Australia, mung

Published online: 09 June 2021

bean is cultivated as one of the main high value summer crops with 129,000 ha grown in 2016–2017 (ABARES 2017). Mung bean has a yield potential of 2.5 to 3.0 t/ha, but this is not achieved due to biotic and abiotic constraints (Nair and Schreinemachers 2020). Biotic constraints limiting mung bean production in the subtropical grain region of eastern Australia include the bacterial pathogens causing tan spot (*Curtobacterium flaccumfaciens*) (Osdaghi et al. 2020) and halo blight (*Pseudomonas savastanoi* pv. *phaseolicola*) (Noble et al. 2019), powdery mildew (*Podosphaera xanthii*) (Weir et al. 2017), root-lesion nematode (*Pratylenchus thornei*) (Owen et al. 2014), and failure of nodulation by rhizobia (Herridge et al. 2005).

Root-lesion nematodes (*Pratylenchus* spp.) are polyphagous migratory endoparasites with a global distribution (Castillo and Vovlas 2007). *Pratylenchus* spp. feed and migrate through the root cortex of host plants, using their characteristic needle-like stylet to destroy plant cell walls via enzymatic degradation and mechanical probing followed by ingestion of the cellular contents (Singh et al. 2013). Destruction of the cortical tissue by the nematodes causes characteristic lesions, which reduce root function and impede the ability of the plant to take up water and nutrients resulting in yield loss in susceptible crops (Jones et al. 2013). *Pratylenchus thornei* is the most important species of *Pratylenchus* attacking grain crops in the subtropical grain region of eastern Australia (Thompson et al. 2008). Mung bean is a susceptible host to *P. thornei* and as such, facilitates the multiplication of the nematode within its roots (Owen et al. 2014).

Arbuscular mycorrhizal fungi (AMF) form a mutualistic symbiotic relationship with the roots of 80% of land plants worldwide, including many agriculturally important species grown in the subtropical grain region of eastern Australia (Thompson 1994), among which mung bean is the most important summer grown legume. Arbuscular mycorrhizal fungi provide many benefits to the plant including; (i) improved acquisition of poorly mobile inorganic nutrients such as phosphorus (P) and zinc (Zn) (Smith and Read 2008), (ii) increased water supply (Augé 2001), (iii) stabilisation of soil aggregates through hyphal binding and the exudation of the fungal glycoprotein glomalin (Rillig et al. 2015), and (iv) bio-protection against biotic stressors such as fungal plant pathogens and plant-parasitic nematodes (Whipps 2004; Yang et al. 2014).

Plant-parasitic nematodes and AMF occupy a similar ecological niche in the roots and rhizosphere of plants (Pinochet et al. 1996). This co-existence in the phytobiome has led to several investigations to determine the effects that AMF colonisation has on nematode population densities. Some analyses showed AMF increased nematode population densities or had no effect (Borowicz 2001; Hol and Cook 2005). However, meta-analyses that grouped the nematodes according to their lifestyle as sedentary or migratory documented a suppressive effect that AMF exert on population densities of some nematode species (Veresoglou and Rillig 2012; Yang et al. 2014). From a specific review of *Pratylenchus* spp., variability in the effects that AMF have on *Pratylenchus* population densities can depend on the taxonomic order and genus of the fungus, and on the plant host functional group (Gough et al. 2020).

Mung bean also forms a symbiotic relationship with bacteria of the genus *Bradyrhizobium* to fix atmospheric nitrogen into the plant-available form of ammonium. Inoculation at sowing with commercially available *Bradyrhizobium* is a common agronomic practice to improve nodulation and nitrogen fixation in the plant. Biological N fixation has benefits in reducing fertilizer requirements while increasing nitrogen uptake in the plant (Mahmud et al. 2020). However, surveys undertaken in the subtropical grain region of eastern Australia have demonstrated that up to 50% of mung bean crops failed to nodulate despite inoculation with *Bradyrhizobium* sp. at sowing (Gentry 2010). Poor nodulation resulted in significant yield losses, where nitrate levels in the soil profile were low (< 50 kg N/ha to 90 cm depth) (Herridge et al. 2005).

Arbuscular mycorrhizal fungi and rhizobia also co-exist intimately in the roots and rhizosphere of their host plant. Root colonisation by AMF can improve the ability of rhizobia to fix nitrogen in the nodules of leguminous plants and increase plant nutrition and growth (Artursson et al. 2006; Barea et al. 2002; Chalk et al. 2006). In a field trial in Pakistan, co-inoculation of mung bean with *Rhizophagus intraradices* and two strains of *Bradyrhizobium japonicum* increased grain yield (20%), shoot dry biomass (24%) nodule dry weight (127%) and shoot N and P concentrations (18% and 27%) compared to inoculation with

one isolate of *Bradyrhizobium* alone (Yasmeen et al. 2012).

There is very little information on how migratory endoparasites such as *Pratylenchus* affect nodulation in legumes. *Pratylenchus penetrans* increased nodule quantity and biomass, but inhibited nitrogen fixing capacity in soybean (*Glycine max*) (Hussey and Barker 1976). In chickpea (*Cicer arietinum*), the number of nodules formed on the roots was reduced under *P. thornei* infestation (Castillo et al. 2008).

The interactions between plant host, beneficial symbionts and plant-parasitic nematodes can be complex. The objectives of this research were to determine (i) how AMF and rhizobia interact in plants challenged or not by *P. thornei*, and the consequent effects on nodulation, biomass, and seed yield, (ii) how AMF and rhizobia affect the population densities of *P. thornei* in the roots of mung bean and, (iii) how these multipartite interactions affect mung bean plant nutrition, growth and seed yield.

Materials and methods

Experimental design

A glasshouse experiment was conducted during spring (September–November, 2018) in Toowoomba, Queensland, Australia (latitude 27.53°S, longitude 151.93°E) with mung bean cv. Jade-AU. The experiment comprised full factorial combinations of treatments of AMF (0 or 16 spores/g soil), *P. thornei* (0, 1 or 10 nematodes/g soil), rhizobia (0 or 6×10^6 CFU/g soil) and times of assessment (6 or 12 weeks after sowing) in a randomised split plot design with four replicates in blocks. The rhizobia treatment was randomised to main plots, with the combinations of factors *P. thornei,* AMF and time of assessment randomised to subplots within the main plots.

Biological materials

The mung bean cultivar Jade-AU used in the experiment accounts for 60% of production of all mung bean cultivars in the subtropical grain region of Australia (www.mungbean.org.au) and is a susceptible host to *P. thornei* (Owen et al. 2016).

The AMF strain used in the experiment was *Funneliformis mosseae* (Nicolson & Gerd) 'Schmelzer 43', originally isolated from Macalister, Queensland (latitude 27.04°S, longitude 151.07°E) (Thompson 1996). This strain originated from a single spore culture and was maintained on maize (*Zea mays*) and chickpea in open pot cultures. Soil containing the cultures was stored after harvest in sealed plastic bags at 4 °C. Arbuscular mycorrhizal fungi spores for inoculum were extracted by wet sieving a suspension of soil and roots from each of six pot cultures of maize of 2.5 kg soil/pot using a modified protocol of Gerdemann and Nicolson (1963). The soil suspension was agitated thoroughly and passed through 250-µm and 63-µm mesh sieves to retain roots and spores respectively. The spores obtained from all cultures were mixed together as a single suspension for inoculation.

The culture of *P. thornei* used in the glasshouse experiments was originally isolated from an experimental trial site near Formartin, Queensland (latitude 27.46°S, longitude 151.43°E) and subsequently multiplied on susceptible wheat (*Triticum aestivum*) cultivars in open pot cultures (Thompson et al. 2015). Soil containing *P. thornei* was stored after harvest in sealed plastic bags at 4 °C. For the experiment, nematodes for inoculum were extracted using a modified Whitehead tray method (Whitehead and Hemming 1965) from the soil and roots of four 18-week old pot cultures of susceptible wheat cultivars Petrie, Suneca and Strzelecki. Briefly, roots and soil from the pot cultures were cut using shears, and nematodes were extracted from the mixed soil and roots spread in thin layers on Kimtech tissues (KIMTECH; Kimberly-Clark Worldwide, Inc) supported on a plastic mesh tray inside a larger plastic tray containing 1 L of water incubated for 48 h at 22 °C.

The *Bradyrhizobium* strain CB 1015 (Eagles and Date 1999), from a culture obtained from the Australian Inoculant Research Group (Menangle, New South Wales), was grown on petri dishes containing yeast mannitol agar at 23.5 °C for 7 days in the dark. After incubation, the *Bradyrhizobium* growth was suspended in 1% sucrose solution and 1 mL/pot was inoculated to the soil around the seeds at

sowing. The concentration of *Bradyrhizobium* in the inoculum suspension was 10^{10} colony forming units per mL (CFU mL^{-1}) determined by the Miles and Misra drop plate method (Vincent 1970).

Plant growth conditions

For the experiment, the 0–15 cm soil depth interval of a black Vertosol (Isbell 1996) was collected from a field at Formartin. The soil was pasteurised for 45 min at 85 °C followed by a fan-forced cooling period of 30 min to bring the soil back to ambient soil temperature using a protocol modified from Thompson (1990). Most soil chemical properties were determined by Australian Precision Ag Laboratory (Hindmarsh, South Australia) while organic carbon and total nitrogen were determined by Southern Cross University (Lismore, New South Wales) using the Australasian Soil and Plant Analysis Council (ASPAC) methods of Rayment and Lyons (2011). Properties of the soil were pH 8.5 (1 soil:5 water suspension), total N 0.09% (LECO TruMac® CNS analyzer), total organic carbon 1.58% (sulphurous acid pretreatment to remove $CaCO_3$ followed by combustion in LECO SC832 CN Analyser), nitrate–N 24.5 mg/kg soil (2 M KCl extraction), P 45 mg/kg soil (Colwell bicarbonate extraction), Zn 1.45 mg/kg soil (DTPA extraction). No fertilizer was added to the pots of soil.

Pots (70-mm wide 150-mm high) designed for bottom watering, with a capacity for 330 g oven dried (OD) equivalent of soil were used in the experiment. Pots were initially filled with a base layer of 70% of the soil, and then placed on strips of capillary matting (Bidim ® Geofabrics Australasia Pty Ltd, Brisbane, Queensland, Australia) running across the glasshouse benches with the ends of the strips in a trough to supply water to the soil at 8 cm of tension (Sheedy and Thompson 2009). The strips of matting were separated by 10 cm gaps to prevent contamination between rhizobia treatments. Two mung bean seeds were placed on the moist soil surface and inoculations of the treatments were made around the seeds before capping with the remaining 30% soil. Seven days after germination, the seedlings were thinned to one per pot, by cutting the stem and leaving the roots of the superfluous seedling behind. The soil temperature was maintained at 22 ± 2 °C, monitored using an iButton (Thermochron®, Australia) placed 2 cm deep within a randomly selected treatment pot, and the air temperature was maintained at ~ 25 °C. At assessment times, the pots were transferred to plastic trays to allow the soil moisture content to reduce to approximately 45%, a moisture content optimal for processing this soil type for *P. thornei* extraction (Sheedy and Thompson 2009). The soil with roots from each pot was broken up into < 5 mm pieces and the roots cut into ~ 10 mm pieces and thoroughly mixed together.

Assessment of plant and microbiota variables

Plant biomass and seed yield

Plant growth was assessed at the vegetative stage at six weeks, and at pod maturity at 12 weeks after sowing. Fresh shoot was weighed to determine biomass of each plant and dry shoot biomass was then determined by drying at 65 °C in a forced draught oven for 96 h. Dried seed yields were hand threshed from pods, and weighed. The dry shoot biomass was recombined with seeds from each plant and finely ground using a Foss CT 193 Cyclotec grinder (FOSS, Hilleroed, Denmark) for chemical analyses.

Plant chemical analyses

The dried shoot tissue was chemically analysed by a commercial laboratory (Australian Precision Ag Laboratory, Hindmarsh, South Australia). Nitrogen was quantified by the Dumas method. The major elements P, K, Ca, S, Mg and trace elements Zn, B, Cu, Mo, Mn, Fe, were determined by microwave digestion and inductively coupled plasma—optical emission spectrometry (ICP-OES) following the methods from ASPAC. To determine fixed N, total $\delta^{15}N$ was quantified by isotope ratio mass spectrometry at the Stable Isotope Laboratory, UC Davis (USA), using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

The percentage of legume N derived from the atmosphere (% Ndfa) used to quantify biological N fixation was calculated with the following equation (Howieson and Dilworth 2016):

$$\%Ndfa = 100 * (x - y) \div (x - B)$$

where x is the $\delta^{15}N$ of the reference plant (a non-inoculated, non-nodulated Jade-AU mung bean plant from the experiment), y is the $\delta^{15}N$ of the experimental sample, and B is the measure of the $\delta^{15}N$ content of mung bean fully dependent on nitrogen fixation for growth calculated as -2.05 (Unkovich et al. 2008). The quantity of fixed N in the plant shoot was calculated using the following equation:

$$Fixed\,N = (N\ uptake) * \frac{\%Ndfa}{100}$$

P. thornei quantification

A 150 g (fresh weight) subsample of homogenised soil and roots was extracted for *P. thornei* by the modified Whitehead tray method described above. The nematodes were collected on a 20-µm aperture mesh sieve and decanted into 28 mL vials, then stored at 4 °C until counted. A 100 g subsample of the homogenised soil and roots was dried at 105 °C for 48 h in a forced draught oven to determine soil moisture content. The nematodes were counted in a 1 mL Peters slide (Chalex Corporation, UT, USA) under a BX53 optical microscope (Olympus, Tokyo, Japan) at 40 X magnification and numbers were expressed as *P. thornei*/kg soil (OD equivalent) and as reproduction factor (RF) calculated using the following equation:

Pratylenchus thornei reproduction factor = final *P. thornei* population ÷ initial *P. thornei* population

Determination of rhizobial nodulation

The 150 g subsample of roots and soil extracted for *P. thornei* was retained on the Kimtech tissue and used for nodule quantification. The sample was agitated in 4 L water and passed through a 250-µm aperture mesh sieve to retain and thoroughly wash the roots free of adhering clay particles. The nodules and roots were carefully separated using forceps and a scalpel and patted dry with paper towelling. The nodules were counted and the roots weighed. The nodules and roots were dried separately in a forced draught oven at 65 °C for 48 h to obtain dry weights.

Determination of AMF colonisation

A 50 g subsample of roots and soil was washed over a 250-µm aperture mesh sieve to retain fine roots (Fiske et al. 1989). Roots were then cut into 1 cm pieces and stained using trypan blue and lactoglycerol by a modification of the method of Phillips and Hayman (1970). Total root length and the percentage root length colonised with AMF were determined by scoring structures including hyphae, arbuscules and vesicles following the grid-intersect method (Giovannetti and Mosse 1980) using an SZM stereomicroscope (Olympus, Tokyo, Japan) at 40 X magnification.

Statistical analyses

Data were analysed using Genstat version 20 (VSN International 2020). There was heterogeneity of variances for nematode data, nodule counts and mycorrhizal root colonisation as assessed by the Shapiro Wilk test for normality. Therefore, nematode data was $\log_e(x+1)$ transformed, nodule counts and nodule biomass were square root transformed, and percentage root length with mycorrhizal colonisation was arcsine $(\sqrt{x}/100)$ transformed. Data were subjected to Analysis of Variance (ANOVA) in a split plot design, followed by Bonferroni's post hoc test where interactions were significant at $P \leq 0.05$. Standard errors of difference (s.e.d) from analysis of the experiment were calculated and plotted using bar markers in the figures (Kozak and Piepho 2020). Correlation analyses were undertaken in Genstat on 16 treatment means to relate plant variables of biomass, seed yield and fixed N at 12 weeks to variables measured at 6 weeks. Relative mycorrhizal dependency (Plenchette et al. 1983) was calculated based on mean values of both total plant biomass and seed yield from the ANOVA using the equation:

$$RMD(\%) = 100 * \frac{biomass\ of\ mycorrhizal\ plant - biomass\ of\ nonmycorrhizal\ plant}{biomass\ of\ mycorrhizal\ plant}$$

Results

Plant parameters: dry shoot biomass, seed yield and nodulation

In the analyses of plant shoot variables, there were no significant three-way interactions at the highest factorial order of the treatments AMF, rhizobia and *P. thornei.* There were significant two-way interactions between AMF and rhizobia ($P<0.001$), and between AMF and *P. thornei* ($P<0.05$) at both 6 and 12 weeks for the plant variables of shoot biomass, seed yield, nodule count per plant and nodule biomass per plant. The *P* values of the *F* statistic from the ANOVA are given in Supplementary Table S1.

Plant response to AMF and rhizobia

At 6 weeks, shoot biomass increased 2.2-fold in plants inoculated with AMF and rhizobia compared to the uninoculated control ($P<0.01$) (Fig. 1a). Nodule biomass increased 22-fold in plants inoculated with AMF and rhizobia as compared to the rhizobia inoculated treatment alone ($P<0.001$) (graph depicts transformed mean) (Fig. 1b).

At 12 weeks, both shoot biomass increased 3.9-fold and seed yield increased 4.4-fold (Fig. 2a and b) in plants inoculated with AMF and rhizobia compared to the uninoculated treatment ($P<0.001$). Inoculation of either AMF or rhizobia alone did not increase shoot biomass or seed yield compared to the uninoculated control. Similarily, nodule counts per plant increased 4.9-fold and nodule weight per plant 2.6-fold ($P<0.001$) when plants were inoculated with AMF and rhizobia as compared to plants inoculated with rhizobia alone (Fig. 2c and d (graphs depict transformed mean)). There was no significant effect of co-inoculation on root weight (data not shown). For plants inoculated with rhizobia, the relative mycorrhizal dependency for shoot biomass was 49.0% at 6 weeks and 83.6% at 12 weeks, and for seed yield was 90.4%. The appearance of the mung bean plants at 12 weeks showing the magnitude of this synergistic response in shoot and pod growth to combined AMF and rhizobia inoculations is illustrated in Fig. 3.

Plant response to the interaction of AMF and P. thornei

At 12 weeks, there was a significant interaction between AMF and *P. thornei* affecting shoot biomass ($P=0.01$) and seed yield ($P<0.05$) as gauged by the *F* tables from ANOVA (please refer to Table S1). Inoculation with *P. thornei* reduced the shoot biomass and seed yield of the plants inoculated with AMF, but this was not statistically significant by the Bonferroni test compared to plants inoculated with AMF alone. Inoculation with *P. thornei* had no effect on the shoot

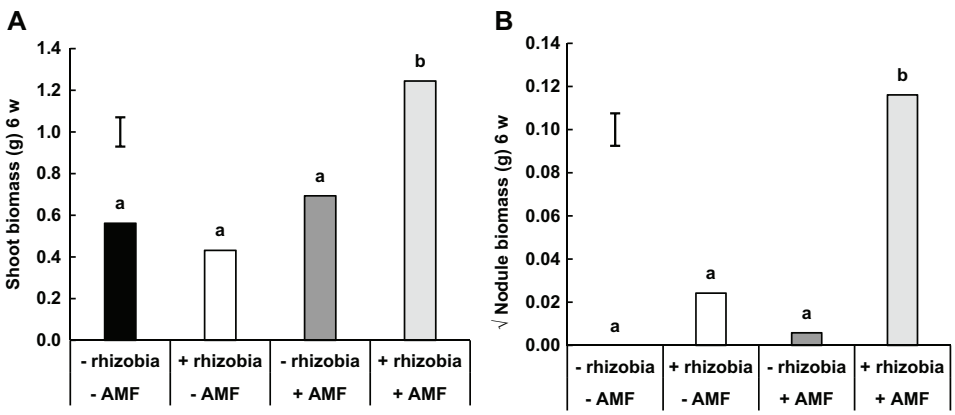

Fig. 1 The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and rhizobia on (**a**) shoot biomass per plant and (**b**) nodule biomass per plant (means of square root transformations from ANOVA) of mung bean at 6 weeks (w) after sowing. Different letters above each bar in each figure indicate significant differences according to the Bonferroni test for multiple comparisons at $P=0.05$ for the interaction of AMF x rhizobia. The vertical bar represents the standard error of difference (s.e.d)

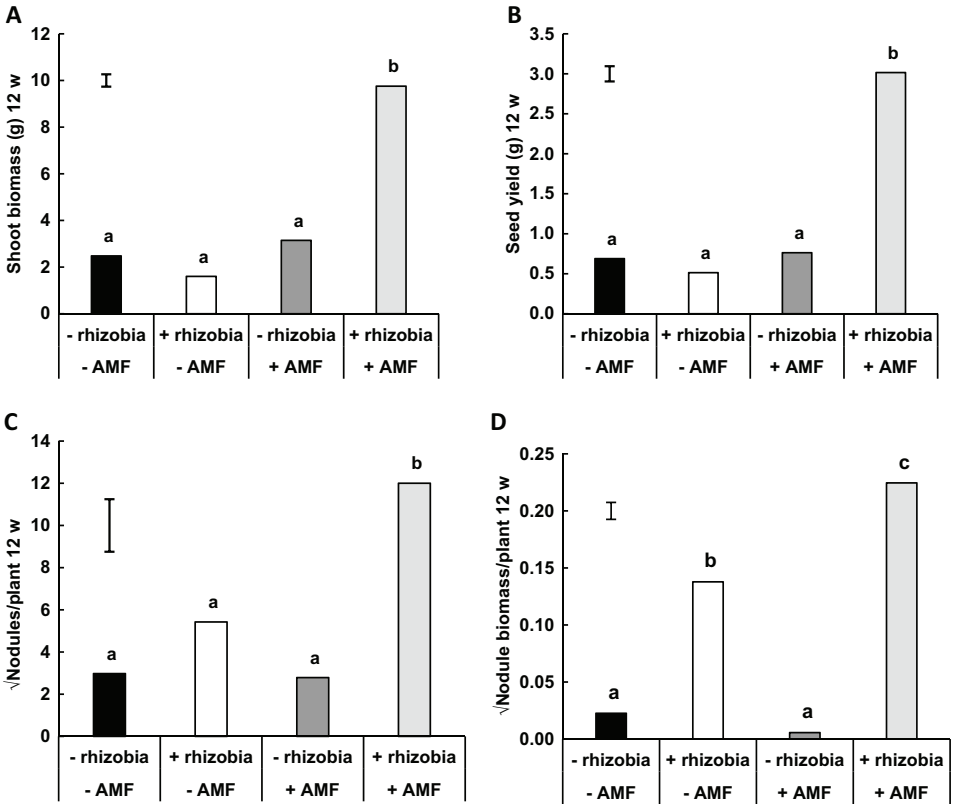

Fig. 2 The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and rhizobia on (**a**) shoot biomass per plant, (**b**) seed yield per plant (**c**) nodule numbers per plant and, (**d**) nodule biomass per plant in mung bean at 12 weeks (w) after sowing. Nodule numbers per plant and nodule biomass per plant are means of square root transformations from ANOVA. Different letters above each bar in each figure indicate significant differences according to the Bonferroni test for multiple comparisons at $P = 0.05$ for the interaction of AMF x rhizobia inoculation. The vertical bar represents the standard error of difference (s.e.d)

biomass and seed yield of the plants not inoculated with AMF (Fig. 4).

Plant response to the main effect of P. thornei

At 6 weeks, plants inoculated with both high and low rates of *P. thornei* had significantly fewer nodule numbers per plant than plants not inoculated with *P. thornei* ($P < 0.05$) (Fig. 5). In plants inoculated with the low rate of *P. thornei,* nodule numbers per plant were reduced 3.8-fold and with the high rate of *P. thornei* nodule numbers per plant were reduced 8.8-fold compared to the nil rate of *P. thornei* treatment. There were no other significant main or interactive effects of *P. thornei* inoculation on other plant variables or any interactive

Fig. 3 Illustration of the synergistic effects of co-inoculation with arbuscular mycorrhizal fungi (AMF) and rhizobia on mung bean growth at 12 weeks. Treatments are as follows; 1: nil AMF, nil rhizobia, 2: + rhizobia, nil AMF, 3: nil rhizobia, + AMF, 4: + rhizobia, + AMF

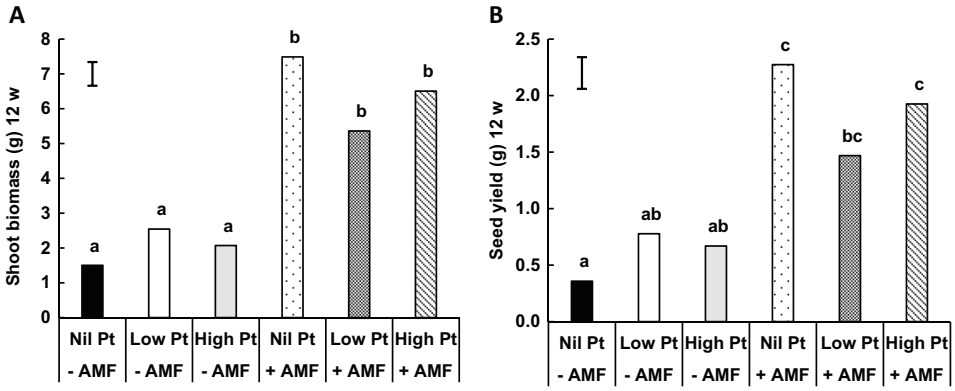

Fig. 4 The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and *Pratylenchus thornei* (Pt) on (**a**) shoot biomass per plant and (**b**) seed yield per plant of mung bean at 12 weeks (w) after sowing. Different letters above each bar in each figure indicate significant differences according to the Bonferroni test for multiple comparisons at $P=0.05$ for the interaction of AMF x *P. thornei*. The vertical bar represents the standard error of difference (s.e.d)

effects between *P. thornei* and rhizobia inoculation at either 6 or 12 weeks ($P>0.05$) (please refer to Table S1).

Mycorrhizal colonisation and *P. thornei* reproduction in mung bean roots

At 6 weeks, there was a significant main effect of AMF inoculation on the proportion of root length colonised with AMF (arcsine transformed) and the total length of AMF-colonised root per plant. There was also a significant main effect of *P. thornei* inoculation on the final population density of *P. thornei*. At 12 weeks, there was a significant interaction between AMF and rhizobia inoculation in the proportion of root length colonised with AMF (arcsine transformed), vesicle intensity, and a significant main effect of AMF inoculation on length of AMF-colonised root/plant. There were also significant main effects of AMF inoculation and *P. thornei* inoculation on *P. thornei* final population density at 12 weeks. The *P* values of the *F* statistic from the ANOVA are given in Supplementary Table S2.

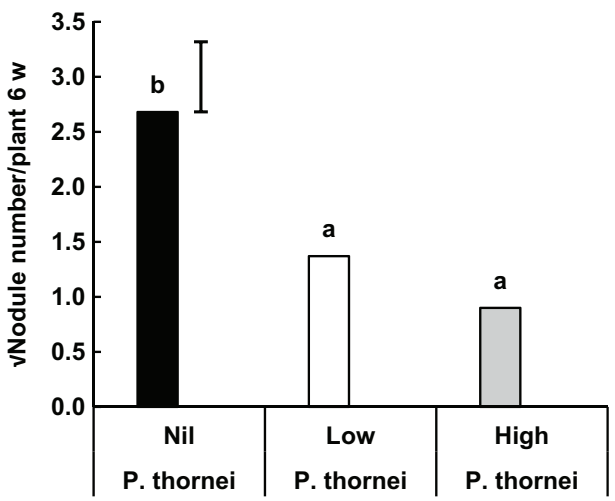

Fig. 5 The main effects of *Pratylenchus thornei* on nodule numbers per plant at low (1 *P. thornei*/g soil) and high rates (10 *P. thornei*/g soil) 6 weeks (w) after sowing. Values are means of square root transformations from ANOVA. Different letters above each bar in each figure indicate significant differences at $P=0.05$. The vertical bar represents the standard error of difference (s.e.d)

Mycorrhizal colonisation

At 6 weeks, mean AMF colonisation of AMF inoculated plants was 59% of the root length and mean mycorrhizal root length was 42 m/plant root system. At 12 weeks, plants inoculated with both AMF and rhizobia had a significantly lower proportion of root length colonisation by AMF (arcsine transformed) (0.66) as compared to roots inoculated with AMF alone (0.90) ($P<0.05$) (Fig. 6a). However, this difference was not reflected in the length of AMF colonised root per plant, there being no significant interaction ($P=0.845$) with a

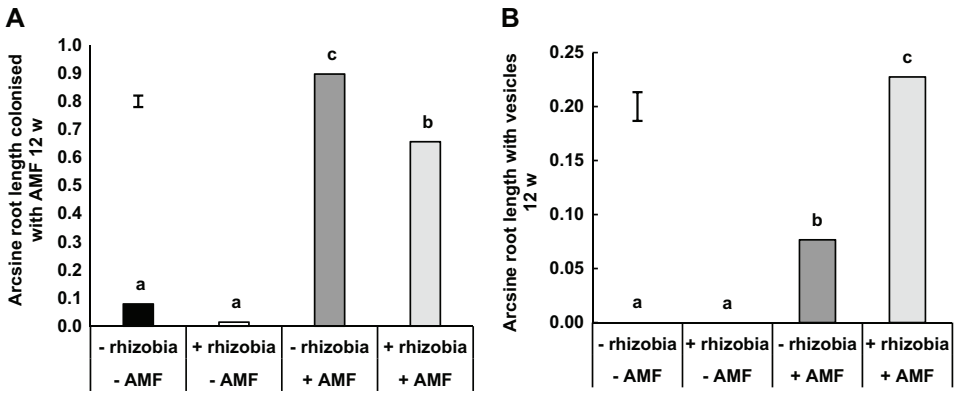

Fig. 6 The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and rhizobia on (**a**) proportion of root length colonised with AMF (arcsine transformed) and (**b**) proportion of root length with vesicles (arcsine transformed) in mung bean at 12 weeks (w). Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at $P=0.05$ for the interaction of AMF x rhizobia. The vertical bar represents the standard error of difference (s.e.d)

mean value of 229 m AMF colonised root/plant for AMF inoculated plants.

At 12 weeks, there were significant interactions between AMF and rhizobia in proportion of root length colonised with vesicles (arcsine transformed). The proportion of root length containing vesicles (arcsine transformed) increased when AMF and rhizobia were inoculated together compared to inoculation with AMF alone ($P<0.001$) (Fig. 6b). The proportion of root length colonisation by AMF (arcsine transformed) or the length of AMF colonised root was not affected by *P. thornei* inoculation at either time of assessment (please refer to Supplementary Table S2).

P. thornei reproduction

At 6 weeks, the *P. thornei* population density and reproduction factor (RF) were greatest in plants inoculated with the high rate of *P. thornei* and AMF, but this was not significantly different from that for plants inoculated with high *P.*

Table 1 The effect of inoculation of arbuscular mycorrhizal fungi (AMF) on *Pratylenchus thornei* population density at low (1 *P. thornei*/g) and high (10 *P. thornei*/g) inoculation rates at 6 and 12 weeks after sowing

		P. thornei/g soil					
		6 weeks after sowing			12 weeks after sowing		
Treatment at inoculation		ln (x + 1)	BTM[a]	RF[b]	ln (x + 1)	BTM[a]	RF[b]
- AMF	Nil *P. thornei*	0.44	1	-	1.38	3	-
- AMF	Low *P. thornei*	2.8	15	15	3.83	45	45
- AMF	High *P. thornei*	3.78	43	4.3	4.38	79	7.9
+ AMF	Nil *P. thornei*	0.66	1	-	2.53	12	-
+ AMF	Low *P. thornei*	2.71	14	14	4.09	59	59
+ AMF	High *P. thornei*	3.98	53	5.3	5.02	150	15
	lsd ($P=0.05$) for AMF	NS[c]			0.645		
	lsd ($P=0.05$) for *P. thornei*	0.654			0.789		

[a]BTM = Back-transformed mean

[b]RF = *Pratylenchus thornei* reproduction factor = final *P. thornei* population ÷ initial *P. thornei* population

[c]NS, non significant $P>0.05$

thornei alone. At 12 weeks, the population density of *P. thornei* and RF were greatest in plants inoculated with AMF and the high initial rate of *P. thornei*, being almost twice the population density for plants inoculated with high *P. thornei* alone ($P < 0.05$) (Table 1).

At 6 weeks, the *P. thornei* population density at the high initial rate of inoculation was positively correlated with shoot biomass (r = 0.75, $P < 0.01$), and negatively correlated with molybdenum concentration in the plant shoot (r = -0.65, $P < 0.05$) (please refer to Table S3). At the low rate of *P. thornei* inoculation, population density at 6 weeks was not significantly correlated with any of the plant variables. At 12 weeks, the *P. thornei* population densitiy at the high initial rate of inoculation was correlated with the proportion of root length colonised with AMF (arcsine transformed) (r = 0.84, $P < 0.001$), P concentration (r = 0.59, $P < 0.05$), Zn concentration (r = 0.59, $P < 0.05$), and Cu concentration (r = 0.59, $P < 0.05$). At the low rate of inoculation with *P. thornei*, the *P. thornei* population density was not significantly correlated with any plant variable at 12 weeks (Supplementary Table S3).

Biological nitrogen fixation

At 12 weeks, there were significant interactions between AMF and rhizobia inoculation for N uptake in the shoot, % N derived from biological N fixation, and the amount of fixed N in the shoot. There were also significant interactions between AMF and *P. thornei* inoculation at 12 weeks for N uptake and biologically fixed N in the shoot. The *P* values of the *F* statistic from the ANOVA are given in Supplementary Table S4.

AMF and rhizobia affecting biological nitrogen fixation

At 12 weeks, N uptake in the plant shoot increased 4.6-fold ($P < 0.001$), % N derived from the atmosphere (% Ndfa) to the plant increased 1.7-fold ($P = 0.002$) and the amount of biologically fixed N in the plant shoot increased 7.8-fold ($P < 0.001$) in

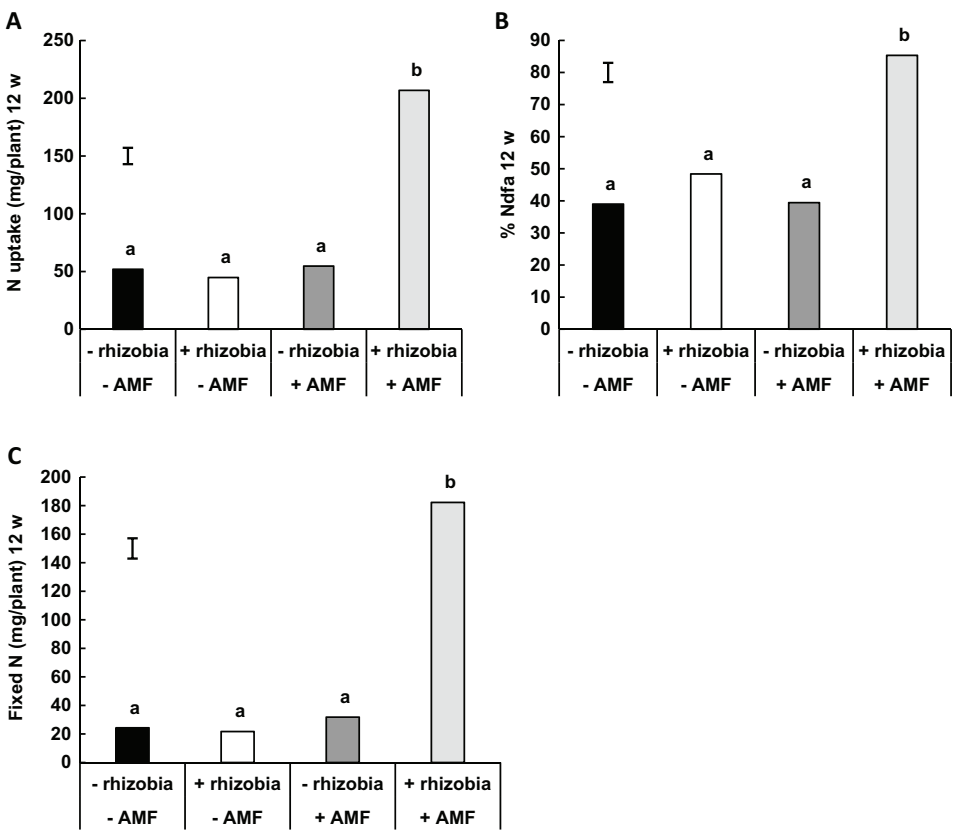

Fig. 7 The interactive effects in mung bean shoot at 12 weeks after sowing of co-inoculation with arbuscular mycorrhizal fungi (AMF) and rhizobia on (**a**) N uptake in the plant (mg), (**b**) percentage N derived from the atmosphere (% Ndfa) in the plant and, (**c**) fixed N from biological N fixation (mg) per plant in mung bean at 12 weeks (w). Different letters above each bar in each figure indicate significant differences according to the Bonferroni test for multiple comparisons at $P = 0.05$ for the interaction of AMF x rhizobia. The vertical bar represents the standard error of difference (s.e.d)

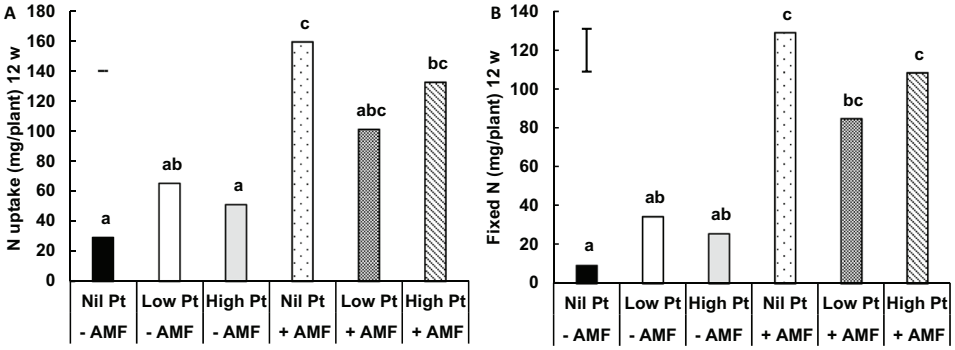

Fig. 8 The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and different rates of *Pratylenchus thornei* (Pt) on (**a**) uptake of N to the plant and (**b**) fixed N to the plant in mung bean at 12 weeks (w). Different letters above each bar in each figure indicate significant differences according to the Bonferroni test for multiple comparisons at $P = 0.05$ for the interaction of AMF x *P. thornei*. The vertical bar represents the standard error of difference (s.e.d)

plants inoculated with AMF and rhizobia, compared to plants inoculated with rhizobia alone (Fig. 7).

AMF and P. thornei affecting biological nitrogen fixation

There were significant interactions between AMF and *P. thornei* with respect to N uptake and the amount of fixed N in the plant ($P < 0.05$). Plants inoculated with AMF and the low rate of *P. thornei* had a 34% reduction in N uptake and a 37% reduction in fixed N ($P < 0.05$) compared to plants inoculated with AMF alone. However, this reduction was not significantly different from plants inoculated with AMF alone using Bonferroni's post hoc test (Fig. 8). Plants inoculated with AMF and the high rate of *P. thornei* had a reduction in N uptake and fixed N to the plant, but this was not significantly different from plants inoculated with AMF with nil or low *P. thornei* using Bonferroni's post hoc test (Fig. 8).

Plant nutrient concentrations

There were significant interactions for various nutrient concentrations at the highest factorial order among AMF, rhizobia and *P. thornei,* and at the second order between AMF and rhizobia and between AMF and *P. thornei* at 6 and 12 weeks. The results for nutrient concentration at 6 weeks are presented here along with correlation analysis between plant variables and nutrients at 6 and 12 weeks. The *P* values of the *F* statistic from the ANOVA at 6 and 12 weeks are given in Supplementary Table S5. Tables showing the effects of the interactions between AMF, rhizobia and *P. thornei,* and AMF

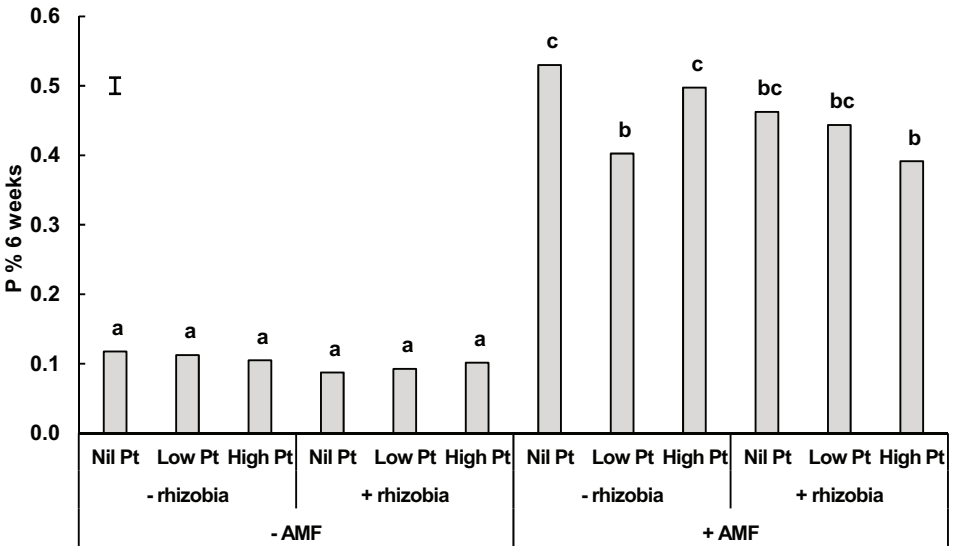

Fig. 9 The interactive effects of arbuscular mycorrhizal fungi (AMF), *Pratylenchus thornei* (Pt) and rhizobia on the concentration of P (%) at 6 weeks (w) in mung bean shoot. Different letters above each bar indicate significant differences according to the Bonferroni test for multiple comparisons at $P = 0.05$ for the interaction of AMF x rhizobia x *P. thornei* for each nutrient. The vertical bar represents the standard error of difference (s.e.d)

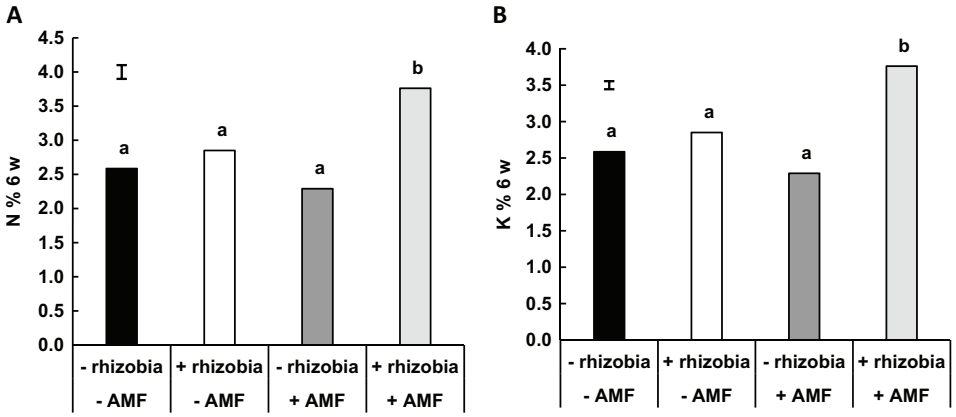

Fig. 10 The interactive effects of arbuscular mycorrhizal fungi (AMF) and rhizobia on the concentration of (**a**) N and (**b**) K in mung bean at 6 weeks (w). Different letters above each bar indicate significant differences according to the Bonferroni test for multiple comparisons at $P = 0.05$ for the interaction of AMF x rhizobia for each nutrient. The vertical bar represents the standard error of difference (s.e.d)

and rhizobia on the means of macro- and micronutrient concentrations at 12 weeks are given in Supplementary Tables S6 and S7 respectively.

Effects of AMF x P. thornei x rhizobia interactions on nutrient concentrations at 6 weeks

At 6 weeks there were significant interactions at the highest order of AMF x rhizobia x *P. thornei* for plant concentrations of P (Fig. 9). The concentration of P increased by 350% in the plants inoculated with AMF ($P < 0.01$) compared to the uninoculated treatment. However, the concentration of P was reduced by 22% when AMF and low *P. thornei* were inoculated together ($P < 0.01$) compared to AMF inoculated alone. There was no statistically significant difference in P concentration between plants co-inoculated with AMF and rhizobia with high, low or nil *P. thornei* levels (Fig. 9).

AMF and rhizobia interactive effects on nutrient concentrations

At 6 weeks, the highest concentration of N was in plants inoculated with both rhizobia and AMF (Fig. 10a). There was no significant difference between plants inoculated with AMF, rhizobia or the uninoculated control. At

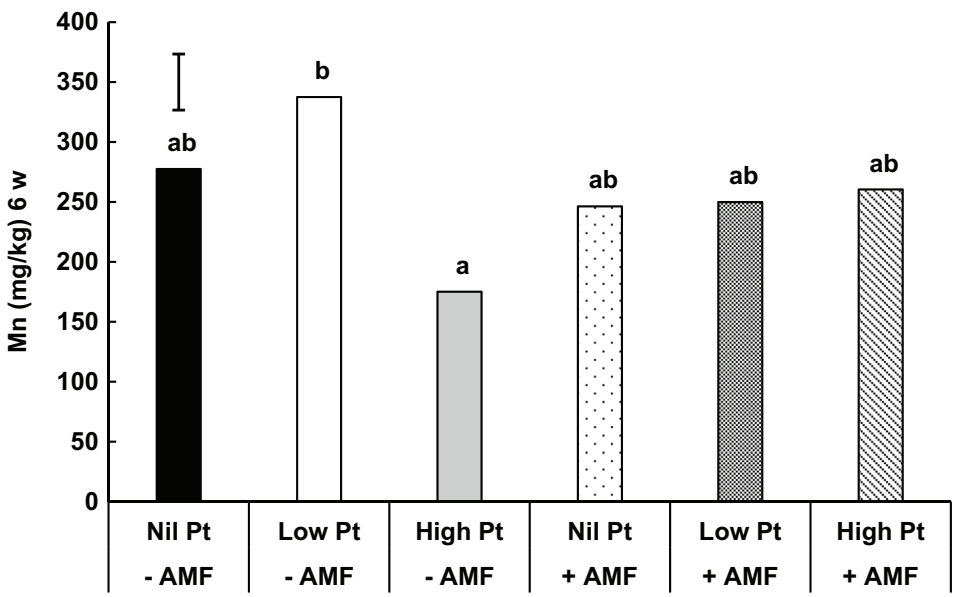

Fig. 11 The interactive effects of arbuscular mycorrhizal fungi (AMF) and *Pratylenchus thornei* (Pt) on the concentration of Mn per plant in mung bean at 6 weeks (w). Different letters above each bar indicate significant differences according to the Bonferroni test for multiple comparisons at $P = 0.05$ for the interaction of AMF x *P. thornei*. The vertical bar represents the standard error of difference (s.e.d).

6 weeks, the highest concentration of K was in plants inoculated with both AMF and rhizobia resulting in a 34% increase compared to the uninoculated treatment (Fig. 10b).

AMF and P. thornei interactive effects on nutrient concentrations

At 6 weeks, inoculation with the high rate of *P. thornei* resulted in a significant reduction (63%) in the concentration of Mn compared to plants inoculated with the low rate of *P. thornei*. AMF inoculated plants had intermediate Mn concentrations regardless of *P. thornei* inoculation rate (Fig. 11).

P. thornei and rhizobia interactive effects on nutrient concentrations

There were no statistically significant main effects of *P. thornei* inoculation on other plant nutrient concentrations or any interactive effects between *P. thornei* and rhizobia inoculation on any nutrient concentration at 6 weeks (Supplementary Table S5).

Correlation between variables at 6 weeks and growth, seed yield and N fixation at 12 weeks

Plant biomass, seed yield and fixed N at 12 weeks were positively correlated with nodule biomass, and K, N, and Cu concentrations at 6 weeks of plant growth (Table 2). Plant biomass at 12 weeks was also correlated with the proportion of mycorrhizal root length (arcsine transformed) and B and P concentrations at 6 weeks, while seed yield was also correlated with the proportion mycorrhizal root length (arcsine transformed) at 6 weeks (Table 2).

Plant nutrient uptakes

There were significant interactions between AMF and rhizobia at 6 weeks, and between AMF and rhizobia, and between AMF and *P. thornei* at 12 weeks for several nutrient uptakes. The *P* values of the *F* statistic from the ANOVA are given in Supplementary Table S8.

AMF and rhizobia effects on nutrient uptakes

At 6 weeks, the uptake of most macronutrients and micronutrients in the plant shoot significantly increased ($P<0.05$) where plants were inoculated with AMF and rhizobia as compared to either treatment alone or the uninoculated control (Fig. 12). Both Zn and Mn uptakes for inoculation with AMF and rhizobia were significantly greater than for inoculation with rhizobia alone, but not with the uninoculated control or AMF inoculation alone (Fig. 12c and d).

At 12 weeks, the uptake of all macronutrients and micronutrients (except Mo) in the plant shoot significantly increased ($P<0.001$) when plants were inoculated with AMF and rhizobia as compared to either treatment alone or the uninoculated control (Fig. 13). Plants inoculated with AMF alone had significantly greater P and Zn uptake ($P<0.001$) in the plant shoot compared to the rhizobia inoculated plant or the uninoculated control (Fig. 13b and c), while plants inoculated with AMF alone had significantly greater Cu uptake than those inoculated with rhizobia alone.

Effects of AMF and P. thornei on nutrient uptakes

At 12 weeks, plants inoculated with AMF and the low rate of *P. thornei* had a reduced uptake of N, Mg, B, and Cu

Table 2 Correlation coefficients (r) between plant variables at 12 weeks and plant variables measured at 6 weeks for treatment means where $n=12$

	Plant variables at 12 weeks		
Variables at 6 weeks	Biomass (g)	Seed yield (g)	Fixed N (mg)
Nodule Biomass (g)	0.95**	0.96**	0.97**
K %	0.92**	0.88**	0.88**
N %	0.81**	0.84**	0.86**
Cu (mg/kg)	0.65*	0.59*	0.59*
AMF root length (arcsine)	0.64*	0.58*	NS
B (mg/kg)	0.62*	NS	NS
P %	0.60*	NS	NS

** $P<0.01$, *$P<0.05$ *NS*, non-significant $P>0.05$

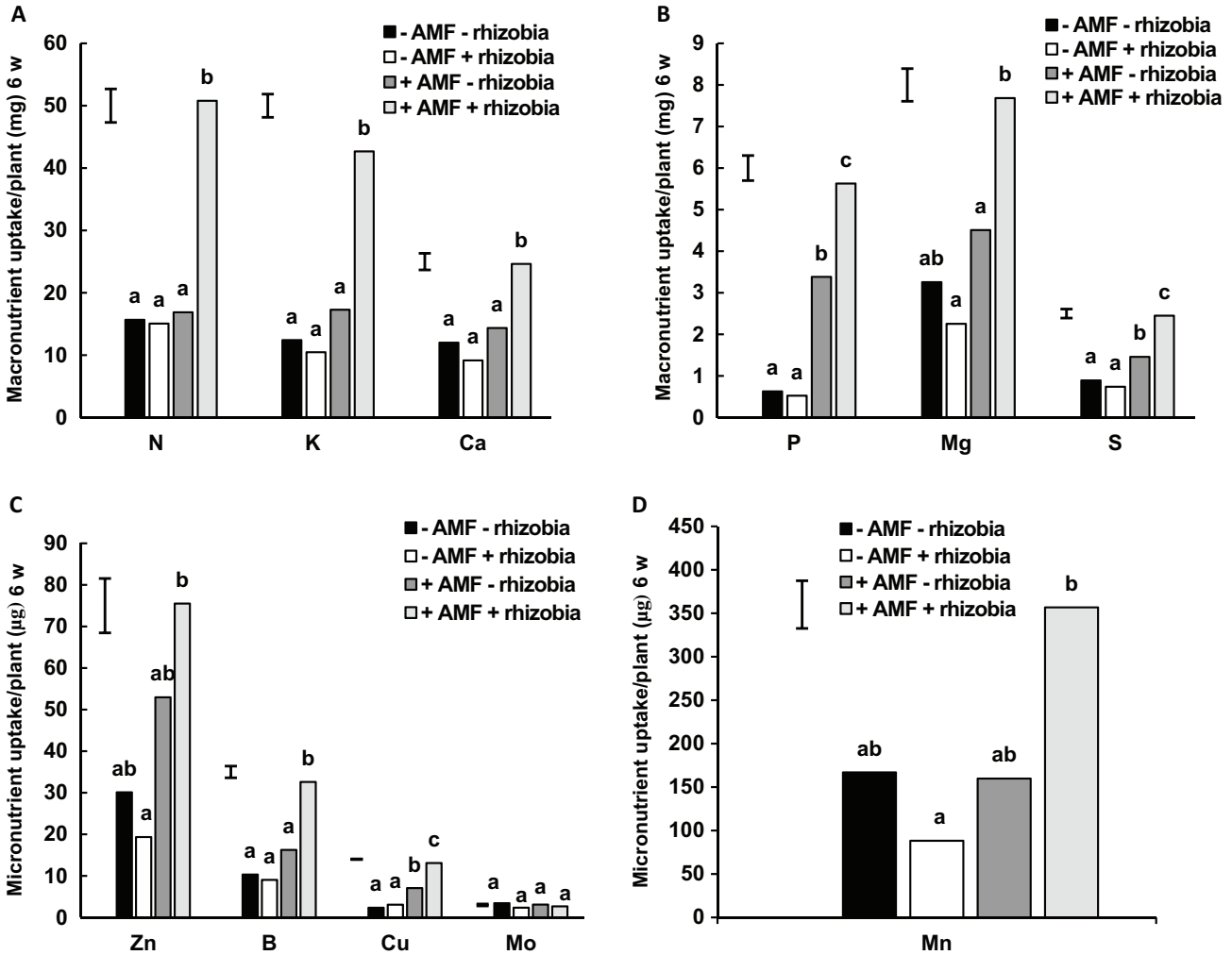

Fig. 12 The interactive effects of co-inoculation with arbuscular mycorrhizal fungi (AMF) and rhizobia on uptakes of (**a**) macronutrients N, K, Ca, (**b**) macronutrients P, Mg and S, (**c**) micronutrients Zn, B, Cu and Mo and, (**d**) micronutrient Mn in mung bean at 6 weeks (w) after sowing. Different letters above each bar indicate significant differences for each given nutrient separately according to the Bonferroni test for multiple comparisons at $P=0.05$ for the interaction of AMF x rhizobia. The vertical bar represents the standard error of difference (s.e.d)

($P<0.05$), compared to plants inoculated with AMF in the absence of *P. thornei* or the high rate of *P. thornei* though this reduction was not significantly different (Fig. 14).

Discussion

AMF and rhizobia interactions on mung bean growth and nutrition

We have demonstrated that co-inoculation with AMF and rhizobia resulted in a clear synergistic effect in mung bean, greatly increasing nodulation, biological N fixation, uptake of essential nutrients, biomass and seed yield. Synergy can be defined as the effects of a combination of two or more agents being greater than their expected additive effects (Greco et al. 1996). This synergistic effect can occur between symbionts that provide different functionalities to the plant, for example nutritional complementarity. Interactions between AMF and rhizobia in other leguminous plants have been described. However, these tend to be additive, rather than synergistic for the majority of parameters investigated (Barea et al. 2005; Chalk et al. 2006), including an additive interaction in mung bean (Yasmeen et al. 2012). In a meta-analysis and review by Larimer et al. (2010), the criteria for synergy as defined by Greco (1996) were not met and their review demonstrated an additive, but not necessarily synergistic,

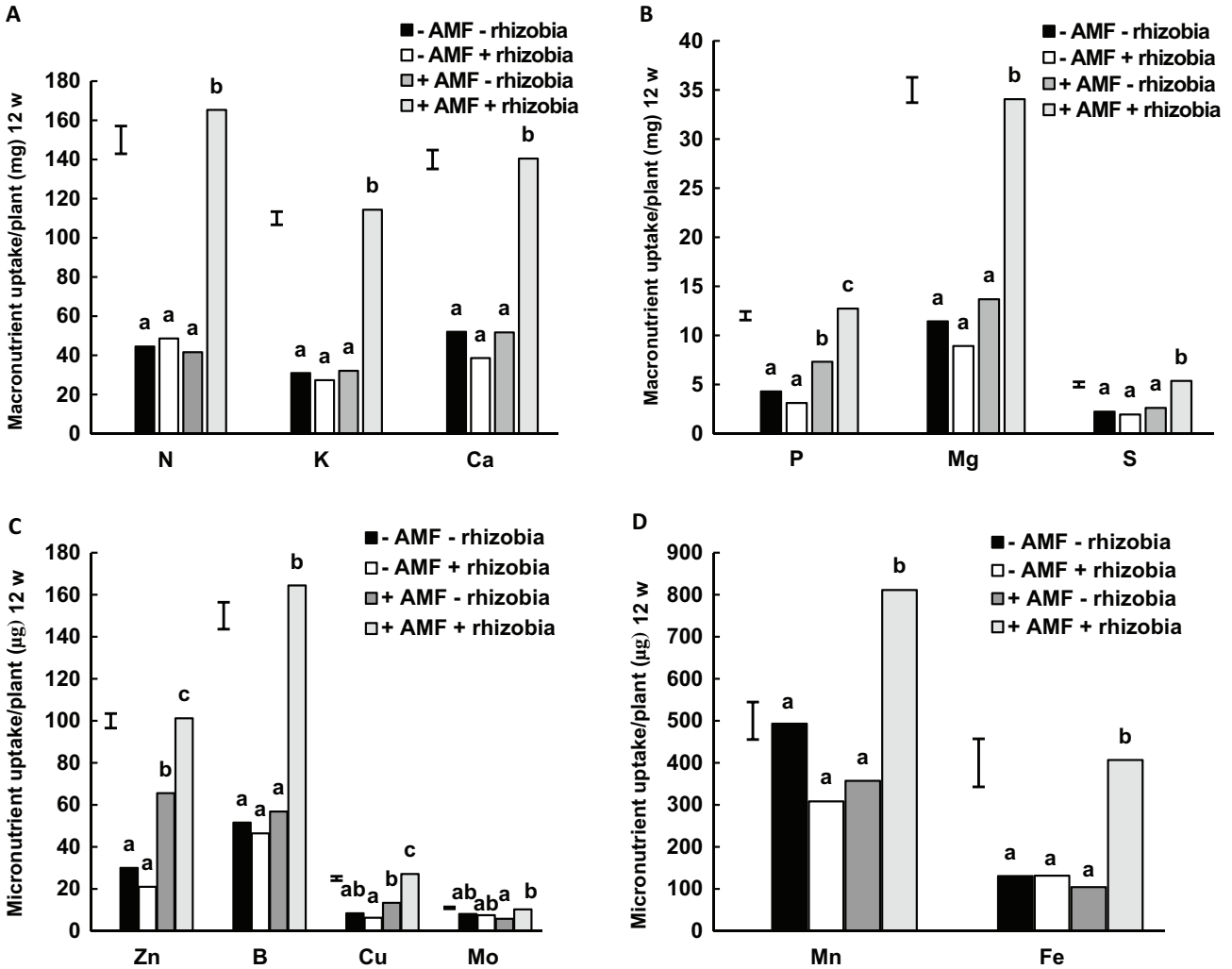

Fig. 13 The interactive effects of co-inoculation with arbuscular mycorrhizal fungi (AMF) and rhizobia on uptakes of (**a**) macronutrients N, K, Ca, (**b**) macronutrients P, Mg, S, (**c**) micronutrients Zn, B, Cu, Mo and (**d**) micronutrients Mn and Fe in mung bean shoot at 12 weeks (w). Different letters above each bar indicate significant differences according to the Bonferroni test for multiple comparisons at $P = 0.05$ for the interaction of AMF x rhizobia for each nutrient seperately. The vertical bar represents the standard error of difference (s.e.d)

effect. The synergism that we have demonstrated between symbionts in mung bean resulted in a greater increase in nodulation, biomass, nutrition and hence crop productivity as compared to an additive effect of the two symbionts acting independently. Variations in the magnitude of the effects may be influenced by specific host-symbiont interactions and by the nutrient status of the soil especially with respect to P and N (Azcón et al. 1992; Larimer et al. 2010; Xavier and Germida 2002).

AMF play an important role in the acquisition of many important plant nutrients (Clark and Zeto 2000) including increasing the uptake and concentration of poorly mobile nutrients in the soil such as P, Zn and Cu (Manjunath and Habte 1988; Smith and Read 2008; Thompson 1990) and as such, have been promoted as a biofertilizer (Baum et al. 2015; Berruti et al. 2016; Smith et al. 2011). The effectiveness of AMF, especially in conjunction with inoculation with rhizobia, in improving the uptake and concentration of essential nutrients for mung bean, has been emphasised in our research, indicating the significance of AMF as a biofertilizer in this important legume. While an increased uptake of N, P and K as a result of co-inoculation with AMF and rhizobia has been documented in the literature, in our study, co-inoculation

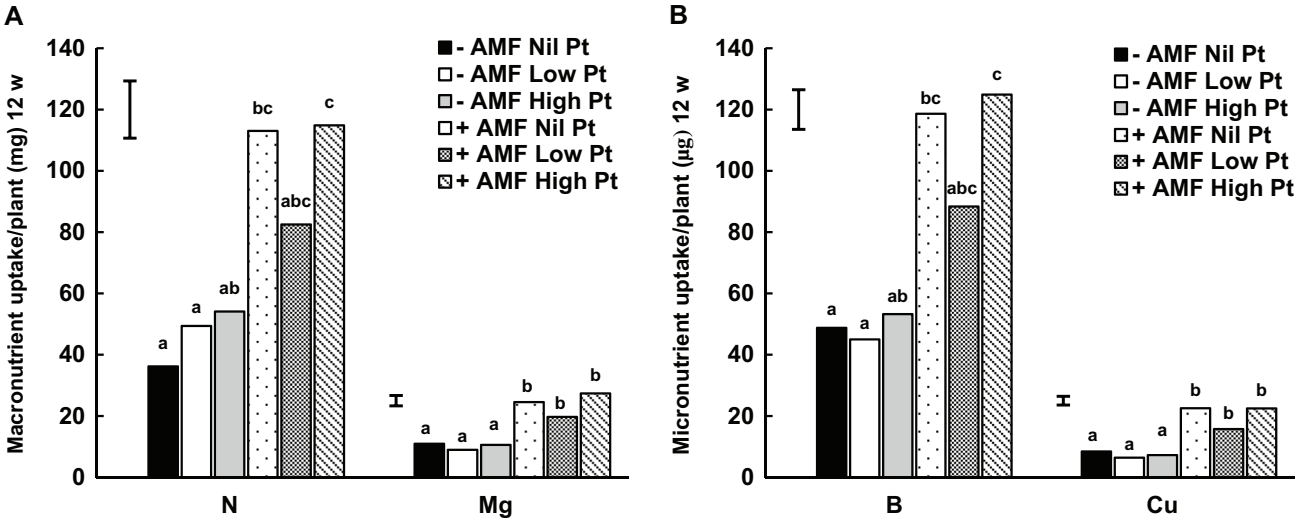

Fig. 14 The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and *Pratylenchus thornei* (Pt) on the uptakes of (**a**) macronutrients N, Mg and (**b**) micronutrients B and Cu in mung bean shoot at 12 weeks (w). Different letters above each bar indicate significant differences for each given nutrient separately according to the Bonferroni test for multiple comparisons at $P=0.05$ for the interaction of AMF x *P. thornei* for each nutrient analysed. The vertical bar represents the standard error of difference (s.e.d)

with AMF and rhizobia increased the uptake of all macronutrients and micronutrients examined, with the exception of Mo. The concentration of nutrients such as P, K, Mg, S, B, Zn and Cu increased in mycorrhizal plants at early stages of plant growth compared with non-mycorrhizal plants. The strain of *F. mosseae* used in our study has been shown to overcome dual deficiencies of P and Zn in linseed growing in vertisol offsetting the requirement for large quantities of these nutrients applied as fertilizers (Seymour et al. 2019). In our research, variables that influenced increases in plant growth, seed production and N fixation at later stages of assessment were all linked to early mycorrhizal colonisation, nodule biomass and crop nutrition. Inoculation with both symbionts, resulted, at later stages of plant growth, in decreased concentrations of Mg in the plant, which can be explained by the 'dilution effect' whereby increasing plant dry biomass results in a subsequent reduction in nutrient concentration (Jarrell and Beverly 1981). Interactions between symbionts and nutrients may influence above-ground or below-ground variables in different manners. For example, in the prairie legume *Amorpha canescens*, interactions between nutrients and microbial symbionts influenced above ground biomass production but not root biomass (Larimer et al. 2014), similar to our results.

Synergistic effects between AMF and rhizobia increasing nodulation and N fixation in legumes are assumed to be predominantly an effect of increased supply of inorganic P by the mycorrhiza (Chalk et al. 2006). Notwithstanding the main contribution of P when the mycorrhizal symbiosis is established in the roots of the plant, there may be other interactions between AMF and rhizobia that initiate and influence the magnitude of the synergism at early stages of infection. There are at least seven genes required for both AMF and root nodule symbiosis and these pathways share a common evolutionary history (Parniske 2008). The role of phytohormones in the symbiosis also needs to be considered. Auxins stimulate mycorrhizal lateral root formation, arbuscule development, promote nodule organogenesis and regulate N fixation, while both auxins and cytokinins are involved in cortical cell divisions and expression of nodulins in rhizobia (as reviewed by Barker and Tagu 2000 and Boivin et al. 2016).

It is well established that P is an essential nutrient for nodulation, N fixation and plant growth, and inorganic P aids N fixation by rhizobia via enhancement of nitrogenase activity (Sa and Israel 1991; Zahran 1999). The increased influx of P to the plant mediated by the fungal partner is likely to have contributed to the effectiveness of nodulation and N fixation

in mycorrhizal plants in our experiment. Inflow of P and Zn into the roots of linseed in a field experiment on a similar vertisol was previously shown to be highly dependent on the percentage colonisation of the roots with AMF (Thompson et al. 2013). However, in addition to increased P concentration and uptake by the fungal symbiont, biological N fixation efficiency in our experiment may also have been stimulated through the direct provision of other inorganic nutrients essential to the establishment of an effective symbiosis such as S, K, Ca, Mg, Zn, B, Fe, Cu, Mo and Mn at both early and later stages of mung bean growth (Azcón-Aguilar and Barea 1992; O'Hara et al. 1988; O'Hara 2001).

Goss and de Varennes (2002), studying the effects of tillage and AMF colonisation in soybean, concluded that the synergistic effects of AMF and rhizobia on improving biological N efficiency in non-disturbed soil was not solely as a result of P uptake improving nodule numbers, but may also have been influenced by molecular aspects of signalling processes between the symbionts. Further research is required to determine if the large increase in biological N fixation in mung bean demonstrated in our experiment is mediated by the influx of P alone. Can inorganic P application with or without other nutrient fertilizers duplicate the effect that AMF has on biological N fixation in mung bean, and if so, are these required rates of fertilizer economic to use? Or does natural mycorrhizal colonisation, manageable by economical agronomic practices, and which leads to increases in the uptake and concentration of nutrients essential for nodulation, result in greater N fixation efficiency in mung bean? Further research is required to answer these questions.

Interactions with *Pratylenchus thornei* and mycorrhiza

Complex interactions between the beneficial symbionts and the plant parasite *P. thornei* occurred in the present factorial experiment. *Pratylenchus thornei* alone decreased nodule numbers at 6 weeks, and reduced the plant biomass and nutrient contents of AMF inoculated plants, but not of plants without AMF. *Pratylenchus thornei* did not affect the percentage root colonisation by mycorrhiza indicating there was no direct antagonism or competition for feeding sites corroborating the conclusions from a previous review (Pinochet et al. 1996). Reductions in P concentration in mycorrhizal plants at early stages of growth may have led to the observed reductions in biological N fixation and fixed N in the plant shoot, culminating in reduced plant biomass and seed yield. Plant parasitic nematodes can affect N dynamics in plants. For example, *P. thornei* infestation resulted in N deficiency in wheat in field experiments (Thompson et al. 1995) and *P. penetrans* inhibited N fixation in soybean (Hussey and Barker 1976). To our knowledge, this paper is the first report of a reduction in biological N efficiency in mung bean due to *Pratylenchus* spp. infestation.

Improved tolerance of mycorrhizal plants to *Pratylenchus* infestation compared with plants without AMF has been previously described and explained as a function of improved plant biomass despite the presence of nematodes, although research on the interaction in legumes is limited (Elsen et al. 2003; Forge et al. 2001; Pinochet et al. 1996). In our experiment, *P. thornei* alone did not affect plant biomass or nutrition. However, in plants inoculated with *P. thornei* and AMF there was a reduced P concentration at early stages of plant growth, and a reduced uptake of nutrients N, Mg, B, and Cu at later stages of plant growth. This may be explained as a function of reduced plant biomass in mycorrhizal plants when inoculated with low rates of *P. thornei*.

Pratylenchus thornei population density at the high inoculation rate in mung bean was positively correlated with plant biomass and negatively correlated with Mo concentration at 6 weeks. Increases in Mo concentration reduced hatching and viability of the root-knot nematode *Meloidogyne incognita* in vitro and reduced galling in pea (*Pisum sativum*) (Chahal and Chahal 1991). To our knowledge, this is the first report of Mo influencing *Pratylenchus* population densities and further research is required to investigate the interactions between Mo and *Pratylenchus* species. At 12 weeks, increases in *P. thornei* population densities were attributable to mycorrhizal root colonisation and host nutrition, namely the increased concentrations of P, Zn and Cu in mung bean shoot. Increases in *Pratylenchus* population density in plants colonised with AMF compared to uncolonised plants have been described in coffee (*Coffea arabica*) for *P. coffeae* (Vaast et al. 1998), maize for *P. brachyurus* (Brito et al. 2018) and wheat for *P. neglectus* (Frew et al. 2018). These increases in *Pratylenchus*

population densities may have resulted from changes in the plant biomass, with direct benefits to *Pratylenchus* from feeding on AMF colonised cortical tissue, or reductions in defensive secondary metabolites in plant roots as a result of mycorrhizal colonisation. For example, increased *P. coffeae* population densities were explained by increased root biomass in mycorrhizal coffee (Vaast et al. 1998). However, increased *P. neglectus* population density in mycorrhizal wheat roots was attributed to mycorrhiza reducing the concentration of benzoxazinoid glucoside compounds, which provided plant defence against the nematode (Frew et al. 2018).

Interactions between AMF and rhizobia in mung bean

Mung bean was an excellent host for mycorrhiza achieving high rates of colonisation of 59% even at our early stage of assessment. In our experiment, the percentage mycorrhizal colonisation of the roots was reduced by rhizobia inoculation, suggesting a potential competition for resources. However, it is noteworthy that in our study the length of mycorrhizal colonised root per plant was not significantly affected by inoculation with rhizobia. Both increased and decreased levels of mycorrhizal colonisation as a result of inoculation with rhizobia have been reported (Chalk et al. 2006; Larimer et al. 2014; Xie et al. 1995). The reductive effect one symbiont can exert on the other through a process called autoregulation has been described previously in other legumes and may also act systemically (Catford et al. 2003). Co-inoculation with AMF and rhizobia increased AMF vesicles in the roots at the later stages of plant growth. As vesicles function as fungal storage organs (Smith and Read 2008), this increase in vesicles in the roots may indicate a propensity for resource hoarding as the crop matured–a conservation of photosynthates–a strategy the fungus may employ when there are limitations to nutrition (Thompson 1986).

The complex and varied effects in this multi-partite interaction may be context dependent and may vary based on genotypic or environmental modifications. It is unknown how other genotypes of mung bean or indeed other species of mycorrhizal fungi and isolates of *Bradyrhizobium* would influence the magnitude and direction of the synergistic effects that were evident in this experiment and further research is required to determine this. Differences in infectivity and effectivity of the symbionts have been documented in other legumes such as soybean (Takács et al. 2018) and this may influence the magnitude of the synergism (Larimer et al. 2010). The order and species of AMF may also influence the outcome of the changes in the population densities of *Pratylenchus* spp. whereby species from the order Diversisporales tended to increase *Pratylenchus* population densities more than species from the Glomerales (Gough et al. 2020).

In the subtropical grain region of eastern Australia, which is characterised by summer dominant rainfall and extended periods of drought, mung bean is integrated into rainfed broadacre cereal cropping systems. AMF are obligate biotrophs and when the land is in bare fallow, spore levels in the soil can be depleted resulting in crops exhibiting symptoms of nutrient deficiency known as Long Fallow Disorder (Thompson 1987). Mung bean inoculated with rhizobia has a demonstrably high dependence on AMF for its nutrient requirements, N fixation capacity, biomass production and seed yield. Therefore, a lack of mycorrhizal spores in the soil could result in significant yield loss in mung bean crops. Double cropping, or sowing after a short (<6 month) fallow after a preceding crop that increases mycorrhizal spore densities in the soil, would be advantageous (Owen et al. 2010) to increase yield. However, AMF colonisation of mung bean may also increase *P. thornei* population densities for a subsequent crop, and as *P. thornei* has a broad distribution in the subtropical grain region of eastern Australia (Thompson et al. 2008), plant breeding to introgress resistance to *P. thornei,* while still retaining the responsive synergism between AMF and rhizobia, is warranted.

Conclusion

Mung bean formed complex multipartite interactions with the beneficially symbiotic arbuscular mycorrhizal fungi and *Bradyrhizobium,* and the pathogenic root-lesion nematode *Pratylenchus thornei.* Mycorrhizal colonisation improved the efficiency of rhizobia in producing nodules and fixing nitrogen, and increased the biomass and seed yield of mung bean. Mycorrhizal colonisation also conferred a tolerance to

P. thornei even though it increased nematode population densities in mung bean roots. Knowing the levels of AMF and *P. thornei* in the soil prior to sowing, and understanding the complex interactive effects of these organisms on plant nutrition, N fixation, biomass and seed yield has important implications for plant breeding and agronomic practices to increase mung bean production efficiently.

Acknowledgements EG acknowledges support from the University of Southern Queensland Research Training Program Scholarship and a Grains Research and Development Corporation (GRDC) research scholarship through Project USQ1912-003RSX. RZ acknowledges support from USQ. KO acknowledges co-funding by the GRDC through Project DJP1907-002RMX and support from the Queensland Department of Agriculture and Fisheries (QDAF) through the Broadacre Cropping Initiative with USQ. The authors wish to thank Daniel Burrell, Biometrician from the University of Southern Queensland for his statistical advice.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CHAPTER 4

THE ROLE OF NUTRIENTS UNDERLYING INTERACTIONS AMONG ROOT-NODULE BACTERIA (*BRADYRHIZOBIUM* SP.), ARBUSCULAR MYCORRHIZAL FUNGI (*FUNNELIFORMIS MOSSEAE*) AND ROOT-LESION NEMATODES (*PRATYLENCHUS THORNEI*) IN NITROGEN FIXATION AND GROWTH OF MUNG BEAN (*VIGNA RADIATA*)

The paper presented in this chapter has been accepted for publication subject to revisions in the international Q1 journal *Plant and Soil* (Springer Nature, The Netherlands), 07 October 2021. The final published version in *Plant and Soil* may differ from that presented in Chapter 4 of this thesis.

Gough EC, Owen KJ, Zwart RS and Thompson JP (2021) The role of nutrients underlying interactions among root-nodule bacteria (*Bradyrhizobium* sp.), arbuscular mycorrhizal fungi (*Funneliformis mosseae*) and root-lesion nematodes (*Pratylenchus thornei*) in nitrogen fixation and growth of mung bean (*Vigna radiata*) *Plant and Soil*, (Q1; Impact Factor: 3.299)

In the study reported in Chapter 3, AMF and rhizobia acted synergistically to improve nodulation, nitrogen fixation, nutrition, biomass and yield. However, AMF also increased the population densities of *P. thornei* in the roots of mung bean.

In this study the role that nutrients play in the processes by which the beneficial AMF improve biological nitrogen fixation (BNF) by *Bradyrhizobium* sp. and how AMF may increase *P. thornei* population densities in mung bean were investigated. A full factorial experiment was carried out among treatments of AMF, rhizobia, *P. thornei*, nitrogen (N), phosphorus (P) and zinc (Zn) in a pasteurised vertisol.

In this study we showed, for the first time, that in mung bean AMF improved nodulation and BNF to a level equal to or greater than the application of fertiliser P. AMF also increased the level of P and Zn to the plant shoot greater than the application of fertiliser alone, while inoculation with rhizobia improved nodulation, shoot N

concentration, biomass and yield greater than the application of fertiliser N. Inoculation with AMF increased the population density of *P. thornei* while the application of N, P and Zn decreased the population density of *P. thornei.*

The comparisons made between the biological symbionts and the application of fertiliser demonstrate the crucial role AMF and rhizobia contribute to sustainable agricultural systems. However, active management to ensure effective AMF colonisation and reduced *P. thornei* reproduction is warranted.

[Supplementary material associated with this Chapter is attached in Appendix C.]

Title: The role of nutrients underlying interactions among root-nodule bacteria (*Bradyrhizobium* sp.), arbuscular mycorrhizal fungi (*Funneliformis mosseae*) and root-lesion nematodes (*Pratylenchus thornei*) in nitrogen fixation and growth of mung bean (*Vigna radiata*)

Authors E.C. Gough[1*], K.J. Owen[1], R.S. Zwart[1], J.P. Thompson[1]

[1]Centre for Crop Health, Institute for Life Sciences and the Environment, University of Southern Queensland, Toowoomba, Queensland, Australia

***Correspondence:**
Corresponding Author
elaine.gough@usq.edu.au

Abstract

Purpose

To investigate the role nutrients play in arbuscular mycorrhizal fungal improvement of biological nitrogen fixation (BNF) by *Bradyrhizobium* sp. and increases in *Pratylenchus thornei* (Sher & Allen) population density in mung bean (*Vigna radiata* (L.) R. Wilczek).

Methods

A glasshouse experiment was conducted on mung bean with 2^6 factorial treatments of AMF, rhizobia, *P. thornei*, nitrogen (N), phosphorus (P) and zinc (Zn) in a pasteurised vertisol. Variates of biomass, yield, nodulation, natural abundance $\delta^{15}N$, mycorrhizal colonisation, nutrients in the plant shoot and *P. thornei* multiplication were assessed at 6 and 11 weeks.

Results

The combination of AMF and P improved BNF in the shoots at 6 weeks, while AMF alone improved BNF and nodulation greater than the addition of P at 11 weeks. Inoculation with AMF increased the shoot concentrations of P and Zn greater than fertilisation with either nutrient alone. Seed yield and biomass were similar when AMF or P was each applied alone with no further increase when combined. Rhizobia increased seed yield greater than the addition of N. Inoculation with AMF and rhizobia increased yield and biomass greater than rhizobia alone,

and to a higher level than inoculation with AMF and fertiliser N. *Pratylenchus thornei* populations in the roots increased with AMF, but the addition of N, P and Zn decreased them.

Conclusion

AMF increase supply of P to mung bean, improving BNF by *Bradyrhizobium*, yield and crop nutrition while reducing fertiliser inputs. Active management to ensure effective AMF colonisation and reduced *P. thornei* reproduction is warranted.

Key words

Arbuscular mycorrhizal fungi, rhizobia, biological nitrogen fixation, *Vigna radiata*, plant nutrition, *Pratylenchus thornei*

Introduction

The production and consumption of legumes are promoted globally to provide nutritious, high-protein plant-based food (Tharanathan and Mahadevamma 2003). Cultivation of legumes has also been advocated due to their role in sustainable agriculture—reducing nitrogen (N) fertiliser use via biological nitrogen fixation (BNF), improving soil organic matter, increasing soil carbon sequestration, and reducing greenhouse gas emissions (Jensen and Hauggaard-Nielsen 2003; Stagnari et al. 2017).

Biological nitrogen fixation occurs in legumes by symbiosis with N fixing bacteria of several genera collectively referred to as rhizobia. Atmospheric dinitrogen (N_2) is converted into plant-available ammonia (NH_3) by the enzyme nitrogenase in rhizobial bacteroids inside root nodules. In exchange, the plant supplies carbohydrates as a food source for the bacteria inside the hospitable environment of the root nodules (Geurts and Bisseling 2002). It has been estimated that BNF by pulse and oilseed legumes contribute 20–22 million tonnes of fixed N annually (Herridge et al. 2008). To quantify BNF, both percentage N derived from the atmosphere (% Ndfa) and quantity of fixed N in the plant are used (Unkovich et al. 2008). Limitations to BNF include high soil nitrate, low phosphorus (P) supply, poor soil moisture, high soil temperatures, soil salinity, inefficient inoculation with rhizobia, incompatible or inefficient rhizobial strains, and poor nodulation (Peoples et al. 2009). Large quantities of P are required by rhizobia for the synthesis of nucleic acids and phospholipids, nodule formation and efficient symbiosis (O'Hara 2001). In soybean (*Glycine max*), P deficiency reduced the efficiency of rhizobial symbiosis by

decreasing nitrogenase activity (Sa and Israel 1991). In agricultural systems, P is typically applied as fertilisers derived from rock phosphate, which is a finite resource with global reserves unequally distributed, and an estimated depletion in 50 to 100 years (Cordell and White 2014). Ideally in agricultural systems, P is obtained from sparingly soluble soil reserves by improving the chemical availability through phosphate solubilising microorganisms or via more efficient root uptake by the symbiotic arbuscular mycorrhizal fungi (AMF) (Berruti et al. 2016; Khan et al. 2007).

Arbuscular mycorrhizal fungi are ancient soil-borne organisms from the phylum Glomeromycota (Schüßler and Walker 2010). They are obligate biotrophs that form symbiotic associations in the roots of up to 80 % of terrestrial plants, including many agriculturally important crops (Parniske 2008). A fully functioning symbiosis involves the hyphae of AMF colonising plant root systems and forming tree-like structures called arbuscules within root cortical cells, which act as points of exchange between the fungus and plant (Khaosaad et al. 2007). Arbuscular mycorrhizal fungi use their fine extraradical hyphae to scavenge poorly mobile nutrients such as P and Zn, from the soil at some considerable distance past the nutrient depletion zone of the root, in exchange for photosynthates from the plant (Smith and Read 2008).

Arbuscular mycorrhizal fungi can interact with rhizobia to improve yield, nodulation and N fixation of legumes such as chickpea (*Cicer arietinum*), common bean (*Phaseolus vulgaris*), soybean and the prairie legume (*Amorpha canescens*) (Chalk et al. 2006; Larimer et al. 2014). In previous research, we found that AMF interacted synergistically with *Bradyrhizobium* improving biomass, seed yield, and BNF in mung bean (*Vigna radiata*) compared to inoculation with rhizobia alone, despite infestation by the root-lesion nematode *Pratylenchus thornei* (Gough et al. 2021). Questions arose regarding the role of nutrition in this improved BNF in mung bean. Did AMF improve BNF due to improving P and/or Zn uptake as shown in vertisols for the non-leguminous broad-leaf crop linseed (*Linum usitatissimum*) (Thompson 1996)? Furthermore, as the stoichiometric relationship between N and P may be used to indicate plant growth limitations (Koerselman and Meuleman 1996), a comparison of the effects of these microsymbionts and the application of fertiliser N and P on the N:P mass ratio of the shoots was also investigated.

The subtropical grain region of eastern Australia is an area of great agricultural productivity characterised by deep fertile soils and summer dominant rainfall (Webb et al. 1997). Soil types in this region include Vertosols, Chromosols and Sodosols with historically high rates of fertility (Isbell 1996). However, continuous cropping and intensive land use have resulted in deficiencies of N, P, Zn, Cu, potassium (K), sulfur (S) and molybdenum (Mo) in the soils, the management of which has become increasingly reliant on the use of inorganic fertilisers to obtain adequate yield (Bell et al. 2010; Dalal et al. 1991; Holloway et al. 2008: McLachlan 1955). To address the costly nutrient deficit in soils, and to manage the build-up of soilborne pathogens of winter cereals such as crown rot (*Fusarium pseudograminearum*), rotations that include N-fixing legumes such as chickpea, mung bean and faba beans (*Vicia faba*) are recommended in the rainfed cropping systems in the region. Mung bean is cultivated as a high-value short-season summer legume. However, some of the biotic constraints to the production of mung bean in the region include failure of nodulation by rhizobia (Herridge et al. 2005) and infestation with the root-lesion nematode *P. thornei* (Owen et al. 2014).

Mung bean, along with other economically important crops in the region, including sorghum (*Sorghum bicolor*), maize (*Zea mays*), sunflower (*Helianthus annus*), faba bean and chickpea, have high levels of dependency upon AMF for optimal yield (Thompson 1987; Thompson et al., 1997). Mycorrhizal dependency can be defined as the level to which a crop depends on AMF to attain sufficient growth or yield at a given level of soil fertility (Gerdemann 1975). In the region, after periods of bare fallow due to insufficient rainfall or changes in cropping sequences, levels of AMF in the soil are low and may lead to Long Fallow Disorder in crops (Thompson 1987). Symptoms of this disorder include poor biomass production and appearance of P or Zn nutrient deficiencies, even when adequate levels of nutrients for normal growth are present in the soil (Thompson 1987; Thompson et al. 2013). The disorder reflects the crucial importance of AMF in accessing these immobile nutrients from the soil.

Pratylenchus thornei is a migratory endo-parasitic nematode with a broad host range (Castillo and Vovlas 2007; Singh et al. 2013) found in 67 % of fields in the subtropical grain region of eastern Australia (Thompson et al. 2010). The nematode enters the roots of host crops destroying cortical tissue as it feeds and migrates through the root, resulting in a loss of root function and characteristic brown lesions (Jones et al. 2013). This degradation of the roots reduces water and nutrient uptake and consequently reduces yield of intolerant crops (Owen et

al. 2014; Thompson et al. 1995; Whish et al. 2014). Furthermore, the multiplication of *Pratylenchus* within the roots of susceptible crops increases the population of the nematode to more damaging levels resulting in yield loss of the current crop. High population densities residual in the soil after susceptible crops can then limit the yield of subsequent crops.

Arbuscular mycorrhizal fungi and *Pratylenchus* interact in the roots of host plants and the direction and magnitude of the effect that AMF have on the population densities of *Pratylenchus* spp. can depend on the order/genus of AMF and on the host plant species (Gough et al. 2020). Changes to *Pratylenchus* population densities as a result of AMF colonisation may be related to enhanced plant nutrient status, increased root biomass, competition for resources or induced systemic responses in the plant (Azcón-Aguilar et al. 2002; Schouteden et al. 2015). In our previous work on the interaction between *P. thornei* and AMF, population densities of *P. thornei* increased when mung bean roots were inoculated with AMF (Gough et al. 2021). This was not related to root biomass, but was correlated with increased plant nutrition, namely P, Zn and Cu concentrations and uptakes in the plant shoot biomass.

In the present study we aimed to investigate whether the interactions among AMF, rhizobia, *P. thornei* and mung bean were mediated via nutritional effects due to the ability of AMF to acquire poorly mobile nutrients from the soil, especially P and Zn, and their effects on increasing N supply to the plant via enhancement of the rhizobial symbiosis. We hypothesised that (a) AMF would improve BNF through increasing supply of P to the nodules and also by increasing supply of other nutrients essential to the nodulation process; (b) AMF would increase *P. thornei* population densities through improved plant nutrition particularly of N, P and Zn; (c) AMF colonisation would increase the concentration of P and Zn nutrition to mung bean shoots comparable to the addition of the nutrients alone, even under constraints of *P. thornei* infestation; (d) inoculation with rhizobia would increase the shoot biomass and N concentration to a level comparable with fertilisation with N.

Materials and Methods

Experimental design

A glasshouse experiment with mung bean (*Vigna radiata* (L.) R. Wilczek) cv. Jade-AU was conducted during summer to autumn (February–April, 2019) in Toowoomba, Queensland,

Australia (latitude 27.53 °S, longitude 151.93 °E). The experiment had 64 treatments that were a 2^6 factorial of AMF (0 and 16 spores/g soil), *Pratylenchus thornei* (Sher & Allen) (0 and 10 nematodes/g soil), rhizobia (0 and $3x10^6$ colony forming units (CFU)/g soil, N (0 and 200 mg/kg soil as $Ca(N0_3)_2$, P (0 and 50 mg/kg soil as NaH_2PO_4), and Zn (0 and 15 mg/kg soil as $ZnCl_2$). The design also comprised two times of assessment, namely at 6 weeks after sowing (flowering) and 11 weeks after sowing (pod maturity). The experiment was arranged on four glasshouse benches as a randomised split plot design with four replicates in blocks, with the two times of assessment randomised to each half of a bench. The eight combinations of biological treatments were randomly allocated to eight main plots, and the eight combinations of nutrient treatments were randomly allocated to the subplots.

Biological materials

The mung bean cultivar Jade-AU used in the study is a susceptible host to *P. thornei* (Owen et al. 2014) and is a good host of AMF (Gough et al. 2021). The AMF strain used in the study was *Funneliformis mosseae* (Nicolson & Gerd) 'Schmelzer 43' a local isolate from a grain farm at Macalister, Queensland (latitude 27.04 °S, longitude 151.07 °E). Previously a similar isolate of *F. mosseae* was shown to have comparable effects to inoculation with mixed field spores on parameters including % mycorrhizal colonisation, biomass and shoot P concentration in linseed (Thompson 1996). This strain originated from a single spore culture and was maintained on maize in pot cultures in the glasshouse. Mycorrhizal spores for inoculum were obtained by wet sieving a suspension of soil and roots from pot cultures of maize using a protocol modified from Gerdemann and Nicolson (1963).

The strain of *Pratylenchus thornei* used in the experiment was originally isolated from a grain farm at Formartin, Queensland (latitude 27.46 °S, longitude 151.43 °E) using an initial inoculation density which is often found after cultivation of susceptible wheat (Owen et al. 2010). The nematodes were multiplied on the roots of susceptible wheat (*Triticum aestivum*) cultivars in pot cultures (Thompson et al. 2015) and extracted from the soil and roots using a modified Whitehead tray method (Whitehead and Hemming 1965) as described in Gough et al. (2021).

The *Bradyrhizobium* strain CB 1015 (Eagles and Date 1999), from a commercially available culture (Nodule N New Edge Microbial, North Albury, Australia), was used in the experiment. For inoculation, a slurry was made using 0.1 g of the peat-based inoculum in 300 mL sterile

distilled water following the manufacturer's instructions. The concentration of *Bradyrhizobium* in the inoculum suspension was 10^9 CFU mL^{-1} quantified using the Miles and Misra drop method (Vincent 1970), and 1 mL of the slurry was inoculated directly onto the seeds in the pots at sowing.

Plant growth conditions

The soil used in the experiment was a Black Vertosol (Isbell 1996) collected from a grain field at Formartin, Queensland and pasteurised for 45 min at 85 °C in an air:steam stream, followed by fan forced air for 30 min to cool the soil to ambient temperature using a protocol modified from Thompson (1990). Soil analyses were conducted by a commercial laboratory (Australian Precision Ag Laboratory, Hindmarsh, Australia and Environmental Analysis Laboratory, Lismore, Australia) using the Australasian Soil and Plant Analysis Council (ASPAC) methods of Rayment and Lyons (2011). Soil chemical properties were as follows; pH 8.5 (1 soil:5 water suspension), total N 0.10 % (LECO TruMac®CNS analyzer), total organic C 1.55 % (acid treatment with combustion by LECO SC832 CN Analyser), nitrate-N 23 mg/kg soil (2M KCl extraction), ammonium-N 16 mg/kg soil (2M KCl extraction), P 46 mg/kg soil (Colwell bicarbonate extraction) and Zn 1.4 mg/kg soil (DTPA extraction).

Glasshouse benches were equipped with a bottom watering system by which pots were watered via polyester capillary matting (Bidim ® Geofabrics Australasia Pty Ltd, Brisbane, Queensland, Australia), with the water tension set at 5 cm (Sheedy and Thompson, 2009). Benches were prepared by covering with plastic sheeting then placing 10 cm wide strips of Bidim® covered with weed matting across the bench with 10 cm spacing between strips to avoid contamination between biological treatments.

Square-based pots (70-mm wide x 150-mm high) designed for bottom watering, with a capacity of 330 g soil oven dried (OD) equivalent were initially filled with a base layer of 70 % of the total soil. For treatments with added fertiliser, nutrients were prepared in solution and 5 mL of each appropriate solution, and 5 mL of tap water for the nil nutrient treatments, were added to pasteurised soil for each pot within a plastic bag, shaken to homogenise, then transferred into the pot according to the experimental nutrient design.

Pots were first grouped according to their nutrient treatments and placed in a tub with 2–3 cm of tap water to moisten the soil by capillarity prior to inoculation and sowing. These pots were then transferred into their designated biological treatment groups prior to inoculation to prevent

cross contamination. Two mung bean seeds were placed on the base layer of soil, and inoculations of the biological treatments were made on or around the seeds prior to adding the remaining 30 % soil, to the pots. The pots were then arranged on the moistened capillary strips in randomised order according to the experimental design. Ten days after germination, the seedlings were thinned to one per pot, by cutting the shoots just above the soil level and leaving the roots behind to minimise disturbance. The soil temperature was maintained at an average temperature of 24 °C and monitored using an iButton (Thermochron®, Australia). The air temperature was maintained between 19 and 25 °C. Three days before the designated assessment times, the capillary matting was removed from the water source to allow the soil moisture content of the pots to reduce to ~ 45 % (Sheedy and Thompson 2009). The soil from each pot was broken into < 5 mm pieces and the roots cut into ~ 10 mm pieces and thoroughly homogenised and stored at 4 °C until further processing.

Assessment of plant and microbiota variates

In total, 29 variates measuring plant biomass (shoot dry weight, root dry weight), seed yield (seed weight), BNF (% Ndfa, fixed N, N uptake), rhizobial nodulation (number of nodules, nodule dry weight), mycorrhizal colonisation (proportion of root length colonised by AMF, root length colonised by AMF per plant, proportion of root length with vesicles, root length containing vesicles per plant), *P. thornei* multiplication (*P. thornei*/kg soil and roots), nutrient concentrations in the shoots (N, P, K, Mg, S, Ca, Zn, B, Mn, Mo, Cu and Fe), nutrient uptakes in the shoots (N, P, Zn) and the N:P mass ratio were assessed.

Plant biomass and seed yield

The growth stage of each plant was recorded at 6 weeks and 11 weeks according to the BBCH scale of Lancashire et al. (1991)—a phenological key of plant growth stages. Fresh shoots were weighed to determine biomass of each plant, and dry shoot biomass was then determined after drying at 60 °C in a forced draught oven for 4 d. The pods were removed from the shoots, counted and weighed, then threshed to obtain the weight of the seeds per plant. The dry shoot biomass (combined shoots, pods and seeds) was finely ground using a Foss CT 193 Cyclotec grinder (FOSS, Hilleroed, Denmark) and stored in sealed containers at room temperature pending chemical analyses.

Plant chemical analyses

A subsample of 5 mg of ground shoot biomass was encapsulated in tin foil cups (LECO, St. Joseph, Michigan, USA) to assess BNF. Total $\delta^{15}N$ was quantified by isotope mass spectrometry using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Laboratory, University of California, Davis (USA).

The percentage of plant nitrogen derived from the atmosphere (% Ndfa) was calculated using the following equation (Howieson and Dilworth 2016):

$$\% \, Ndfa = 100 * \frac{(x - y)}{(x - B)}$$

where x is the average value of $\delta^{15}N$ from cv. Jade-AU mung bean from the experiment for treatments that were not inoculated and not nodulated with *Bradyrhizobium*, y is the $\delta^{15}N$ of the experimental sample, and B is the $\delta^{15}N$ content of mung bean fully dependent on nitrogen fixation for growth, determined to be -2.05 (Unkovich et al. 2008).

The quantity of biologically fixed N per plant shoot was calculated using the following equation:

$$Fixed \; N = (N \; uptake) * \frac{\% \, Ndfa}{100}$$

The dried shoot biomass was analysed for major and trace elements by a commercial laboratory (Australian Precision Ag Laboratory, Hindmarsh, Australia). Nitrogen was quantified by the Dumas method. Concentrations of P, K, Ca, S, Mg, Zn, B, Cu, Mo, Mn and Fe were determined by microwave digestion and inductively coupled plasma-optical emission spectrometry (ICP-OES) following the methods from ASPAC (Australasian Soil and Plant Analysis Council) (Rayment and Lyons 2011).

Nematode quantification

Pratylenchus thornei were extracted for 48 hours from a 150 g subsample of the mixed soil and root samples by the modified Whitehead tray method (Whitehead and Hemming 1965) as described in Gough et al. (2021). This method allowed a maximal extraction rate of 88 % at 48 hours (Thompson et al. 2017). A further 100 g subsample of mixed soil and roots was dried at 105 °C for 48 h in a fan forced oven to determine the soil moisture content. The nematodes were

counted in a 1 mL Peters slide (Chalex Corporation, Portland, USA) under a BX53 optical microscope (Olympus, Tokyo, Japan) at 40 X magnification and numbers were expressed as *P. thornei*/kg oven dried soil equivalent.

Determination of rhizobial nodulation

Following *P. thornei* extraction, the soil and roots were agitated in 4 L of water and the suspension was poured through a 250-µm mesh aperture sieve. The roots, collected on the sieve, were thoroughly washed and nodules were removed from the roots using a scalpel and forceps and then counted. The nodules and roots were patted dry with paper towels, and weighed separately to obtain fresh weights. They were then dried separately in a fan forced oven at 65 °C for 48 h to obtain dry weights. Number and weight of nodules and weight of roots were expressed on a per plant basis.

Determination of AMF colonisation

From the mixed soil and roots, a 50 g subsample was washed over a 250-µm aperture mesh sieve to retain fine roots. A fresh root weight was obtained and then the roots were placed in stain tubes (Fiske et al. 1989) and stained using trypan blue in lactoglycerol by a modified method of Phillips and Hayman (1970). Colonisation of AMF and root length were determined by the grid-intersect method (Giovannetti and Mosse 1980). Arbuscules and mycorrhizal hyphae were counted together and vesicles were counted separately using an SZM stereomicroscope (Olympus, Tokyo, Japan) at 40X magnification.

Statistical analyses

Data were analysed using Genstat version 20 (VSN International 2020). Data were subject to distribution normality checks using the Wilks-Shapiro test. Nematode data were $\log_e (x+c)$ transformed, where x is *P.thornei*/kg soil and roots, and c is a constant chosen to normalise the variances in the residuals in the data set (Marks and Proctor 1974). Nodule number and nodule dry weights were square root transformed; proportional root length with mycorrhizal colonisation was arcsine ($\sqrt{x}$) transformed; and nutrient concentration was $\log(x+1)$ transformed to ensure homogeneity of variance over the range of the data. The N:P mass ratio was calculated by dividing N % by P % per plant.

Data were then subjected to Analysis of Variance (ANOVA) to test for main effects and interactions by the *F* statistic. Where treatment interactions were significant at $P \leq 0.05$, *post hoc*

multiple comparisons of means were conducted by the Bonferroni test. Where only main effects were significant at $P \leq 0.05$, *post hoc* comparisons of means were made using Fishers protected l.s.d. The standard errors of difference (s.e.d.) from ANOVA were determined and presented where appropriate (Kozak and Piepho 2020). Gaussian regression in Genstat was also used to identify the N:P ratio for maximum dry matter production at both times of assessment based on mean values for the 64 treatment combinations.

To analyse and interpret multivariate trends in the complex data set, principal components analyses (PCA) were conducted (Abdi & Williams 2010). As the % Ndfa and fixed N values were derived from the rhizobia-inoculated treatments only, two PCAs were conducted. The first was based on the correlation matrix of 32 treatment combinations for only rhizobia-inoculated treatments. Mean values were obtained from the ANOVA for variates of BNF and all other variates of plant biomass, seed yield, mycorrhizal colonisation, *P. thornei* multiplication, and nutrient concentrations at both times of assessment (23 variates total). The second PCA was based on the correlation matrix for all (64) treatment combinations obtained from the ANOVA for all variates, except those for BNF at both times of assessment (20 variates total). The same two data matrices used for PCA were used for hierarchical cluster analyses based on Euclidean Distance as a similarity measure with clustering by Average Linkage to further delineate treatment relationships displayed on the PCA biplots.

Results

Principal components analysis for rhizobia-inoculated treatments

In the rhizobia-inoculated data set, PC1 accounted for 44.7 % of the variation and PC2 accounted for 15.1 % (Fig. 1). There were strong positive loadings for PC1 of shoot biomass, seed yield, variates of BNF including % Ndfa, fixed N and N concentration, and variates of mycorrhizal colonisation as well as the nutrients increased by the addition of AMF namely P, Zn and Cu. In contrast, strong negative loadings for PC1 were high concentrations of Ca, Mo, Mg, S and Mn in the shoots at 6 weeks, and high concentration of Mo in the shoots at 11 weeks, which were associated with low plant biomass production. There were strong positive loadings for PC2 for root biomass at 11 weeks, and concentrations of B, Fe and K at 6 weeks, and Mn and S at 11 weeks. Strong negative loadings for PC2 were associated with variates of root biomass and concentrations of N at 6 weeks and Ca at 11 weeks.

Variates of BNF, namely % Ndfa and fixed N, were positively correlated with mycorrhizal colonisation, nodule number and nodule dry weight, shoot biomass and P, Zn and Cu concentrations in the shoots at 6 and 11 weeks. The vectors of these variates encompassed the treatments with large positive scores on PC1, namely most treatments inoculated with AMF (Fig. 1, Cluster D), with the exception of the treatment of AMF alone which had an intermediate PC1 treatment score (Treatment 5, Fig. 1, Cluster C). Treatments inoculated with AMF in the absence of *P. thornei* or N were less closely associated with these variates (Fig. 1 Cluster C). Treatments including the addition of P in the absence of AMF, were not as strongly associated with shoot biomass, seed yield and biological N fixation as those with AMF (Fig. 1, Cluster B). Treatments with added N and without AMF clustered together (Fig. 1, Cluster A) and were not closely associated with shoot biomass, seed yield and BNF compared to those treatments with added N and AMF. Treatments that had large negative scores on PC1 included combinations of treatments of N, Zn and *P. thornei*, in the absence of P and AMF, along with the uninoculated control. Treatments that had large negative scores on PC2 included combinations of P, N and *P. thornei* in the absence of AMF.

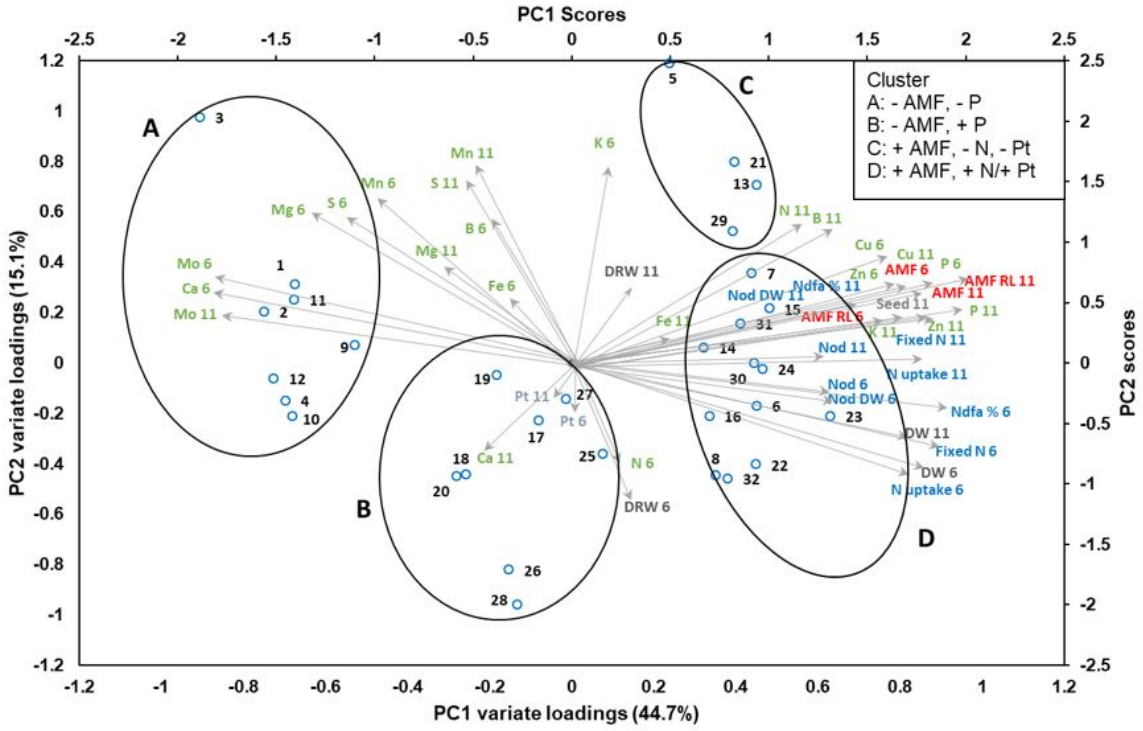

Fig. 1. Biplot from principal components analysis of the rhizobia-inoculated treatments for mung bean after 6 and 11 weeks growth. Small blue open circles represent treatment scores, with the code numbers referring to treatment

designations listed in Supplementary Table S1, while numerical values of treatment scores and variate loadings are given in Supplementary Table S3. Variate loadings are plotted as vectors with abbreviated names followed by the time point in weeks indicated as 6 or 11, respectively. Abbreviations of variates: shoot and root biomass and yield (DW=dry shoot weight, DRW=dry root weight, Seed=seed weight), biological N fixation (% Ndfa=% N fixed from the atmosphere, fixed N=biologically fixed N/plant), rhizobial nodulation (Nod=number of nodules, Nod DW=nodule dry weight), mycorrhizal colonisation (AMF=proportion of root length colonised by AMF (arcsine transformed), AMF RL=root length/plant colonised by AMF in cm), Pt=log (*Pratylenchus thornei*/kg + c). All elements represented are concentrations- N, P, K, Mg, S, Ca are percentages and Zn, B, Mn, Mo, Cu and Fe are mg/kg in the plant shoot biomass. Boundaries of four clusters (A-D) of treatments delineated in the hierarchical cluster analysis (Fig. S1) are demarcated by black circles on Fig. 1 with treatment designations outlined in Supplementary Table S1.

Principal components analysis of all treatments

In the analysis of all treatments, except variates of BNF which were encapsulated in the added rhizobia-inoculated dataset, PC1 accounted for 43.3 % and PC2 accounted for 23.2 % of the variation. Strong positive loadings on PC1 were for variates of shoot biomass, seed yield, nodulation and the concentration of N % in the shoot at 6 and 11 weeks. Conversely, there were strong negative loadings on PC1 for concentrations of Ca, Mg, S, Mo and Mn in the shoot at 6 and 11 weeks, which were negatively associated with shoot biomass, seed yield, nodulation and concentration of N.

The treatments with large negative scores on PC1 included P and Zn fertilisers in the absence of N, AMF or rhizobia (Fig. 2, Cluster A), and also those treatments where *P. thornei* was combined with P or Zn fertilisation. Large positive scores on PC1 were associated with treatments that combined rhizobia and P in the absence of AMF, and were associated with N concentration in the shoot and nodule dry weights and numbers (Fig. 2, Cluster B), and treatments that combined rhizobia and AMF (Fig. 2, Cluster C), which were strongly associated with greater shoot biomass and seed yield.

Variates of mycorrhizal colonisation at both time points, namely proportion of root length colonised with AMF, root length per plant colonised with AMF, proportion of root length with vesicles and root length per plant with vesicles had strong negative loadings on PC2. These variates were all strongly correlated with each other, and also with the concentrations of P, Zn and Cu in the shoot at 6 and 11 weeks. Treatments with large negative scores on PC2 were those with added AMF and rhizobia (Fig. 2, Cluster C), added AMF and N, in the absence of

rhizobia (Fig.2 Cluster D), and added AMF in the absence of N or rhizobia (Fig. 2 Cluster E). Treatments with large postive scores on PC2 included fertilisation with P or Zn in the presence of *P. thornei* and absence of AMF or rhizobia (Fig. 2, Cluster A).

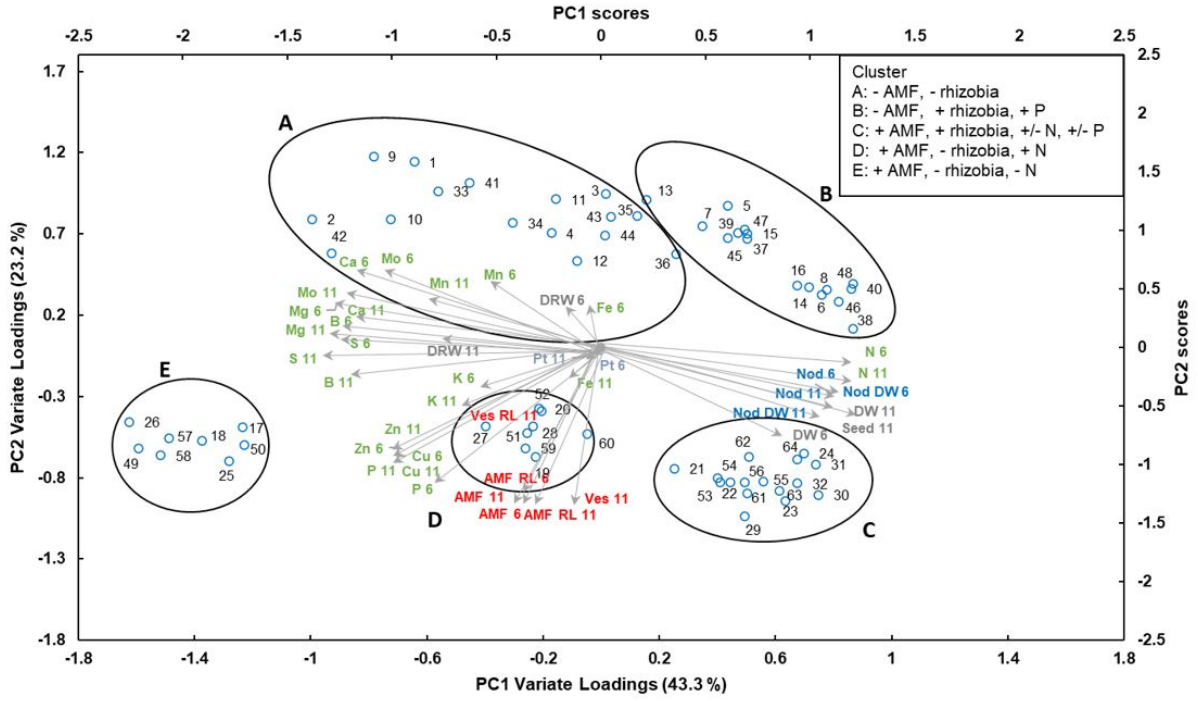

Fig. 2. Biplot from principal components analysis of the treatments for mung bean after 6 and 11 weeks growth. Small blue open circles represent treatment scores, with the code numbers referring to treatment designations listed in Supplementary Table S2, while numerical values of treatment scores and variate loadings are given in Supplementary Table S4. Variate loadings are plotted as vectors with abbreviated names followed by the time point in weeks indicated as 6 or 11, respectively. Abbreviations of variates: shoot and root biomass and yield (DW=dry shoot weight, DRW=dry root weight, Seed=seed weight), rhizobial nodulation (Nod = number of nodules, Nod DW = nodule dry weight), mycorrhizal colonisation (AMF= proportion of root length colonised by AMF (arcsine transformed), AMF RL= root length colonised by AMF in cm, Ves= proportion of root length with vesicles (arcsine transformed), Ves RL= root length with vesicles in cm, Pt= log (x+c) *Pratylenchus thornei*/kg. All elements represented are concentrations- N, P, K, Mg, S, Ca are percentages and Zn, B, Mn, Mo, Cu and Fe are mg/kg in the plant shoot biomass. Boundaries of five clusters (A-E) of treatments delineated in the hierarchical cluster analysis (Fig. S2) are demarcated by black circles on Fig. 2 with treatment designations in Supplementary Table S2.

Significant interactions at various factorial orders between treatments

The *P* values of the *F* statistic from ANOVAs of rhizobia inoculated plants are given in Supplementary Table S5 and the *P* values of the *F* statistic from ANOVAs within all inoculated

plants are given in Supplementary Tables S6–S10. The figures below present the mean values of these interactive effects when there were significant differences in the Bonferroni *post hoc* test. Where significant interactions for each variate had a similar pattern of effects, only one representative interaction is presented, with the remaining included in Supplementary Figures S4–S32.

Interactions between treatments for biological N fixation in rhizobia-inoculated plants

In rhizobia-inoculated plants, variates from the treatment interaction of AMF x P at 6 and 11 weeks are presented in Fig. 3. All other interactions for the variates of biological nitrogen fixation are presented in Supplementary Fig. S3.

Interactions between AMF x P in rhizobia-inoculated plants

Biological N fixation in rhizobia-inoculated plants with added AMF and P

The addition of AMF or P to rhizobia-inoculated plants significantly (P<0.05) increased the % Ndfa by 79–88 % and fixed nitrogen per plant by 193–219 % compared to plants inoculated with rhizobia alone. This effect was consistent for plants at 6 and 11 weeks with a further significant (P<0.05) increase of 12 % Ndfa at 11 weeks from inoculation with AMF, compared to the addition of P alone. There were similar levels of % Ndfa at both 6 and 11 weeks and fixed N at 11 weeks in plants treated with AMF and P together compared to plants with AMF alone (Fig. 3A, B).

Shoot biomass and seed yield in rhizobia-inoculated plants with added AMF and P

At 6 weeks, the growth stages ranged from first flower buds visible to full flowering with 50 % of flowers open (BBCH scale 5.1–6.5). At 11 weeks, all plants were harvest–fully ripe, with nearly all pods brown (BBCH scale 8.9). The addition of either P or AMF alone to rhizobia-inoculated plants significantly (P<0.05) increased the shoot biomass by 63–74 % at 6 weeks, and by 60 % at 11 weeks compared to rhizobia inoculation of plants alone. At 6 weeks, the combination of AMF and P increased shoot biomass by 17 % which was significantly (P<0.05) greater than P alone, though this was not significantly different from AMF alone. There were no further increases in shoot biomass with added AMF and P together at 11 weeks compared to AMF or P alone. A similar effect was observed for seed yield where the addition of P or AMF alone significantly (P<0.05) increased the yield by 52–70 %, with no additional significant increases in yield with treatments of AMF and P together (Fig. 3C).

Nodulation in rhizobia-inoculated plants with added AMF and P

At 6 weeks, there were significant main effects of addition of P (P<0.001) and AMF (P<0.05) increasing nodule number/plant, and significant main effects of the addition of P increasing nodule dry weight/plant (P<0.01). At 11 weeks, the addition of P or AMF alone significantly (P<0.05) increased nodule number/plant by 95 % and 172 % and nodule dry weight/plant by 130 % and 303 %, respectively, compared to rhizobia-inoculated plants, with no further significant increase in nodulation from adding AMF and P together compared to AMF alone (Fig. 3D, E).

Shoot nutrient concentration in rhizobia-inoculated plants with added AMF and P

In rhizobia-inoculated plants, the addition of AMF significantly (P<0.05) increased the concentration of P in the shoots by 86 % and 64 % greater than the addition of P alone at 6 and 11 weeks respectively. The combination of AMF and P did not significantly further increase the P concentration (Fig. 3F).

In contrast to P concentration, the addition of AMF or P alone and combined, significantly (P<0.05) decreased the concentrations of Mg, Ca, and S in the shoots at 6 weeks, and significantly (P<0.05) decreased the concentrations of Mo and Mn in the shoots at 6 and 11 weeks compared to inoculation with rhizobia alone (Supplementary Fig. S4A-E). The addition of P or AMF significantly (P<0.05) increased the concentration of K in the shoots at 11 weeks, with AMF increasing K concentration significantly (P<0.05) greater than the addition of P with no further increase on the addition of both together (Supplementary Fig. S4F).

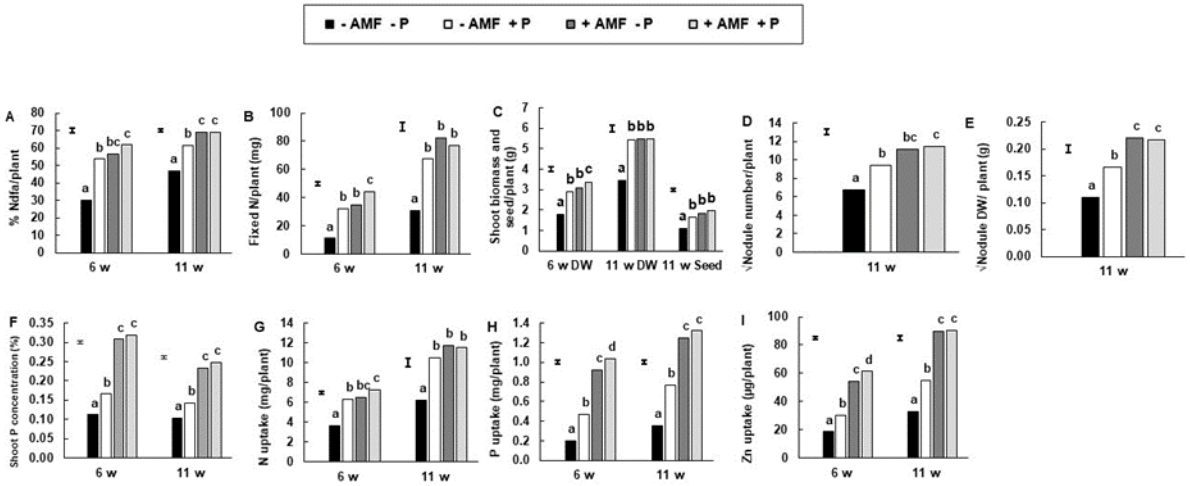

Fig. 3. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and P in rhizobia-inoculated plants at 6 and/or 11 weeks (w) on **(A)** % nitrogen derived from the atmosphere (% Ndfa), **(B)** fixed nitrogen (N) (mg/kg) in the plant, **(C)** shoot biomass/plant (DW) and seed weight/plant, **(D)** nodule number per plant, **(E)** nodule dry weight per plant and, **(F)** shoot P concentration (%). Nodule number per plant and nodule dry weight/ plant are means of square root transformations. Different letters above each bar graph indicate significant differences for each variate separately according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction of AMF x P. The vertical bar represents the standard error of difference (s.e.d.).

Significant interactions between all treatments in the complete data set

For all treatment combinations, when >20 variates analysed had a P value of <0.01 in the ANOVA, these interactions are presented in Figs. 4–6. All other significant interactions at various factorial orders are shown in the Supplementary Fig. S8–S32, including a table of P values from the F statistic of the main effects in Supplementary Table S5.

Interactions between rhizobia x N

Shoot biomass and seed yield in rhizobia-inoculated and N-fertilised plants

The addition of N or rhizobia, alone or combined, significantly (P<0.05) increased shoot biomass 41–65 % at 6 weeks and 110 % at 11 weeks compared to the uninoculated control. The addition of N or rhizobia alone significantly (P<0.05) increased seed yield 201–423 % at 11 weeks compared to the uninoculated control. However, a significant (P<0.05) reduction in yield of 15 % was observed in plants with added N and rhizobia together compared to rhizobia alone (Fig. 4A).

Nodulation of rhizobia-inoculated and N-fertilised plants

The addition of rhizobia significantly (P<0.05) increased the nodule dry weight/plant at 11 weeks compared to the uninoculated control. However, N fertiliser significantly (P<0.05) decreased nodule dry weight of rhizobia-inoculated plants by 31 % compared to rhizobia alone (Fig. 4B).

Shoot nutrient concentration and uptakes in rhizobia-inoculated and N-fertilised plants

The addition of N or rhizobia significantly (P<0.05) increased the concentration of N by 40–59 % at 6 weeks and 40–108 % at 11 weeks compared to the uninoculated control. At 6 weeks, the addition of rhizobia and N together significantly (P<0.05) increased the concentration of N to its largest amount, with a 72 % increase compared to the uninoculated control. However, at 11 weeks the addition of rhizobia and N together significantly (P<0.05) reduced the concentration of N by 19 % compared to plants inoculated with rhizobia alone (Fig. 4C).

The addition of N or rhizobia either alone or combined significantly (P<0.05) reduced the concentrations of P, Zn, Mg, Ca, S, Mo and, B at both 6 and 11 weeks, and K and Cu at 11 weeks compared to the uninoculated control (Fig. 4D and 4E for P and Zn, respectively; Supplementary Fig. S5 for all other nutrients). The addition of N significantly (P<0.05) reduced the concentrations of K and Mn greater than the addition of rhizobia (Supplementary Fig. S5).

The addition of rhizobia or N either alone or combined significantly (P<0.05) increased the N uptake in the plant shoot at 6 and 11 weeks. At 11 weeks, the addition of rhizobia alone increased the N uptake in the shoot to its largest amount, with a 321 % increase compared to the uninoculated control (Fig. 4F). At 11 weeks, the uptake of both P and Zn in the plant shoot significantly (P<0.05) increased by 32 % for P uptake, and 9–17 % for Zn uptake following inoculation with rhizobia or N, with no further increase from both together (Fig. 4G and 4H).

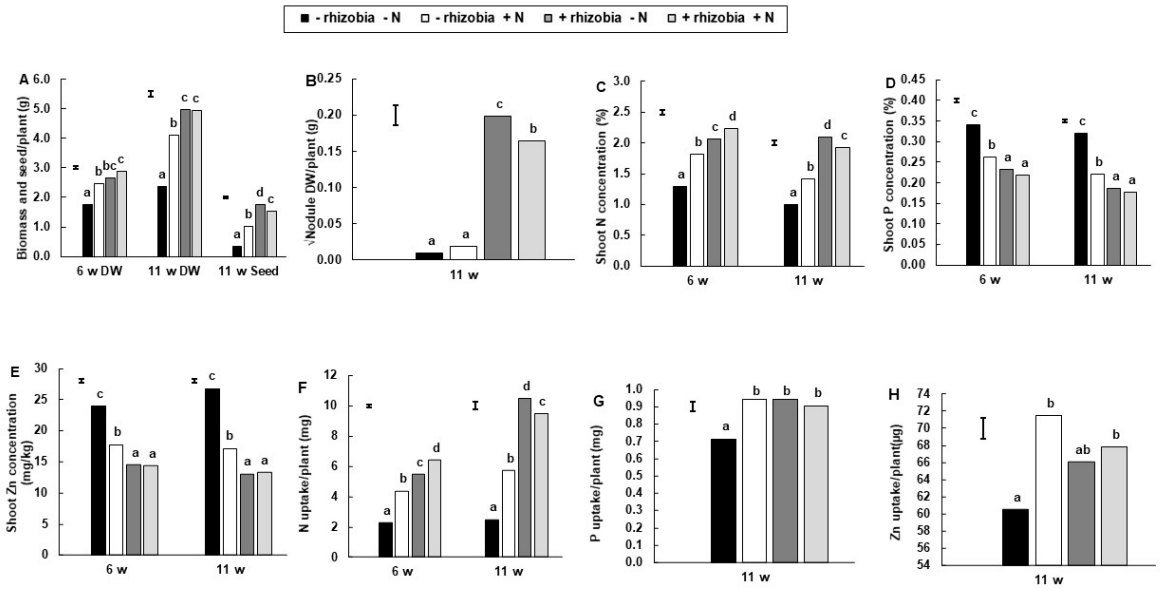

Fig. 4. The interactive effects of rhizobia and nitrogen (N) at 6 and/or 11 weeks (w) on **(A)** shoot biomass (DW) and seed yield, **(B)** nodule dry weight/plant, **(C)** shoot N concentration (%), **(D)** shoot P concentration (%), **(E)** shoot Zn concentration (mg/kg), **(F)** N uptake/plant (mg), **(G)** P uptake/plant (mg) and, **(H)** Zn uptake/plant (µg). Nodule dry weights are means of square root transformations. Different letters above each bar graph indicate significant differences for each variate seperately according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction of rhizobia x N. The vertical bar represents the standard error of difference (s.e.d.).

Interactions between AMF and rhizobia

Shoot biomass and seed yield of AMF and rhizobia-inoculated plants

At 6 weeks, for shoot biomass, the addition of AMF or rhizobia alone did not differ from the uninoculated control. However, the addition of AMF and rhizobia together significantly (P<0.05) increased shoot biomass by 60 % compared to the uninoculated control. At 11 weeks, the addition of rhizobia significantly (P<0.05) increased shoot biomass by 38 % and seed yield by 96 % compared to the uninoculated control, while the addition of AMF and rhizobia together significantly (P<0.05) increased shoot biomass by 71 % and seed yield 173 % the value of the uninoculated control (Fig. 5A)

Nodulation in AMF and rhizobia-inoculated plants

At 11 weeks, the addition of both AMF and rhizobia significantly (P<0.05) increased the nodule dry weight by 165 % and nodule number per plant by 95 % compared to the addition of rhizobia alone (Fig. 5B).

Shoot nutrient concentration and uptakes in AMF and rhizobia-inoculated plants

At 6 and 11 weeks, the addition of AMF alone significantly (P<0.05) increased the concentrations of P, S, Zn and Cu compared to the uninoculated control (Fig. 5C and 5D for P and Zn, respectively; Supplementary Fig. S6 for the other nutrients). The addition of rhizobia alone significantly (P<0.05) reduced the concentrations of P, Zn, Ca, Cu and Mo and increased the concentrations of N and Mg by 43 % and 25 % (Fig. 5D–F, Supplementary Fig. S6). The addition of rhizobia and AMF together significantly (P<0.05) reduced concentrations of P, S, Cu, and Mo at both 6 and 11 weeks, Ca at 6 weeks and Zn at 11 weeks, compared to AMF alone (Fig. 5D and E for P and Zn; Supplementary Fig. S6 for the other nutrients). However, there was no further reduction in Zn concentration when AMF and rhizobia were added together compared to AMF alone at 6 weeks (Fig. 5E).

The uptake of N in the plant shoot was significantly (P<0.05) increased by 52–88 % from inoculation with rhizobia and was significantly (P<0.05) increased by 110–161 % from inoculation of both AMF and rhizobia at both 6 and 11 weeks (Fig. 5G). The uptake of P in the plant shoot was significantly (P<0.05) increased by 106–127 % from inoculation with AMF at 6 and 11 weeks, with a further significant increase (P<0.05) of 138–153 % from inoculation of both AMF and rhizobia at both 6 and 11 weeks (Fig. 5H).

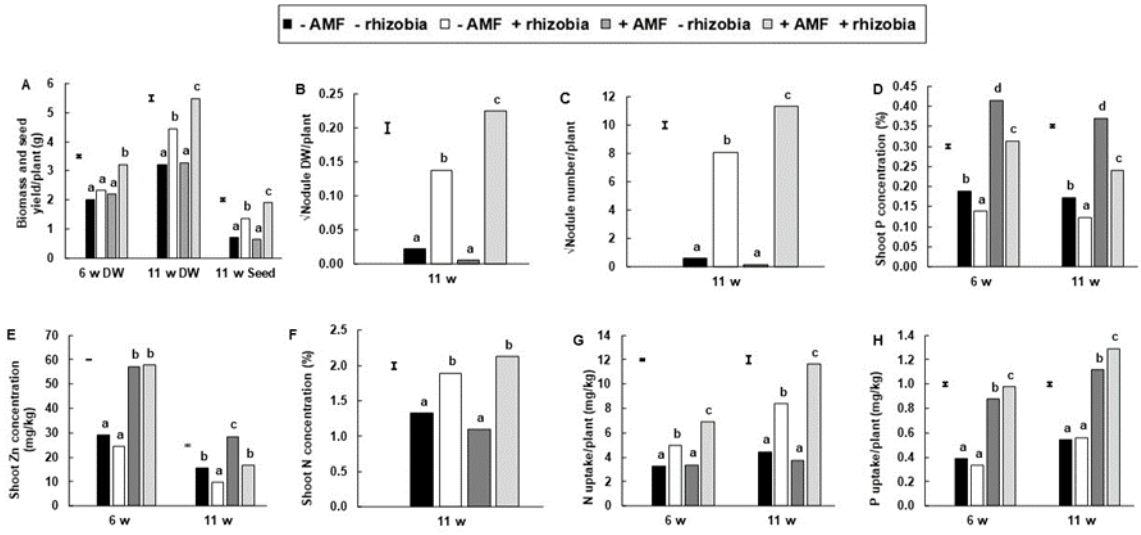

Fig. 5. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and rhizobia at 6 and/or 11 weeks (w) on **(A)** shoot biomass and seed yield, **(B)** nodule dry weight/plant, **(C)** nodule number/plant, **(D)** shoot P concentration (%), **(E)** shoot Zn concentration (mg/kg) and, **(F)** shoot N concentration (%), **(G)** N uptake/plant (mg), and **(H)** P uptake/plant (mg). Nodule number and dry weights are means of square root transformations.

Different letters above each bar graph indicate significant differences for each given variate seperately according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction of AMF x rhizobia. The vertical bar represents the standard error of difference (s.e.d.).

Interactions between AMF and N

Shoot biomass and seed yield in AMF-inoculated and N-fertilised plants

Adding AMF and N, alone and combined, had a similar effect to that in plants inoculated with both AMF and rhizobia resulting in a significantly (P<0.05) increased plant shoot biomass and seed yield. However, the increases in shoot biomass and seed yield from combined AMF and N were not as great as from combined AMF and rhizobia treatments. In plants with added AMF and N together, shoot biomass increased by 50 % and 39 % at 6 and 11 weeks respectively, and seed yield by 60 % more than the uninoculated control (Fig. 6A).

Shoot nutrient concentrations and uptakes in AMF-inoculated and N-fertilised plants

The addition of AMF significantly (P<0.05) increased the concentrations of P, Zn, K, S and Cu compared to the addition of N alone or the uninoculated control at 6 and 11 weeks (Fig 6B and C for P and Zn, respectively; Supplementary Fig. S7 for the other nutrients). The addition of N significantly (P<0.05) increased the N concentration by 13 %, which was lower than that from addition of rhizobia and AMF together (Fig. 6D).

The addition of AMF and N significantly (P<0.05) reduced the concentrations of all nutrients compared to AMF alone, with the exception of N at 11 weeks (P<0.05) (Fig. 6D for N, Supplementary Fig. S7 for the other nutrients). Inoculation with AMF and N combined significantly (P<0.05) reduced the Zn concentration at 6 weeks compared to AMF alone (Fig. 6C), although this effect did not occur in plants co-inoculated with AMF and rhizobia, which had a similar concentration of Zn to AMF alone.

At 6 weeks, the addition of AMF or N significantly (P<0.05) increased the N uptake in the plant shoots by 18–33 %, while the addition of both significantly (P<0.05) increased the N uptake in the plant shoots by 71 % compared to the uninoculated control (Fig. 6E). At both time points, the addition of AMF significantly (P<0.05) increased P and Zn uptake with increases of between 96–138 % for P uptake and 70–100% for Zn uptake, depending on time of assessment. There was a further significant (P<0.05) increase on combining both AMF and N with a range of

increases of 134–171 % for P uptake and 103–129 % for Zn uptake depending on time of assessment (Fig. 6F and 6G).

Other contrasts in interactions between treatments of AMF and rhizobia compared to treatments of AMF and N include; (a) no significant interactive effects on Ca concentration in the AMF x N treatment compared to AMF x rhizobia treatment, (b) no significant interactive effects on B or Mn concentration in the AMF x rhizobia treatment compared to AMF x N treatments, (c) the concentration of Mg at 6 weeks was increased by rhizobia and decreased by N.

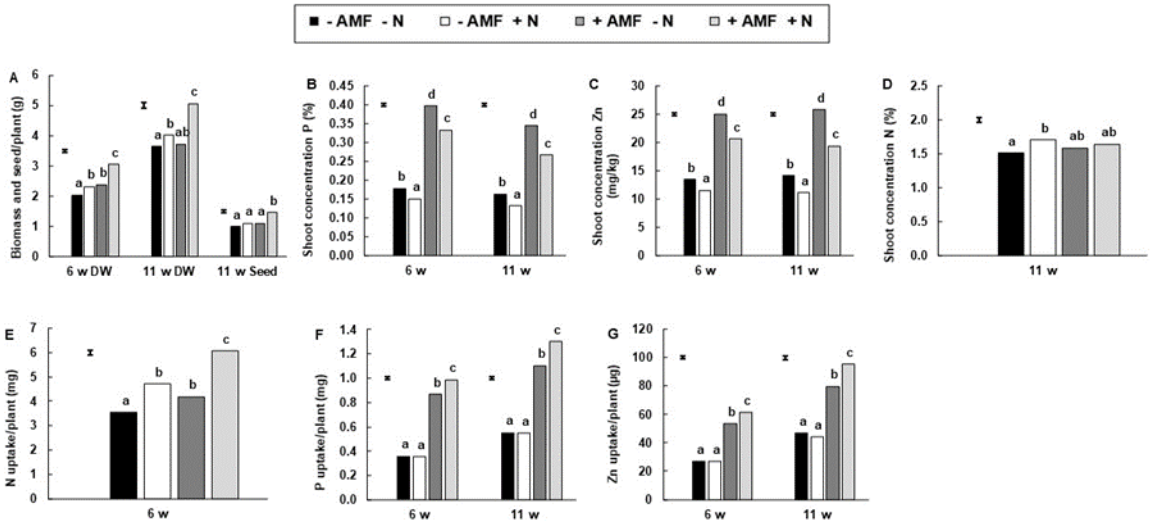

Fig. 6. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and N at 6 and/or 11 weeks (w) on **(A)** plant biomass and seed yield, **(B)** shoot P concentration (%), **(C)** shoot Zn concentration (mg/kg) and, **(D)** shoot N concentration (%), **(E)** N uptake/plant (mg), **(F)** P uptake/plant (mg) and, **(G)** Zn uptake/plant (µg). Different letters above each bar graph indicate significant differences for each given variate seperately according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction of AMF x N. The vertical bar represents the standard error of difference (s.e.d.).

Interactions between AMF, rhizobia, *P. thornei*, N, P and Zn on seed yield, shoot biomass, root biomass, mycorrhizal colonisation and *P. thornei* population densities

Interactive effects on seed yield

The F values from the ANOVAs of seed yield per plant at 11 weeks were significant at P<0.05 for the following interactions; (a) AMF x rhizobia x P and, (b) AMF x rhizobia x Zn.

In the AMF x rhizobia x P interaction, plants inoculated with rhizobia had greater seed yield compared to the uninoculated control. Plants with added AMF and rhizobia, or added P and

rhizobia had similar yields. The addition of P to plants with added AMF and rhizobia did not significantly increase seed yield any further compared to the addition of AMF and rhizobia together (Fig. 7A)

In the AMF x rhizobia x Zn interaction, plants with added rhizobia alone or rhizobia and Zn together had a significantly (P<0.05) increased seed yield compared to the uninoculated control. The seed yield of plants with added AMF and rhizobia without Zn had the greatest increase in seed yield but the addition of Zn significantly (P<0.05) reduced the seed yield (Fig. 7B).

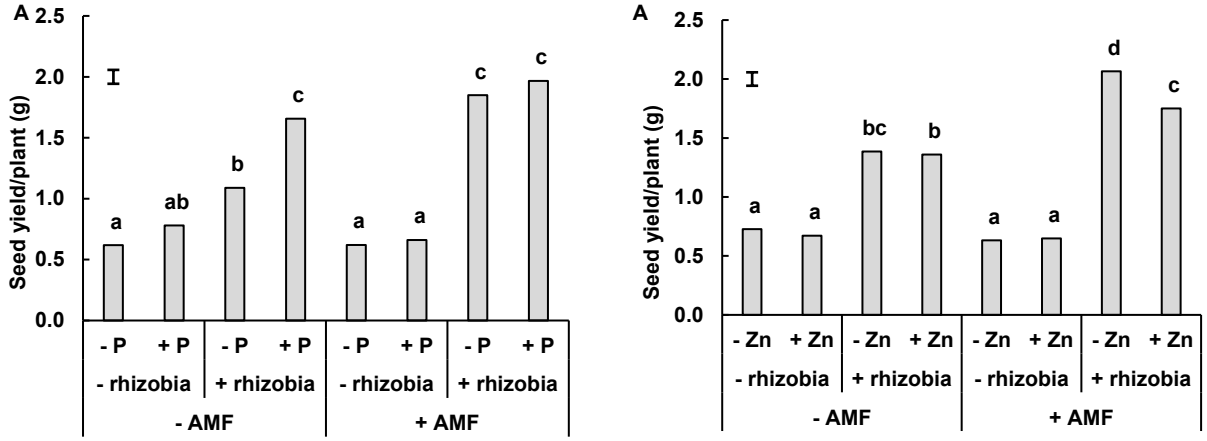

Fig. 7. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia x P, **(B)** arbuscular mycorrhizal fungi (AMF) x rhizobia x Zn on seed yield per plant of mung bean at 11 weeks after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Interactive effects on shoot biomass

At 6 weeks, in the AMF x rhizobia x *P. thornei* x N interaction, the addition of *P. thornei* or N to plants with added AMF and rhizobia had the greatest increase in shoot biomass compared to the uninoculated control, and had significantly (P<0.05) increased shoot biomass compared to AMF and rhizobia together. Plants with added AMF and N together also had significantly (P<0.05) increased shoot biomass, though this was not to the same level as those with added AMF and rhizobia (Fig. 8A).

At 11 weeks, in the AMF x rhizobia x P interaction, plants with added AMF and rhizobia together significantly (P<0.05) increased shoot biomass compared to either symbiont alone. Plants inoculated with rhizobia and P together had a similar biomass to plants inoculated with

rhizobia and AMF together. There were no further increases when adding P, AMF and rhizobia together compared to AMF and rhizobia together (Fig. 8B).

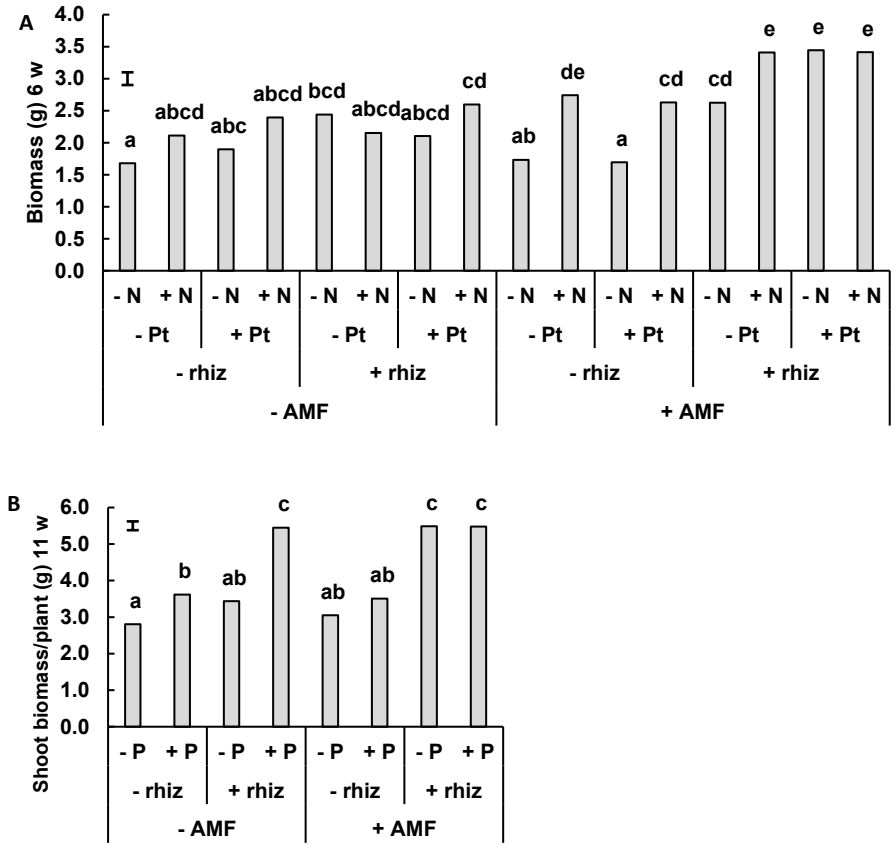

Fig. 8. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (Pt) x N on shoot biomass per plant of mung bean at 6 weeks (w) after sowing, **(B)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P on shoot biomass of mung bean at 11 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d).

Interactive and main effects on root biomass

In the rhizobia x N interaction, the addition of N alone significantly (P<0.05) increased root biomass by 41–46 % compared to all other treatments at 6 weeks (data not shown). There were significant main effects on root biomass at 11 weeks, whereby rhizobia reduced root biomass by 31 % (P<0.05) (data not shown).

Main effects on mycorrhizal colonisation

In the complete data set, there was very low mycorrhizal colonisation in non-mycorrhizal treatments at 6 and 11 weeks (a mean value of 0.00028 and 0.02 for the proportion of root length

colonised by AMF (arcsine transformation) respectively. Therefore ANOVA were carried out on the AMF inoculated data alone at both time points.

At 6 weeks, there was a significant main effect of rhizobia decreasing the proportion of root length colonised by AMF (arcsine transformation) by 13 % at 6 weeks (P<0.01) (data not shown). There were no main or interactive effects on the length of root colonised by AMF.

At 11 weeks, there were significant main effects of N and rhizobia whereby the addition of these significantly (P<0.05) reduced the proportion of root length colonised by AMF (arcsine transformation) by 9–14 % compared to AMF inoculated alone, and where the addition of N significantly (P <0.05) reduced root length colonised with AMF (cm) by 18 % compared to AMF alone (data not shown).

At 11 weeks, there were significant main effects of P and rhizobia on the proportion of root length containing vesicles (arcsine transformation), and significant main effects of N on root length containing vesicles (cm). The addition of P significantly (P<0.05) reduced the proportion of root length containing vesicles by 14 %, while the addition of rhizobia significantly (P<0.05) increased the proportion of root length containing vesicles (arcsine transformation) by 27 % compared to AMF alone. The root length containing vesicles (cm) was significantly (P<0.05) decreased by 19 % with the addition of N compared to AMF alone (data not shown).

Significant effects on P. thornei *population densities*

In the complete data set, there were very low *P. thornei* population densities in the non-inoculated nematode treatments at 6 and 11 weeks (<1 *P. thornei*/kg soil), therefore ANOVA were carried out on the *P. thornei* inoculated data alone at both time points.

At 6 weeks, there were significant interactive effects between rhizobia x N on the population densities of *P. thornei*. In *P. thornei* inoculated plants, inoculation with rhizobia significantly (P<0.05) reduced the *P. thornei* population densities by 29 % in the absence of N fertiliser but not in its presence (data not shown). At 11 weeks, there were significant main effects of AMF, N, P and Zn on *P. thornei* population densities. In plants with added AMF, *P. thornei* population densities significantly (P<0.05) increased by 53 % compared to plants inoculated with *P. thornei* alone. The addition of N, P or Zn alone significantly (P<0.01) decreased *P. thornei* population densities by 23–27 % compared to plants inoculated with *P. thornei* alone (Supplementary Fig S10).

Interactions between AMF, rhizobia, P. thornei, *N, P and Zn on plant P and Zn nutrition at 6 weeks*

Phosphorus concentration

In the AMF x rhizobia x *P. thornei* x N interaction, plants inoculated with AMF had the greatest increase in P % at 6 weeks. The addition of N or rhizobia significantly (P<0.05) decreased P concentration 21–24 % in AMF inoculated plants. In plants inoculated with AMF and rhizobia, the addition of *P. thornei* also significantly (P<0.05) reduced the P concentration by 17 % compared to plants inoculated with AMF and rhizobia alone (Fig. 9A).

In the AMF x rhizobia x P interaction, plants inoculated with AMF had a significant increase in P concentration of 78 % compared to addition of P and a 193 % increase compared to the uninoculated control with no further significant increase when inoculated with AMF and P. The addition of rhizobia significantly (P<0.05) reduced P concentration by 25–30 % in AMF and P inoculated plants compared to AMF and P inoculated plants alone (Fig. 9B).

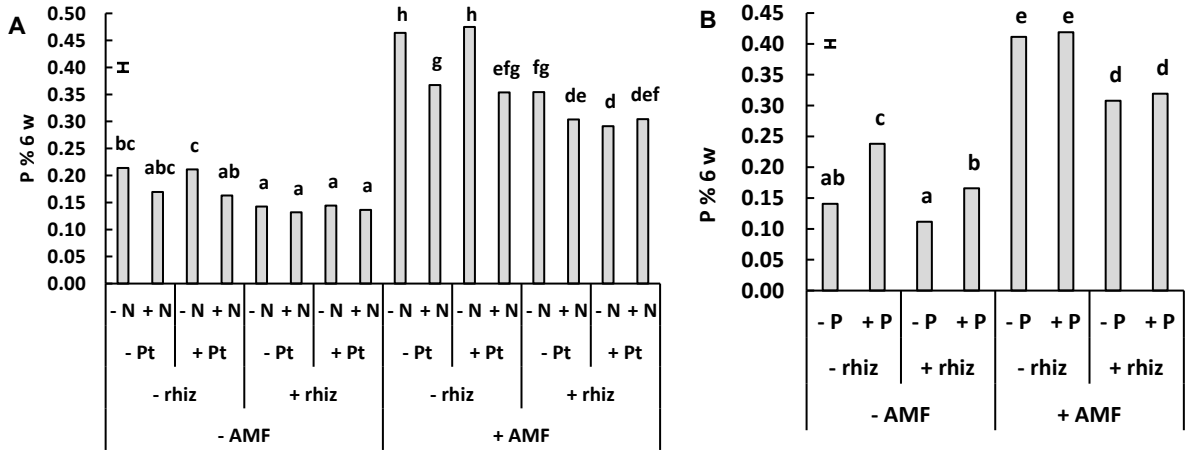

Fig. 9. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (Pt) x N and **(B)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P on P concentration (%) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Zn concentration

In the AMF x rhizobia x *P. thornei* x N interaction, inoculation with AMF increased Zn concentration compared to all treatments without AMF. The addition of N or rhizobia significantly (P<0.05) decreased Zn concentration in all treatments. The concentration of Zn

significantly (P<0.05) decreased further when AMF plants had *P. thornei* and rhizobia added together (Fig. 10A).

In the AMF x *P. thornei* x N x Zn interaction, inoculation with AMF significantly (P<0.05) increased the Zn concentration by 65 % compared to the addition of Zn. In AMF inoculated plants, the addition of N or *P. thornei* alone and combined resulted in a significant (P<0.05) reduction in Zn concentration by 15–26 % (Fig. 10B).

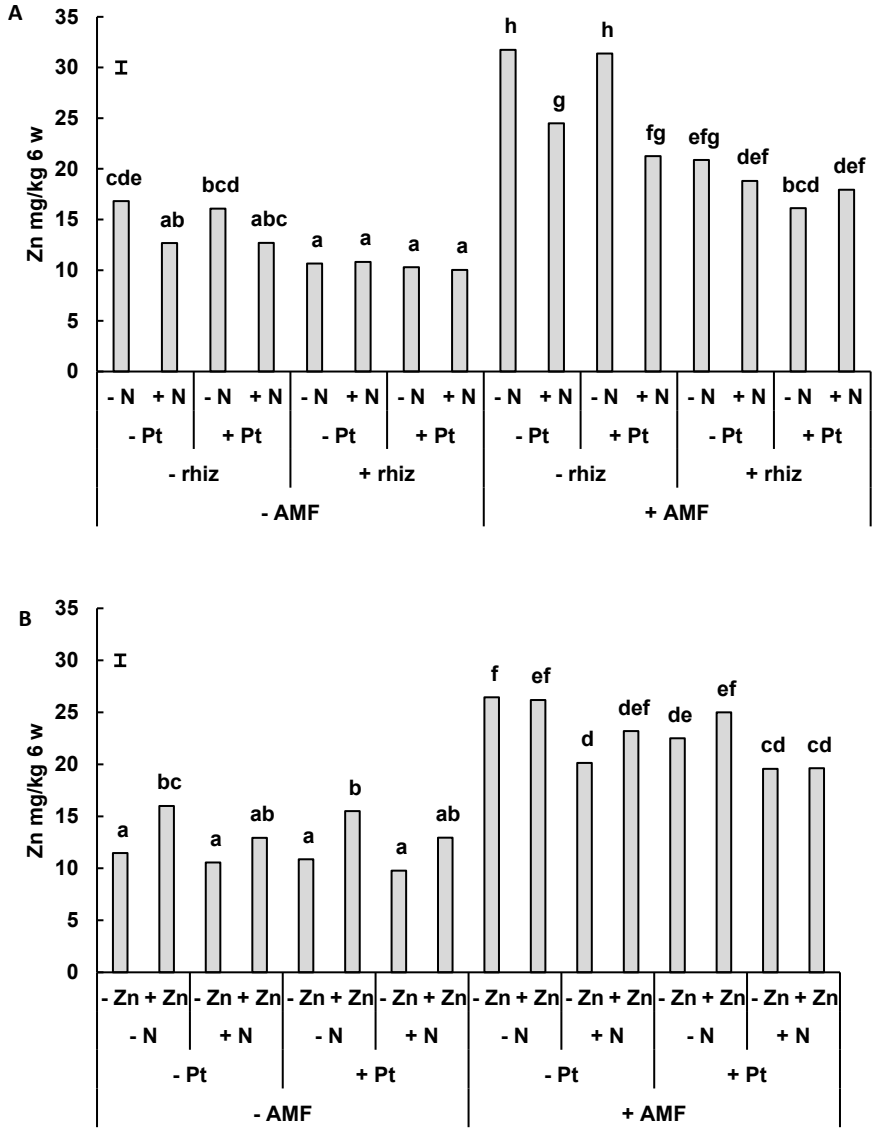

Fig. 10 The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (Pt) x N and **(B)** arbuscular mycorrhizal fungi (AMF) x *Pratylenchus thornei* (Pt) x N x Zn on Zn concentration (mg/kg) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each

bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Shoot N:P ratio

There was no relationship between dry weight and N:P ratio at 6 weeks of plant growth (Supplementary Table S11). At 11 weeks, the optimal N:P ratio for dry weight across all treatment combinations was determined as 11.67 from Gaussian regression (R^2= 0.43, P<0.001).

In the interaction between AMF x rhizobia x *P. thornei* x Zn, the addition of rhizobia significantly increased (P<0.05) the N:P ratio compared to the uninoculated control (N:P ratio of 15.11 compared to 7.12), while the addition of AMF reduced it (N:P ratio of 2.98). The addition of both AMF and rhizobia resulted in an N:P ratio not significantly different from that of the control (N:P ratio of 9.50). The addition of *P. thornei* did not change the N:P ratio, while the addition of Zn alone significantly (P<0.05) increased the N:P ratio compared to the control (Fig. 11A).

In the interaction between AMF x P x N, the addition of P or AMF resulted in significantly (P<0.05) reduced N:P ratio (N:P ratio of 9.69 and 6.02 respectively) compared to the uninoculated control (N:P ratio of 11.87), while the addition of N significantly (P<0.05) increased the N:P ratio to its highest level (N:P ratio of 16.76). The addition of P and N reduced the N:P ratio to the level of the control, while the addition of AMF and N had an N:P ration similar to AMF alone (N:P ratio of 6.74) (Fig. 11B).

In the interaction between rhizobia x N, the addition of either rhizobia or N significantly (P<0.05) increased the N:P ratio compared to the uninoculated control (N:P ratio of 4.2), with a significantly greater N:P ratio when inoculated with rhizobia (N:P ratio of 12.31) compared to fertilisation with N (N:P ratio of 7.95).The addition of rhizobia and N did not increase the N:P ratio further (Fig. 11C).

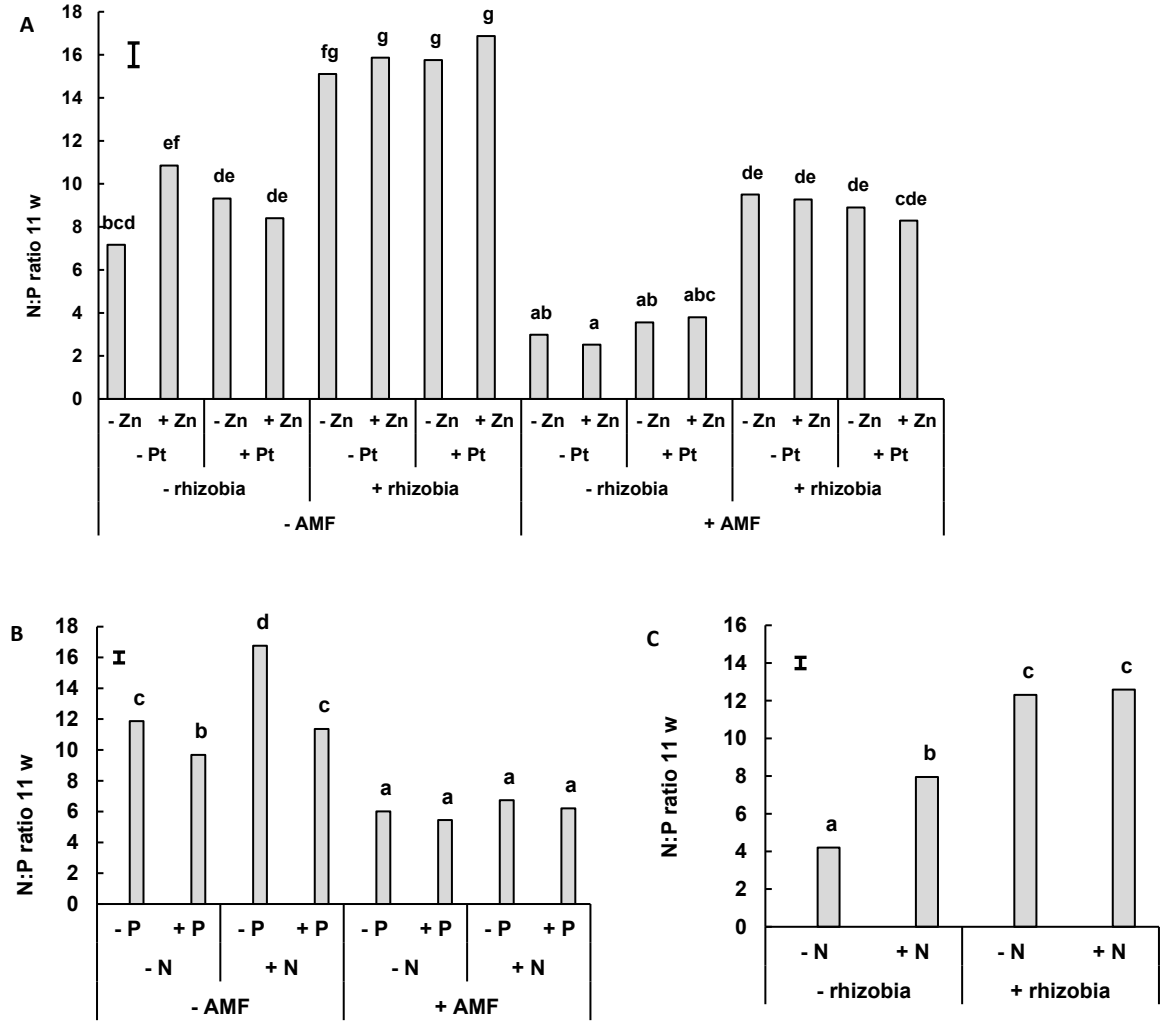

Fig. 11. The interactive effects on the shoot N:P ratio at 11 weeks of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia x *P. thornei* (Pt) x Zn, **(B)** arbuscular mycorrhizal fungi (AMF) x N x P, **(C)** rhizobia x N at 11 weeks (w). Different letters above each bar graph indicate significant differences for each given variate seperately according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Discussion

Rotations with legumes have been promoted to improve long-term sustainable agricultural systems due to benefits such as improved soil fertility through nitrogen fixation, sequestration of carbon increasing soil organic matter, improved soil nutrient circulation, along with yielding an economical high-protein food source (Stagnari et al. 2017). It is therefore imperative that we understand the complex interactions that occur between micro-organisms that associate with legume roots including rhizobia, AMF and plant parasitic nematodes. This study has provided

insights into the role that nutrients contribute to the improvement of biological nitrogen fixation, plant growth and yield by AMF and has highlighted the complex interactions that occur between symbionts and plant-parasites in the rhizosphere and inside the roots of mung bean.

The effects of nutrients and AMF on biological nitrogen fixation

We have demonstrated that inoculation with AMF can improve the efficiency of biological N fixation by *Bradyrhizobium* sp. in mung bean, leading to improved biomass, seed yield, nodulation and plant nutrition equal to or greater than, the application of fertiliser P with no further benefit to BNF from the addition of both. These results increase our understanding of how these microsymbionts may interact both with each other, and with the application of exogenous fertiliser to improve legume cultivation systems.

Phosphorus is an essential nutrient for plant growth and for biological N fixation as the enzyme nitrogenase, which reduces N_2 to NH_3 in the bacteroids, has a high demand for ATP (Simpson and Burris 1984). The P concentration in root rhizobial nodules can be up to three times the concentration found in other plant tissues (Sa and Israel 1991). Poor BNF and nodulation have been linked to P deficiencies in other legumes including white clover (*Trifolium repens*), soybean and barrel medic (*Medicago truncatula*) (Høgh-Jensen et al. 2002; Sa and Israel 1991; Sulieman et al. 2013). In our research, we found strong positive associations between BNF and the concentration of P in mung bean shoots from both the application of fertiliser P and mycorrhizal colonisation. Furthermore, our research showed that plants inoculated with AMF increased % Ndfa greater than those where P alone was added, demonstrating the importance of AMF in improving BNF in mung bean. Strong positive correlations have been found between total plant P content and % Ndfa in other legumes such as the barrel medic (*M. truncatula*) and alfalfa (*M. sativa*), but with no further benefit from AMF in improving % Ndfa when grown under high P supply (Püschel et al. 2017). In our research, the combination of AMF and P increased BNF and P content at 6 weeks. However, at 11 weeks, the addition of AMF alone supplied sufficient P for BNF in the plant, which confirms the high level of mycorrhizal dependency of mung bean for the acquisition of P and the value of AMF to the N fixation process in mung bean. Biological N fixation rates in grain legumes can vary over the growing season and may depend on the growth stage of the crop. For example, soybean fixed the most N at the beginning of the reproductive stage but only continued for 20 days, while faba bean had a much longer period of N fixation (Zapata et al. 1987a, b). Further investigation is

warranted to determine the growth stage and length of time required to fix most N in mung bean. However, it is well established that in early stages of plant growth in N fixing grain legumes the demand for P by the developing nodules is high and colonisation with AMF can enhance P inflow to the legume, improving nodulation and BNF by rhizobia (Thompson 1991).

In the mycorrhizal symbiosis, the exchange of nutrients and metabolites occurs in the plant-derived peri-arbuscular membrane—the zone around the arbuscule that acts as the intimate interface between plant and fungus (Pumplin and Harrison 2009). Plants that form associations with mycorrhizal fungi gain access to larger soil reserves of P, due to the mycorrhizal hyphae that extend further past the nutrient depletion zone of the root (Miller et al. 1995). Furthermore, AMF also form associations with phosphate solubilizing bacteria (PSB) that can increase the release of soluble P, improving mycorrhizal P acquisition (Kobae 2019; Zhang et al. 2016). Some plants can obtain their total P requirements via the mycorrhizal association, depending on host/fungal combinations (Smith et al. 2003).

Arbuscular mycorrhizal fungi transport P in the form of polyphosphate from the rhizosphere, through the intraradical hyphae, into the arbuscule and subsequently across the peri-arbuscular membrane —a process controlled by membrane phosphate transporters including the well conserved phosphate transporter MtPT4, which is highly expressed in cells with arbuscules (MacLean et al. 2017; Sawers et al. 2017; Yang et al. 2012). We observed significantly greater increases in the concentration and uptake of P in plant shoots when inoculated with AMF compared with fertilisation with P, likely as a result of the increased efficiency of the mycorrhizal nutrient uptake pathway at improving plant P nutrition compared to the direct uptake pathway by the roots (Smith et al. 2011).

The quantity of phosphorus uptake by AMF may depend on the soil phosphorus content, interactions with PSBs, mycorrhizal dependency of the host plant species, the fungal species, and the complex relationships between these parameters (Burleigh et al. 2002; Kobae 2019; Smith et al. 2003; Smith and Read 2008). While high levels of P may reduce the colonisation and functionality of the symbiosis (Breuillin et al. 2010; Püschel et al. 2017), in our experiment the application of P did not reduce the proportion or the total length of root colonised with AMF. The experimental soil used originated from a rainfed research trial site, under a well-managed fertilisation regime, therefore the P and Zn levels were higher than the average levels in vertisols

of the region at P <10 mg/kg soil (Colwell bicarbonate extraction) and Zn <1 mg/kg soil (DTPA extraction) (Holloway et al. 2008; O'Mara 2015). Notwithstanding the higher nutrient levels of the soil, the benefits that AMF provided in increasing P and Zn concentrations and uptakes to the shoots, and the subsequent increases in biomass, yield and BNF suggest that the value of the symbiosis is likely to be even greater to mung bean growing in soil with typically lower P levels (Hoeksema et al. 2010).

In addition to improved P concentration, our research also demonstrated mycorrhizal colonisation was highly associated with greater Zn and Cu concentrations in the plant shoots at both 6 and 11 weeks, despite infestation with *P. thornei*. We demonstrated that mycorrhizal colonisation increased the concentration and uptake of Zn in mung bean shoots greater than the application of fertiliser Zn, which we understand to be a novel finding in mung bean. In the roots of the plant, Zn^{2+} is taken up from the rhizosphere via ZIP transporters (Zinc-regulated, Iron-regulated transporter-like Protein family), which have also been implicated in the transport of Fe^{2+} and Mn^{2+}, while Cu^{2+} is mainly transported by the Copper Transporter Family (CTR) (Ajeesh Krishna et al. 2020; Casieri et al. 2013; Guerinot 2000). Expressions of the ZIP transporter genes MtZIP5 and MtZIP14 were upregulated in mycorrhizal medic (*Medicago truncatula*) (Nguyen et al. 2019; Cardini et al. 2021). Also, candidate metal transporter genes from the ZIP and CTR families have been identified in the AMF species *Rhizophagus irregularis* (Tamayo et al. 2014). Our finding of increased concentrations of Zn and Cu in mung bean shoots is likely due to the increased efficiency of mycorrhizal roots improving Zn and Cu uptake by accessing these micronutrients which may be positionally unavailable to the root (Smith and Read 2008), though increases in the upregulation of ZIP and CTR transporters in mycorrhizal mung bean are also likely to play a role. Further research is required to identify the mechanism for the increase in Zn and Cu in mung bean by *F. mosseae*.

Zinc deficiency is believed to be one of the main causes of human morbidity in developing countries with an estimated 17.3 % of the world's population having insufficient dietary intake (Bailey et al. 2015). While efforts are undertaken to improve the nutritional content of legume varieties, including mung bean, by plant breeding (Jha and Warkentin 2020; Nair et al. 2015), Zn biofortification by AMF can improve the nutritional content of a crop both sustainably and economically, while reducing the use of fertilisers (Cavagnaro 2008; Lehmann et al. 2014). For example, the grain contents of protein, Fe and Zn increased in chickpea inoculated with AMF

(Pellegrino and Bedini 2013), while AMF improved the nutritional value of many horticultural crops (as reviewed by Baum et al. 2015). Vertisols in the subtropical grain region of eastern Australia are often low in phytoavailable P and Zn due to soil properties including high cation exchange capacity, alkaline pH and high clay contents (Holloway et al. 2008). Agronomic systems in the region rely on the application of fertiliser P, primarily imported and typically applied via deep P placement (~20 cm deep in the soil profile) to increase crop yields (Bell et al. 2010; Cordell et al. 2013). Utilising the increased efficacy of AMF for uptake of these poorly mobile nutrients should be advocated in soils under legume cultivation.

The effects of rhizobia and fertiliser N on plant growth with AMF

Our results demonstrated that the addition of rhizobia was highly associated with increased biomass, nodulation and seed yield. Furthermore, the addition of rhizobia increased biomass, seed yield, nodulation and the concentration and uptake of N in the plant shoots to a greater level than the addition of fertiliser N alone. At 11 weeks, there was a reduction in yield when rhizobia and N had been added together, potentially caused by a reduction in nodule dry weight resulting in a lower concentration of N to the shoots.

In our previous research, mung bean plants inoculated with AMF and rhizobia resulted in increased biomass, yield, nodulation and plant nutrient uptakes compared with plants not inoculated or inoculated with only one microsymbiont (Gough et al. 2021). In the current research we investigated if the increased supply of N by rhizobia was the underlying reason for the beneficial effects on growth and yield. The multifactorial design of the current experiment allowed us to compare the effect of N sources on mung bean growth, yield and nutrition and also compare how these N sources interacted with AMF.

The combination of AMF and rhizobia was more effective than the combination of AMF and fertiliser N on shoot biomass and yield. Our results demonstrated that the inoculation of AMF and rhizobia together increased the shoot biomass and seed yield in mung bean by 1.8 and 3.6 times the values obtained when inoculated with AMF and N. This effect is potentially due to the increase in nodulation and BNF, along with greater improvements in plant nutrition including N and P in plants inoculated with both AMF and rhizobia. It is established that AMF increases the concentration and uptake of the nutrients Zn and Cu in other plants (Clark and Zeto 2000) and both these nutrients are required for enzyme function and N fixation by rhizobia

(O'Hara 2001). To our knowledge this is the first report on AMF increasing Cu in mung bean in a vertisol.

The nodulation process is believed to have evolved from the mycorrhization process and both symbionts share at least seven genes for initiation and control of the symbiosis known as the "common symbiosis pathway" (Parniske 2008). Recent transcriptomics research has investigated the overlap between genes upregulated in mycorrhizal, rhizobial and co-colonised soybean (Sakamoto et al. 2019). In their research, 56 host genes were specifically up-regulated on co-inoculation with the AMF species *Gigaspora rosea* and *Bradyrhizobium diazoefficiens*. These included nodulin genes, which Sakamoto et al. (2019) concluded led to increased rhizobial nodule number and biomass, and transporter genes including the bidirectional sugar transporter SWEET1, which plays a role in nutrient exchange between the host plant and both microsymbionts. It is likely that co-inoculation with AMF and rhizobia should result in the upregulation of similar genes leading to the increased biomass, yield and nodulation observed in other legumes (Chalk et al. 2010). It remains to be investigated if metal transporter genes from the ZIP and CTR families, which are upregulated by mycorrhiza, are also involved in benefitting the symbiosis with rhizobia.

In the subtropical grain region, fertilisation with N represents >65 % of the fertiliser budget for grain cropping (Bell et al. 2010). Rotation with leguminous crops is encouraged to improve the balance of the N nutrient budget in the farming system via BNF. However, reductions in nodulation and BNF occur under P deficient conditions, where legumes preferentially utilise soil NO_{3-} or $NH_{4+,}$ which is less energy demanding than N fixation, thereby conserving C compounds for growth (Valentine et al. 2017). This has the undesired effect of reducing the soil N budget for the crops under cultivation and for subsequent crops in the sequence. The N:P ratio can be used to indicate nutrient limitations impacting plant production and growth. The N:P ratio may vary dependent upon the plant species, growth rate, age of the plant and tissue sampled (Güsewell 2004). We observed that plants inoculated with rhizobia had an N:P ratio closer to optimal for dry biomass production (determined as 11.67) compared to the ratio in plants fertilised with N. On the other hand, plants inoculated with AMF had a greatly reduced N:P compared to fertilisation with P. This effect was most likely due to increased P concentration and uptake following mycorrhizal colonisation resulting in P surplus to that required for plant growth. The addition of AMF and rhizobia together resulted in an N:P ratio closer to optimal

for biomass production, compared to the addition of AMF and N. Mung bean grown under natural conditions is likely to be mycorrhizal. Mung bean inoculated with rhizobia and cultivated in soils with adequate levels of AMF may result in an optimised N:P ratio compared to fertilisation with P. Management of AMF in the cropping systems, alongside inoculation of legumes with rhizobia, should lead to increases in (i) the rates of BNF, (ii) improved nutrient use efficiency and, (iii) an optimal N:P ratio for biomass production. Subsequent improvements in balancing both the N and P nutrient budget should reduce requirements for fertiliser inputs by growers.

Effects of plant nutrition and *P. thornei* population densities in mung bean

Notwithstanding increased plant nutrition in mung bean due to mycorrhizal colonisation, we also observed complex interactions between *P. thornei*, AMF and rhizobia with respect to plant growth and nutrition, whereby *P. thornei* reduced the concentration of P and Zn in the shoots of mung bean inoculated with AMF and rhizobia together. Infestation with *Pratylenchus* spp. can result in nutrient deficiencies in other crop species including reductions in the uptakes and concentrations of N and P in wheat by *P. thornei* (Thompson and Clewett 2021), and reductions in the concentrations of N, P, K, Ca, Mg, Mn and Cu in plum rootstock (*Prunus cerasifera* X *P. munsoniana*) by *P. vulnus* (Pinochet et al. 1998). Nutrient deficiencies can arise as a consequence of the destructive feeding and migration of the nematodes, which destroys cortical tissue, reduces root biomass and disrupts the uptake of nutrients and water to the plant (Pinochet et al. 1996; Thompson et al. 2012; Whish et al. 2014). Interestingly, we observed increases in mung bean shoot biomass in the interaction between AMF, rhizobia and *P. thornei* at 6 weeks of growth, but this was not evident on inoculation with *P. thornei* alone. The increase in biomass may have been as a result of *P. thornei* increasing the concentration of N in plants inoculated with AMF and rhizobia, reducing the concentration of P, K, Mg, Zn, Ca, B and Mo potentially via the growth and dilution effect (Jarrell and Beverly 1981). Soil nitrogen dynamics may be altered as a result of nematode infestation. For example, in cotton (*Gossypium hirsutum*), N mineralisation and soil extractable soil N increased under *Rotylenchus reniformis* infestation (Tu et al. 2003). The authors attributed this increased mineralisation due to leakage of photoassimilates from damaged root cells, along with increased degradation of cellulose by nematodes, resulting in increased microbial biomass. Increased soil N may have been as a result of a reduction in root extraction efficiency under nematode infestation resulting in soil N

remaining in the rhizosphere. Stimulations of plant biomass have been reported by Wallace (1971) following infestation by pin nematode (*Paratylenchus* sp.) and cyst nematode (*Heterodera schachtii*), and it was postulated that low levels of nematode infestation may stimulate an increase in the production of lateral root biomass, leading to subsequent increases in shoot biomass. This increase in shoot biomass may be perceived as a tolerance response to nematode infestation, where the plant maintains biomass and yields well despite infestation (Roberts 2002). However, the population densities of plant-parasitic nematodes, such as *P. thornei*, may increase in the roots to very high levels affecting subsequent intolerant crops in the cropping sequence (Owen et al. 2014: Thompson et al. 2008; Thompson et al. 2020). Nevertheless, despite the increase in shoot biomass, *P. thornei* significantly reduced Zn concentration in the mung bean shoots. In the subtropical grain region of eastern Australia where mung bean is produced, low soil Zn levels are common (Holloway et al. 2008), and *P. thornei* was found in 67 % of grain fields surveyed (Thompson et al. 2010). High pre-sowing population densities of *P. thornei* coinciding with low Zn in soils under cultivation may lead to plant Zn deficiencies with negative consequences for mung bean growth and yield.

In our previous research, population densities of *P. thornei* increased in mycorrhizal mung bean, which correlated with the proportion of mycorrhizal root length and subsequent increases in the uptakes of P and Zn in the plant as a function of efficient mycorrhizal colonisation. This increase in *P. thornei* population densities was not correlated with root biomass as was found, for example in quince (*Cydonia oblonga*), where increased root biomass increased *P. vulnus* population densities (Calvet et al. 1995). We had hypothesised that increases in nutrient concentration of the shoots as a result of mycorrhizal colonisation would lead to a subsequent increase in *P. thornei* population densities. However, we observed decreases in population densities of *P. thornei* on addition of N, P and Zn, while inoculation with AMF increased the multiplication of the nematode.

Reductions in population densities of *Pratylenchus* sp. following the application of N fertilisers may be influenced by both the source of N and soil pH levels with greater nematicidal properties of ammonia in alkaline soils, compared to acidic soils (de Melo Santana-Gomes et al., 2013; Walker 1971). Reductions in *Pratylenchus* population densities following high P fertilisation were reported in plum rootstocks (*Prunus cerasifera* x *P. musoniana*) infested with *P. vulnus* (Pinochet et al. 1998) and wheat infested with *P. neglectus* (Vanstone et al. 2002). In rice,

populations of *P. zeae* were negatively correlated with Zn and Fe (Coyne et al. 2004). While the mechanism underlying the reduction of *Pratylenchus* sp. following Zn remains to be determined, it was hypothesized that Zn may alter the physiology of the plant and also reduce penetration of plant parasitic nematodes into the roots (Siddiqui et al., 2002). To our knowledge, the present study is the first report of the application of fertiliser Zn and P reducing *P. thornei* populations.

Investigations of the interaction between mycorrhizal fungi and *Pratylenchus* spp. have indicated that mycorrhizal colonisation may suppress or increase *Pratylenchus* populations within the roots of the colonised host depending on nematode species, AMF taxonomic order and host functional group (Gough et al. 2020). *Pratylenchus* spp. and AMF may compete for space and photosynthates within the root, resulting in reduced mycorrhizal colonisation, with subsequent reductions in nutrient uptake by mycorrhizal plants (Hol and Cook 2004; Pinochet et al. 1996).

Mycorrhizal fungi may alter plant defence responses as reviewed by Schouteden et al. (2015). In brief, on initial penetration by the mycorrhizal hyphae, the plant may perceive the fungus as pathogenic, and the microbe-associated molecular pattern (MAMP) molecules may induce an immune response in the plant, termed the MAMP triggered immunity (MTI) response. This MTI response can result in alterations in the transcriptome and modifications to secondary metabolite production such as flavonoids and phenolics (Kaur and Suseela 2020). In wheat, AMF reduced the benzoxazinoid compounds—plant secondary metabolites associated with defence against biotic stressors, resulting in the multiplication of *P. neglectus* (Frew et al. 2018). The mechanisms behind how AMF increase *P. thornei* population densities in mung bean, and if the MTI response altered defence metabolites, resulting in increased nematode populations, remain to be investigated.

Selection of mung bean germplasm that associates efficiently with beneficial symbionts such as mycorrhizal fungi and *Bradyrhizobium* sp. to receive the benefits of the symbioses, but is resistant to *P. thornei,* is warranted in future breeding programmes for mung bean improvement. Additionally, the directed manipulation of both beneficial symbiont and plant-parasitic nematodes will benefit mung bean production while reducing fertiliser inputs and economic costs. For example, within the Australian subtropical grain region, strategic rotations with crops

that are hosts of AMF but are generally resistant to *P. thornei* including, but not limited to, sorghum, sunflower, linseed and pigeon pea (*Cajanus cajan*) can increase levels of mycorrhizal inoculum and reduce population densities of *P. thornei* in the soil (Thompson 1994, Owen et al., 2014).

Conclusion

The valuable contribution of the beneficial symbionts AMF and rhizobia has been demonstrated in our research with AMF increasing P supply to mung bean, improving biological N fixation by *Bradyrhizobium*, yield and crop nutrition while reducing fertiliser inputs. Management of AMF by adoption of agricultural practices that encourage the proliferation of AMF in vertisols, along with inoculation with optimal strains of *Bradyrhizobium*, should result in improved biological N fixation optimising plant nutrient acquisition, biomass production, and grain yield, while reducing the reliance on excessive applications of exogenous fertilisers. It is imperative that we acknowledge the benefits that AMF provide to the legume cultivation system, but concurrently, it is also essential to understand that complex interactions between AMF and other soil-borne organisms occur, and AMF may have the unwanted effect of increasing the proliferation of *P. thornei*.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

EG acknowledges support from the University of Southern Queensland Research Training Program Scholarship and a Grains Research and Development Corporation (GRDC) research scholarship through Project USQ1912-003RSX. RZ acknowledges support from USQ. KO acknowledges co-funding by the GRDC through Project DJP1907-002RMX. KO and RZ acknowledge support from the Queensland Department of Agriculture and Fisheries (QDAF) through the Broadacre Cropping Initiative with USQ.

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CHAPTER 5

DISCUSSION AND CONCLUSION

"It is in the roots, not the branches, that a tree's greatest strength lies."

—Matshona Dhliwayo

Roots are a host to a multitude of species of bacteria, fungi, protists and animals all of which can affect the physiology of the plant and the biological, chemical and physical properties of the soil. Interactions occur between micro-organisms in the rhizosphere and inside the roots themselves, as they compete for host photosynthates, nutrition for growth and a more hospitable environment in which to thrive. The research presented in this thesis investigated the interactions between the beneficial symbionts, arbuscular mycorrhizal fungi and rhizobia, and the plant-parasitic nematode *Pratylenchus thornei* and determined how these interactions influenced plant productivity and nutrition in mung bean. The research hypotheses addressed in this thesis and the outcomes of the investigation are discussed below.

5.1 The interactions between root-lesion nematodes (*Pratylenchus* spp.) and arbuscular mycorrhizal fungi in different plant species

Arbuscular mycorrhizal fungi and *Pratylenchus* spp. co-exist in close proximity to each other in the root cortical cells of plant hosts (Pinochet et al., 1996), and interactions that occur between these organisms impact plant host growth and nutrition, level of mycorrhizal colonisation and *Pratylenchus* population densities in the roots. There are contradictory reports of outcomes of interactions between plant-parasitic nematodes and AMF. In existing reviews in the literature, nematodes were classified broadly into their mode of feeding. In this manner, *Pratylenchus* spp., were assessed in the same category as other migratory endo-parasites such as *Radopholus* spp. and *Hirschmanniella* spp. In the systematic review presented in Chapter 2 it was hypothesized that by utilising data from research carried out on *Pratylenchus* spp. alone, the effects that AMF have on population densities of *Pratylenchus* spp. could

be clarified. In this manner, the effect of AMF inoculation on plant biomass, under *Pratylenchus* spp. infestation could also be determined. Furthermore, taking into consideration the functional diversity of AMF species, it was also hypothesized that categorising AMF species taxonomically, should result in clearer conclusions on the interactions between AMF and *Pratylenchus* spp. as distinct from other endo-parasitic nematodes.

A systematic review of studies with *Pratylenchus* spp. analysed the effects of *Pratylenchus* spp. with and without inoculation with AMF. The interactions with AMF taxa were reviewed according to the revised classification based on molecular analysis of AMF species by Schüßler and Walker (2010). The systematic review, presented in Chapter 2, provided evidence that:

a) the population densities of *Pratylenchus* spp. in the roots and soil increased to a greater level on inoculation with AMF species from the order Diversisporales, than from the order Glomerales,
b) inoculation of plants with AMF species from the genera *Funneliformis* or *Glomus* had a neutral to reductive effect on the population densities of *Pratylenchus* species,
c) plants inoculated with AMF exhibited greater tolerance to the negative effects of infestation by *Pratylenchus* spp. as assessed by plant biomass,
d) increased population densities of *Pratylenchus* spp. may be influenced by host functional group, with increased population densities following inoculation with AMF more commonly observed in grass species, compared with tree or herb species.

The results from this systematic review indicated the predominantly beneficial effects of inoculation with AMF on maintaining shoot and root biomass of a host crop under *Pratylenchus* spp. infestation. This study also provided evidence for considering the taxonomic order of AMF when assessing the impact of AMF on altering population densities of the nematode. There is a lack of research on interactions between AMF and *Pratylenchus* spp. in leguminous hosts, therefore the experimental work carried out in this thesis on mung bean has made several contributions to the current literature on interactions between these organisms in legumes.

5.2 The interactions between arbuscular mycorrhizal fungi and population densities of the root-lesion nematode *P. thornei* in mung bean

Mung bean is a susceptible host to *P. thornei* and is highly dependent on AMF for growth and yield (Owen et al., 2014; Thompson, 1994). As both organisms co-exist in a very similar ecological niche, and are dependent on photosynthetically produced organic compounds from the plant host, it was hypothesized that an interaction could occur between both organisms. This interaction may result in AMF altering population densities of *P. thornei* in mung bean. Investigations carried out in Chapters 3 and 4, found that inoculation with AMF significantly increased the population density of *P. thornei* in the roots of mung bean. Increased population densities of *P. thornei* after inoculation with *F. mosseae* was not anticipated based on the outcome of the systematic review, whereby AMF species from the genera *Funneliformis* had a neutral to reductive effect on population densities of the nematode in previous publications. However, there is a paucity of reports in the literature on interactions between AMF and *Pratylenchus* spp. in leguminous plants.

Mycorrhizal fungi may increase population densities of *Pratylenchus* spp. by improvement of plant nutrition resulting in increased plant vigour and resulting in a perceived tolerance to nematode infestation (Pinochet et al., 1996: Roberts, 2002). Higher population densities of *P. thornei* in mung bean presented in Chapter 3 were not correlated with increased root biomass, but were initially positively correlated with increased plant nutrition in mycorrhizal plants, namely increased shoot concentrations of P, Zn and Cu.

Further investigations in Chapter 4 determined that the addition of nutrients N, P and Zn to plants inoculated with *P. thornei* was highly significant in reducing population densities of *P. thornei* which has not previously been reported in mung bean. In the literature, there are reports of reduced population densities of plant-parasitic nematodes following the application of fertilisers. Application of N fertilisers can reduce the population densities of plant-parasitic nematodes including *Pratylenchus*. These reductions may depend on the source of N, along with the soil properties with stronger nematicidal properties of ammonia in alkaline soils, compared to acidic soils (de Melo Santana-Gomes et al., 2013; Oka et al., 2007). Nitrogenous amendments to

soil reduced the population densities of *P. penetrans* due to increased ammonification (Walker, 1971). In glasshouse pot experiments, the application of rates of nitrate from 100–400 mg N kg^{-1} resulted in a decline in the population density of *P. thornei* in a vertisol in the absence of wheat plants but an increase in the population density where susceptible wheat was grown over a 16 week period (Thompson et al., 2015). In field experiments, a reduction of population densities of nematodes was observed in wheat following application of high rates of N (100 kg ha^{-1}), which is uneconomical in the broad-acre cultivation of legumes (Coyne et al., 2004; Vanstone et al., 2008). The application of moderate levels of P fertiliser (20–30 kg ha^{-1}) reduced population densities of *P. neglectus* in wheat (Vanstone et al., 2002), while in plum rootstocks, the application of P reduced the population densities of *P. vulnus* (Pinochet et al., 1998). The application of Zn also reduced the population densities of the root-knot nematode *M. javanica* in tomato. While the mechanism for this reduction remains unknown, the authors hypothesized that Zn may alter the physiology of the plant and also reduce penetration of the nematode into the roots (Siddiqui et al., 2002). Due to low P and Zn levels in vertisols of the Australian sub-tropical grain region, fertilisers including P and Zn are often applied prior to planting (Bell et al., 2010; Dalal et al., 1991). Both P and Zn are poorly mobile in the soil due to sorption to colloids while *P. thornei* can be found at depth in the soil profile (Owen et al., 2014). The magnitude of the effect of application of these fertilizers on reducing population densities of *P. thornei* in mung bean cultivated under field conditions may differ from that demonstrated in these pot experiments, however further investigation is warranted.

Mycorrhizal colonisation could be affected by *P. thornei* infestation in the roots of mung bean as both organisms inhabit the root cortex and migration by the nematode through the root cortical cells could destroy cells for arbuscular colonisation. *Pratylenchus thornei* had no effect on the level of mycorrhizal colonisation in mung bean in my experiments which indicated that there was no direct competition for feeding sites or photosynthates between these two organisms, a result which is in agreement with Pinochet et al. (1996). Improved plant nutrition or increased root biomass was not the cause of increased population densities of *P. thornei* in mung bean, raising the question of what was the cause of increased reproduction in *P. thornei*. The effect of modifications to root exudation and the alterations to the plants

defence system may yet play a role in explaining increased population densities of *P. thornei* in mycorrhizal roots of mung bean.

Experiments carried out *in vitro* with banana demonstrated that exudates from mycorrhizal roots reduced the penetration and attraction of the migratory nematode *Radopholus similis* (Vos et al., 2012c). It is therefore feasible that mycorrhizal inoculation could alter root exudation concentrations to increase the numbers of nematode penetrating the root. In wheat, population densities of *P. neglectus* increased further on inoculation with soil-borne wheat pathogens including *Rhizoctonia solani, Pythium irregulare*, *F. equiseti, Microdochium bolleyi* and *Gaeumannomyces graminis* which the authors attributed to pathogen infection rendering the root more favourable to nematode multiplication (Taheri et al., 1994). Doncaster (1971) reported that migratory endoparasitic nematodes *Ditylenchus destructor* and *D. dipsaci* also feed on fungal hyphae of *Chaetomium indicum* and *Botrytis cinera*. Therefore, it is plausible that *Pratylenchus* sp. may use the organic contents of fungal hyphae or the lipid filled mycorrhizal spores and vesicles as an additional food source.

Root colonisation with AMF may alter the concentrations of phytohormones that modify plant response to nematode infestation. In leek, concentration of the phytohormone indole acetic acid (IAA) increased after inoculation with AMF (Torelli et al., 2000). Increased IAA concentrations were observed in wheat genotypes susceptible to *P. thornei* and IAA may serve as an attractant for the nematode, promoting infestation (Rahaman et al., 2021). In a metabolomic study in wheat, AMF improved plant nutrition but increased the population densities of *P. neglectus*, due to a reduction in plant defence metabolites (Frew et al., 2018). Future research utilising multi-omics approaches including, but not limited to, studies on genomics, transcriptomics, proteomics and metabolomics may prove more fruitful in elucidating the causes of these increases in nematode population densities in mung bean.

5.3 The effects of *P. thornei* infestation on mung bean growth and nutrition

As both AMF and *P. thornei* depend on the plant host for photosynthates, it was also hypothesized that interactions between these organisms could alter the nutrient uptake, biomass and yield of mycorrhizal mung bean. There was no reduction in mung bean

shoot or root biomass observed when plants were inoculated with *P. thornei* alone. Interestingly, significant increases in biomass were observed in plants with added *P. thornei*, AMF and rhizobia, compared to AMF and rhizobia alone. Stimulations to plant biomass have been observed on inoculation with plant-parasitic nematodes including the pin nematode (*Paratylenchus* sp.) and the cyst nematode (*Heterodera schachtii*) (Wallace, 1971), and it was proposed that increased shoot biomass was due to increased lateral root formation under nematode infestation. This stimulation in root biomass and lateral root formation could be explained by increased leakage of photoassimilates as a result of herbivory impacting the availability of nutrients for the plant. Gebremikael et al. (2016) found that herbivory by nematodes increased root exudation, resulting in increased microbial densities and more diverse community composition in the rhizosphere. The improved microbial biomass resulted in an increased abundance of microbivorous nematodes and increased soil organic matter, with the subsequent effect of increasing nutrients available for plant uptake in a 'priming effect' (Kuzyakov et al., 2000).

With respect to the hypothesis that *P. thornei* could alter mung bean nutrition, there was no reduction in nutrient concentration observed under infestation by *P. thornei* alone. However, when co-inoculated with both AMF and rhizobia, *P. thornei* significantly reduced the nutrient concentrations of P, Zn, K, Mg, Ca and B and significantly increased the concentration of N in shoots of mung bean. In the experiment presented in Chapter 4, there was increased biomass in plants inoculated with AMF, rhizobia and *P. thornei* compared with plants inoculated with AMF and rhizobia. This effect may have reduced nutrient concentrations in the mung bean shoots due to a growth and dilution effect (Jarrell & Beverly, 1981). However, the greater reductions of Zn concentration in the shoot could not be explained by growth and dilution alone. This reduction could be related to decreased efficiency of roots infested with *P. thornei* which limited their uptake of the uptake of the poorly mobile Zn from the bulk soil. Following years of intensive cropping, vertisols in the Australian sub-tropical grain region are typically low in P and Zn (Dalal et al., 1991), and further reductions in nutrient concentration caused by *P. thornei* infestation may have negative repercussions on plant nutrition, biomass and yield of mung bean.

5.4 The interactions between AMF and rhizobia and their effects on nodulation, nitrogen fixation, biomass and yield in mung bean

Biological N fixation (BNF) can reduce the reliance on N fertilisation and improve soil N budgets, not only for the legume crop but also for subsequent crops in the sequence. Increasing the efficiency of N fixation by rhizobia, can reduce the reliance on exogenous N fertiliser application and improve the long-term sustainability of agricultural systems (Stagnari et al., 2019). One of the constraints to mung bean production in the Australian sub-tropical grain region is a failure to nodulate despite inoculation with the commercially available strain of *Bradyrhizobium* CB1015. A lack of efficient nodulation causes reduced seed yield, biomass and biological nitrogen fixation (BNF) leading to a net deficit in the soil N budget (Herridge et al., 2005). It was hypothesized in this thesis that a cause of nodulation failure in mung bean could be explained by poor root colonisation with the beneficial soil-borne AMF and/or by the invasion of the root-lesion nematode *P. thornei.*

The experiment reported in Chapter 3 demonstrated that co-inoculation with AMF and *Bradyrhizobium* in glasshouse experiments in a vertisol resulted in a highly significant synergistic effect, greatly increasing nodulation, BNF, plant nutrition, shoot biomass and seed yield of mung bean. The research provided strong evidence of the significant effect mycorrhizal fungi have on increasing nodulation rates and BNF efficiency in mung bean by rhizobia, which contributes to our understanding of one of the potential causes of nodulation failure in mung bean. It was hypothesized that AMF and rhizobia would interact positively to benefit mung bean productivity and nutrition. The positive synergistic effect between AMF and rhizobia that was demonstrated highlights the benefit of co-inoculation with both symbionts, which has practical implications for improving mung bean yields.

5.5 The interactions between *P. thornei* and rhizobia and their effects on nodulation and nitrogen fixation

With respect to the hypothesis that *P. thornei* could affect nodulation in mung bean, in the experiment in Chapter 3, it was demonstrated that *P. thornei* significantly

reduced nodule number/plant at six weeks compared to rhizobia inoculated alone. In Chapter 4, the addition of *P. thornei* to the treatment combinations of AMF, nitrogen fertiliser and rhizobia reduced fixed N and % Ndfa, compared to the combination of AMF, nitrogen fertiliser and rhizobia in the absence of *P. thornei*.

Increased N concentration in mung bean shoots was observed following *P. thornei* infestation, which could contribute to explaining the reduction in nodulation and BNF observed. The production of nodules and the process of N fixation is an energy intensive process, with a high photosynthetic cost to the plant. It has been estimated that up to 14% of photosynthetic C is consumed in the symbiosis with rhizobia (Kaschuk et al., 2010). When legumes are supplied with sufficient N, it is more efficient, in terms of photosynthetic expenditure, to utilize the available N present in the soil compared to utilizing the costly N fixation by rhizobia in root nodules. Consequently, N fixation in legumes can be decreased where the soil contains high quantities of available N (Hardarson et al., 1984).

Pratylenchus spp. can alter the concentration of N in the shoots of plants, with both increased (Gilarte et al., 2020) and decreased concentration of N reported (Thompson & Clewett, 2021). Feeding by plant-parasitic nematodes including *Pratylenchus* sp. may cause roots to leak photo-assimilates high in C that can increase microbial activity in soil. This increased microbial activity combined with a reduction in N uptake due to reduce extraction efficiency of the damaged roots can alter soil N dynamics and increase net N mineralisation. (Gebremikael et al., 2016; Tu et al., 2003). Increased N concentrations in the shoots of oats and chickpea following infestation by *P. thornei* have been demonstrated, although nodule number in chickpea increased with no change to N fixation efficiency (Gilarte et al., 2020). Increased nodule number in soybean infested with *P. penetrans* has been reported, although N fixation was reduced (Hussey & Barker, 1976). This research highlights the effects of *P. thornei* have on altering N concentrations in the plant shoots, which result in negative impacts for nodulation and N fixation in mung bean.

5.6 The contributions of AMF and rhizobia compared to the application of fertilisers for improving mung bean biomass, yield, nodulation, N fixation, and nutrition of mung bean

Facilitated by the full factorial designed experiment, the relative contribution of AMF to improvements in biomass and yield was assessed when mung bean was supplied with alternative sources of N—either via the symbiotic relationship with rhizobia or via fertiliser N application. The interaction between AMF and rhizobia resulted in greater increases in yield and biomass compared to the interaction between AMF and N. It was demonstrated that rhizobia significantly increased shoot biomass and yield compared to the addition of fertiliser N, which was likely due to improved nodulation and BNF by rhizobia increasing N concentration in the shoot greater than the application of fertiliser N.

Additionally, it was investigated if AMF could improve the concentration of P and Zn in the shoot to a level comparable to the application of fertiliser P and Zn. The results demonstrated that inoculation with AMF resulted in a highly significant increase in the concentration of P and Zn in the shoots of mung bean. Furthermore, the concentration of both P and Zn within plant shoots was significantly greater from inoculation with AMF, than from the addition of fertiliser P and Zn. Analysis of the nutrient concentrations in the shoots provided the first evidence for increased concentration of Cu in shoots of mung bean by AMF in a vertisol. Reports of Cu deficiency in vertisols in the sub-tropical grain region of eastern Australia have been linked to reduced biomass and yield in wheat (Grundon, 1980). In legumes, Cu is essential to enzyme function, nodulation and N fixation (O'Hara, 2001) and contributes to positive effects on biomass and yield (Seliga, 1998). Increased Cu uptake by AMF has been reported (Smith & Read, 2008) and it is likely that the increased concentration of Cu observed in mycorrhizal mung bean may have contributed to the improved nodulation, N fixation, biomass and yield observed in this research.

The effects of increased P in mung bean shoots are likely to be explained by mycorrhizal Pi transporters which facilitate the transfer of inorganic phosphate (Pi) via the intraradical hyphae and directly into the arbuscule where it can be exchanged

across the peri-arbuscular membrane. The mycorrhizal pathway for Pi uptake can be a more efficient method of acquisition of P, compared to the slower diffusion into the roots across the P deficient gradient in the rhizosphere (Smith et al., 2011). Additionally, nodules require high concentrations of P for efficient symbiosis (Simpson & Burris, 1984). It was hypothesized that AMF could improve biological N fixation by rhizobia, to a level comparable to the application of fertiliser P. The results of the experiments presented in Chapter 4 demonstrated that AMF supplied additional P to the plant, which could then be accessed by the bacteroids in the nodules, further improving the rate of biological N fixation, and increasing N supply to the plant. Moreover, inoculation of AMF significantly increased nodulation and BNF greater than fertilisation of P. This is a noteworthy contribution to research on AMF and rhizobia interactions, as it has demonstrated that inoculation with AMF is as effective, as fertiliser P for efficient biological N fixation. Phosphorus is a finite resource with an estimated depletion in 50 to 100 years (Cordell & White, 2014), and a global phosphorus crisis has been predicted if this resource is not efficiently managed (Vance et al., 2003). It has been estimated that only 10–45% of P fertiliser is taken up by crops (Adesemoye & Kloeppe, 2009). By demonstrating the improved P acquisition by AMF resulting in improved biological N fixation in mung bean, this research contributes further evidence of the crucial role AMF contribute to sustainable agricultural production systems.

It is likely that the nutritional complementarity between both symbionts resulted in the great increases in biomass and seed yield observed in the research presented in this thesis. Interactions between AMF and rhizobia have been demonstrated in several grain legumes including chickpea, soybean, faba bean and mung bean resulting in increased yield, biomass, nodulation, BNF and crop nutrition (Chalk et., al 2006; Yasmeen et al., 2012). The interactions between AMF and rhizobia can be additive or synergistic and the research carried out in this thesis aimed to determine the direction and magnitude of the interactions between the symbionts in mung bean. In field experiments in Pakistan, inoculation with AMF and two strains of *Bradyrhizobium japonicum* indicated an additive effect on yield and a synergistic effect on crop nutrition of N and P in mung bean (Yasmeen et al., 2012). Synergism in this context, can be defined as an observed response from inoculating with both symbionts together that is greater than the sum of responses from each symbiont inoculated individually

(Greco, 1996). In the research in this thesis, synergistic effects between AMF and rhizobia on biomass, yield, nodulation, N fixation and nutrient uptake was demonstrated in Chapter 3 and additive effects on these variates were demonstrated in Chapter 4. A variation in the magnitude of the positive effects may be explained by a seasonal difference between the experiments. The first experiment was conducted in late spring and the subsequent experiment in late summer suggesting that light intensity and temperature (soil temperature was maintained at an average temperature of 22°C in spring and 24°C in summer) may influence biomass and yield by altering rates of photosynthesis in mung bean (Karim et al., 2003).

In a meta-analysis carried out of interactions between plant microbial symbionts by Larimer et al. (2010), interactions between AMF and rhizobia were deemed to be additive and not synergistic. The authors acknowledged limitations in their analysis that soil fertility, especially in relation to N and P levels, may have influenced the magnitude of the interaction, and they hypothesized that in less fertile soils, synergism would be expected. In the experiments on mung bean in the present research, the soil fertility levels with respect to levels of P and Zn, were higher than anticipated for typical vertisols in the sub-tropical grain region of Australia. Furthermore, mung bean cultivation in this region may also occur on other soil types including those from the soil Orders Chromosols and Sodosols (Isbell 1996). These soils are characteristically lower in fertility and have lower levels of P, Zn and Cu compared to Vertosols (Dalal et al., 1991; Isbell 1996). Nevertheless, results presented in Chapter 4 indicated the essential role that AMF play in increasing the concentrations of P and Zn in the plant shoots and the value of the symbiosis even at these higher soil nutrient levels to improving mung bean biomass and yield, which is likely to increase in soil with typically lower concentration of P (Hoeksema et al., 2010). While higher levels of P may suppress mycorrhization to varying levels depending on plant host and AMF species (Kobae, 2019), the rate of P fertiliser added to the soil in the present research did not decrease mycorrhizal colonisation in mung bean. Future research on the symbiosis of AMF and rhizobia under different rates of P and N, is also warranted to quantify the value of the microsymbionts to mung bean biomass and yield and correlate this value to the economic cost of the addition of fertilisers N, P and Zn as demonstrated by Seymour et al. (2019) in linseed.

Apart from nutritional complementarity, it is feasible that co-inoculation with both AMF and rhizobia resulted in modifications to the upregulation of genes involved in initiating and maintaining the symbioses. There are a number of genes conserved in the common symbiosis pathway of both symbionts (Genre & Russo, 2016; Oldroyd, 2013). Co-inoculation with *B. diazoefficiens* and AMF species *Gigaspora rosea* upregulated genes for nodulation and transport of nitrate and sugars, resulting in increased nodulation and biomass in soybean (Sakamoto et al., 2019). The upregulation of genes on co-inoculation with both symbionts may have increased the efficiency of the symbiosis.

5.7 Interactions between the symbionts AMF and rhizobia in the host

Research on the dynamic relationship between plant host and mycorrhizal symbiont has indicated that the control of the symbiosis is bi-directional and dictated by a 'biological market' for resources. In experiments carried out by Kiers et al. (2011) more host carbon was directed to mycorrhizal hyphae that supplied greater amounts of P to the roots of barrel medic (*M. truncatula*), and likewise, the mycorrhizal hyphae supplied more P to those roots that provided higher amounts of carbon compounds. In mycorrhizal barrel medic, the plant host may degrade arbuscules that do not supply sufficient P (Javot et al., 2007). Similarly, in the rhizobial symbiosis, the plant host can allocate photosynthates preferentially to nodules that are more productive with respect to N fixation, along with reducing nodulation in response to ample N supply from the soil (Kiers et al., 2003; Oono et al., 2011). The concept that the plant host can regulate both the mycorrhizal and rhizobial symbiosis, and impose host sanctions on those symbionts that do not provide sufficient benefit in the form of N or P to the plant, is of interest. This indicates that there is potential to select for mycorrhizal isolates and rhizobial strains that are deemed more 'cooperative' and select for a more efficient symbiosis.

Indigenous *Bradyrhizobium* strains obtained from soils to the north of the Australian sub-tropical grain region were as effective as the commercial strain CB1015 for nodulation, N fixation and biomass production of mung bean (Christopher et al., 2018). However, these indigenous strains may outcompete the commercial strain in

certain environments. It would be of interest to investigate further how AMF interacts with indigenous strains of *Bradyrhizobium,* and if the observed improvements to nodulation, N fixation, biomass and yield observed in the experiments carried out in this thesis, would be reproduced or even improved upon using these endemic strains.

Plant host regulatory processes termed 'autoregulation of the symbiosis', can control the density of colonisation of the symbionts, potentially due to competition for photosynthates (Vierheilig & Piché, 2002). Regulation of the symbiosis can also be modified by the symbionts themselves through the systemic regulatory mechanisms of the plant host (Catford et al., 2003). In the experiments on mung bean in the present research, inoculation with rhizobia significantly reduced the proportion of root length colonised with AMF indicating that plant host regulatory mechanisms could be involved in reducing mycorrhizal colonisation. Interestingly, significant increases in the proportion of mycorrhizal root length containing vesicles were observed on inoculation with AMF and rhizobia. As vesicles function as storage organs for the fungus (Smith & Read, 2008), it is possible that competition for host photosynthates between AMF and rhizobia resulted in AMF moving into a resource conservation phase in response to the reduction of mycorrhizal colonisation as a result of inoculation with rhizobia. It may be of interest to determine the influence of plant host regulatory processes under harsher environmental conditions when carbon may be more limited, for example under lower light conditions, or water stress, on both rhizobial and mycorrhizal colonisation.

5.8 Summary of the multipartite interactions on mung bean biomass, yield, nodulation, N fixation, and nutrition

Inoculation with AMF and rhizobia resulted in a synergistic effect improving nodulation, N fixation, nutrition, biomass and yield in mung bean. AMF improved the concentrations of P and Zn greater than, or equal to, fertilisation with P and Zn, and this increased concentration of P resulted in improved N fixation in mycorrhizal mung bean. Inoculation with rhizobia improved biomass, yield, nodulation, and N concentration greater than fertilisation with N. Infestation with *P. thornei* reduced nodulation and N fixation, potentially by increasing the concentration of N in the plant

shoots, which may have resulted in a stimulation to shoot biomass at early stages of plant growth. Infestation with *P. thornei* also resulted in a reduction in the concentrations of P and Zn in the shoot which may have impacted mung bean production resulting in nutrient deficiencies.

From investigations in this thesis, it is likely that nodulation failure reported in mung bean in the subtropical grain region could be explained by a lack of mycorrhizal inoculum in the soil. The magnitude of improvement in nodulation levels and BNF efficiency rates was greater in plants inoculated with AMF compared to the magnitude of the reduction observed following inoculation with *P. thornei.* Results from this research contribute towards understanding the complex multipartite interactions that occur within the roots of plant hosts which impact biomass, nutrition and yield.

5.9 The effects of agricultural practices on mycorrhizal inoculum and population densities of *P. thornei* in the soils of the sub-tropical grain region

The strategies of crop rotations and breeding programmes currently in place for management of *P. thornei,* may also offer growers opportunities to increase mycorrhizal inoculum levels in the rotation. In the Australian sub-tropical grain region, a diverse range of crops are resistant to *P. thornei* include sorghum, linseed, sunflower, pigeon pea, millets (*Setaria italica*, *Echinochloa* spp*., Pennisetum* spp. and *Panicum* spp.) and cotton (Owen et al., 2014; Owen et al., 2015; Thompson et al., 1994). Furthermore, these species may also increase AMF inoculum density in the soil for subsequent crops (Thompson, 1994). However, crop rotations carried out with the intention of returning an economic gain, along with manipulating the populations of AMF inoculum levels while reducing population densities of *P. thornei* can only be achieved when there is sufficient moisture in the soil profile.

In the sub-tropical grain region of eastern Australia, crop cultivation, particularly for winter crops, is dependent on moisture stored in the soil profile after the summer-dominant rainfall events (Webb et al., 1997). Weed-free fallows are used to increase plant available water (PAW) in the profile, accumulate soil nitrate and can also contribute to reductions in the population densities of *P. thornei,* which improves yields for subsequent crops in the rotation (Owen et al., 2014; Thompson et al., 2021).

Stubble retention of crop residues are also promoted to increase water infiltration, reduce water run-off and soil erosion improving the PAW (Thomas et al., 2007). However, prolonged weed-free fallows reduce mycorrhizal inoculum in the soil resulting in reduced nutritional and yield benefits for crops with high mycorrhizal dependency known as Long Fallow Disorder (Thompson et al., 1987; Thompson et al., 2013). Mycorrhizal inoculum in vertisols of the sub-tropical grain region can be in the form of spores, hyphae or infected roots (Pattison & McGee et al., 1997). However, periodic rainfall in a long fallow may reduce infectivity of spores compared to rates of infection obtained in a drier long fallow (Pattison & McGee et al., 1997). Furthermore, the high proportion of clay particles in vertisols, may aid in the generation of biofilm mineral structures encapsulating the mycorrhizal spores and resulting in a protective barrier against desiccation (Cuadros, 2017). Nevertheless, after a long fallow, when spores are low, the cultivation of crops with a lower mycorrhizal dependency, such as wheat and barley, should be encouraged. These crops may improve mycorrhizal inoculum in the soil, benefiting the subsequent cultivation of crops with higher mycorrhizal dependency, including mung bean (Thompson, 1994).

Cover crops may play a role in low-input sustainable agricultural system where stubble density is low, for example after cultivation of a chickpea crop, and can further improve mycorrhizal inoculum for subsequent crops in the rotation (Bowles et al., 2017). Cover crops were reported to increase soil microbial diversity and biomass, improve mycorrhizal colonisation and enhance P nutrient cycling efficiency resulting in benefits to crop P nutrition and yield (Hallama et al., 2019). When crop cultivation is dependent on sufficient PAW in the soil profile the benefit of cultivating cover crops needs to be counter-balanced with the disadvantage of water extracted from the soil profile which would otherwise contribute towards improving yields of subsequent crops in the rotation. In the sub-tropical grain region of eastern Australia, investigations using French millet (*Panicum miliaceum*) as a cover crop improved water infiltration, soil biological activity and yield of wheat that followed in the rotation. Whish et al. (2009) reported that removing the French millet cover crop just before flowering, resulted in benefits of improved water infiltration and reduced soil erosion, with no reduction in PAW or negative impacts to subsequent wheat yield. However, if the millet cover crop was grown to maturity, the yield of the subsequent

wheat crop was negatively impacted due to reduced PAW. Short-term cover crops grown into longer fallows in a dry season resulted in no net deficit in PAW compared to those grown in a shorter fallow, which incurred a penalty in PAW and a reduction in yield of subsequent wheat (Erbacher et al., 2020).

The impact on cultivating a cover crop for a short duration on mycorrhizal inoculum should also be taken into account. Mycorrhizal fungi have the capacity to propagate from hyphae, vesicles and spores, however propagation and colonisation of a plant host species may be dependent on the source of mycorrhizal inoculum, with reports of successful colonisation from spores, and not hyphae, in the AMF genera *Gigaspora* and *Scutellospora* (Klironomos & Hart, 2002). Cultivation of a short-term cover crop may therefore have an impact on the diversity of AMF species found in the cropping soil. Choice of cover crop may also influence the colonisation of subsequent crops with species identity being more important than species diversity in impacting the percentage mycorrhizal colonisation (Njeru et al., 2014). Targeted research is required to investigate the impact of cover cropping on mycorrhizal inoculum levels and on the population densities of plant parasitic nematodes in the soil, along with optimisation of management of PAW to benefit subsequent crops in the sequence.

Plant breeding has historically focused on traits such as biomass, yield and disease resistance with little focus on below ground associations with mycorrhiza and other beneficial microorganisms which influence these traits. Under continual selection of breeding lines grown under high soil P, plant breeders may inadvertently select for varieties of wheat with a reduced dependency on the mycorrhizal symbiosis (Hetrick et al., 1993). As a result, concerns have been raised regarding the effects of plant breeding on the mycorrhizal dependency of the crop, though it has been argued that modern plant varieties still retain the capacity to form associations with mycorrhiza (Lehmann et al., 2012). Long term sustainable agricultural systems will require plant varieties that have the capacity to thrive under more adverse climactic conditions, with reduced fertiliser and pesticide application. To that end, plant breeders have been considering improvement of mycorrhizal dependency, to take advantage of the benefits of the association including drought resistance, phosphorus acquisition, resistance to pathogens, resilience to abiotic stressors and improved yield (Jacott et al., 2017). Low-input selective breeding has improved the mycorrhizal dependency of switch grass (*Panicum virgatum*) and improved biomass production (Cobb et al.,

2021). The authors argue that breeding for mycorrhizal reliance may reduce dependence on fertilisers resulting in cultivars that are more effective in sustainable agricultural systems.

Recent investigations on crop rotations reported that the rotational sequence of sorghum/fallow/mung bean/wheat/fallow/chickpea with four crops in three years was advocated as being optimal at the cropping system level for revenue throughout the sub-tropical grain region of eastern Australia (Hochmann et al., 2020). However, the majority of these crops are hosts to *P. thornei*, and their cultivation will increase population densities of *P. thornei* in the soil. Furthermore, the consequences of AMF further increasing *P. thornei* population densities in the roots of mung bean, greater than anticipated through cultivation of mung bean with no AMF, may impact crop choice, and extend the duration of cultivation of non-hosts in the rotation to reduce *P. thornei* population densities. Increased population densities as a result of mycorrhizal inoculation may have implications in how resistance and tolerance to *P. thornei* is assessed in mung bean germplasm both in glasshouse and field trials.

From the systematic review undertaken in Chapter 2, variation was recorded between plant varieties in their response to mycorrhizal inoculation for plant biomass, mycorrhizal colonisation and changes in population densities of *Pratylenchus* species. The research presented in this thesis was conducted with mung bean cultivar Jade-AU and *F. mosseae*–an isolate species of AMF with a global distribution. Subsequent research has confirmed increased *P. thornei* multiplication from inoculation with the same isolate of *F. mosseae* used in this thesis in the majority of six other cultivars of mung bean. (J Sheedy, University of Southern Queensland, personal communication, June 2021). Soils under mung bean production in the sub-tropical grain region are likely to contain AMF species of great phylogenetic diversity, with a range of functional traits including root colonisation efficiency, P uptake, effect on plant biomass, and effect on biotic stressors including plant-parasitic nematodes. Agricultural practices including fertilisation and tillage may reduce the abundance and diversity of AMF species compared to undisturbed soils (Jansa et al., 2002; Williams et al., 2017) and abiotic stressors including drought, application of fungicides, and increased salinity may result in an increased number of species from the family Glomeraceae (Lenoir et al., 2016). A meta-analysis carried out by Yang et al. (2017) found that while family richness increased plant performance, alterations to nematode

population densities or plant growth under nematode infestation did not vary significantly between AMF families. Nevertheless, further investigations are required to determine the interactions between AMF species that are both taxonomically and functionally diverse and their impact on population changes in *Pratylenchus* sp. and level of synergism when combined with *Bradyrhizobium*. Furthermore, it is unknown how AMF may impact the biomass, yield or the population densities of *P. thornei* in other economically important crops used in rotational sequences in the region and future research in this direction is strongly advocated.

6. Conclusion

Research undertaken in the course of this thesis has contributed towards our understanding of the multipartite interactions that occur among microbiota in the roots of mung bean. Interactions between the beneficial arbuscular mycorrhizal fungi and rhizobia, and the plant-parasitic nematode *P. thornei,* link below and above-ground processes including nutrient flow, primary production and reproduction, which impact plant nutrition, biomass, and yield in mung bean. The problem of nodulation failure reported in the subtropical grain region in mung bean could be explained by a lack of mycorrhizal inoculum in the soil. Through increased P and Zn uptake, and improvement of biological nitrogen efficiency mediated by a synergistic effect with *Bradyrhizobium*, arbuscular mycorrhizal fungi have a demonstrable intrinsic value in contributing to long-term sustainable agriculture, reducing the reliance on excessive fertilisers, while maintaining yields in mung bean. Sustainable agriculture will require promotion of the benefits that AMF offer to mung bean production and the active conservation of AMF inoculum levels. However, it is crucial to recognise and acknowledge that AMF may increase population densities of *P. thornei* in the roots of economically important crops, which will have implications for breeding programmes and for management of the nematode in the soils of the sub-tropical grain region of eastern Australia.

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APPENDIX A

Due to size restrictions, the supplementary material for Appendix A of the PhD thesis entitled **"**The interaction between arbuscular mycorrhizal fungi, rhizobia and root-lesion nematodes (*Pratylenchus thornei*) in mung bean (*Vigna radiata*)" by Elaine C Gough, and Chapter 2 entitled "A systematic review of the effects of arbuscular mycorrhizal fungi on root-lesion nematodes *Pratylenchus* spp." published in *Frontiers in Plant Science* 11:923 is too large to be added to Appendix A.

The Supplementary material can be accessed at:
https://zenodo.org/record/5139664#.YP_u9OgzY2w

The Supplementary material can also be found online at:
https://www.frontiersin.org/articles/10.3389/fpls.2020.00923/full#supplementary-material

APPENDIX B

REPRODUCTION OF ELECTRONIC SUPPLEMENTARY INFORMATION OF CHAPTER 3

Article title

Arbuscular mycorrhizal fungi acted synergistically with *Bradyrhizobium* sp. to improve nodulation, nitrogen fixation, plant growth and seed yield of mung bean (*Vigna radiata*) but increased the population density of the root-lesion nematode *Pratylenchus thornei*

Journal

Plant and Soil (Springer Nature, The Netherlands)

Authors

Elaine C. Gough, Kirsty J. Owen, Rebecca S. Zwart, John P. Thompson

Affiliations

Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD 4350, Australia

Supplementary Figure

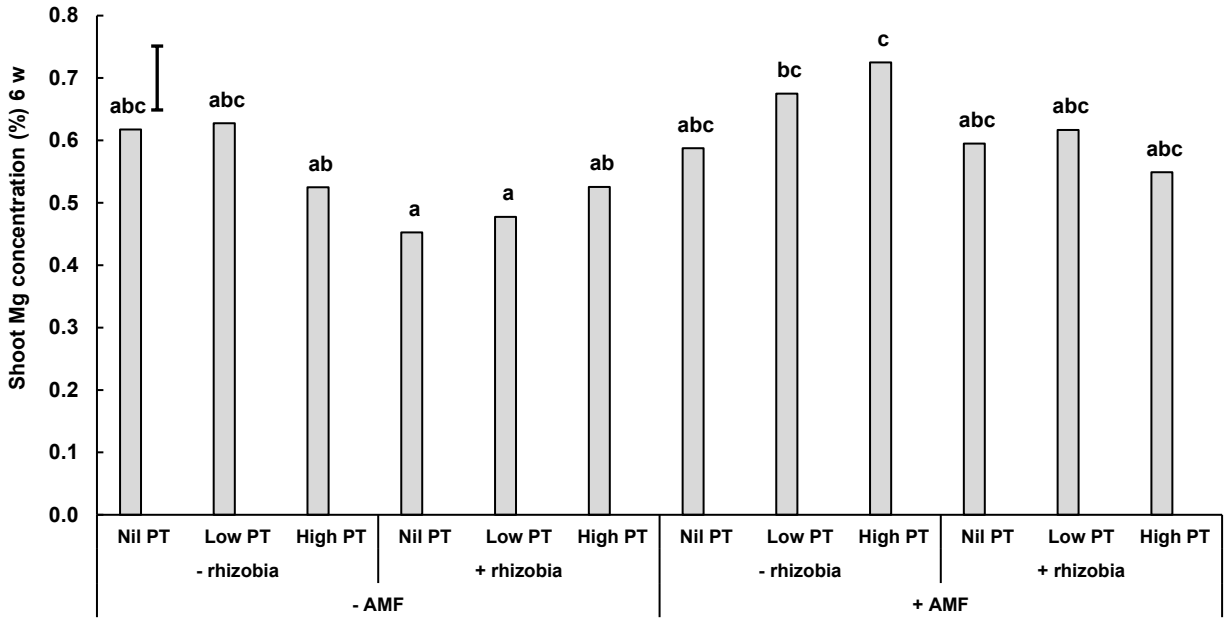


Fig. S1. The interactive effects of arbuscular mycorrhizal fungi (AMF) x rhizobia x *Pratylenchus thornei* (PT) on the concentration of Mg per plant (%) in mung bean at 6 weeks (w). Different letters above each bar indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction of AMF x *P. thornei*. The vertical bar represents the standard error of difference (s.e.d).

Supplementary Tables

Supplementary Table S1. Probabilities of *F* values from factorial analysis of variance of plant parameters at 6 and 12 weeks after sowing. All biomasses are dry weights expressed on a per plant basis. Highlighted in bold are significant interactions from the highest factorial orders.

Inoculation treatments	6 weeks				12 weeks				
	Shoot biomass	Root biomass	Nodule numbers	Nodule biomass	Shoot biomass	Root biomass	Seed yield	Nodule numbers	Nodule biomass
Rhizobia	0.059	0.062	**0.008**	0.006	0.004	0.724	0.002	0.005	0.616
AMF	<.001	0.547	0.071	<.001	<.001	**0.038**	<.001	<.001	<.001
P. thornei	0.456	0.183	**0.025**	0.208	0.532	0.284	0.569	0.523	0.208
AMF x *P. thornei*	0.345	0.834	0.971	0.847	**0.010**	0.751	**0.016**	0.118	0.847
AMF x rhizobia	**0.009**	0.962	0.153	**0.001**	**<.001**	0.091	**<.001**	**<.001**	**0.001**
P. thornei x rhizobia	0.933	0.513	0.081	0.440	0.154	0.692	0.815	0.247	0.440
AMF X *P. thornei* x rhizobia	0.677	0.330	0.786	0.624	0.768	0.655	0.727	0.333	0.624

Supplementary Table S2. Probabilities of *F* values from factorial analysis of variance of AMF root colonisation and *Pratylenchus thornei* population density at 6 and 12 weeks after sowing. RL= root length, PT=*P. thornei*/kg $\log_e$ transformed. Highlighted in bold are significant interactions from the higher factorial orders.

	6 weeks after sowing				12 weeks after sowing				
Inoculation treatments	Arc sin RL colonised	RL colonised with AMF (cm/plant)	RL (cm/ plant)	PT ln(x+1)	RL colonised (arcsine)	RL colonised with AMF (cm/plant)	RL (cm/ plant)	Vesicle Intensity	PT ln(x+1)
Rhizobia	0.451	0.64	0.318	0.643	0.007	0.166	0.378	0.075	0.714
AMF	**<.001**	**<.001**	0.845	0.662	<.001	**<.001**	0.998	<.001	**0.039**
P. thornei	0.246	0.14	0.679	**<.001**	0.089	0.052	0.657	0.662	**<.001**
AMF x *P. thornei*	0.204	0.085	0.154	0.864	0.933	0.273	0.148	**<.001**	0.521
AMF x rhizobia	0.653	0.666	0.713	0.778	**0.015**	0.845	0.222	0.645	0.897
P. thornei x rhizobia	0.117	0.639	0.539	0.572	0.438	0.568	0.493	0.135	0.994
AMF X *P. thornei* x rhizobia	0.224	0.861	0.765	0.397	0.752	0.905	0.767	0.155	0.989

Supplementary Table S3. Table of correlation coefficients (r values) with probability (*P*) between *Pratylenchus thornei* final population density as ln(*P .thornei*/kg soil and roots +1) and plant parameters including nutrient concentrations and uptakes in the tops at 6 and 12 weeks for low and high initial inoculation rates of *P. thornei* takes. Highlighted in bold are the significant values where *n*=16. NA=not available.

	6 weeks				12 weeks			
	Low *P. thornei*		High *P. thornei*		Low *P. thornei*		High *P. thornei*	
Plant parameters	r	*P*	r	*P*	r	*P*	r	*P*
Root length colonised (arcsine)	0.44	0.098	0.22	0.445	0.36	0.186	**0.84**	**<0.001**
Weight of mycorrhizal root (g)	0.39	0.152	0.42	0.103	0.32	0.250	**0.53**	**0.041**
Length of mycorrhizal root (cm)	0.4432	0.0980	0.286	0.3206	-0.1158	0.6810	0.4432	0.0980
Total root length (cm)	-0.3870	0.1541	0.269	0.352	0.3108	0.2595	-0.3870	0.1541
Shoot biomass/plant (g)	0.32	0.244	**0.75**	**0.002**	0.21	0.585	0.36	0.165
Nodule biomass/plant (g)	0.11	0.703	0.28	0.340	-0.11	0.942	0.34	0.209
Nodules/plant	0.19	0.504	0.27	0.344	0.13	0.702	0.30	0.262
Root biomass/plant (g)	0.16	0.566	0.38	0.176	0.38	0.154	0.29	0.282
Seed weight/plant (g)	NA	NA	NA	NA	0.07	0.167	0.41	0.113

Nutrients	Concentration		Uptake		Concentration		Uptake		Concentration		Uptake		Concentration		Uptake	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
B	0.31	0.269	0.36	0.184	-0.21	0.466	**0.70**	**0.005**	-0.28	0.488	0.20	0.477	-0.05	0.846	0.39	0.138
Ca	-0.22	0.432	0.31	0.267	-0.21	0.467	**0.77**	**0.001**	-0.33	0.305	0.09	0.760	0.21	0.431	0.43	0.095
Cu	0.05	0.859	0.28	0.318	0.30	0.299	**0.71**	**0.004**	0.16	0.235	0.30	0.280	**0.59**	**0.023**	**0.55**	**0.028**
Fe	-0.14	0.617	0.32	0.244	-0.06	0.835	0.34	0.228	0.01	0.568	0.12	0.669	-0.44	0.090	-0.40	0.125
Mg	0.12	0.675	0.34	0.217	-0.22	0.448	**0.73**	**0.003**	-0.12	0.984	0.18	0.525	0.40	0.129	0.44	0.088
Mn	-0.34	0.209	0.14	0.610	0.33	0.251	**0.66**	**0.011**	-0.16	0.677	0.22	0.428	0.02	0.928	0.44	0.086
Mo	-0.46	0.086	-0.28	0.306	**-0.65**	**0.012**	**0.66**	**0.010**	-0.41	0.562	-0.03	0.904	-0.22	0.413	**0.51**	**0.043**
N	0.02	0.932	0.29	0.292	-0.06	0.832	**0.61**	**0.021**	-0.02	0.129	0.25	0.374	-0.25	0.347	0.36	0.169
P	0.43	0.113	0.38	0.157	0.26	0.377	**0.62**	**0.018**	0.15	0.648	0.29	0.292	**0.59**	**0.020**	**0.64**	**0.007**
K	0.36	0.183	0.36	0.190	0.20	0.487	**0.68**	**0.007**	-0.39	0.455	0.14	0.612	0.34	0.200	0.40	0.126
S	0.30	0.279	0.38	0.162	-0.32	0.260	**0.72**	**0.003**	0.02	0.804	0.26	0.348	0.38	0.148	**0.58**	**0.019**
Zn	0.02	0.933	0.20	0.468	0.38	0.182	**0.67**	**0.008**	0.27	0.943	0.38	0.160	**0.59**	**0.021**	**0.65**	**0.007**

Supplementary Table S4. Probabilities of *F* values from factorial analysis of variance of N uptake, percentage of plant N derived from the atmosphere (% Ndfa) and biological N fixation to the plant 12 weeks after sowing. Highlighted in bold are significant interactions from the higher factorial order.

Inoculation treatments	N uptake (mg/plant)	% Ndfa	Fixed N (mg/plant)
Rhizobia	<0.001	0.003	<0.001
AMF	<0.001	0.001	<0.001
P. thornei	0.773	0.514	0.677
AMF x *P. thornei*	**0.029**	0.106	**0.048**
AMF x rhizobia	**<0.001**	**0.002**	**<0.001**
P. thornei x rhizobia	0.624	0.351	0.711
AMF X *P. thornei* x rhizobia	0.795	0.075	0.825

Supplementary Table S5. Probabilities of *F* values from factorial analysis of variance of plant nutrient concentrations in shoot biomass 6 and 12 weeks after sowing. Highlighted in bold are significant interactions from higher factorial orders.

	Concentrations (%)						Concentrations (mg/kg)					
6 weeks	N	P	K	Ca	Mg	S	Zn	B	Fe	Cu	Mn	Mo
Rhizobia	0.002	0.037	0.0200	**0.040**	0.022	**0.051**	0.526	0.85	0.869	0.471	0.638	0.041
AMF	0.113	<.001	0<.001	0.434	<.001	**<.001**	**0.011**	**<.001**	**0.05**	**<.001**	0.682	**<.001**
P. thornei	0.939	0.014	0.757	0.620	0.387	0.546	0.061	0.337	0.818	0.513	0.088	0.656
AMF x rhizobia	**0.004**	0.187	**0.002**	0.500	0.492	0.176	0.523	0.673	0.709	0.135	**0.002**	0.492
AMF x *P. thornei*	0.826	0.014	0.847	0.811	0.553	0.590	0.061	0.464	0.894	0.388	**0.042**	0.768
P. thornei x rhizobia	0.887	0.019	0.970	0.551	0.883	0.406	0.753	0.904	0.186	0.155	0.558	0.428
AMF x *P. thornei* x rhizobia	0.817	**0.007**	0.554	0.248	**0.006**	0.305	0.126	0.272	0.250	0.063	0.143	0.187
12 weeks												
Rhizobia	0.013	0.014	0.160	**0.001**	<.001	0.012	0.006	0.058	0.389	0.041	0.047	0.007
AMF	0.006	0<.001	0.451	0.562	0.035	0.425	<.001	0.868	0.308	<.001	0.005	<.001
P. thornei	0.323	0.610	0.758	0.909	0.570	0.173	0.798	0.163	0.395	0.097	0.553	0.978
AMF x rhizobia	0.664	**<.001**	**<.010**	0.674	0.014	<.001	**<.001**	0.009	0.377	**0.003**	0.934	**0.015**
AMF x *P. thornei*	**0.025**	0.123	0.379	0.439	0.118	0.191	0.199	0.703	0.358	0.486	0.035	0.084
P. thornei x rhizobia	0.799	0.862	0.109	0.165	0.279	0.283	0.580	0.030	0.392	0.190	0.002	0.567
AMF x *P. thornei* x rhizobia	0.122	0.239	0.207	0.056	**0.023**	**0.021**	0.098	**0.005**	0.363	0.103	**<.001**	0.478

Supplementary Table S6. Table of means of plant nutrient concentrations at 12 weeks after sowing for the highest factorial order of arbuscular mycorrhizal fungi (AMF) x *P. thornei* (PT) x rhizobia. Different letters after each figure indicate significant differences at $P<0.05$ for the interaction of AMF x *P. thornei* x rhizobia and probabilites of the *F* tests for factors are given in the last four rows of the table.

			Nutrient concentrations									
			6 weeks		12 weeks							
Inoculation Treatments			Mg %		Mg %		S %		B mg/kg		Mn mg/kg	
- AMF	- rhizobia	Nil PT	0.6175	abc	0.63	bcde	0.100	abc	25.3	ab	320	b
- AMF	- rhizobia	Low PT	0.6275	abc	0.49	abcde	0.098	ab	22.8	ab	193	ab
- AMF	- rhizobia	High PT	0.525	ab	0.47	abc	0.095	ab	21.3	ab	110	a
- AMF	+ rhizobia	Nil PT	0.4525	a	0.42	a	0.086	ab	21.3	ab	112	a
- AMF	+ rhizobia	Low PT	0.4775	a	0.42	a	0.095	ab	20.8	ab	131	a
- AMF	+ rhizobia	High PT	0.5253	ab	0.48	abcd	0.095	ab	26.5	b	203	ab
+ AMF	- rhizobia	Nil PT	0.5875	abc	0.58	abcde	0.098	ab	23.3	ab	127	a
+ AMF	- rhizobia	Low PT	0.675	bc	0.66	ce	0.108	bc	26.3	b	188	ab
+ AMF	- rhizobia	High PT	0.725	c	0.66	ce	0.138	c	26.3	b	177	a
+ AMF	+ rhizobia	Nil PT	0.595	abc	0.44	a	0.075	ab	22.0	ab	106	a
+ AMF	+ rhizobia	Low PT	0.6167	abc	0.39	a	0.063	a	19.0	a	77	a
+ AMF	+ rhizobia	High PT	0.5489	abc	0.46	ab	0.068	a	21.8	ab	125	a
			Probability of *F* value									
Rhizobia			0.022		<.001		0.012		0.058		0.047	
AMF			<.001		0.035		0.425		0.868		0.005	
P. thornei			0.387		0.57		0.173		0.163		0.553	
AMF x *P. thornei* x rhizobia			**0.006**		**0.023**		**0.021**		**0.005**		**<0.001**	

Supplementary Table S7. Table of means from factorial analysis of variance of plant nutrient concentrations at 12 weeks for the next highest factorial order of AMF x rhizobia. Different letters after each figure indicate significant differences at $P<0.05$ for the interaction of AMF x rhizobia and probabilites of the *F* tests for factors are given in the last four rows of the Table.

		Nutrient concentrations 12 weeks									
Inoculation treatments		P %		K %		Zn mg/kg		Cu mg/kg		Mo mg/kg	
- AMF	- rhizobia	0.19	a	1.41	ab	13.2	a	3.9	a	3.6	b
- AMF	+ rhizobia	0.15	a	1.34	ab	10.0	a	3.0	a	3.5	b
+ AMF	- rhizobia	0.34	b	1.34	a	31.5	b	5.8	b	2.8	b
+ AMF	+ rhizobia	0.16	a	1.46	b	12.8	a	3.4	a	1.3	a
		Probability of *F* value									
Rhizobia		0.014		0.16		0.006		0.041		0.007	
AMF		0<.001		0.451		<.001		<.001		<.001	
P. thornei		0.61		0.758		0.798		0.097		0.978	
AMF x rhizobia		**<.001**		**<.010**		**<.001**		**0.003**		**0.015**	

Supplementary Table S8. Probabilities of *F* values from factorial analysis of variance of plant nutrient uptakes in shoot biomass at 6 and 12 weeks after sowing. Highlighted in bold are significant interactions from the higher factorial order.

	Uptakes (mg/plant)						Uptakes (µg/plant)					
	N	P	K	Ca	Mg	S	Zn	B	Cu	Mn	Mo	Fe
6 weeks												
Rhizobia	0.034	0.111	0.024	0.125	0.113	0.061	0.630	0.029	0.108	0.288	0.075	0.130
AMF	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.983	0.895
P. thornei	0.829	0.821	0.696	0.805	0.959	0.445	0.347	0.892	0.820	0.698	0.378	0.804
AMF x rhizobia	**<.001**	**0.003**	**<.001**	**0.003**	**0.001**	**0.002**	**0.026**	**<.001**	**<.001**	**<.001**	0.381	0.778
AMF x *P. thornei*	0.727	0.985	0.550	0.269	0.273	0.547	0.176	0.396	0.875	0.151	0.086	0.804
P. thornei x rhizobia	0.736	0.624	0.712	0.821	0.589	0.464	0.538	0.679	0.595	0.621	0.652	0.320
AMF x *P. thornei* x rhizobia	0.638	0.608	0.848	0.837	0.729	0.563	0.865	0.738	0.426	0.797	0.857	0.226
12 weeks												
Rhizobia	<.001	0.027	0.002	0.004	0.012	0.009	0.043	0.008	0.006	0.056	0.025	0.210
AMF	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.842
P. thornei	0.799	0.476	0.341	0.790	0.292	0.531	0.141	0.264	0.099	0.420	0.500	0.437
AMF x rhizobia	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	0.880
AMF x *P. thornei*	**0.037**	0.224	**0.006**	0.082	**0.029**	**0.039**	0.086	**0.011**	**0.032**	**0.021**	0.754	0.322
P. thornei x rhizobia	0.492	0.149	0.294	0.105	**0.044**	0.439	**0.046**	**0.045**	0.099	**0.003**	0.437	0.371
AMF x *P. thornei* x rhizobia	0.720	0.715	0.984	0.861	0.376	0.852	0.420	0.491	0.877	0.150	0.814	0.337

APPENDIX C

REPRODUCTION OF ELECTRONIC SUPPLEMENTARY INFORMATION OF CHAPTER 4

Article title

The role of nutrients underlying interactions among root-nodule bacteria (*Bradyrhizobium* sp.), arbuscular mycorrhizal fungi (*Funneliformis mosseae*) and root-lesion nematodes (*Pratylenchus thornei*) in nitrogen fixation and growth of mung bean (*Vigna radiata*)

Journal

Submitted to *Plant and Soil* (Springer Nature, The Netherlands)

Authors

Elaine C. Gough, Kirsty J. Owen, Rebecca S. Zwart, John P. Thompson

Affiliations

Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD 4350, Australia

Supplementary Figures

Hierarchical classification

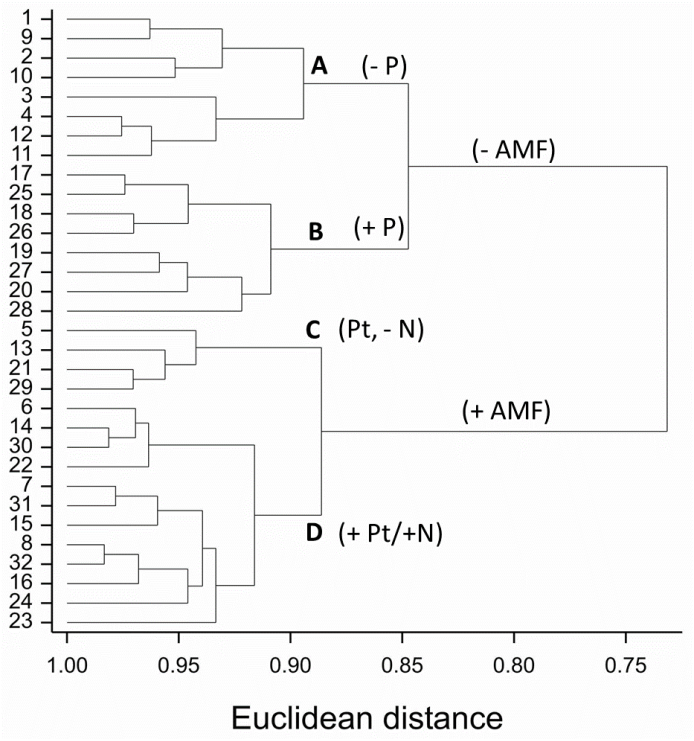


Fig. S1. Dendrogram from a hierarchical cluster analysis of the rhizobia inoculated data for 32 treatment combinations and 23 variates. Treatment codes from 1 to 32 on the Y axis represent the combination of the full factorial of treatments in rhizobia-inoculated plants (code numbers in Supplementary Table S1). Four groups (A to D) are delineated at Euclidean distance of 0.9. Treatment factors associated with major furcations in the dendrogram are indicated as ±AMF: arbuscular mycorrhizal fungi, ±P: phosphorus, ±Pt: *P. thornei* and, ±N: nitrogen. -N,-Pt indicates both absent while +N/+Pt indicates one or both present.

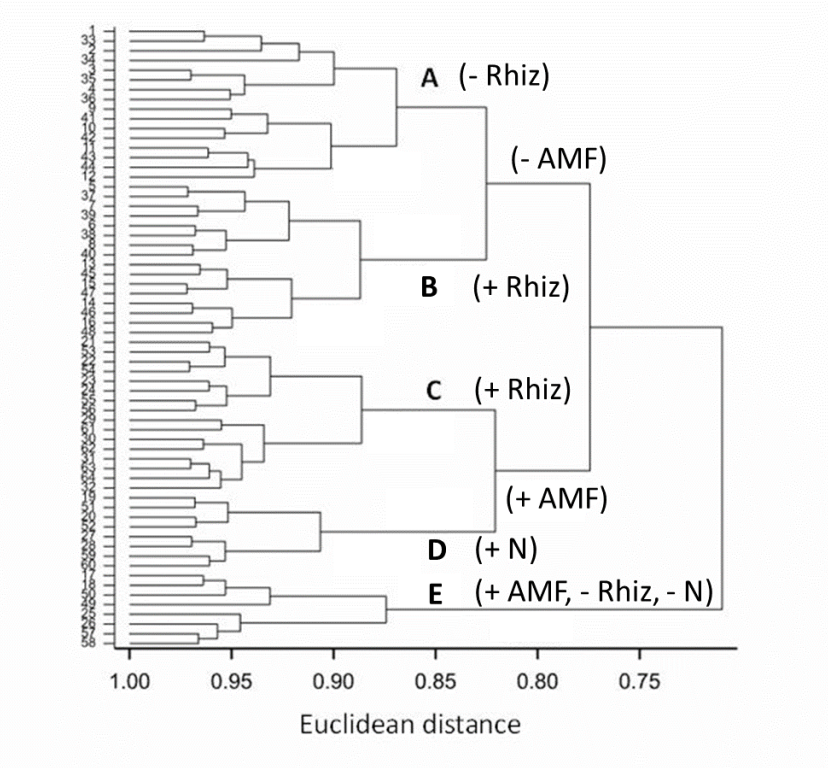
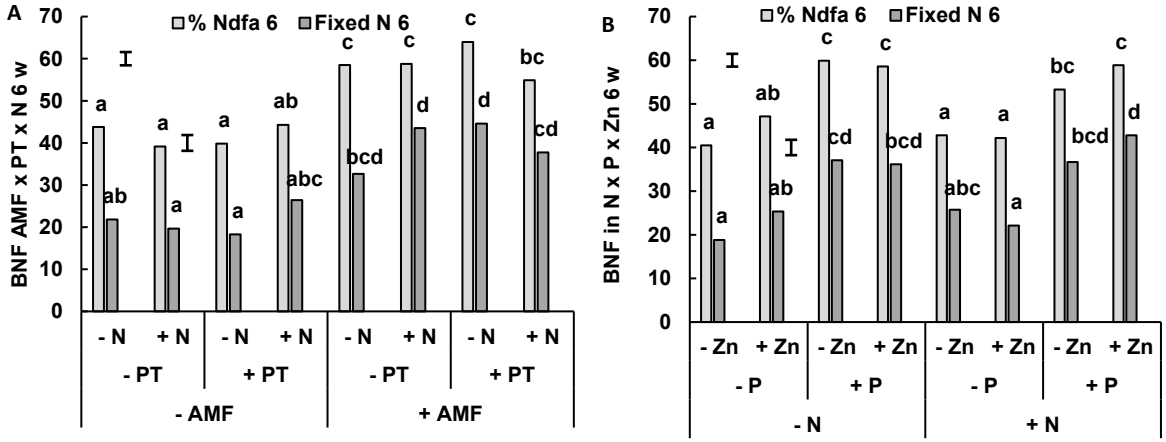


Fig. S2. Dendrogram from a hierarchical cluster analysis of the complete data for 64 treatment combinations and 20 variates set. Treatment codes from 1 to 64 on the Y axis represent the combination of the full factorial of treatments (code numbers in Supplementary Table S2). Five groups (A to E) are delineated at Euclidean distance of 0.85. Treatment factors associated with major furcations in the dendrogram are indicated as ±AMF: arbuscular mycorrhizal fungi, ±Rhiz: rhizobia: ±Pt: *Pratylenchus thornei*, or ±N: nitrogen

Biological Nitrogen Fixation 6 and 11 weeks



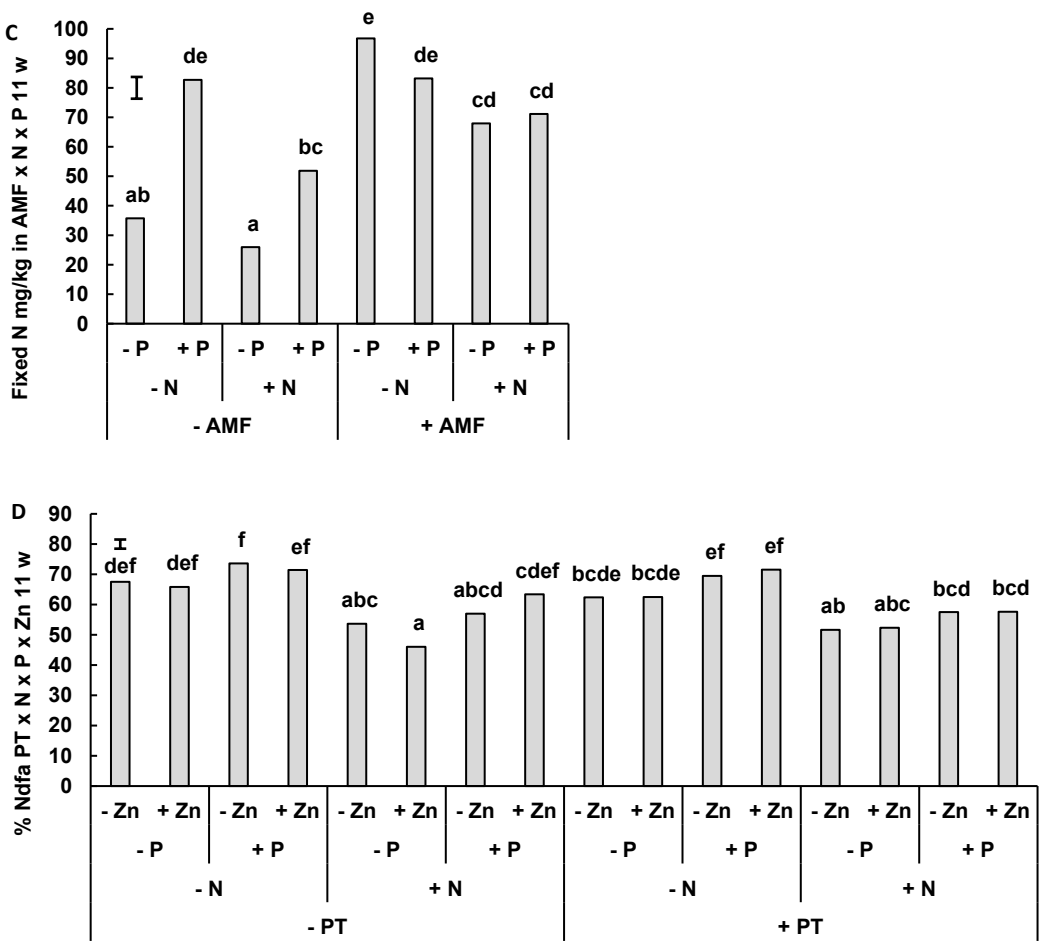


Fig. S3. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF), *Pratylenchus thornei* (PT) and N and, **(B)** N, P and Zn on % nitrogen derived from the atmosphere (% Ndfa) and fixed nitrogen (N) (mg/kg) at 6 weeks (w), **(C)** arbuscular mycorrhizal fungi (AMF), N and P on fixed N at 11 weeks and, **(D)** *Pratylenchus thornei* (PT), N, P, Zn on % nitrogen derived from the atmosphere (% Ndfa) at 11 weeks. All interactions are in rhizobia inoculated plants. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for each different interaction. The vertical bar represents the standard error of difference (s.e.d.).

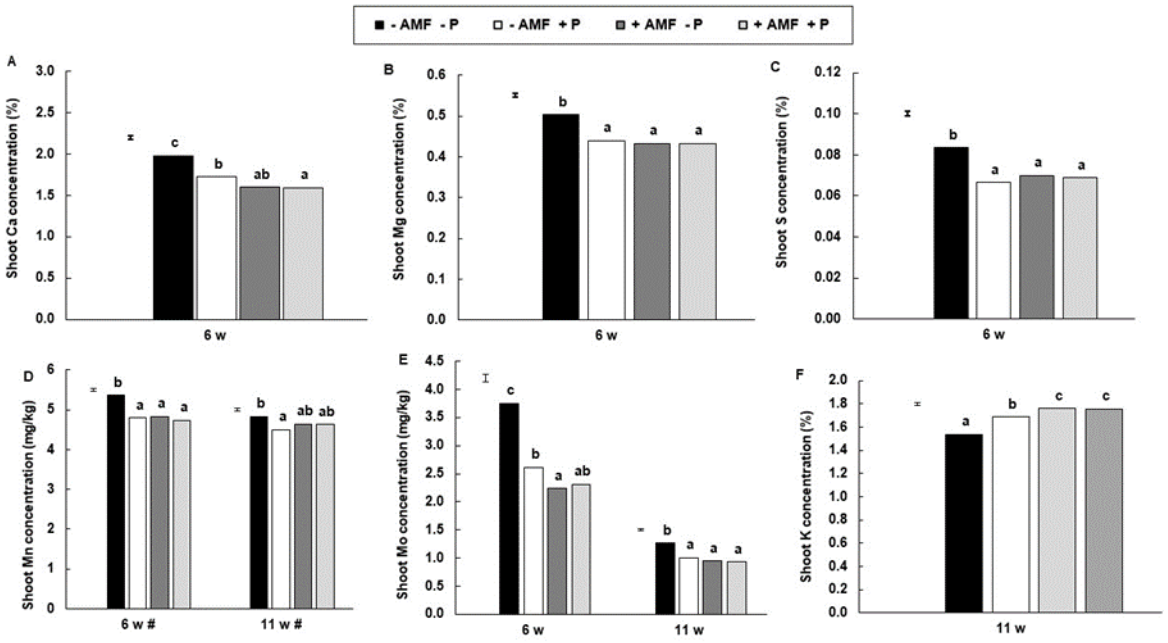


Fig. S4. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and phosphorus (P) in rhizobia inoculated plants on the shoot concentration at 6 and/or 11 weeks (w) of **(A)** Ca (%), **(B)** Mg (%), **(C)** S (%), **(D)** Mn (mg/kg), **(E)** Mo (mg/kg), **(F)** K (%) . The symbol # signifies a transformed data set. Different letters above each bar graph indicate significant differences for each nutrient according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction of AMF x phosphorus. The vertical bar represents the standard error of difference (s.e.d.).

Significant interactions between rhizobia and N in complete data set

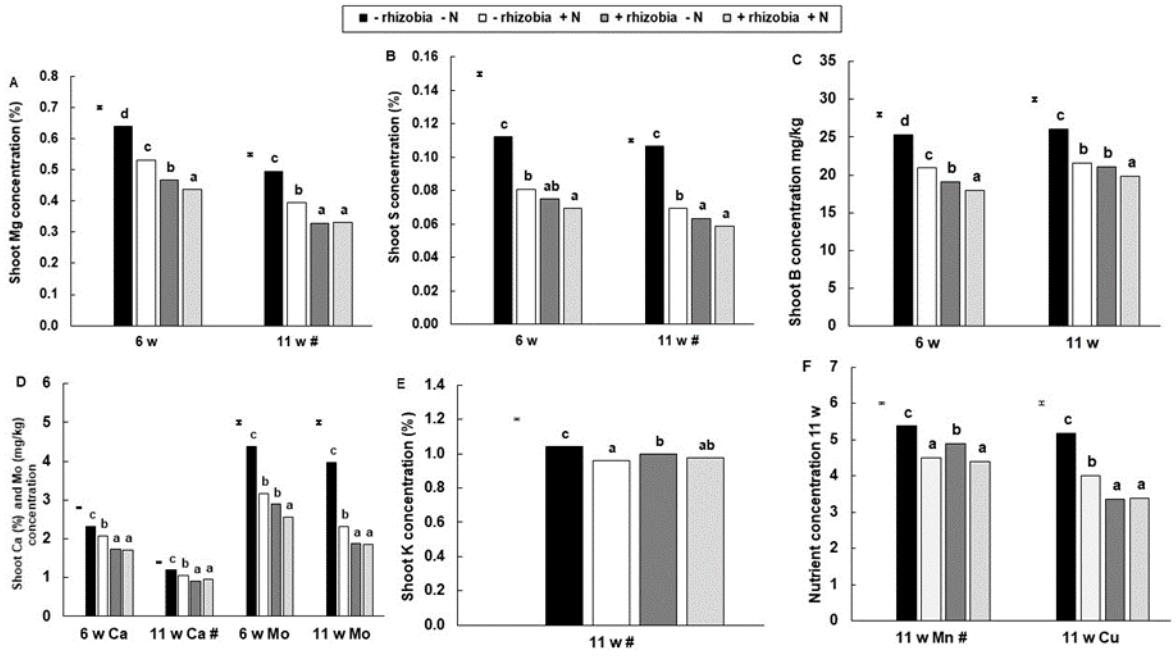


Fig. S5. The interactive effects of co-inoculation of rhizobia and N on the shoot concentration at 6 and/or 11 weeks (w) of **(A)** Mg (%), **(B)** S (%), **(C)** B (mg/kg) **(D)** Ca % and Mo (mg/kg), **(E)** K % and, **(F)** Mn and Cu (mg/kg). The symbol # signifies a transformed data set. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction of rhizobia x N. The vertical bar represents the standard error of difference (s.e.d.).

Significant interactions between AMF and rhizobia in complete data set

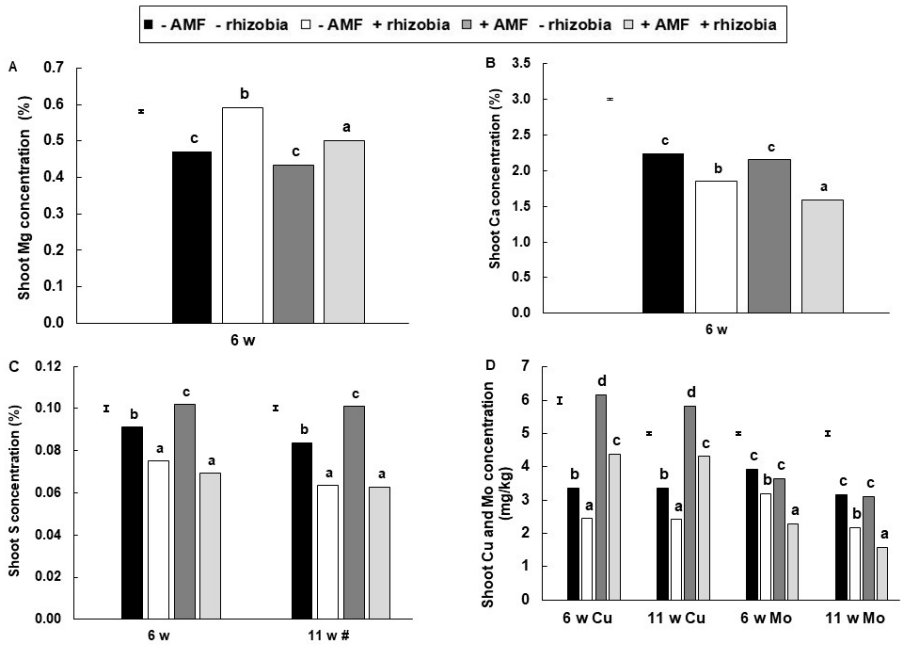


Fig. S6. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and rhizobia on the concentration of **(A)** Mg (%), **(B)** Ca % at in mung bean shoots at 6 weeks (w), **(C)** S (%) in mung bean shoots at 6 and 11 weeks and, **(D)** Cu and Mo (mg/kg) in mung bean shoots at 6 and 11 weeks. The symbol # signifies a transformed data set. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction of rhizobia x N. The vertical bar represents the standard error of difference (s.e.d.).

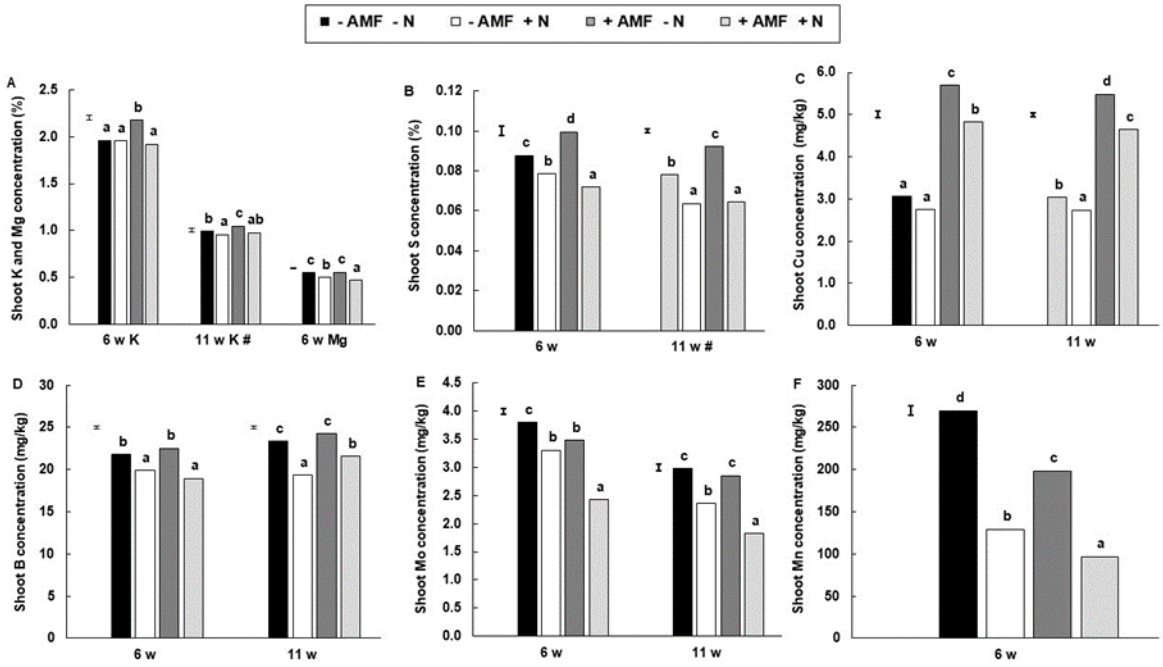


Fig. S7. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and N on the concentration of **(A)** K (%) and Mg (%), **(B)** S (%), **(C)** Cu (mg/kg), **(D)** B (mg/kg), **(E)** Mo (mg/kg) in mung bean shoots at 6 and 11 weeks (w) and **(F)** Mn (mg/kg) in mung bean shoots at 6 weeks. The symbol # signifies a transformed data set. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction of arbuscular mycorrhizal fungi (AMF) x N. The vertical bar represents the standard error of difference (s.e.d.).

Significant interactions between AMF, rhizobia, P. thornei*, N, P and Zn on plant parameters of shoot biomass, nodulation, and nutrient concentration in the shoots 6 and 11 weeks after sowing*

Biomass 6 weeks

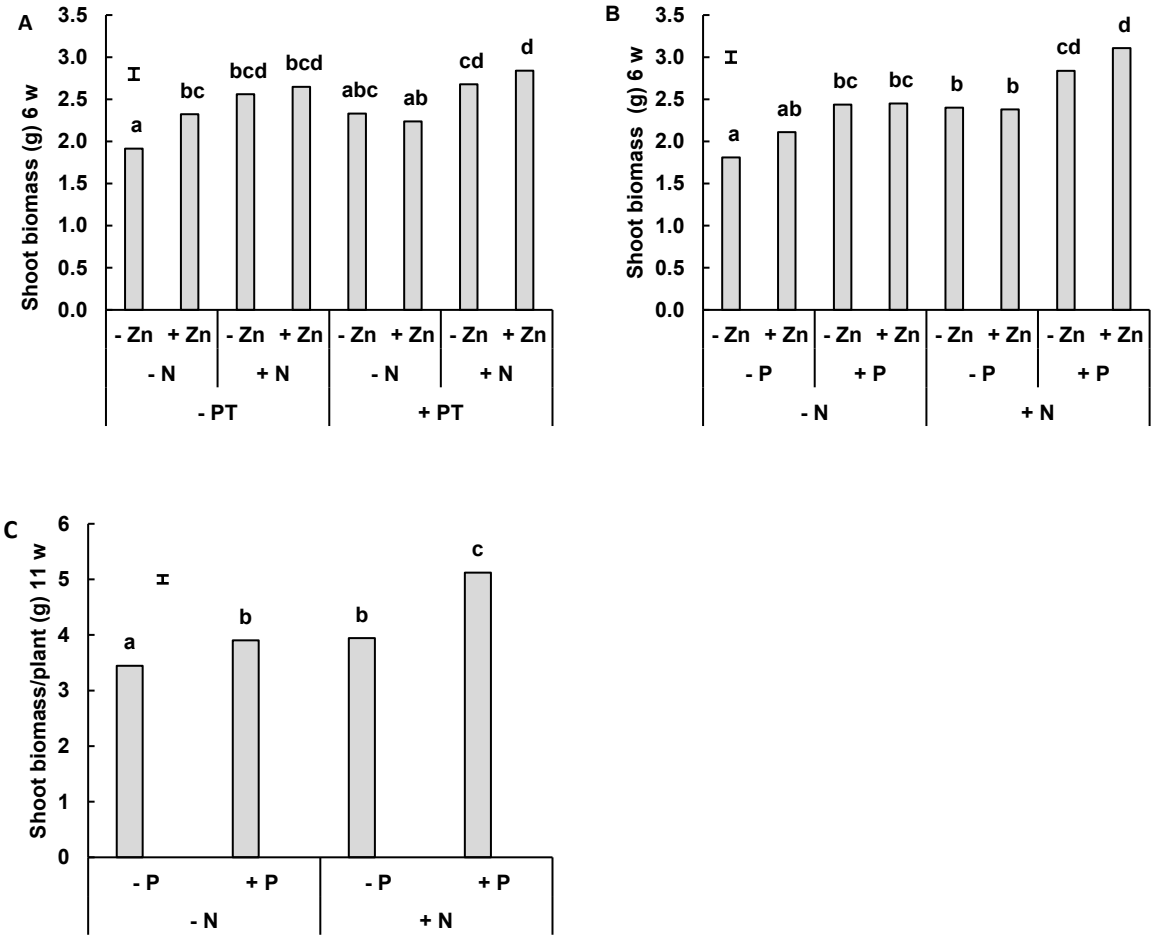


Fig. S8. The interactive effects of co-inoculation of **(A)** *Pratylenchus thornei* (PT) x N x Zn and **(B)** N x P x Zn and, **(C)** N x P on shoot biomass per plant of mung bean at 6 weeks (w). Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Nodulation 11 weeks

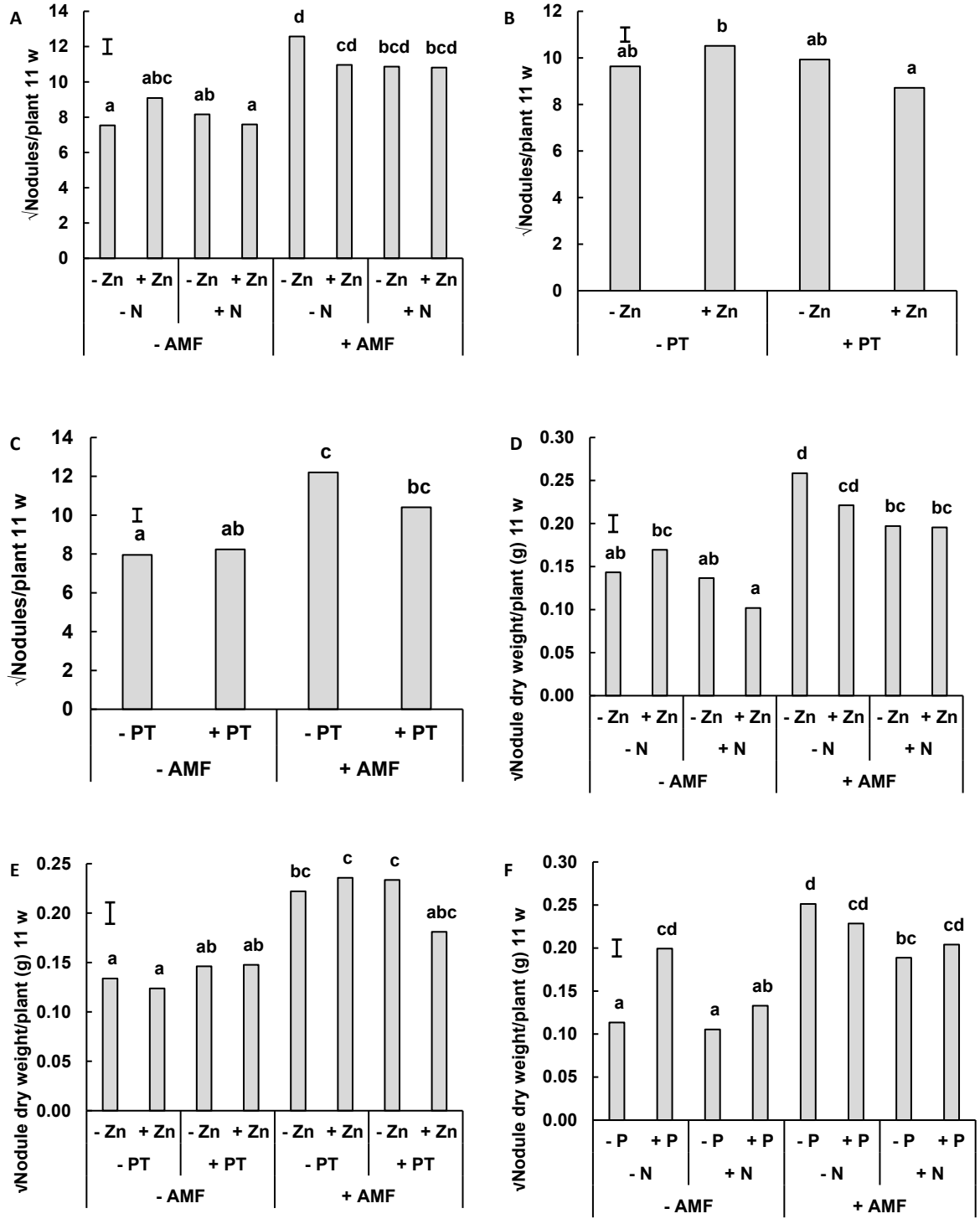


Fig. S9. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x N x Zn, **(B)** *Pratylenchus thornei* (PT) x Zn, **(C)** arbuscular mycorrhizal fungi (AMF) x *Pratylenchus thornei* (PT) on nodule

numbers per plant and, **(D)** arbuscular mycorrhizal fungi (AMF) x N x Zn, **(E)** arbuscular mycorrhizal fungi (AMF) x *Pratylenchus thornei* (PT) x Zn and, **(F)** arbuscular mycorrhizal fungi (AMF) x N x P on nodule dry weight per plant at 11 weeks (w). Nodulation data is square root transformed. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

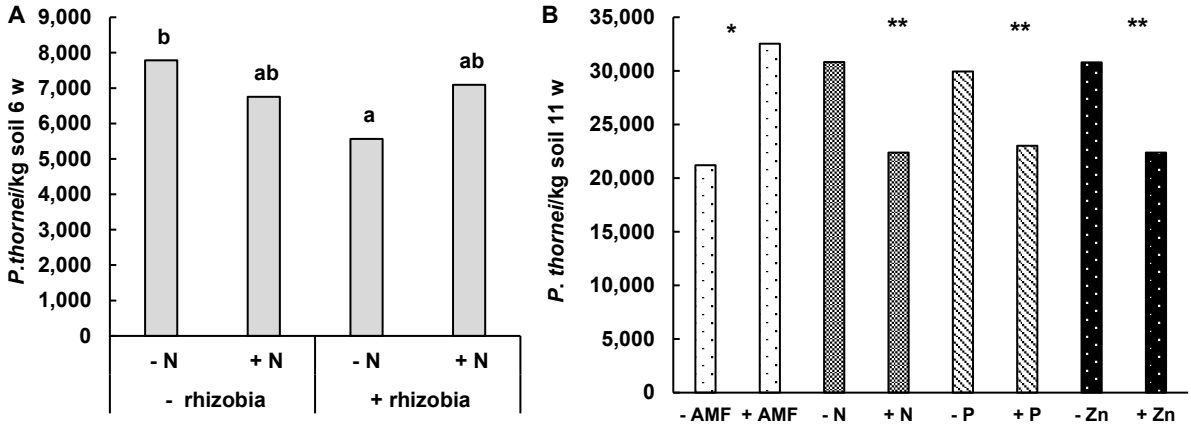
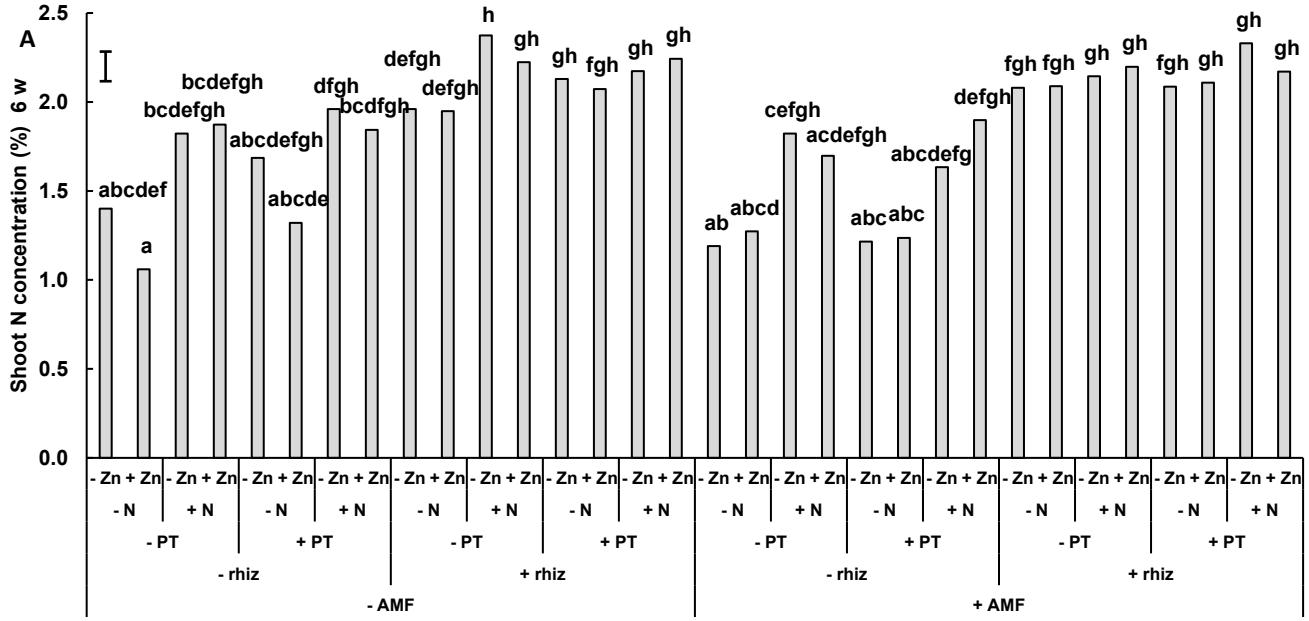
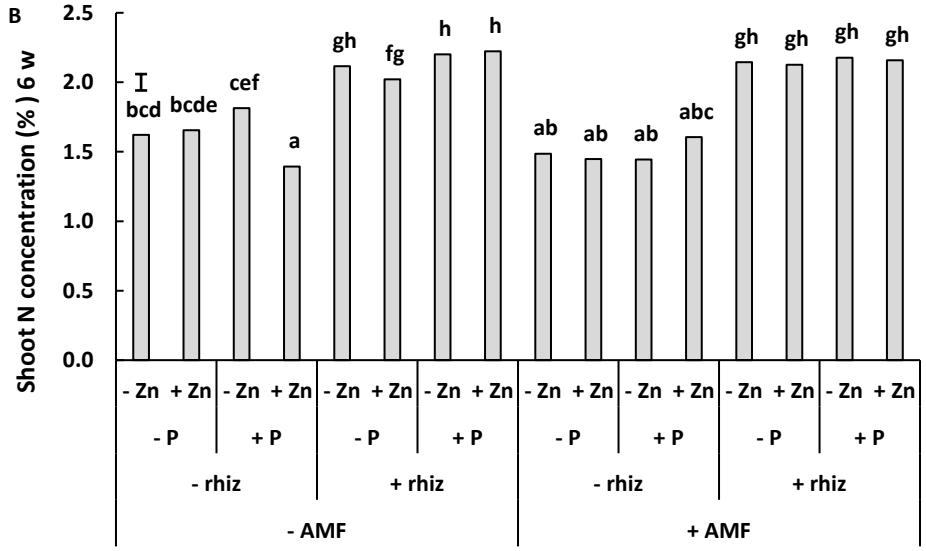


Fig. S10. The interactive effects of inoculation of **(A)** rhizobia and N on the population densities of *Pratylenchus thornei* in mung bean at 6 weeks after sowing and **(B)** the main effects of arbuscular mycorrhizal fungi (AMF) (-/+), nitrogen (N) (-/+), phosphorus (P) (-/+), and zinc (Zn) (-/+) on the population densities of *Pratylenchus thornei* in mung bean at 11 weeks after sowing. *Pratylenchus thornei* values expressed are back transformed means from ANOVA. * denotes significance levels at P<0.05, ** denotes significance at P<0.01.

N concentration in the shoot 6 weeks after sowing





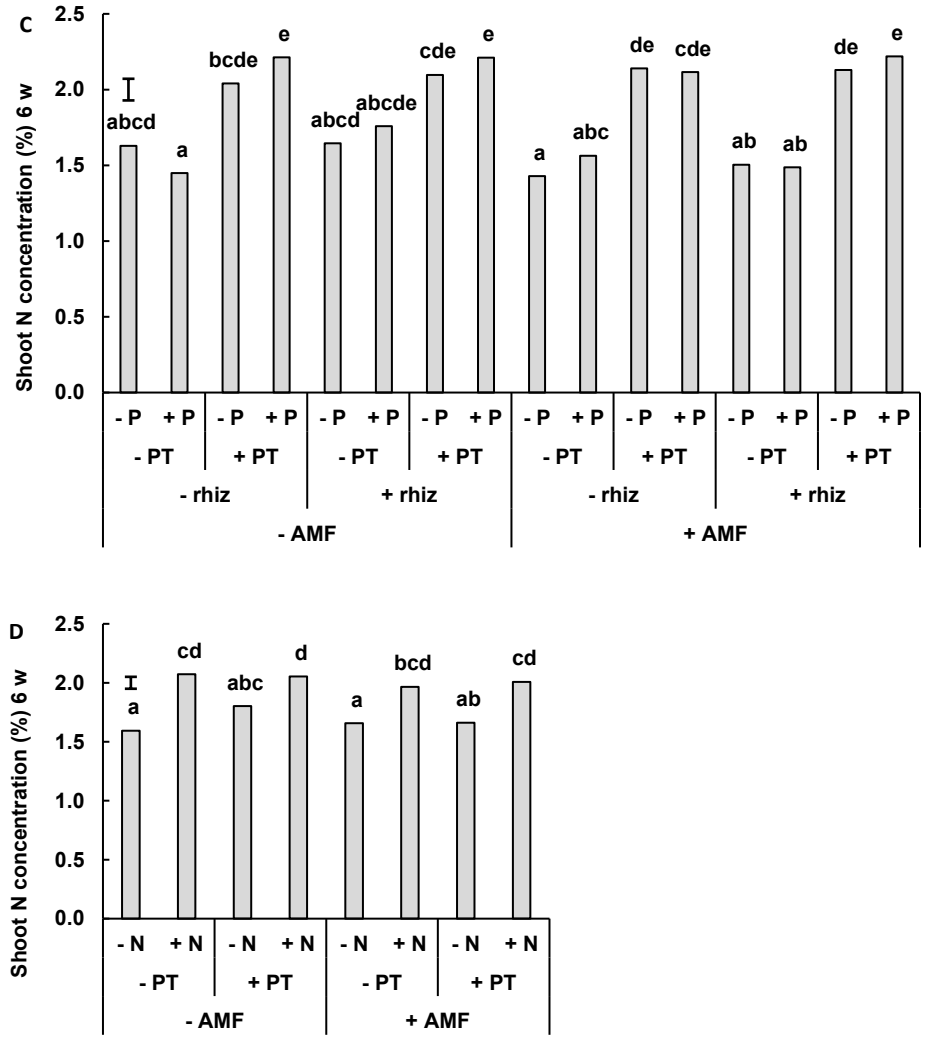


Fig. S11. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N x Zn, **(B)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P and Zn, **(C)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x P and, **(D)** arbuscular mycorrhizal fungi (AMF) x *Pratylenchus thornei* (PT) x N on N concentration (%) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

P concentration in the shoot 6 weeks after sowing

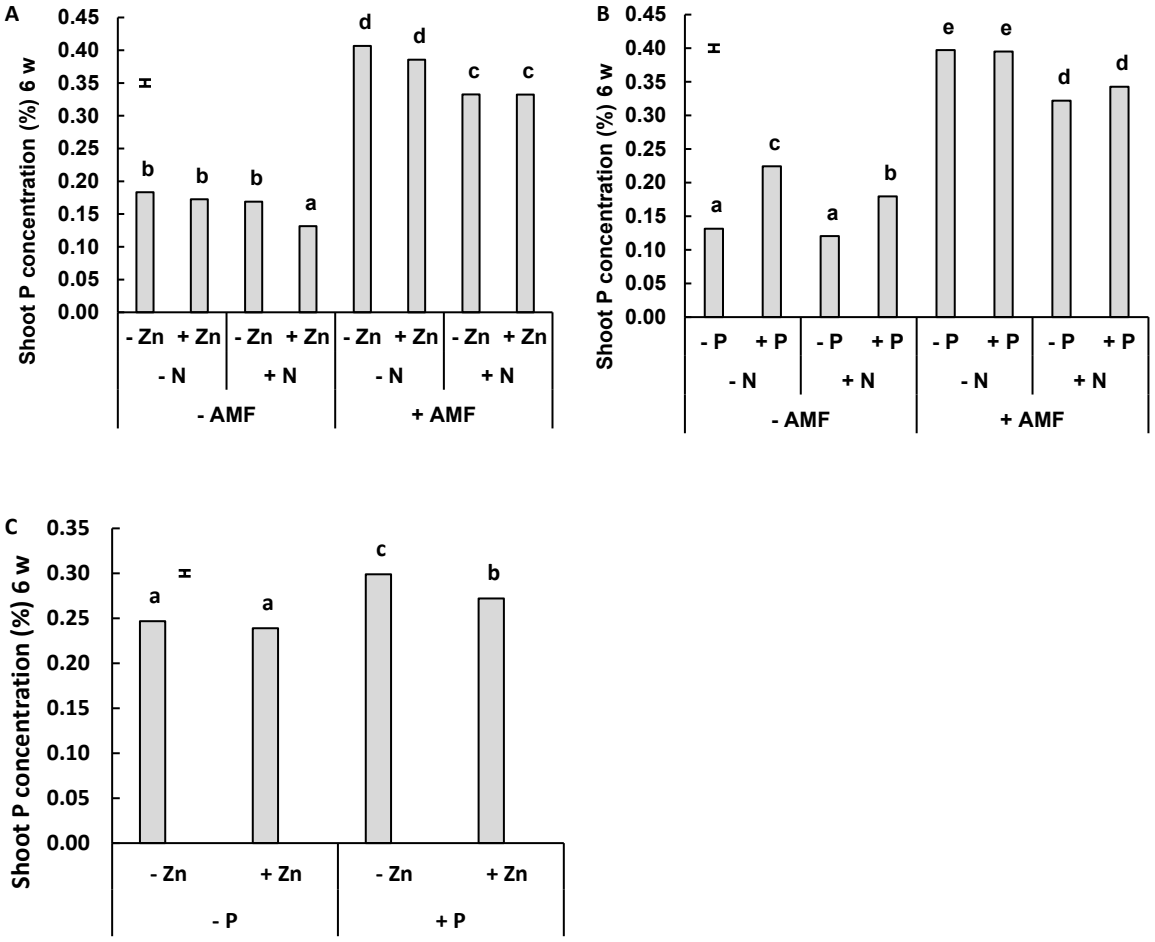


Fig. S12. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x N x Zn, **(B)** arbuscular mycorrhizal fungi (AMF) x N x P and, **(C)** P x Zn on P concentration (%) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

K concentration in the shoot 6 weeks after sowing

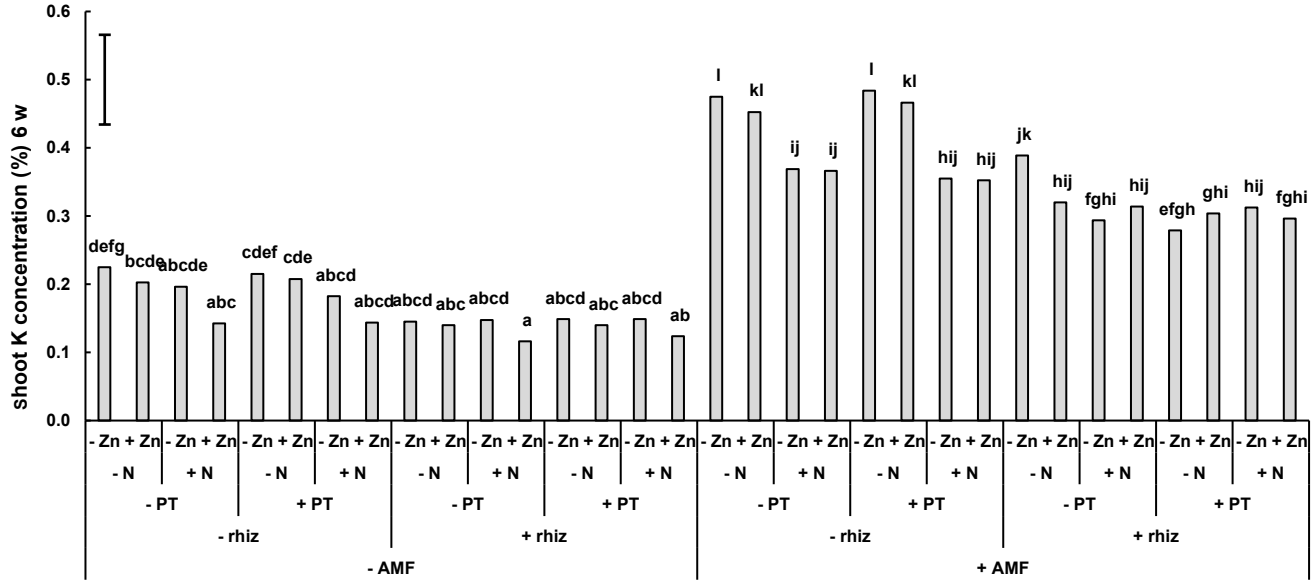
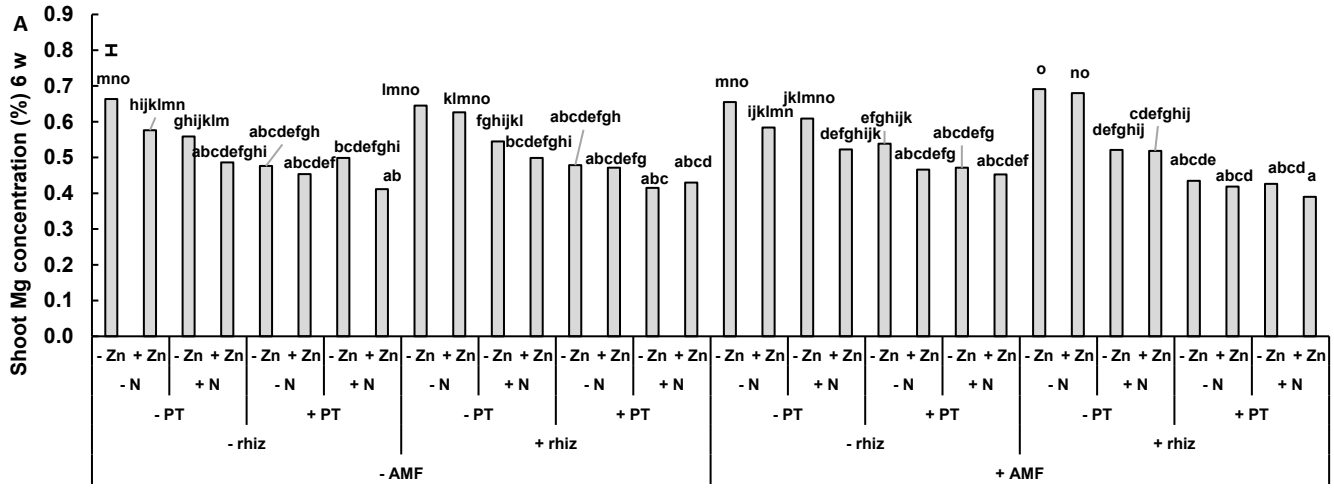


Fig. S13. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N x Zn on K concentration (%) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Mg concentration in the shoot 6 weeks after sowing



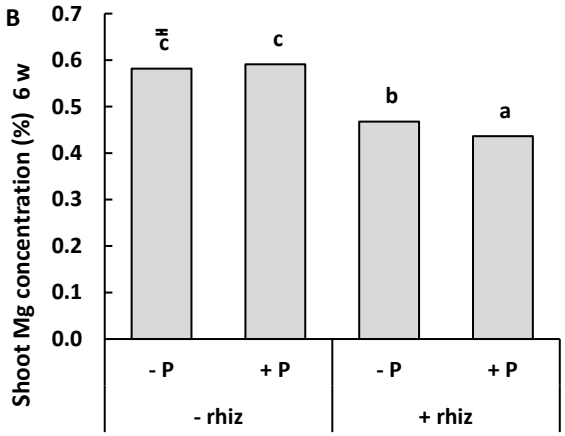
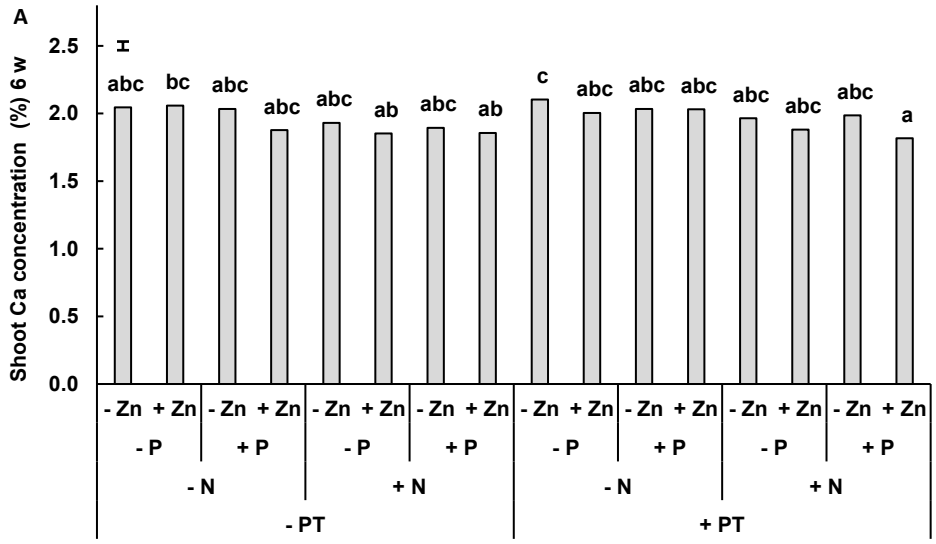


Fig. S14. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N x Zn and **(B)** rhizobia (rhiz) x P on Mg concentration (%) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Ca concentration in the shoot 6 weeks after sowing



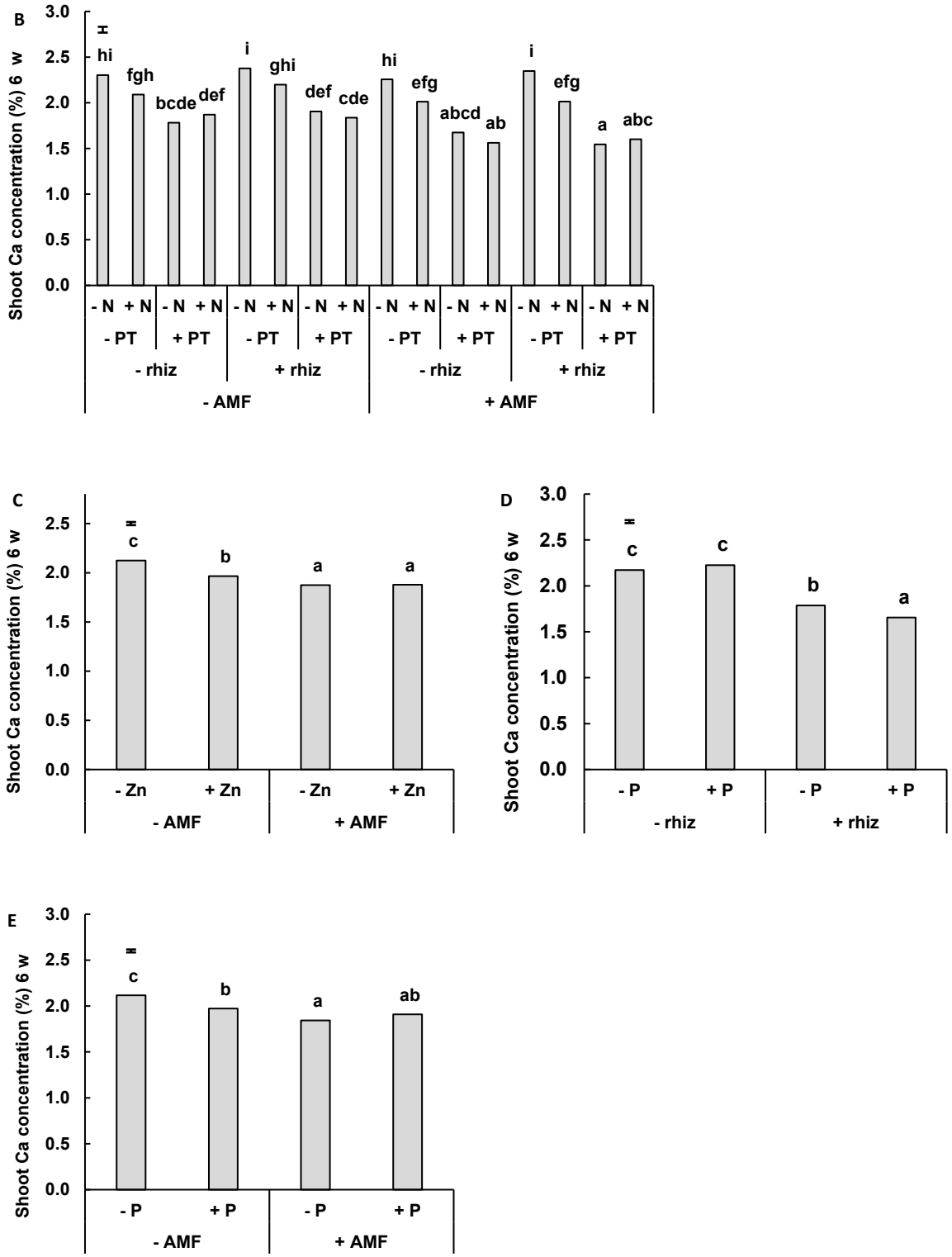


Fig. S15. The interactive effects of co-inoculation of **(A)** *Pratylenchus thornei* (PT) x N x P x Zn, **(B)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N, **(C)** arbuscular mycorrhizal fungi

(AMF) x Zn, **(D)** rhizobia (rhiz) x P, **(E)** arbuscular mycorrhizal fungi (AMF) x P on Ca concentration (%) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

S concentration in the shoot 6 weeks after sowing

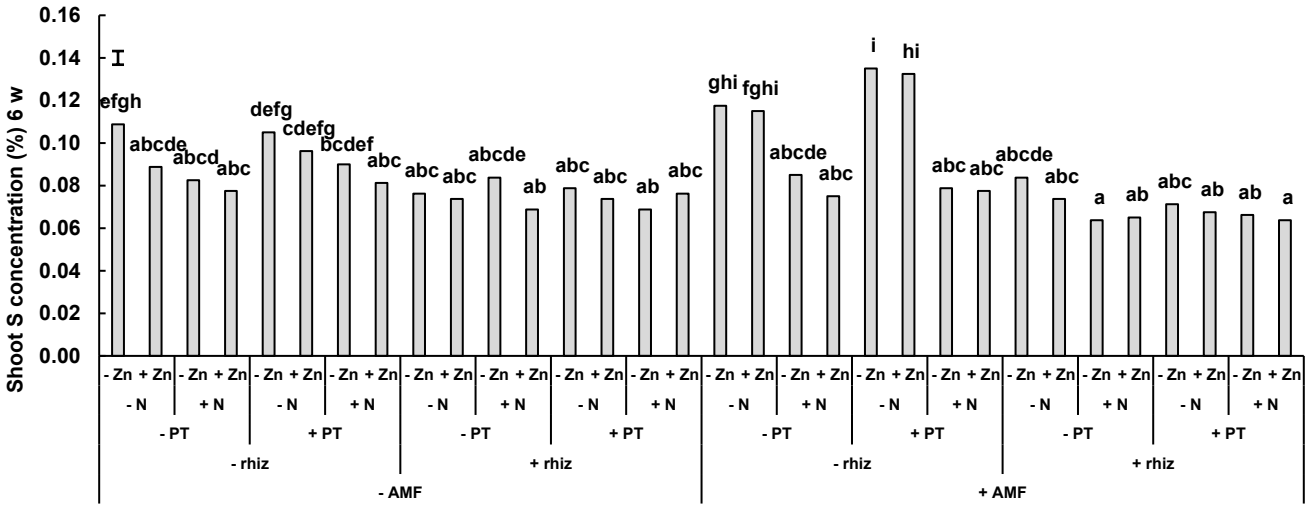


Fig. S16. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N x Zn on S concentration (%) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Zn concentration in the shoot 6 weeks after sowing

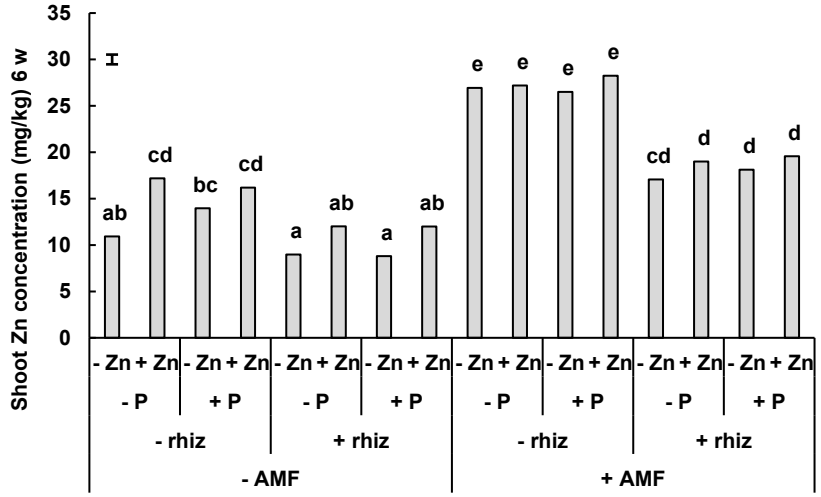
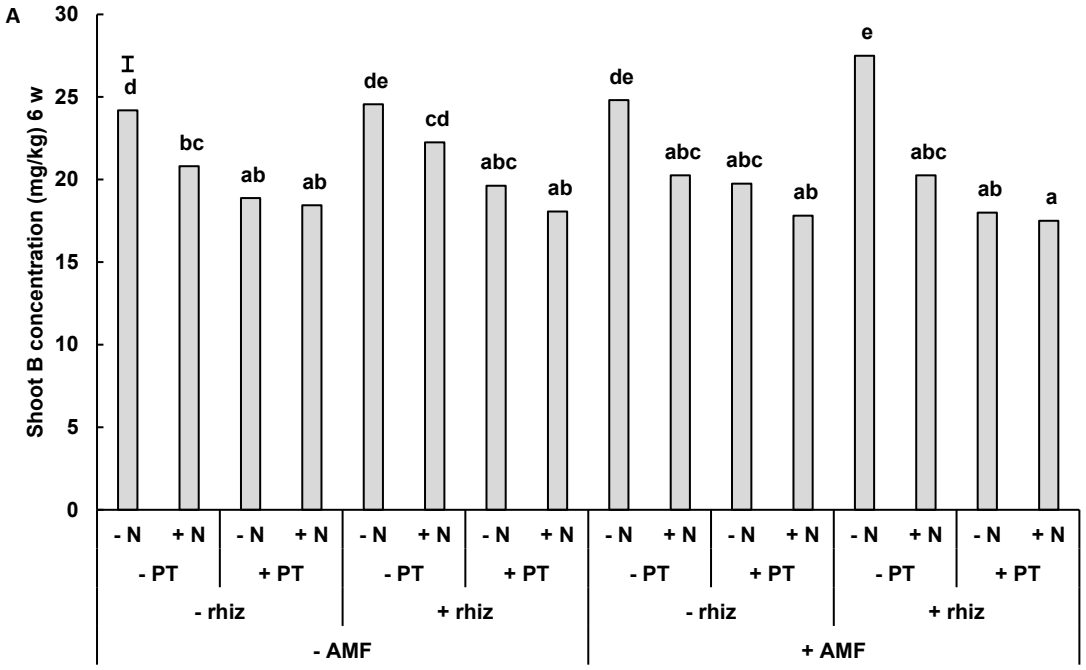


Fig. S17. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P x Zn on Zn concentration (mg/kg) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

B concentration in the shoot 6 weeks after sowing



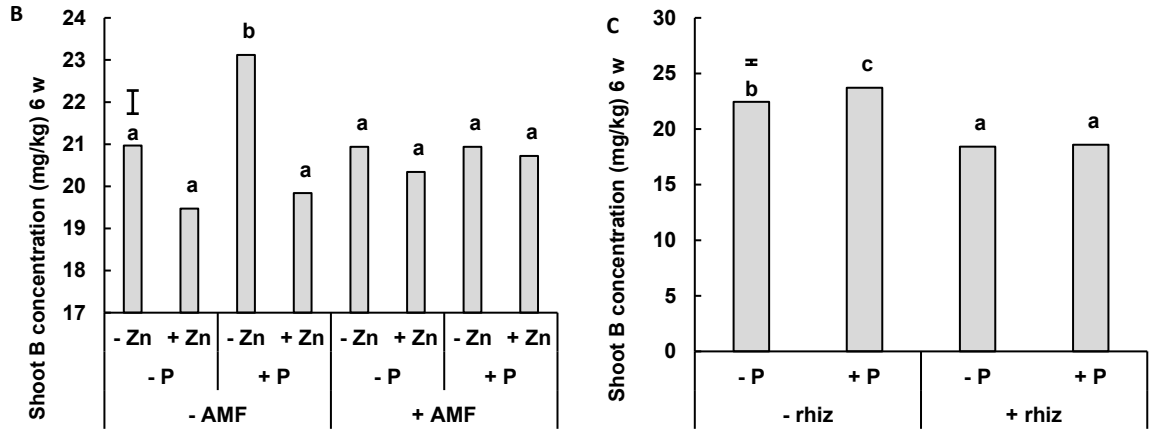


Fig. S18. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N, **(B)** arbuscular mycorrhizal fungi (AMF) x P x Zn and, **(C)** rhizobia (rhiz) x P on B concentration (mg/kg) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Cu concentration in the shoot 6 weeks after sowing

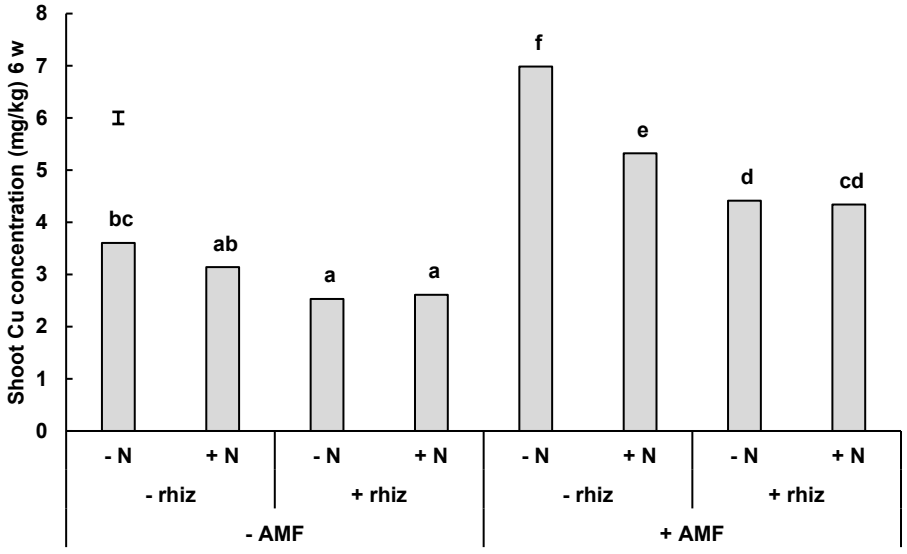


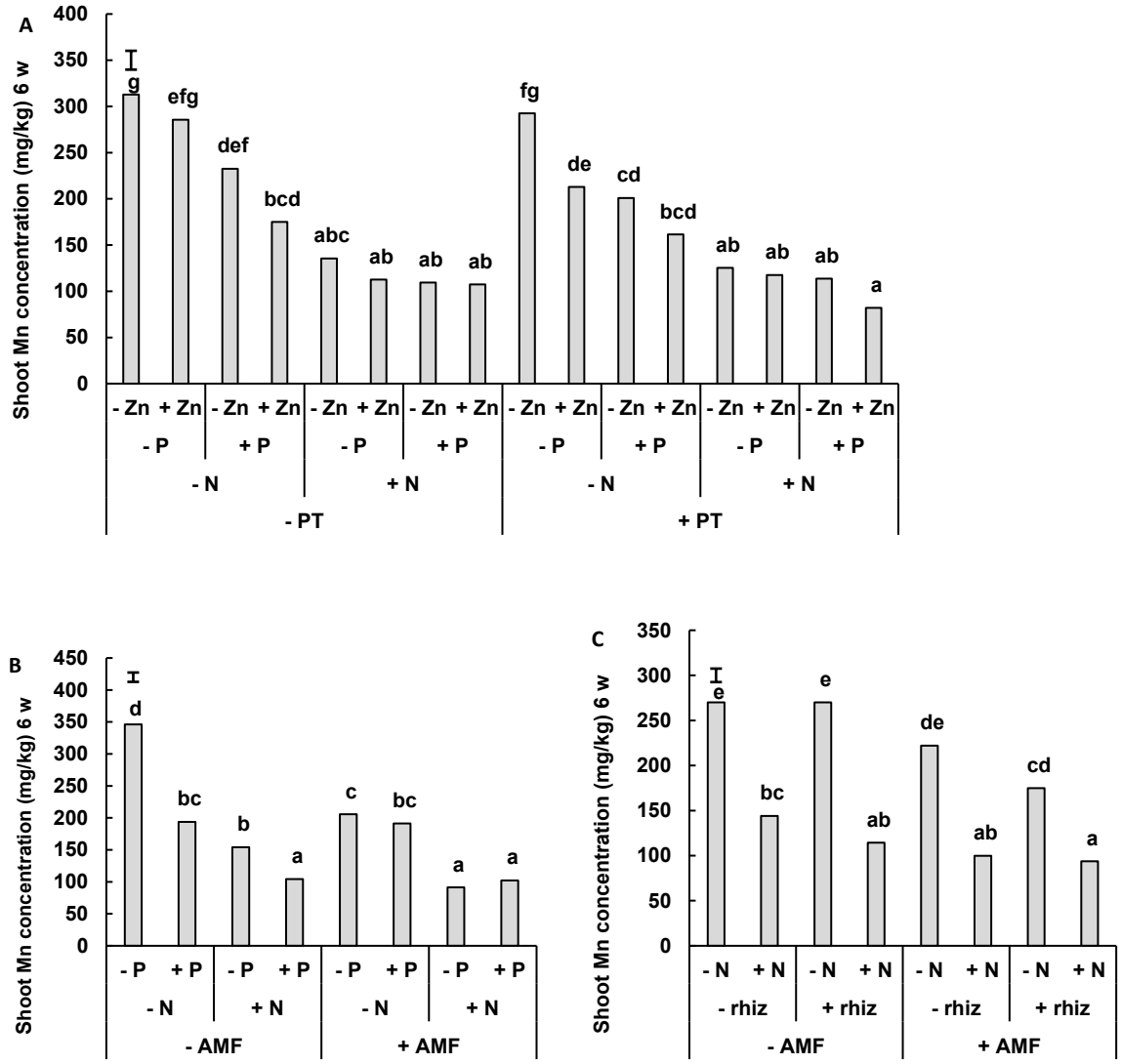
Fig. S19. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x N on Cu concentration (mg/kg) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each

bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Fe concentration in the shoot 6 weeks after sowing

At 6 weeks there were significant interactive effects between of AMF x rhizobia (rhiz) x *P. thornei* x Zn on Fe concentration at 6 weeks (w) However, after Bonferroni's post hoc test, the interaction was not significant.

Mn concentration in the shoot 6 weeks after sowing



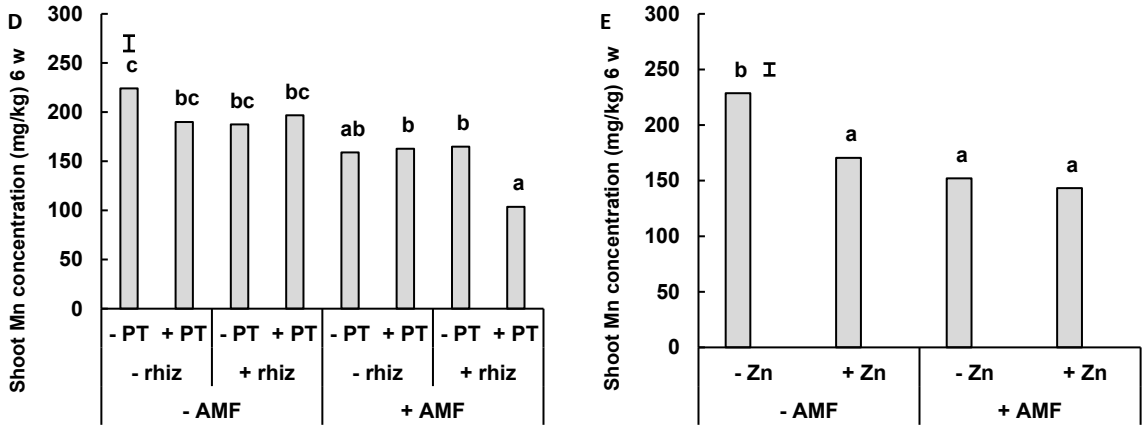
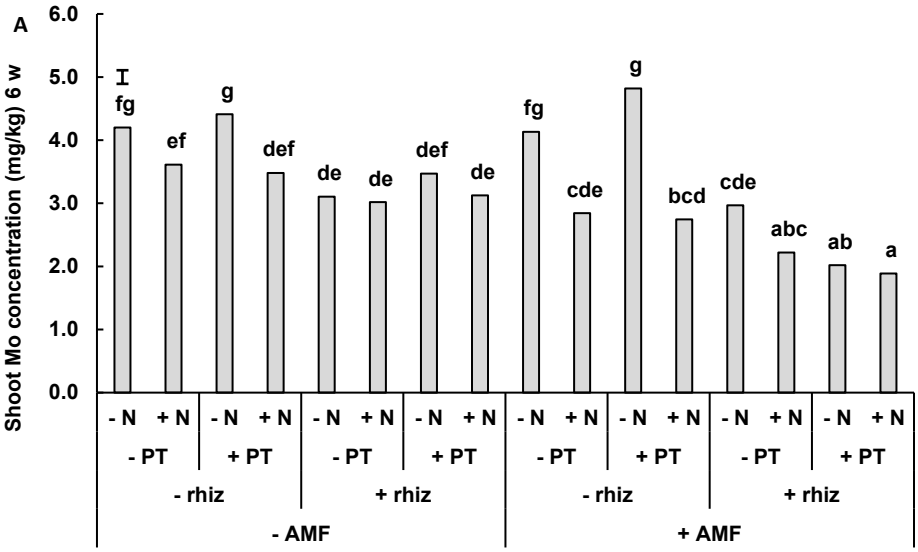


Fig. S20. The interactive effects of co-inoculation of **(A)** *Pratylenchus thornei* (PT) x N x P x Zn, **(B)** arbuscular mycorrhizal fungi (AMF) x N x P, **(C)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x N, **(D)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *P. thornei* (PT) and, **(E)** arbuscular mycorrhizal fungi (AMF) x Zn on Mn concentration (mg/kg) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Mo concentration in the shoot 6 weeks after sowing



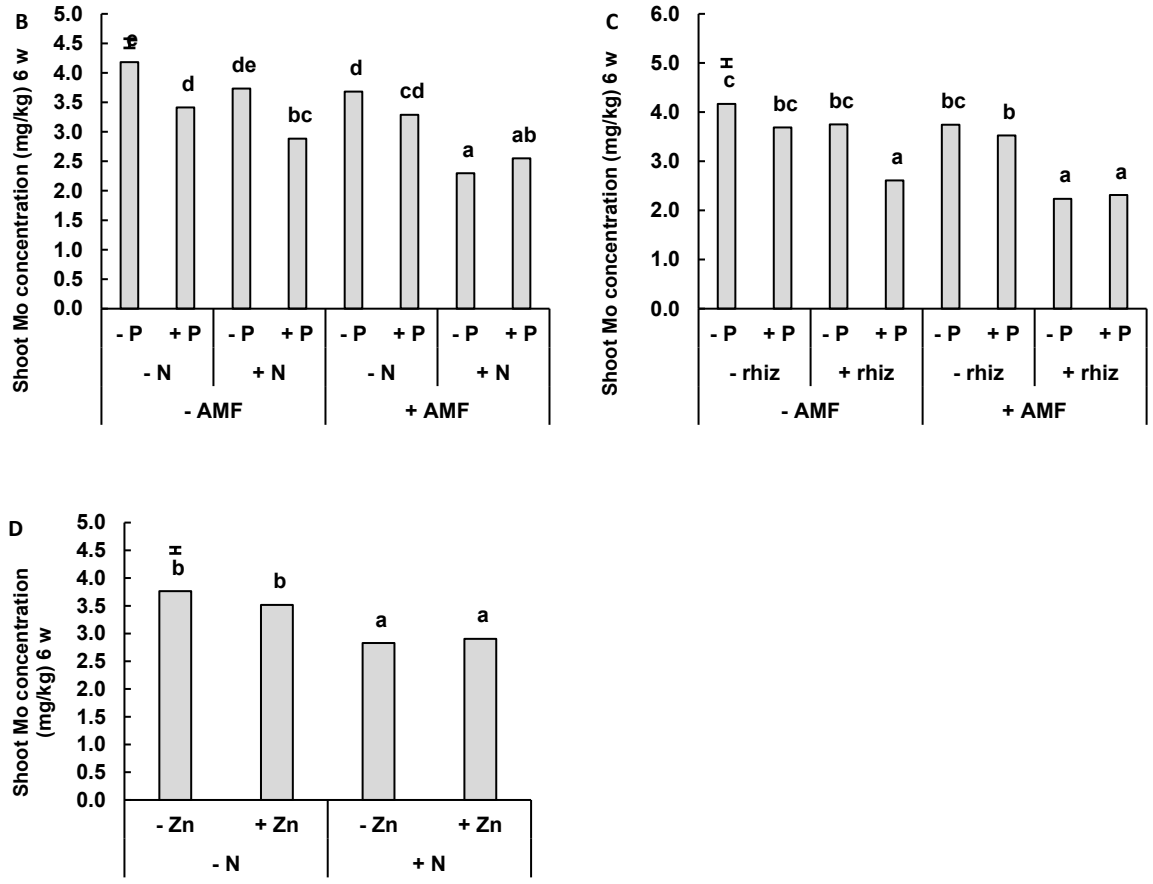
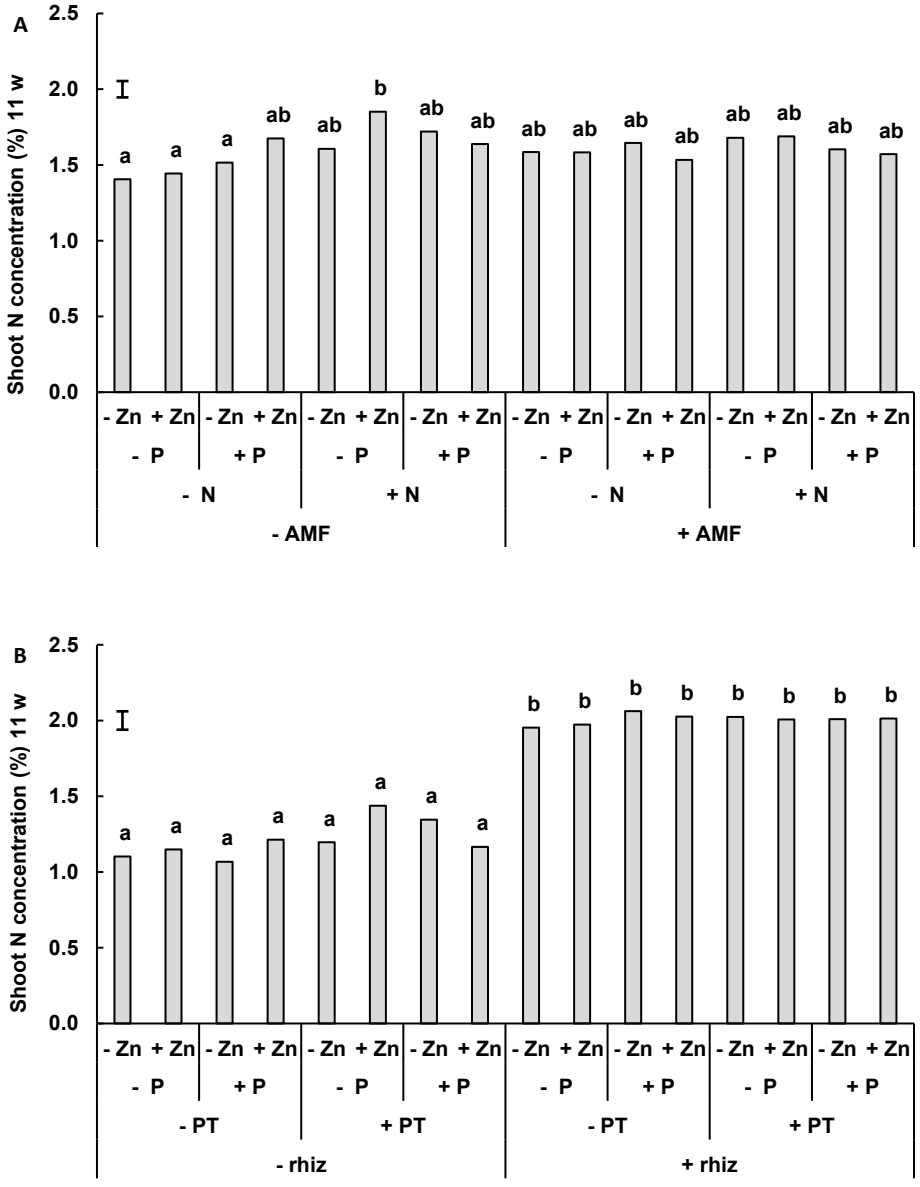


Fig. S21. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N, **(B)** arbuscular mycorrhizal fungi (AMF) x N x P, **(C)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P and, **(D)** N x Zn on Mo concentration (mg/kg) in the shoots of mung bean at 6 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

N concentration in the shoot 11 weeks after sowing



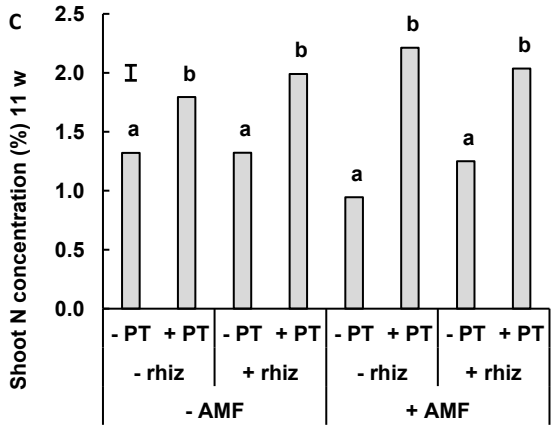
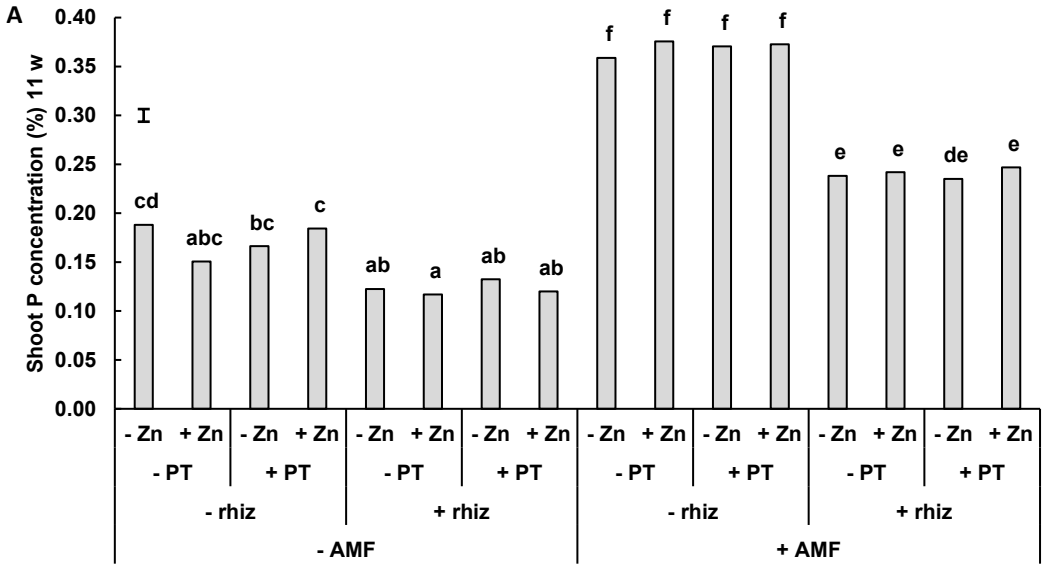


Fig. S22. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x N x P x Zn, **(B)** rhizobia (rhiz) x *Pratylenchus thornei* (PT) x P x Zn and **(C)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) on N concentration (%) in the shoots of mung bean at 11 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

P concentration in the shoot 11 weeks after sowing



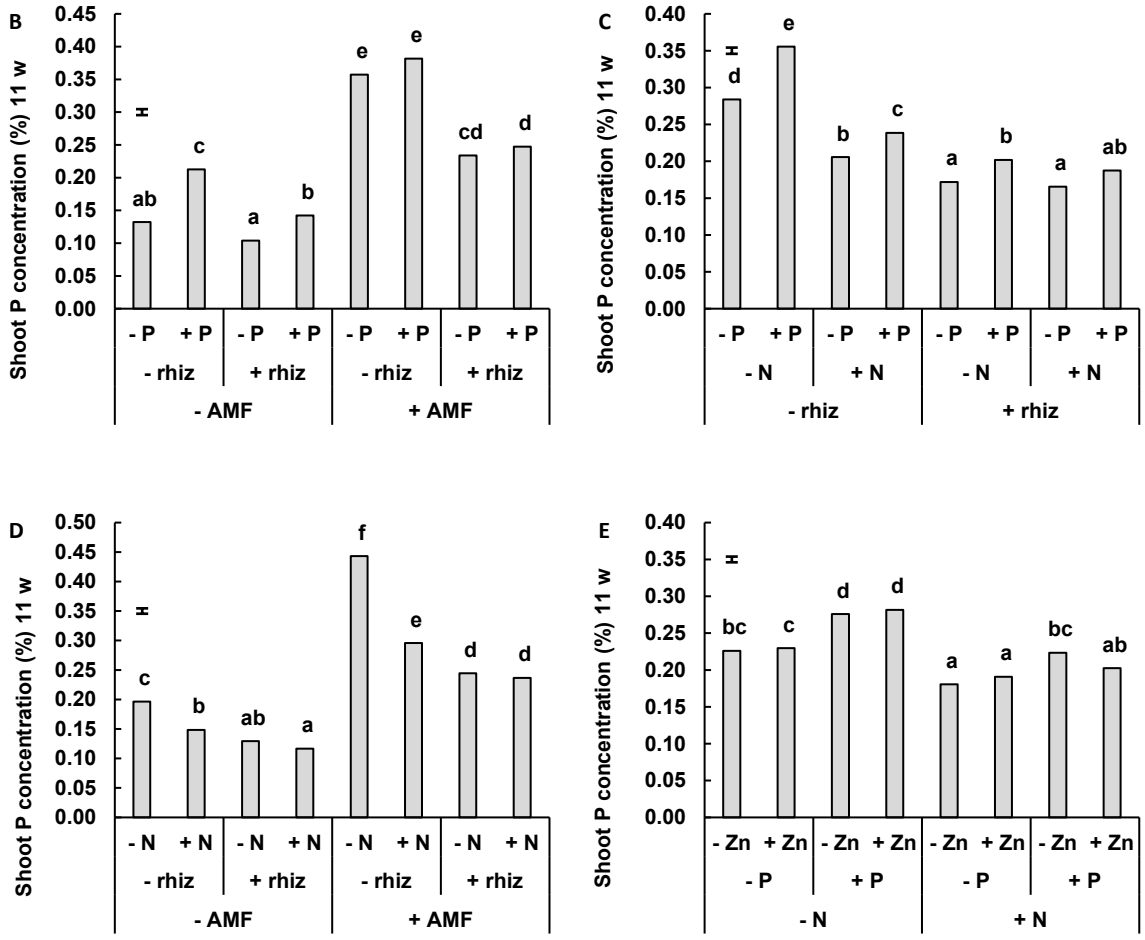


Fig. S23. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x Zn, **(B)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P, **(C)** rhizobia (rhiz) x N x P, **(D)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x N and, **(E)** N x P x Zn on P concentration (%) in the shoots of mung bean at 11 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

K concentration in the shoot 11 weeks after sowing

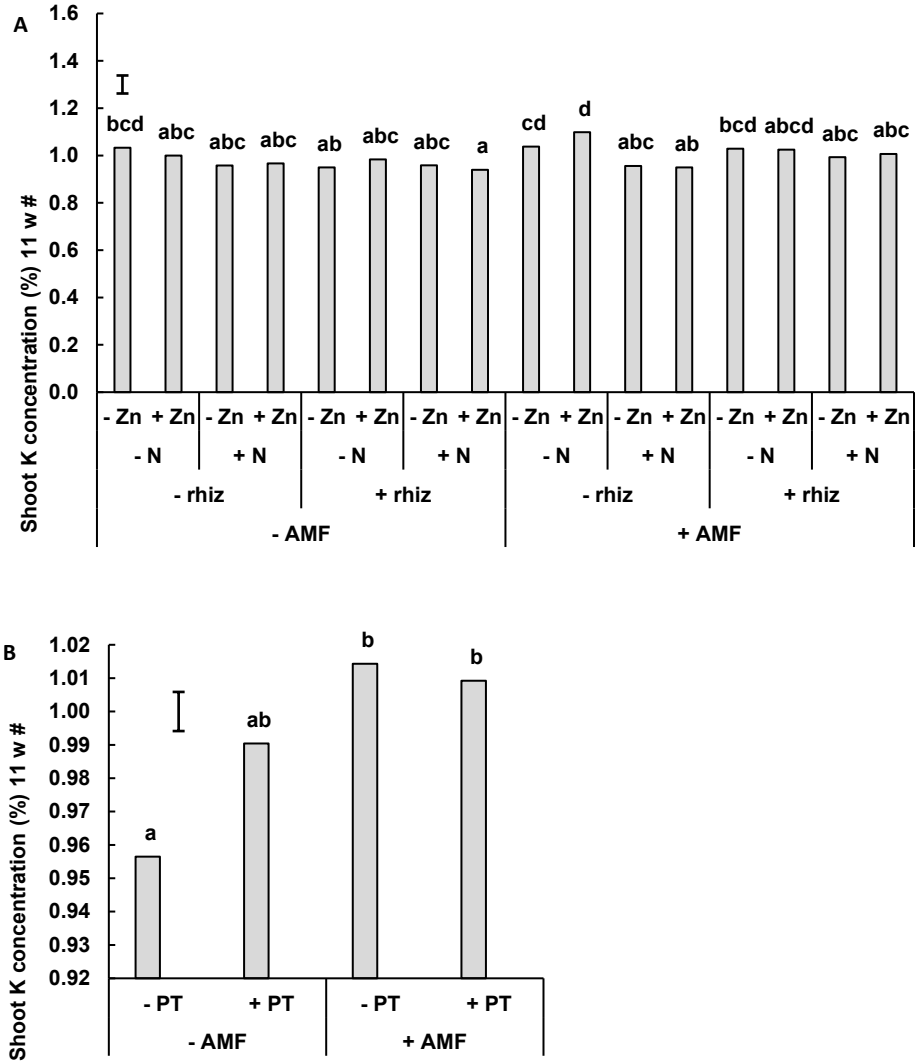
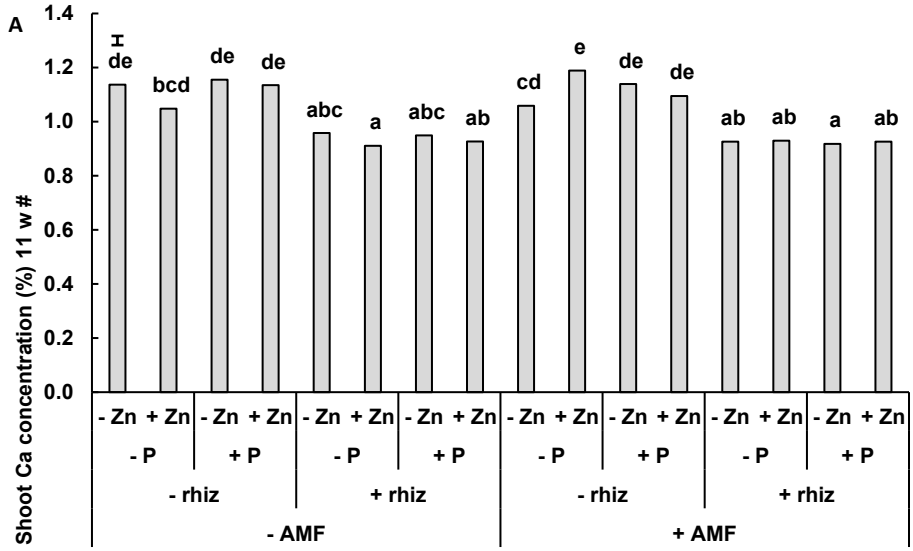


Fig. S24. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x N x Zn and **(B)** arbuscular mycorrhizal fungi (AMF) x *Pratylenchus thornei* (PT) on K concentration (%) in the shoots of mung bean at 11 weeks (w) after sowing. The symbol # signifies a log transformed data set. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Ca concentration in the shoot 11 weeks after sowing



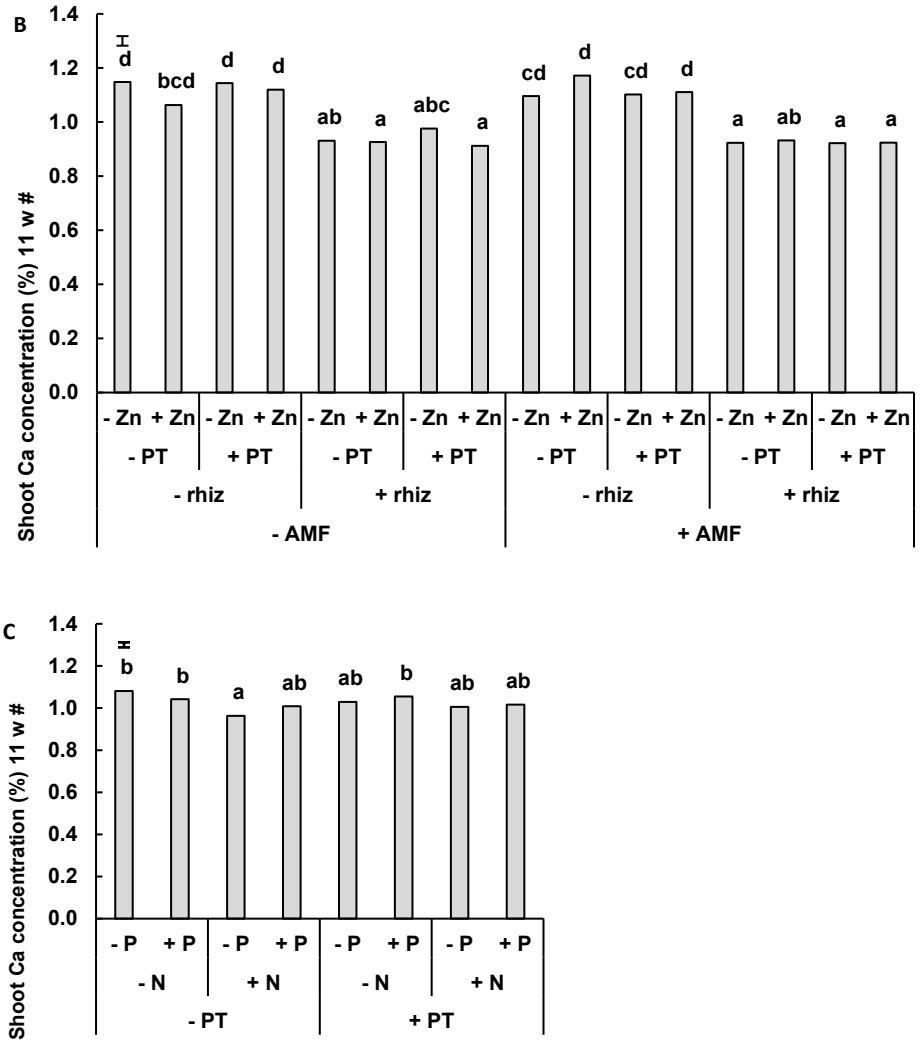
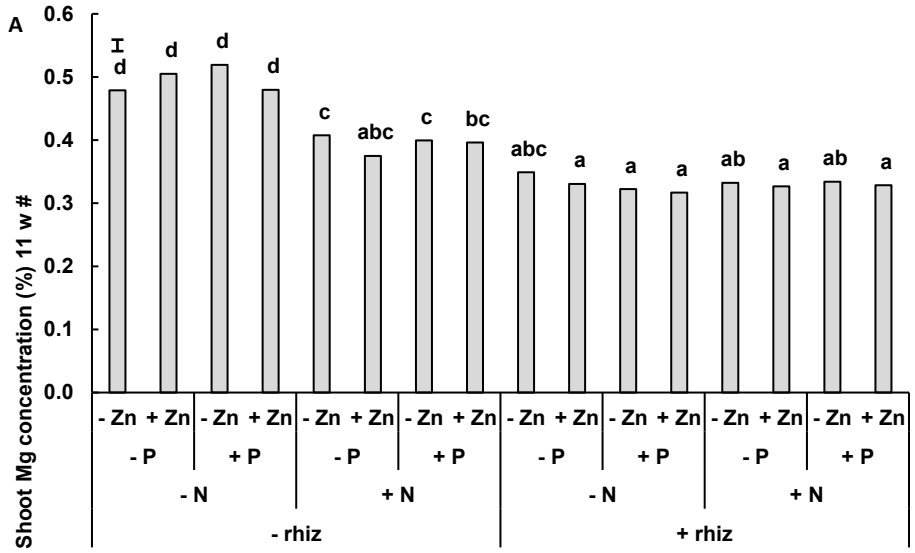
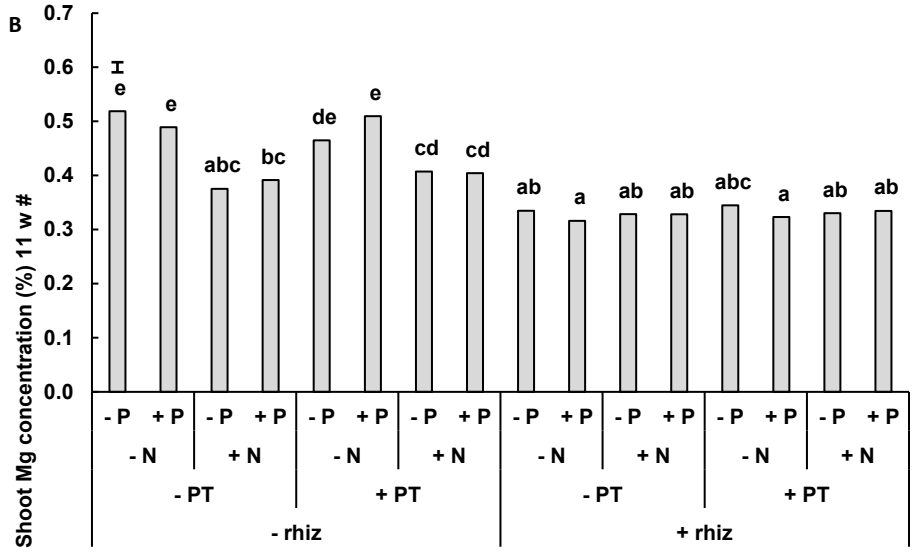


Fig. S25. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P x Zn, **(B)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz)x *Pratylenchus thornei* (PT) x Zn and, **(C)** *Pratylenchus thornei* (PT) x N x P on Ca concentration (%) in the shoots of mung bean at 11 weeks (w) after sowing. The symbol # signifies a log transformed data set. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Mg concentration in the shoot 11 weeks after sowing

In the interaction between AMF x P x Zn, that was significant in the *F* table had no significant interaction after Bonferroni's post hoc test.


A
Shoot Mg concentration (%) 11 w #
0.6
0.5
0.4
0.3
0.2
0.1
0.0
I
d
d
d
d
c
abc
c
bc
abc
a
a
a
ab
a
ab
a
- Zn + Zn - Zn + Zn - Zn + Zn - Zn + Zn - Zn + Zn - Zn + Zn - Zn + Zn - Zn + Zn
- P + P - P + P - P + P - P + P
- N + N - N + N
- rhiz + rhiz


B
Shoot Mg concentration (%) 11 w #
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
I
e
e
abc
bc
de
e
cd
cd
ab
a
ab
ab
abc
a
ab
ab
- P + P - P + P - P + P - P + P - P + P - P + P - P + P - P + P
- N + N - N + N - N + N - N + N
- PT + PT - PT + PT
- rhiz + rhiz

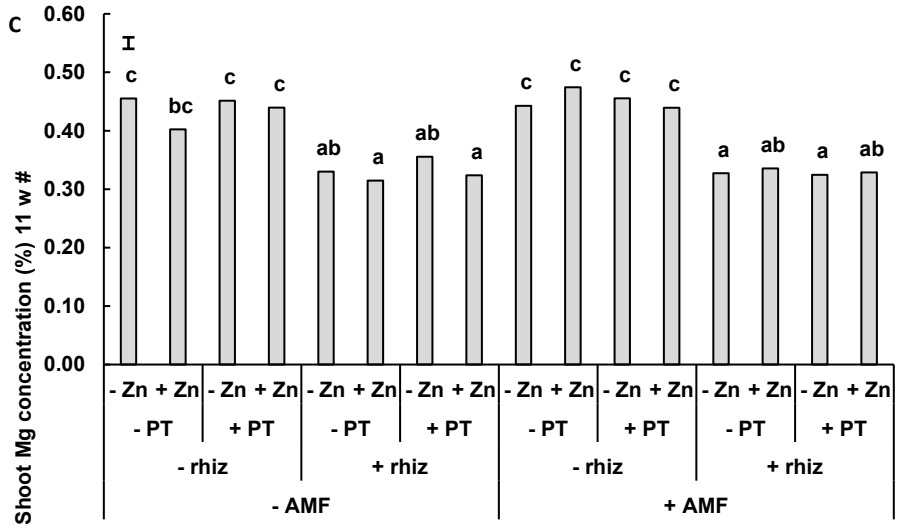
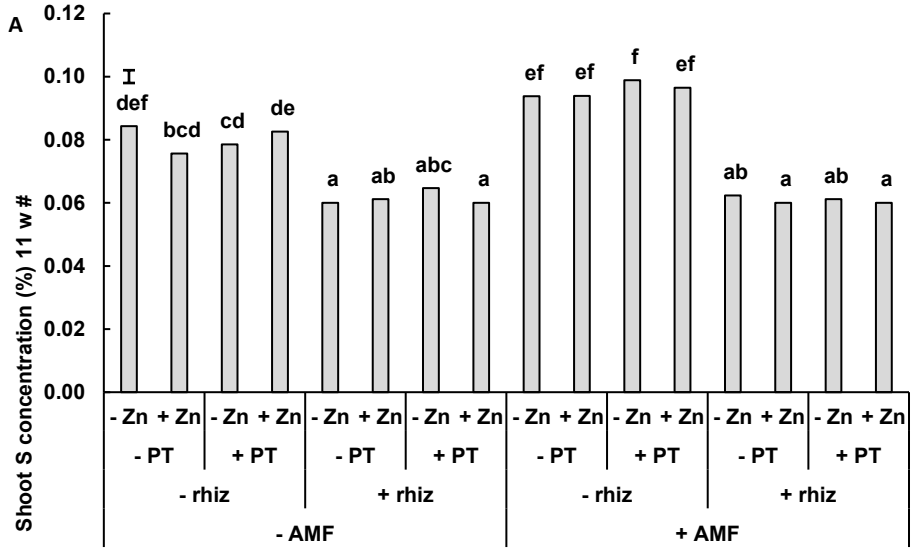


Fig. S26. The interactive effects of co-inoculation of **(A)** rhizobia (rhiz) x N x P x Zn, **(B)** rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N x P and, **(C)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x Zn on Mg concentration (%) in the shoots of mung bean at 11 weeks (w) after sowing. The symbol # signifies a log transformed data set. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

S concentration in the shoot 11 weeks after sowing



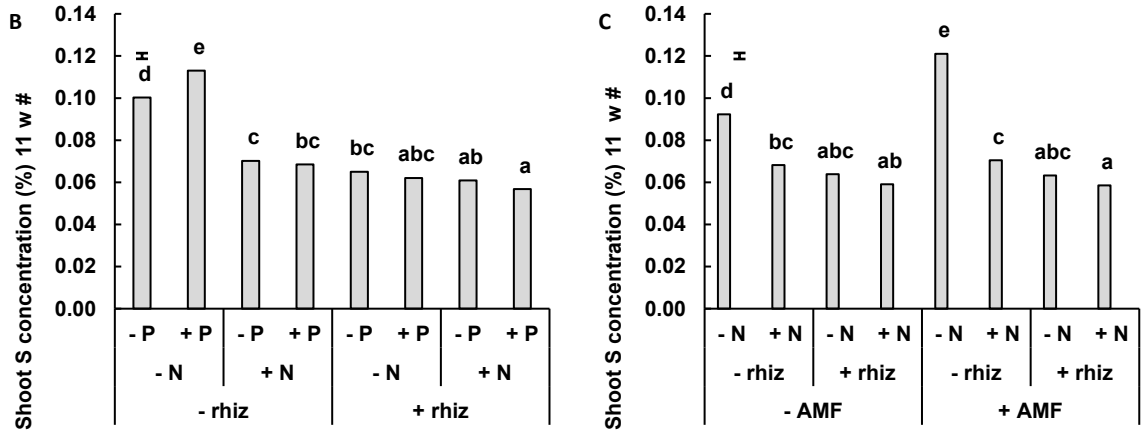
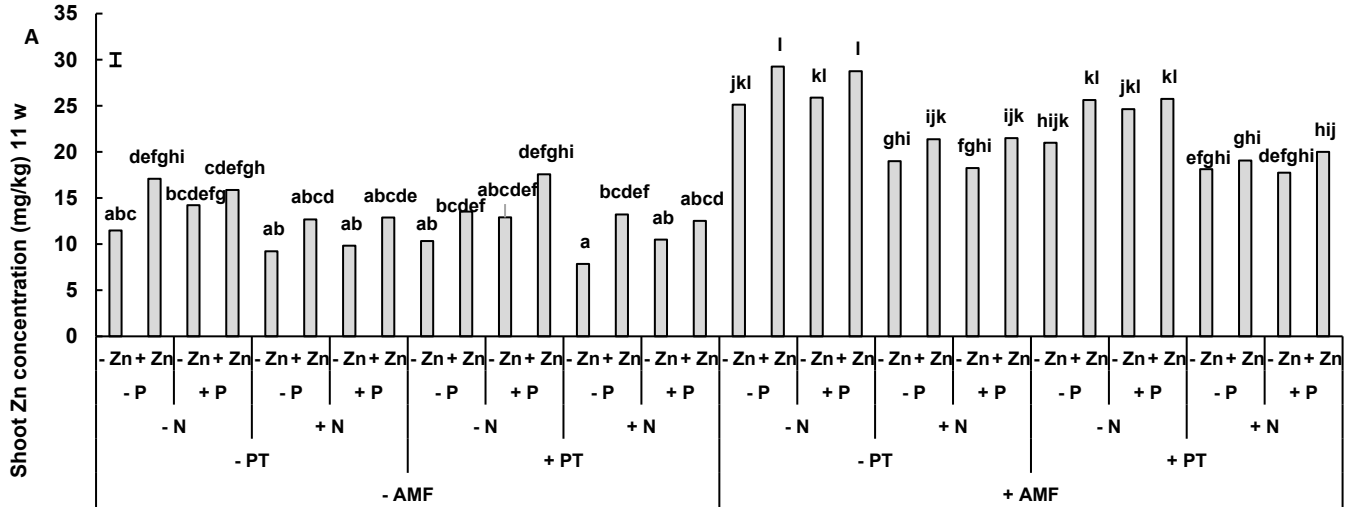


Fig. S27. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x Zn, **(B)** rhizobia (rhiz) x N x P, **(C)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x N on S concentration (%) in the shoots of mung bean at 11 weeks (w) after sowing. The symbol # signifies a log transformed data set. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Zn concentration in the shoot 11 weeks after sowing



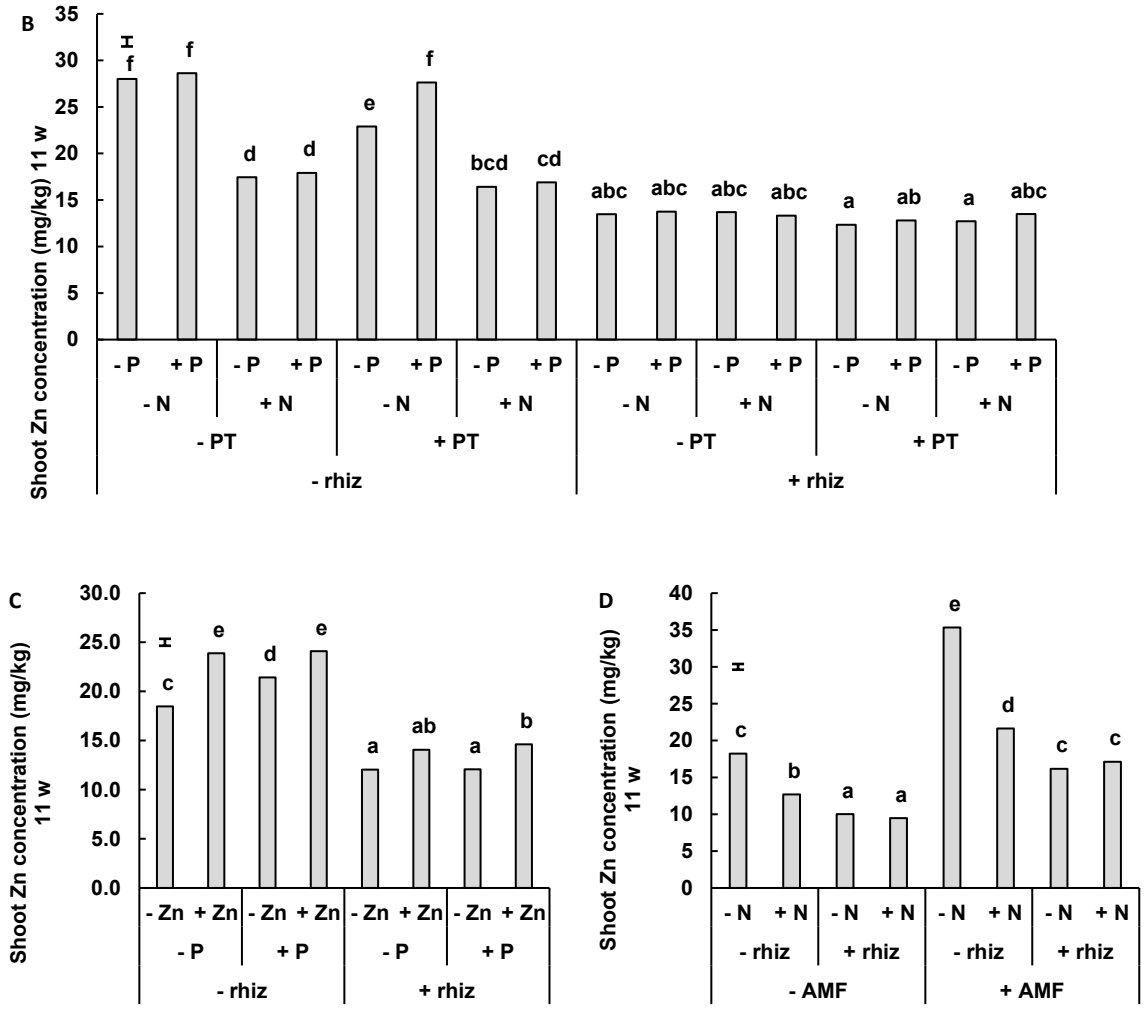


Fig. S28. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x *Pratylenchus thornei* (PT) x N x P x Zn, **(B)** rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N x P, **(C)** rhizobia (rhiz) x P x Zn, and **(D)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x N on Zn concentration (mg/kg) in the shoots of mung bean at 11 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Fe concentration in the shoot 11 weeks after sowing

The interactions between Fe concentration in the shoot at 11 weeks after sowing that were significant in the *F* table had no significant interaction after Bonferroni's post hoc test.

B concentration in the shoot 11 weeks after sowing

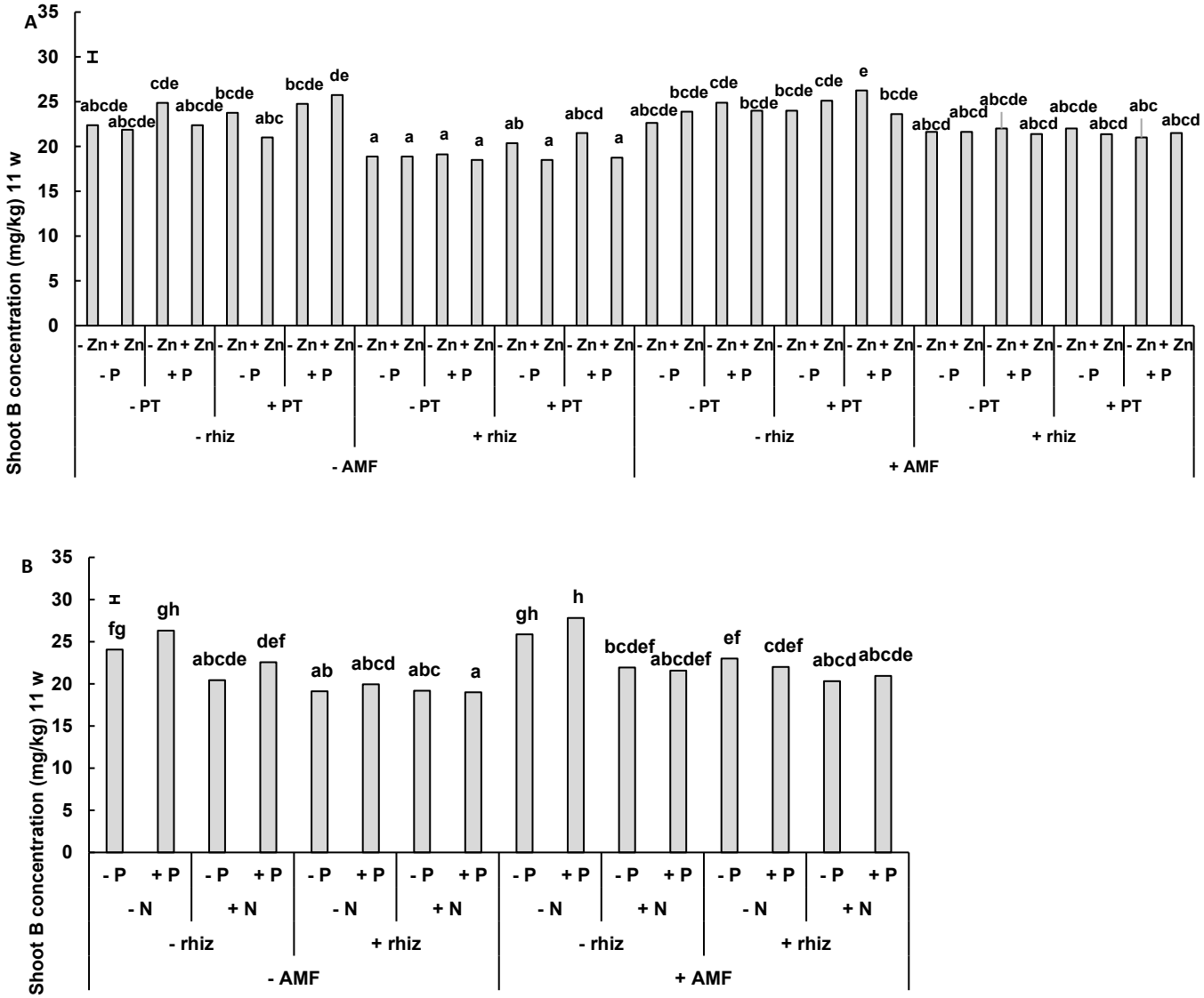


Fig. S29. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x P x Zn and **(B)** arbuscular mycorrhizal fungi (AMF) rhizobia (rhiz) x N x P on B concentration (mg/kg) in the shoots of mung bean at 11 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Cu concentration in the shoot 11 weeks after sowing

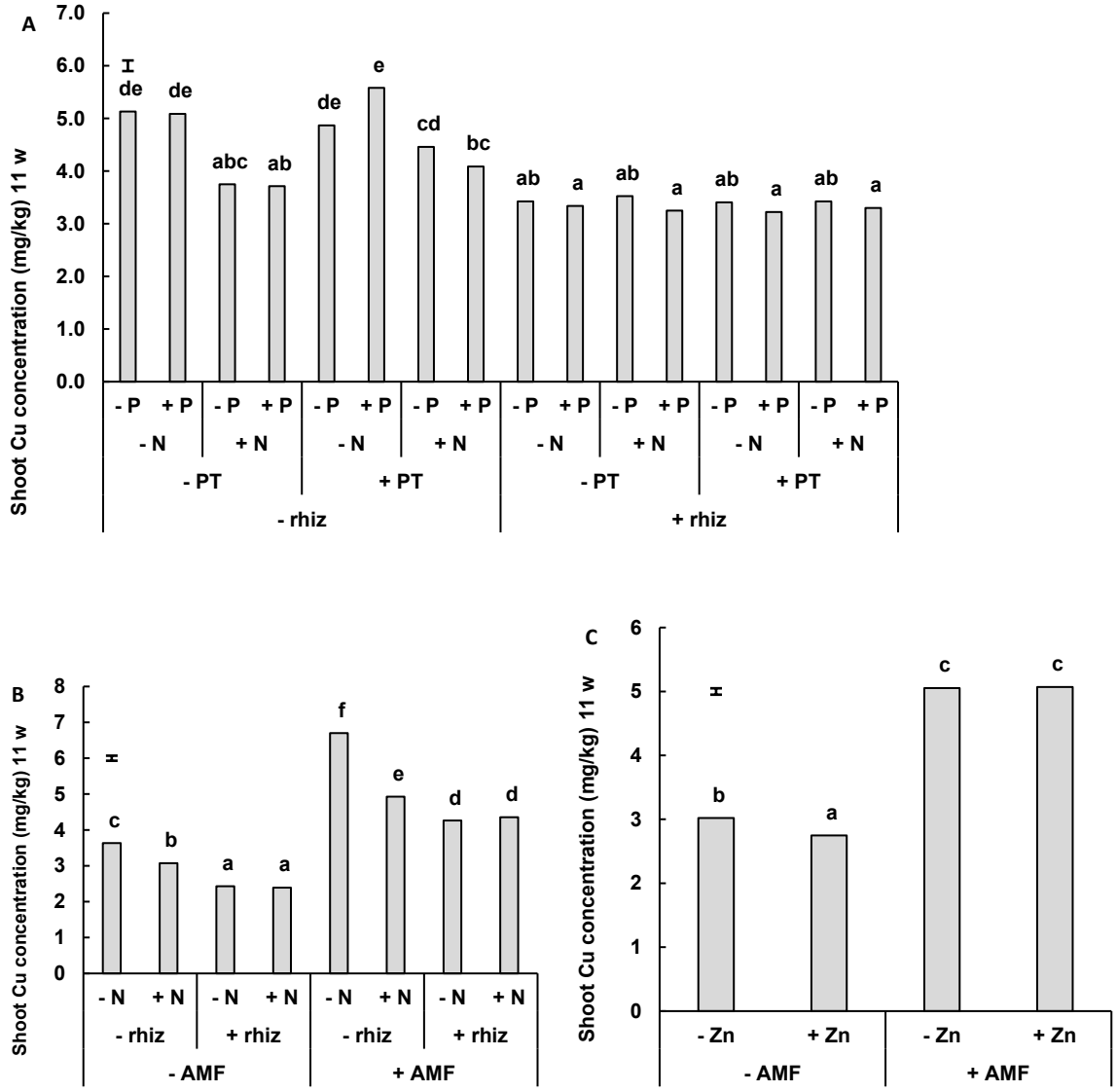


Fig. S30. The interactive effects of co-inoculation of **(A)** rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N x P, **(B)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x N and, **(C)** arbuscular mycorrhizal fungi (AMF) x Zn on Cu concentration (mg/kg) in the shoots of mung bean at 11 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Mn concentration in the shoot 11 weeks after sowing

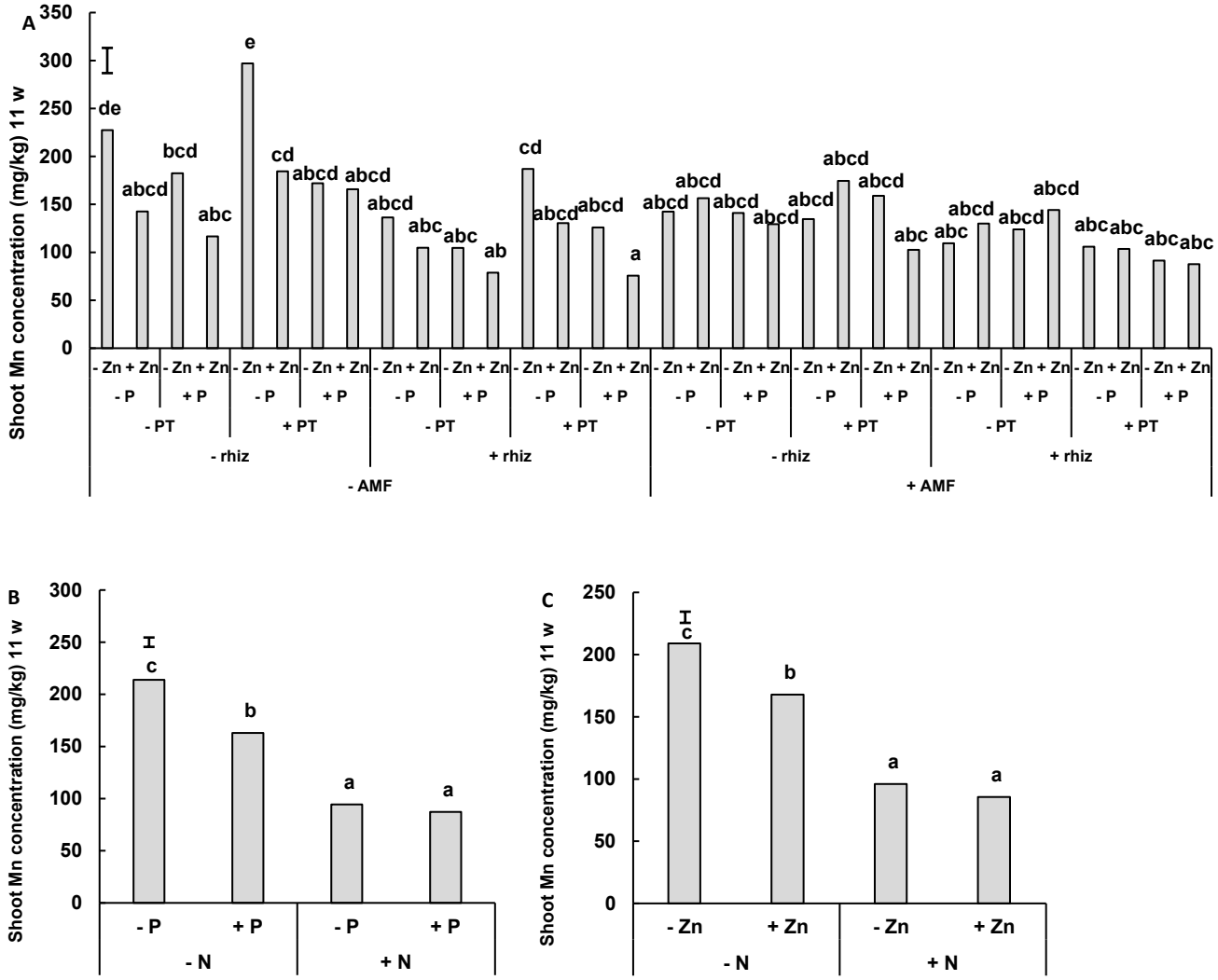


Fig. S31. The interactive effects of co-inoculation of **(A)** arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x P x Zn, **(B)** N x P and **(C)** N x Zn on Mn concentration (mg/kg) in the shoots of mung bean at 11 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Mo concentration in the shoot 11 weeks after sowing

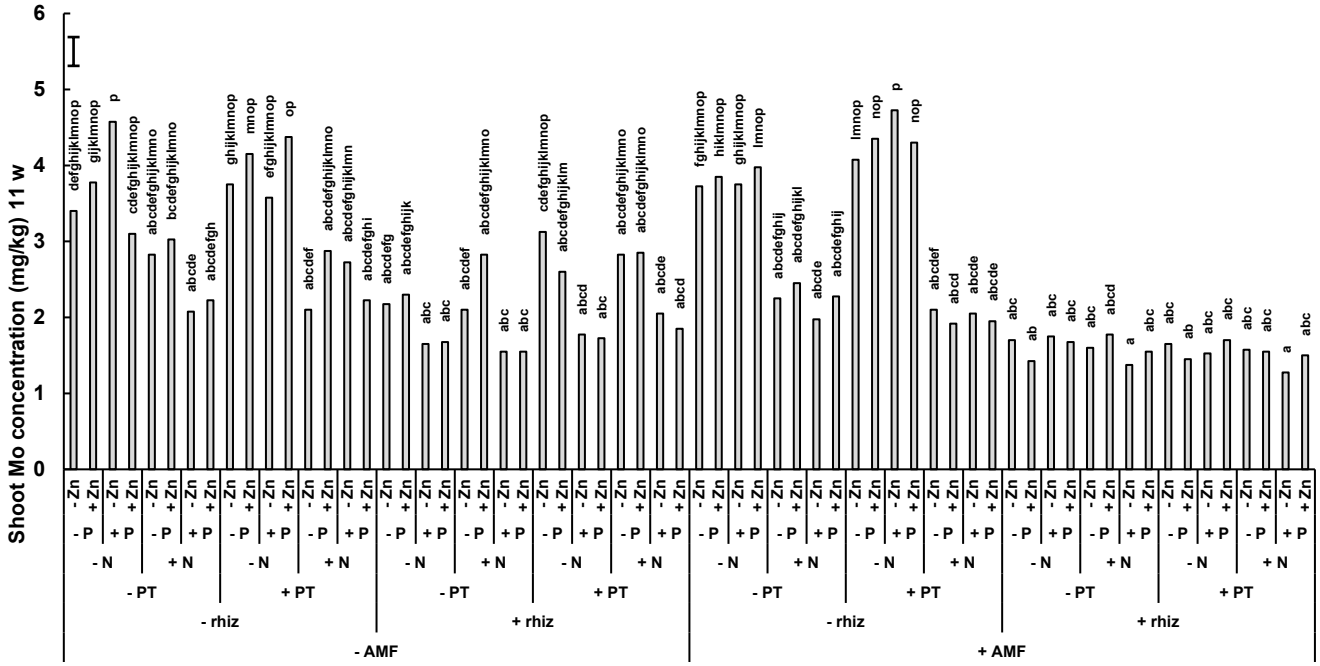


Fig. S32. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (PT) x N x P x Zn on Mo concentration (mg/kg) in the shoots of mung bean at 11 weeks (w) after sowing. Different letters above each bar graph indicate significant differences according to the Bonferroni test for multiple comparisons at P=0.05 for the interaction. The vertical bar represents the standard error of difference (s.e.d.).

Supplementary Tables

Table S1. Table of the treatment designations used in the principal components analysis in Fig. 1. Treatment codes from 1 to 32 represent the combination of the full factorial of treatments in rhizobia-inoculated plants. Letters for treatment codes designated "TRT" are based on the following; A=Arbuscular mycorrhizal fungi; Pt=*Pratylenchus thornei*; N=nitrogen; P=phosphorus; Z=zinc. Four major clusters delineated by the dendrogram in Fig. S1 are demarcated by black circles on Fig. 2 and labelled using letters A-D.

#	TRT	#	TRT	#	TRT	#	TRT
Cluster A: - AMF, - P				Cluster B: - AMF, + P			
1	Nil	9	Z	17	P	25	PZ
2	N	10	NZ	18	NP	26	NPZ
3	Pt	11	PtZ	19	PtP	27	PtPZ
4	PtN	12	PtNZ	20	PtNP	28	PtNPZ
Cluster C: + AMF, - N, - Pt							
5	A	13	AZ	21	AP	29	APZ
Cluster D: + AMF, + N/+ Pt							
6	AN	14	ANZ	22	ANP	30	ANPZ
7	APt	15	APtZ	23	APtP	31	APtPZ
8	APtN	16	APtNZ	24	APtNP	32	APtNPZ

Table S2. Table of the treatment designations used in the principal component analysis in Fig. 2. Treatment codes from 1 to 64 represent the combination of the full factorial of treatments. Letters for treatment codes designated "TRT" are based on the following: A=Arbuscular mycorrhizal fungus; Pt=*Pratylenchus thornei;* R=rhizobia; N=nitrogen; P=phosphorus; Z=zinc. Clusters delineated by the hierarchical cluster analyses in Fig. S2 are demarcated by black circles on Fig. 2 and labelled using letters A-E.

#	TRT	#	TRT	#	TRT	#	TRT	#	TRT	#	TRT	#	TRT	#	TRT
Cluster A: - AMF, - rhizobia								Cluster E: + AMF, - rhizobia, - N							
1	Nil	9	Pt	33	Z	41	PtZ	17	A	25	APt	49	AZ	57	APtZ
2	P	10	PtP	34	PZ	42	PtPZ	18	AP	26	APtP	50	APZ	58	APtPZ
3	N	11	PtN	35	NZ	43	PtNZ	Cluster D: + AMF, - rhizobia, + N							
4	NP	12	PtNP	36	NPZ	44	PtNPZ	19	AN	27	APtN	51	ANZ	59	APtNZ
								20	ANP	28	APtNP	52	ANPZ	60	APtNPZ
Cluster B: - AMF, + rhizobia, + P/N								Cluster C: + AMF, + rhizobia							
5	R	13	PtR	37	RZ	45	PtRZ	21	AR	29	APtR	53	ARZ	61	APtRZ
6	RP	14	PtRP	38	RPZ	46	PtRPZ	22	ARP	30	APtRP	54	ARPZ	62	APtRPZ
7	RN	15	PtRN	39	RNZ	47	PtRNZ	23	ARN	31	APtRN	55	ARNZ	63	APtRNZ
8	RNP	16	PtRNP	40	RNPZ	48	PtRNPZ	24	ARNP	32	APtRNP	56	ARNPZ	64	APtRNPZ

Table S3. Table of the treatment designations used in the principal components analysis in Fig. 1. Treatment codes from 1 to 32 represent the combination of the full factorial of treatments in rhizobia-inoculated plants. Letters for treatment codes designated "TRT" are based on the following; A=Arbuscular mycorrhizal fungi; Pt=*Pratylenchus thornei*; N=Nitrogen; P=Phosphorus; Z=Zinc.

Loadings for PCA 1 and PCA 2 on Rhizobia inoculated dataset				Scores for PCA 1 and PCA 2 on Rhizobia inoculated dataset		
	Latent vectors (loadings)				Principal component scores	
Variate	PC1	PC2	Treatment	Code	PC1	PC2
DW 6	0.827	-0.436	Nil	1	-1.401	0.651
DW 11	0.802	-0.280	N	2	-1.560	0.421
Seed 11	0.829	0.156	Pt	2	-1.887	2.029
Nod 6	0.604	-0.115	PtN	4	-1.449	-0.316
Nod 11	0.601	0.031	A	5	0.496	2.477
Nod DW 6	0.616	-0.138	AN	6	0.942	-0.356
Nod DW 11	0.677	0.245	APt	7	0.915	0.744
DRW 6	0.157	-0.510	APtN	8	0.731	-0.932
DRW 11	0.142	0.320	Z	9	-1.098	0.148
AMF 6	0.874	0.291	NZ	10	-1.415	-0.444
AMF 11	0.885	0.285	PtZ	11	-1.407	0.524
AMF RL 6	0.745	0.174	PtNZ	12	-1.512	-0.130
AMF RL 11	0.870	0.319	AZ	13	0.940	1.470
Ndfa % 6	0.914	-0.182	ANZ	14	0.673	0.126
Ndfa % 11	0.811	0.302	APtZ	15	1.004	0.448
Fixed N 6	0.865	-0.316	APtNZ	16	0.700	-0.444
Fixed N 11	0.841	0.194	P	17	-0.165	-0.476
N uptake 6	0.820	-0.441	NP	18	-0.536	-0.922
N uptake 11	0.845	0.019	PtP	19	-0.380	-0.102
Pt 6	-0.009	-0.170	PtNP	20	-0.583	-0.939
Pt 11	-0.052	-0.126	AP	21	0.829	1.665
B 6	-0.203	0.581	ANP	22	0.937	-0.841
B 11	0.624	0.537	APtP	23	1.314	-0.439
Ca 6	-0.901	0.281	APtNP	24	0.973	-0.052
Ca 11	-0.228	-0.344	PZ	25	0.159	-0.756
Cu 6	0.775	0.412	NPZ	26	-0.317	-1.709
Cu 11	0.843	0.307	PtPZ	27	-0.026	-0.298
Fe 6	-0.153	0.256	PtNPZ	28	-0.275	-2.001
Fe 11	0.236	0.101	APZ	29	0.819	1.091
K 6	0.079	0.789	ANPZ	30	0.926	0.001
K 11	0.798	0.191	APtPZ	31	0.859	0.322
Mg 6	-0.646	0.608	APtNPZ	32	0.793	-0.956
Mg 11	-0.316	0.375				
Mn 6	-0.475	0.662				
Mn 11	-0.253	0.783				
Mo 6	-0.823	0.436				
Mo 11	-0.864	0.200				
N 6	0.115	-0.378				
N 11	0.561	0.556				
P 6	0.893	0.326				
P 11	0.936	0.223				
S 6	-0.557	0.579				
S 11	-0.264	0.749				
Zn 6	0.790	0.314				
Zn 11	0.856	0.182				

Table S4. Table of the treatment designations used in the principal component analysis in Fig. 2. Treatment codes from 1 to 64 represent the combination of the full factorial of treatments. Letters for treatment codes designated "TRT" are based on the following: A=Arbuscular mycorrhizal fungus; Pt= Pratylenchus thornei; R=rhizobia; N=Nitrogen; P=Phosphorus; Z=Zinc

Loadings for PCA 1 and PCA 2 on complete dataset			Scores for PCA 1 and PCA 2 on complete dataset			
	Latent vectors (loadings)				Principal component scores	
Variate	PC1	PC2	Treatment	Code	PC1	PC2
DW 6	0.608	-0.535	Nil	1	-0.894	1.587
DW 11	0.816	-0.387	P	2	-1.382	1.098
DRW 6	-0.117	0.243	N	3	0.023	1.311
DRW 11	-0.547	0.071	NP	4	-0.238	0.977
Nod DW 6	0.778	-0.270	R	5	0.606	1.216
Nod DW 11	0.734	-0.385	RP	6	1.078	0.494
Nod 6	0.788	-0.277	RN	7	0.484	1.037
Nod 11	0.782	-0.350	RNP	8	1.054	0.450
Seed 11	0.854	-0.383	Pt	9	-1.089	1.633
AMF 6	-0.260	-0.936	PtP	10	-1.004	1.098
AMF 11	-0.292	-0.936	PtN	11	-0.216	1.268
AMF RL 6	-0.245	-0.872	PtNP	12	-0.115	0.744
AMF RL 11	-0.231	-0.930	PtR	13	0.215	1.262
Ves 11	-0.090	-0.938	PtRP	14	0.996	0.517
Ves RL 11	-0.325	-0.482	PtRN	15	0.699	0.976
Pt 6	-0.025	-0.012	PtRNP	16	0.936	0.534
Pt 11	-0.040	-0.052	A	17	-1.716	-0.680
B 6	-0.878	0.143	AP	18	-1.907	-0.795
B 11	-0.842	-0.165	AN	19	-0.312	-0.934
Ca 6	-0.827	0.478	ANP	20	-0.295	-0.524
Ca 11	-0.851	0.175	AR	21	0.351	-1.035
Cu 6	-0.704	-0.668	ARP	22	0.558	-1.112
Cu 11	-0.711	-0.674	ARN	23	0.881	-1.309
Fe 6	-0.047	0.247	ARNP	24	0.970	-0.903
Fe 11	-0.085	-0.159	APt	25	-1.779	-0.971
K 6	-0.407	-0.242	APtP	26	-2.257	-0.636
K 11	-0.473	-0.351	APtN	27	-0.554	-0.670
Mg 6	-0.912	0.265	APtNP	28	-0.355	-0.727
Mg 11	-0.923	0.111	APtR	29	0.688	-1.437
Mn 6	-0.368	0.409	APtRP	30	1.037	-1.257
Mn 11	-0.590	0.312	APtRN	31	1.026	-0.998
Mo 6	-0.747	0.489	APtRNP	32	0.938	-1.159
Mo 11	-0.868	0.330	Zn	33	-0.779	1.338
N 6	0.882	-0.092	PZn	34	-0.423	1.065
N 11	0.852	-0.201	NZn	35	0.173	1.127

Table S4 (cont.). Table of the treatment designations used in the principal component analysis in Fig. 2. Treatment codes from 1 to 64 represent the combination of the full factorial of treatments. Letters for treatment codes designated "TRT" are based on the following: A=Arbuscular mycorrhizal fungus; Pt= Pratylenchus thornei; R=rhizobia; N=Nitrogen; P=Phosphorus; Z=Zinc

Loadings for PCA 1 and PCA 2 on complete dataset			Scores for PCA 1 and PCA 2 on complete dataset			
	Latent vectors (loadings)				Principal component scores	
	PC1	PC2	Treatment	Code	PC1	PC2
P 6	-0.569	-0.783	NPZn	36	0.360	0.802
P 11	-0.702	-0.672	RZn	37	0.699	0.930
S 6	-0.906	0.080	RPZn	38	1.204	0.160
S 11	-0.942	-0.050	RNZn	39	0.653	0.981
Zn 6	-0.700	-0.627	RNPZn	40	1.198	0.503
Zn 11	-0.796	-0.499	PtZn	41	-0.631	1.411
			PtPZn	42	-1.291	0.805
			PtNZn	43	0.047	1.121
			PtNPZn	44	0.018	0.960
			PtRZn	45	0.606	0.936
			PtRPZn	46	1.137	0.392
			PtRNZn	47	0.688	1.006
			PtRNPZn	48	1.204	0.543
			AZn	49	-2.211	-0.859
			APZn	50	-1.708	-0.829
			ANZn	51	-0.325	-0.670
			ANPZn	52	-0.285	-0.541
			ARZn	53	0.569	-1.150
			ARPZn	54	0.618	-1.148
			ARNZn	55	0.777	-1.143
			ARNPZn	56	0.686	-1.149
			APtZn	57	-2.066	-0.773
			APtPZn	58	-2.109	-0.921
			APtNZn	59	-0.363	-0.861
			APtNPZn	60	-0.067	-0.735
			APtRZn	61	0.699	-1.243
			APtRPZn	62	0.707	-0.933
			APtRNZn	63	0.852	-1.220
			APtRNPZn	64	0.937	-0.957

Table S5 *P* values from the F statistic in the ANOVA for all parameters in the + rhizobia dataset at 6 and 11 weeks after sowing. Abbreviations of variates: shoot and root biomass and yield (DW=dry shoot weight, DRW=dry root weight, Seed=seed weight), biological N fixation (% Ndfa=% N fixed from the atmosphere, fixed N=biologically fixed N/plant), rhizobial nodulation (Nod=number of nodules, Nod DW=nodule dry weight), mycorrhizal colonisation (AMF=proportion of root length colonised by AMF (arcsine transformed), AMF RL=root length/plant colonised by AMF in cm). All elements represented are concentrations- N, P, K, Mg, S, Ca are percentages and Zn, B, Mn, Mo, Cu and Fe are mg/kg in the plant shoot biomass.

6 weeks																			
Table S.3	**Plant Parameters**								**%**						**mg/kg**					
Treatments	**DW**	**DRW**	**Nod DW***	**Nod #**	**% Ndfa**	**N fixed**	**AMF prop**	**AMF RL cm**	**N**	**P**	**K**	**Mg**	**Ca**	**S**	**Zn**	**B**	**Fe***	**Cu**	**Mn***	**Mo**
AMF	<.001	0.752	0.098	0.021	<.001	<.001	<.001	<.001	0.896	<.001	0.285	<.001	<.001	0.021	<.001	0.077	0.466	<.001	0.002	<.001
PT	0.077	0.355	0.786	0.283	0.683	0.269	0.251	0.340	0.652	0.071	0.270	0.566	0.993	0.198	0.006	0.117	0.167	0.064	0.036	0.044
AMF.PT	0.160	0.933	0.743	0.203	0.956	0.720	0.251	0.340	0.908	0.034	0.053	0.007	0.201	0.460	0.041	0.033	0.825	0.731	0.003	<.001
N	0.022	0.163	0.021	0.107	0.130	0.162	0.196	0.232	<.001	0.039	<.001	<.001	0.739	0.003	0.839	<.001	0.453	0.696	<.001	<.001
P	<.001	<.001	0.004	<.001	<.001	<.001	0.705	0.449	0.010	<.001	0.371	<.001	<.001	<.001	0.423	0.451	0.272	0.017	<.001	<.001
Zn	0.126	0.944	0.734	0.544	0.083	0.251	0.466	0.376	0.408	0.044	0.399	<.001	0.085	0.036	<.001	0.002	0.998	0.041	0.014	0.939
AMF.N	0.183	0.268	0.394	0.084	0.145	0.787	0.196	0.232	0.118	0.487	0.020	0.501	0.513	0.024	0.942	0.631	0.308	0.465	0.018	0.174
PT.N	0.931	0.579	0.886	0.413	0.961	0.297	0.444	0.541	0.204	0.016	0.926	0.919	0.906	0.480	0.060	0.731	0.833	0.569	0.061	0.273
AMF.P	<.001	0.697	0.150	0.126	<.001	0.001	0.705	0.449	0.102	0.002	0.549	<.001	<.001	<.001	0.324	0.537	0.547	0.245	<.001	<.001
PT.P	0.612	0.796	0.317	0.054	0.503	0.663	0.853	0.286	0.675	0.676	0.872	0.759	0.159	0.291	0.642	0.194	0.020	0.174	0.977	0.289
N.P	0.572	0.642	0.582	0.464	0.531	0.724	0.747	0.421	0.176	0.676	0.222	0.187	0.231	0.480	0.407	0.731	0.064	0.439	0.015	0.325
AMF.Zn	0.770	0.403	0.253	0.511	0.751	0.576	0.466	0.376	0.787	0.578	0.423	0.012	0.024	1.000	0.118	0.001	0.573	0.643	0.069	0.909
PT.Zn	0.095	0.964	0.731	0.487	0.060	0.020	0.103	0.261	0.933	0.267	0.442	0.501	0.780	0.114	0.603	0.945	0.918	0.093	0.281	0.226
N.Zn	0.580	0.535	0.727	0.283	0.947	0.653	0.085	0.271	0.577	0.926	0.452	0.886	0.897	0.378	0.893	0.304	0.137	0.261	0.119	0.002
P.Zn	0.912	0.681	0.931	0.209	0.766	0.745	0.553	0.456	0.393	0.117	0.899	0.698	0.940	0.009	0.850	0.731	0.976	0.650	0.487	0.622

(Continued)

6 weeks																				
Table S5 Cont.	Plant parameters								%						mg/kg					
Treatments	**DW**	**DRW**	**Nod DW***	**Nod #**	**% Ndfa**	**N fixed**	**AMF prop**	**AMF RL cm**	**N**	**P**	**K**	**Mg**	**Ca**	**S**	**Zn**	**B**	**Fe***	**Cu**	**Mn***	**Mo**
AMF.PT.N	<.001	0.002	0.356	0.001	0.002	<.001	0.444	0.541	0.026	0.025	0.007	0.038	0.006	0.036	0.019	0.006	0.555	0.047	0.984	0.009
AMF.PT.P	0.260	0.594	0.807	0.875	0.977	0.681	0.853	0.286	0.204	0.963	0.219	0.318	0.715	1.000	0.642	0.837	0.909	0.438	0.963	0.344
AMF.N.P	0.127	0.912	0.495	0.862	0.219	0.919	0.747	0.421	0.036	0.088	0.266	0.668	0.314	0.724	0.682	0.117	0.710	0.924	0.530	0.384
PT.N.P	0.220	0.928	0.139	0.386	0.552	0.131	0.953	0.387	0.424	0.379	0.427	0.263	0.983	0.378	0.213	0.373	0.507	0.716	0.223	0.098
AMF.PT.Zn	0.463	0.427	0.729	0.807	0.653	0.114	0.103	0.261	0.168	0.308	0.577	0.200	0.897	0.218	0.975	0.451	0.136	0.809	0.032	0.273
AMF.N.Zn	0.174	0.889	0.864	0.888	0.129	0.076	0.085	0.271	0.642	0.097	0.866	0.822	0.621	0.378	0.411	0.537	0.591	0.088	0.237	0.344
PT.N.Zn	0.022	0.462	0.246	0.224	0.507	0.091	0.943	0.279	0.882	0.028	0.266	0.215	0.479	0.291	0.986	0.631	0.023	0.526	0.298	0.131
AMF.P.Zn	0.710	0.097	0.112	0.025	0.725	0.312	0.553	0.456	0.382	1.000	0.636	0.230	0.434	0.218	0.717	0.631	0.117	0.922	0.853	0.939
PT.P.Zn	0.657	0.122	0.873	0.471	0.437	0.734	0.598	0.313	0.669	0.781	0.735	0.427	0.772	0.291	0.493	0.089	0.541	0.885	0.954	0.909
N.P.Zn	0.014	0.741	0.235	0.577	0.018	0.017	0.933	0.292	0.254	0.140	0.273	0.527	0.409	1.000	0.529	0.013	0.337	0.886	0.545	0.141
AMF.PT.N.P	0.091	0.128	0.622	0.718	0.871	0.052	0.953	0.387	0.867	0.180	0.953	0.791	0.379	0.055	0.341	0.373	0.701	0.259	0.350	0.849
AMF.PT.N.Zn	0.197	0.567	0.711	0.429	0.372	0.433	0.943	0.279	0.071	0.011	0.007	0.010	0.110	0.015	0.176	0.451	0.268	0.150	0.037	0.047
AMF.PT.P.Zn	0.123	0.797	0.493	0.968	0.689	0.343	0.598	0.313	0.116	0.308	0.549	0.886	0.182	1.000	0.785	0.631	0.454	0.586	0.999	0.071
AMF.N.P.Zn	0.468	0.562	0.140	0.673	0.480	0.874	0.933	0.292	0.570	0.140	0.080	0.281	0.872	0.015	0.018	0.245	0.304	0.232	0.954	0.761
PT.N.P.Zn	0.554	0.535	0.636	0.726	0.488	0.349	0.821	0.208	0.648	0.404	0.376	0.128	0.047	0.860	0.970	0.089	0.497	0.189	0.093	0.519
AMF.PT.N.P.Zn	0.532	0.083	0.207	0.026	0.463	0.440	0.821	0.208	0.583	0.578	0.636	0.984	0.675	0.596	0.317	0.245	0.068	0.536	0.495	0.595

(Continued)

11 weeks																							
Table S5 Cont.		**Plant parameters**										**%**						**mg/kg**					
Treatments	**DW**	**Seed**	**DRW**	**Nod DW***	**Nod ***	**% Ndfa**	**N fixed**	**AMF Prop**	**AMF RL cm**	**Prop Ves**	**Ves RL**	**N**	**P**	**K**	**Mg**	**Ca**	**S**	**Zn**	**B**	**Fe***	**Cu**	**Mn***	**Mo***
AMF	0.003	0.002	0.491	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.026	<.001	<.001	0.659	0.240	0.725	<.001	<.001	0.353	<.001	0.609	<.001
PT	0.986	0.364	0.366	0.897	0.137	0.159	0.929	0.595	0.058	0.789	0.457	0.915	0.538	0.021	0.161	0.535	0.725	0.120	0.151	0.498	0.291	0.586	0.296
AMF.PT	0.200	0.835	0.424	0.153	0.050	0.590	0.674	0.401	0.687	0.932	0.565	0.062	0.643	<.001	0.026	0.323	0.487	0.655	0.043	0.264	0.727	0.021	0.079
N	0.880	<.001	0.655	<.001	0.089	<.001	<.001	0.408	0.022	0.013	0.021	0.026	0.013	0.886	0.005	<.001	0.477	<.001	0.937	0.689	<.001	0.622	0.004
P	<.001	<.001	0.435	0.003	0.004	<.001	<.001	0.665	0.650	0.085	0.760	<.001	0.003	0.020	0.904	0.011	0.348	0.841	0.135	0.015	<.001	<.001	<.001
Zn	0.548	0.006	0.877	0.171	0.669	0.797	0.841	0.472	0.456	0.796	0.538	0.891	0.480	0.030	0.218	0.198	<.001	0.018	0.499	0.461	0.003	0.614	0.422
AMF.N	0.019	0.012	0.330	0.713	0.535	0.347	0.977	0.725	0.235	0.040	0.052	0.060	0.585	0.566	0.512	0.178	1.000	0.014	0.023	0.396	0.324	0.959	0.176
PT.N	0.091	0.107	0.369	0.638	0.292	0.182	0.167	0.613	0.788	0.516	0.652	0.830	0.585	0.041	0.549	0.709	1.000	0.298	0.091	0.375	0.759	0.402	0.942
AMF.P	<.001	<.001	0.210	<.001	<.001	<.001	<.001	0.196	0.164	0.027	0.483	0.150	0.007	0.002	0.106	0.685	0.088	0.530	0.424	0.965	0.299	<.001	<.001
PT.P	0.673	0.717	0.994	0.741	0.363	0.552	0.517	0.342	0.330	0.143	0.575	0.250	0.682	0.953	0.932	0.602	0.667	0.270	0.841	0.732	0.835	0.025	0.465
N.P	0.255	0.872	0.837	0.558	0.450	0.634	0.723	0.184	0.060	0.823	0.233	0.029	0.375	0.716	0.006	0.301	0.667	0.783	0.617	0.289	0.630	0.041	0.077
AMF.Zn	0.153	0.019	0.353	0.381	0.101	0.191	0.075	0.645	0.710	0.775	0.859	0.594	0.068	0.890	<.001	0.096	1.000	0.302	0.074	0.770	0.044	<.001	0.951
PT.Zn	0.495	0.576	0.874	0.116	0.010	0.347	0.256	0.141	0.176	0.495	0.514	0.990	0.946	0.963	0.182	0.199	0.390	0.847	0.163	0.348	0.198	0.037	0.215
N.Zn	0.970	0.453	0.813	0.470	0.721	0.870	0.565	0.923	0.520	0.949	0.740	0.830	0.891	0.333	0.408	0.962	0.198	0.427	0.920	0.689	0.289	0.059	0.022
P.Zn	0.518	0.868	0.463	0.258	0.282	0.083	0.330	0.549	0.705	0.449	0.750	0.817	0.340	0.584	0.408	0.610	0.390	0.381	0.689	0.998	0.306	0.372	0.767
AMF.PT.N	0.851	0.120	0.402	0.388	0.116	0.117	0.703	0.482	0.157	0.232	0.376	0.282	0.733	0.266	0.797	0.415	0.390	0.759	0.617	0.470	0.977	0.688	0.669
AMF.PT.P	0.247	0.101	0.120	0.816	0.202	0.733	0.770	0.931	0.976	0.334	0.879	0.588	0.838	0.314	0.627	0.242	0.667	0.557	0.318	0.884	0.835	0.407	0.648
AMF.N.P	0.292	0.074	0.213	0.006	0.712	0.109	0.003	0.623	0.330	0.806	0.418	0.122	0.585	0.111	0.512	0.724	0.390	0.044	0.038	0.087	0.114	0.844	0.112
PT.N.P	0.185	0.466	0.309	0.947	0.554	0.107	0.474	0.106	0.108	0.641	0.692	0.052	0.340	0.266	0.627	0.566	0.198	0.433	0.617	0.052	0.365	0.484	0.064
AMF.PT.Zn	0.668	0.864	0.131	0.026	0.296	0.286	0.768	0.745	0.795	0.827	0.774	0.963	0.413	0.869	0.408	0.291	0.198	0.767	0.074	0.732	0.357	0.679	0.069
AMF.N.Zn	0.472	0.701	0.393	0.006	0.023	0.937	0.942	0.204	0.629	0.573	0.955	0.086	0.453	0.052	0.119	0.331	0.667	0.888	0.617	0.639	0.431	0.038	0.745
PT.N.Zn	0.905	0.988	0.005	0.417	0.324	0.636	0.210	0.896	0.234	0.294	0.161	0.468	0.453	0.848	0.221	0.287	0.667	0.348	0.920	0.229	0.612	0.283	0.543
AMF.P.Zn	0.288	0.946	0.594	0.788	0.753	0.448	0.200	0.160	0.614	0.768	0.953	0.950	0.196	0.827	0.841	0.639	0.390	0.194	0.424	0.036	0.705	0.564	0.047
PT.P.Zn	0.608	0.671	0.785	0.848	0.983	0.159	0.404	0.115	0.291	0.651	0.906	0.606	0.375	0.995	0.587	0.440	0.667	0.759	0.548	0.443	0.638	0.339	0.160
N.P.Zn	0.345	0.889	0.202	0.042	0.192	0.164	0.420	0.498	0.580	0.703	0.210	0.114	0.221	0.367	0.408	0.912	0.034	0.938	0.272	0.342	0.385	0.055	0.068
AMF.PT.N.P	0.379	0.717	0.940	0.506	0.053	0.522	0.495	0.445	0.492	0.942	0.981	0.943	0.375	0.736	0.221	0.655	1.000	0.439	0.617	0.420	0.136	0.492	0.301
AMF.PT.N.Zn	0.195	0.104	0.164	0.607	0.712	0.783	0.989	0.192	0.951	0.587	0.321	0.830	0.785	0.668	0.347	0.321	0.198	0.537	0.920	0.182	0.489	0.232	0.608
AMF.PT.P.Zn	0.183	0.776	0.425	0.122	0.546	0.518	0.678	0.456	0.868	0.926	0.585	0.510	0.682	0.984	0.182	0.484	0.198	0.530	0.424	0.844	0.859	0.580	0.770
AMF.N.P.Zn	0.559	0.788	0.27	0.598	0.946	0.955	0.212	0.870	0.146	0.374	0.099	0.009	0.196	0.464	0.267	0.837	0.088	0.141	0.764	0.310	0.604	0.545	0.318
PT.N.P.Zn	0.830	0.103	0.685	0.112	0.476	0.050	0.291	0.504	0.514	0.323	0.227	0.164	0.539	0.566	0.977	0.987	0.198	0.530	0.368	0.278	0.753	0.295	0.884
AMF.PT.N.P.Zn	0.874	0.671	0.568	0.066	0.575	0.987	0.973	0.135	0.121	0.139	0.109	0.936	0.682	0.995	0.886	0.804	0.390	0.751	0.764	0.664	0.684	0.159	0.752

Table S6. *P* values from the *F* statistic in the ANOVA for all parameters in the complete dataset (except % Ndfa and Fixed N) at 6 and 11 weeks (w) after sowing. Abbreviations of variates: shoot and root biomass and yield (DW=dry shoot weight, DRW=dry root weight, Seed=seed weight), rhizobial nodulation (Nod = number of nodules, Nod DW = nodule dry weight), mycorrhizal colonisation (AMF= proportion of root length colonised by AMF (arcsine transformed), AMF RL= root length colonised by AMF in cm, Ves= proportion of root length with vesicles (arcsine transformed), Ves RL= root length with vesicles in cm. All elements represented are concentrations- N, P, K, Mg, S, Ca are percentages and Zn, B, Mn, Mo, Cu and Fe are mg/kg in the plant shoot biomass.

	6 weeks																					
Table S6	**Plant parameters**						**%**						**mg/kg**						**mg/plant**		**µg/plant**	
Treatment	**DW**	**# Nod**	**Nod DW**	**DRW**	**AMF prop**	**AMF RL cm**	**N**	**P**	**K**	**Mg**	**Ca**	**S**	**Zn**	**B**	**Cu**	**Fe**	**Mn**	**Mo**	**N uptake**	**P uptake**	**Zn uptake**	**N:P ratio**
AMF	<.001	0.052	0.264	0.226	<.001	<.001	0.386	<.001	0.058	0.017	<.001	0.187	<.001	0.732	<.001	0.192	<.001	<.001	<.001	<.001	<.001	<.001
PT	0.047	0.219	0.952	0.608	0.080	0.946	0.370	0.198	0.860	0.120	0.141	0.441	0.006	0.310	0.952	0.852	0.016	0.808	0.006	0.49	0.406	0.375
Rhiz	<.001	<.001	<.001	0.014	0.011	0.460	<.001	<.001	0.403	<.001	<.001	<.001	<.001	<.001	<.001	0.389	0.015	<.001	<.001	0.271	0.156	<.001
AMF.PT	0.911	0.151	0.752	0.865	0.085	0.945	0.582	0.232	0.068	0.035	0.151	0.902	0.053	0.570	0.961	0.747	0.309	0.045	0.739	0.485	0.102	0.932
AMF.Rhiz	<.001	0.236	0.144	0.435	0.012	0.461	0.306	<.001	0.462	<.001	0.001	<.001	<.001	0.290	0.002	0.331	0.461	<.001	<.001	0.003	0.066	<.001
PT.Rhiz	0.345	0.890	0.647	0.212	0.982	0.860	0.732	0.386	0.154	0.024	0.137	0.034	0.506	0.033	0.107	0.250	0.497	0.019	0.435	0.678	0.884	0.798
AMF.PT.Rhiz	0.035	0.972	0.429	0.981	0.959	0.861	0.489	0.155	0.589	0.020	0.618	0.352	0.669	0.234	0.847	0.853	0.002	<.001	0.006	0.244	0.182	0.25
N	<.001	0.193	0.015	0.002	0.061	0.529	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.533	<.001	<.001	<.001	<.001	<.001	<.001
P	<.001	<.001	0.002	0.298	0.215	0.023	0.101	<.001	0.248	0.110	0.087	0.024	0.132	0.004	0.102	0.481	<.001	<.001	<.001	<.001	<.001	<.001
Zn	0.028	0.982	0.714	0.858	0.273	0.387	0.122	<.001	0.063	<.001	<.001	<.001	<.001	<.001	<.001	0.467	<.001	0.285	0.282	0.759	<.001	0.019
AMF.N	0.002	0.035	0.272	0.376	0.059	0.526	0.518	<.001	<.001	0.007	0.146	<.001	<.001	0.001	<.001	0.563	0.007	<.001	0.004	<.001	<.001	<.001
PT.N	0.932	0.870	0.768	0.579	0.194	0.093	0.119	0.322	0.384	0.927	0.818	0.370	0.627	0.513	0.775	0.993	0.052	0.228	0.416	0.673	0.644	0.017
Rhiz.N	<.001	0.370	0.169	0.012	0.855	0.013	<.001	<.001	0.573	<.001	<.001	<.001	<.001	<.001	<.001	0.917	0.692	<.001	<.001	0.086	0.501	<.001
AMF.P	<.001	0.302	0.397	0.644	0.202	0.022	0.879	<.001	0.272	0.009	<.001	0.268	0.891	0.033	0.267	0.641	<.001	<.001	<.001	<.001	0.002	<.001
PT.P	0.066	0.326	0.289	0.049	0.833	0.317	0.415	0.509	0.461	0.855	0.436	0.561	0.272	0.732	0.598	0.722	0.576	0.111	0.031	0.015	0.02	0.952
Rhiz.P	0.009	0.050	0.094	0.098	0.522	0.507	0.213	0.039	0.978	0.003	<.001	<.001	0.657	0.028	0.451	0.132	0.074	0.252	<.001	0.477	0.175	0.669
N.P	0.432	0.655	0.677	0.369	0.561	0.584	0.457	0.575	0.554	0.927	0.378	0.429	0.595	0.250	0.569	0.570	<.001	0.077	0.032	0.579	0.211	0.502
AMF.Zn	0.550	0.435	0.642	0.517	0.262	0.390	0.026	0.157	0.009	<.001	<.001	0.268	<.001	<.001	0.892	0.486	<.001	0.761	0.818	0.092	0.112	0.043
PT.Zn	0.097	0.157	0.183	0.469	0.535	0.105	0.815	0.210	0.082	0.909	0.609	0.103	0.808	0.925	0.095	0.118	0.393	0.731	0.072	0.375	0.165	0.821
Rhiz.Zn	0.782	0.641	0.848	0.631	0.949	0.747	0.525	0.448	0.454	0.171	0.243	0.226	0.748	0.009	0.187	0.385	0.091	0.252	0.24	0.381	0.287	0.642
N.Zn	0.791	0.691	0.475	0.128	0.408	0.540	0.281	0.766	0.627	0.801	0.495	0.370	0.316	0.128	0.575	0.177	0.016	0.043	0.988	0.026	0.163	<.001
P.Zn	0.992	0.489	0.575	0.372	0.746	0.873	0.562	0.045	0.521	0.631	0.526	0.370	0.291	0.162	0.575	0.575	0.908	0.450	0.988	0.012	0.636	0.288
AMF.PT.N	<.001	0.004	0.428	0.098	0.202	0.094	0.029	0.291	0.065	0.567	0.269	0.493	0.805	0.554	0.297	0.818	0.387	0.498	0.003	0.027	0.012	0.173
AMF.Rhiz.N	0.365	0.139	0.764	0.678	0.865	0.013	0.274	0.006	0.172	0.050	0.535	<.001	<.001	0.005	0.002	0.193	0.014	0.030	0.313	0.184	0.713	0.503
PT.Rhiz.N	0.957	0.828	0.952	0.506	0.935	0.321	0.879	0.013	0.321	0.837	0.703	0.083	0.043	0.335	0.148	0.930	0.179	0.020	0.788	0.232	0.115	0.101
AMF.PT.P	0.211	0.341	0.134	0.774	0.872	0.319	0.261	0.947	0.973	0.631	0.926	0.874	0.927	0.732	0.733	0.522	0.110	0.341	0.074	0.366	0.298	0.852

(Continued)

							6 weeks															
Table S6 cont.	**Plant parameters**						**%**						**mg/kg**						**mg/plant**		**µg/plant**	
Treatment	**DW**	**# Nod**	**Nod DW**	**DRW**	**AMF prop**	**AMF RL cm**	**N**	**P**	**K**	**Mg**	**Ca**	**S**	**Zn**	**B**	**Cu**	**Fe**	**Mn**	**Mo**	**N uptake**	**P uptake**	**Zn uptake**	**N:P ratio**
AMF.Rhiz.P	0.304	0.141	0.128	0.587	0.499	0.510	0.096	0.014	0.759	0.050	0.325	<.001	0.240	0.113	0.106	0.444	0.925	0.003	0.018	0.164	0.44	0.917
PT.Rhiz.P	0.306	0.035	0.559	0.186	0.933	0.255	0.724	0.947	0.343	0.599	0.302	0.493	0.631	0.400	0.220	0.140	0.721	0.617	0.451	0.079	0.076	0.967
AMF.N.P	0.012	0.396	0.461	0.980	0.545	0.587	0.087	0.003	0.448	0.766	0.683	0.268	0.194	0.202	0.822	0.812	0.007	0.023	0.101	0.697	0.369	0.529
PT.N.P	0.679	0.404	0.106	0.514	0.562	0.131	0.671	0.792	0.716	0.101	0.382	0.370	0.985	0.225	0.361	0.506	0.265	0.268	0.374	0.721	0.753	0.546
Rhiz.N.P	0.090	0.527	0.627	0.165	0.946	0.147	0.448	0.249	0.305	0.164	0.512	0.103	0.567	0.400	0.117	0.367	0.298	0.450	0.286	0.317	0.562	0.406
AMF.PT.Zn	0.977	0.359	0.439	0.241	0.527	0.106	0.763	0.692	0.588	0.784	0.349	0.493	0.670	0.596	0.434	0.143	0.098	0.059	0.989	0.878	0.423	0.783
AMF.Rhiz.Zn	0.900	0.269	0.158	0.979	0.969	0.750	0.053	0.530	0.119	0.450	0.535	0.268	0.185	0.367	0.787	0.812	0.175	0.673	0.34	0.672	0.195	0.289
PT.Rhiz.Zn	0.293	0.824	0.441	0.651	0.047	0.349	0.744	0.741	0.464	0.385	0.877	0.792	0.654	0.975	0.844	0.134	0.902	0.362	0.151	0.339	0.771	0.457
AMF.N.Zn	0.047	0.736	0.939	0.359	0.429	0.543	0.134	0.014	0.862	0.963	0.390	0.712	0.101	0.436	0.228	0.542	0.784	0.129	0.007	0.491	0.186	<.001
PT.N.Zn	0.025	0.631	0.081	0.426	0.467	0.390	0.476	0.100	0.704	0.450	0.325	0.712	0.066	0.400	0.902	0.124	0.728	0.498	0.01	0.256	0.161	0.687
Rhiz.N.Zn	0.529	0.105	0.888	0.760	0.054	0.152	0.091	0.668	0.611	0.927	0.397	0.874	0.410	0.554	0.877	0.769	0.764	0.236	0.104	0.998	0.943	0.403
AMF.P.Zn	0.091	0.701	0.794	0.191	0.737	0.877	0.028	0.122	0.231	0.963	0.416	0.792	0.075	0.033	0.273	0.286	0.193	0.776	0.867	0.268	0.076	0.829
PT.P.Zn	0.837	0.986	0.544	0.102	0.212	0.856	0.108	0.468	0.539	0.283	0.444	0.874	0.278	0.732	0.873	0.586	0.650	0.372	0.57	0.42	0.171	0.275
Rhiz.P.Zn	0.850	0.085	0.675	0.948	0.535	0.257	0.128	0.817	0.418	0.360	0.465	0.024	0.421	0.276	0.240	0.554	0.663	0.806	0.633	0.874	0.295	0.161
N.P.Zn	0.025	0.523	0.260	0.796	0.738	0.428	0.400	0.071	0.048	0.507	0.877	0.958	0.724	0.067	0.353	0.092	0.820	0.576	0.019	0.501	0.081	0.86
AMF.PT.Rhiz.N	0.005	0.010	0.470	0.194	0.954	0.323	0.763	0.030	0.079	0.004	0.013	0.002	0.004	0.002	0.194	0.511	0.072	0.040	0.008	0.251	0.879	0.863
AMF.PT.Rhiz.P	0.566	0.807	0.270	0.787	0.893	0.257	0.012	0.895	0.111	0.522	0.576	0.874	0.598	0.596	0.412	0.522	0.088	0.976	0.511	0.553	0.306	0.574
AMF.PT.N.P	0.247	0.538	0.344	0.134	0.553	0.130	0.895	0.509	0.375	0.164	0.499	0.006	0.912	0.100	0.115	0.098	0.356	0.462	0.203	0.68	0.486	0.172
AMF.Rhiz.N.P	0.969	0.703	0.701	0.562	0.927	0.148	0.528	0.575	0.475	0.855	0.378	0.493	0.450	0.876	0.462	0.999	0.776	0.167	0.887	0.987	0.796	0.372
PT.Rhiz.N.P	0.116	0.766	0.412	0.904	0.626	0.706	0.645	0.130	0.492	0.697	0.367	0.874	0.094	0.685	0.875	0.195	0.492	0.536	0.066	0.597	0.768	0.832
AMF.PT.Rhiz.Zn	0.247	0.827	0.837	0.972	0.049	0.346	0.068	0.291	0.202	0.244	0.440	0.429	0.640	0.225	0.917	0.009	0.572	0.450	0.02	0.374	0.422	0.487
AMF.PT.N.Zn	0.200	0.539	0.877	0.480	0.497	0.393	0.831	0.057	0.409	0.385	0.118	0.635	0.019	0.685	0.271	0.110	0.644	0.631	0.17	0.619	0.784	0.168
AMF.Rhiz.N.Zn	0.831	0.880	0.721	0.980	0.050	0.151	0.325	0.921	0.960	0.766	0.818	0.155	0.583	0.827	0.613	0.969	0.501	0.589	0.989	0.712	0.568	0.794
PT.Rhiz.N.Zn	0.133	0.176	0.871	0.804	0.401	0.492	0.583	0.130	0.065	0.522	0.937	0.370	0.070	0.685	0.844	0.930	0.407	0.372	0.29	0.86	0.015	0.394
AMF.PT.P.Zn	0.413	0.390	0.516	0.109	0.203	0.852	0.532	0.741	0.114	0.132	0.829	0.635	0.773	0.513	0.123	0.501	0.106	0.961	0.832	0.916	0.79	0.596
AMF.Rhiz.P.Zn	0.274	0.005	0.019	0.403	0.543	0.259	0.002	0.122	0.565	0.193	0.071	0.083	0.024	0.088	0.475	0.819	0.613	0.837	0.268	0.801	0.797	0.134
PT.Rhiz.P.Zn	0.356	0.072	0.731	0.787	0.681	0.944	0.255	0.741	0.867	0.855	0.693	0.155	0.862	0.225	0.514	0.259	0.682	0.313	0.891	0.077	0.172	0.29
AMF.N.P.Zn	0.906	0.927	0.045	0.715	0.701	0.430	0.928	0.339	0.102	0.583	0.840	0.103	0.066	0.128	0.279	0.669	0.531	0.589	0.683	0.475	0.398	0.301
PT.N.P.Zn	0.825	0.760	0.545	0.332	0.878	0.217	0.372	0.552	0.700	0.105	0.033	0.127	0.413	0.304	0.514	0.580	0.045	0.362	0.319	0.523	0.5	0.32
Rhiz.N.P.Zn	0.079	1.000	0.407	0.973	0.639	0.877	0.671	0.766	0.592	0.963	0.227	0.958	0.627	0.640	0.717	0.305	0.642	0.332	0.129	0.14	0.287	0.966
AMF.PT.Rhiz.N.P	0.111	0.794	0.891	0.527	0.618	0.702	0.957	0.210	0.418	0.273	0.653	0.635	0.247	0.400	0.818	0.190	0.752	0.352	0.07	0.181	0.279	0.415
AMF.PT.Rhiz.N.Zn	0.417	0.250	0.650	0.071	0.429	0.489	0.027	0.076	0.005	0.038	0.624	0.014	0.576	0.779	0.648	0.301	0.086	0.111	0.793	0.466	0.425	0.75
AMF.PT.Rhiz.P.Zn	0.091	0.385	0.634	0.414	0.663	0.948	0.261	0.076	0.428	0.178	0.055	0.635	0.516	0.276	0.456	0.315	0.265	0.054	0.262	0.349	0.016	0.502
AMF.PT.N.P.Zn	0.945	0.038	0.541	0.317	0.908	0.215	0.831	0.692	0.907	0.437	0.512	0.874	0.996	0.513	0.479	0.151	0.184	0.498	0.827	0.94	0.478	0.709
AMF.Rhiz.N.P.Zn	0.291	0.753	0.671	0.524	0.604	0.881	0.473	0.249	0.489	0.507	0.683	0.188	0.179	0.640	0.649	0.351	0.726	0.821	0.565	0.717	0.821	0.715
PT.Rhiz.N.P.Zn	0.461	0.984	0.863	0.375	0.837	0.591	0.163	0.552	0.431	0.927	0.678	0.188	0.385	0.596	0.562	0.825	0.614	0.806	0.951	0.567	0.4	0.644
AMF.PT.Rhiz.N.P.Zn	0.346	0.033	0.143	0.924	0.808	0.587	0.412	0.235	0.458	0.423	0.235	0.429	0.185	0.088	0.175	0.899	0.878	0.898	0.507	0.038	0.019	0.324

(Continued)

11 weeks																										
Table S6 cont.	**Plant parameters**									**%**						**mg/kg**						**mg/plant**		**µg/plant**	
Treatment	**DW**	**Seed**	**# Nod**	**Nod DW**	**DRW**	**AMF prop**	**AMF RL cm**	**Ves Prop**	**Ves RL cm**	**N**	**P**	**K**	**Mg**	**Ca**	**S**	**Zn**	**B**	**Cu**	**Fe**	**Mn**	**Mo**	**N uptake**	**P uptake**	**Zn uptake**	**N:P ratio**
AMF	<.001	0.003	<.001	0.003	0.933	<.001	<.001	<.001	<.001	0.953	<.001	<.001	0.717	0.371	<.001	<.001	0.485	<.001	<.001	0.021	0.002	0.011	<.001	<.001	<.001
PT	0.695	0.593	0.117	0.492	0.262	0.575	0.755	0.899	0.641	0.217	0.395	0.097	0.850	0.561	0.390	0.005	0.754	0.070	0.054	0.854	0.154	0.494	0.216	0.069	0.696
Rhiz	<.001	<.001	<.001	<.001	<.001	0.038	0.628	0.165	0.256	<.001	<.001	0.098	<.001	<.001	<.001	<.001	0.630	<.001	<.001	<.001	<.001	<.001	<.001	0.652	<.001
AMF.PT	0.571	0.879	0.406	0.687	0.892	0.713	0.773	0.870	0.487	0.792	0.735	0.028	0.183	0.116	0.887	0.098	0.340	0.401	0.674	0.014	0.294	0.833	0.955	0.305	0.794
AMF.Rhiz	0.002	<.001	<.001	<.001	0.203	0.212	0.666	0.049	0.096	0.002	<.001	0.053	0.641	0.257	<.001	<.001	0.266	0.069	<.001	0.103	0.009	<.001	0.001	0.048	0.261
PT.Rhiz	0.720	0.288	0.364	0.937	0.06	0.314	0.240	0.765	0.475	0.274	0.889	0.649	0.825	0.818	0.611	0.150	0.321	0.431	0.013	0.310	0.904	0.474	0.568	0.353	0.719
AMF.PT.Rhiz	0.062	0.834	0.014	0.047	0.77	0.717	1.000	0.977	0.832	0.015	0.842	0.888	0.518	0.855	0.385	0.209	0.216	0.431	0.529	0.136	0.183	0.553	0.012	0.015	0.204
N	<.001	<.001	0.280	0.134	0.665	0.042	0.025	0.047	0.021	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.965	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
P	<.001	<.001	0.001	0.056	0.736	0.781	0.572	0.256	0.938	0.825	<.001	0.011	0.340	0.867	0.422	0.004	0.058	0.003	0.479	<.001	<.001	<.001	<.001	<.001	<.001
Zn	0.489	0.015	0.987	0.709	0.155	0.993	0.712	0.457	0.690	0.396	0.925	0.409	0.383	0.094	0.173	<.001	0.840	0.004	0.078	<.001	0.473	0.729	0.112	<.001	0.08
AMF.N	<.001	0.001	0.359	0.815	0.17	0.257	0.100	0.291	0.082	0.029	<.001	0.034	0.519	0.116	<.001	<.001	0.005	0.005	<.001	0.762	0.002	0.089	<.001	<.001	<.001
PT.N	0.082	0.115	0.441	0.959	0.045	0.347	0.737	0.620	0.972	0.536	0.208	0.491	0.054	0.167	0.675	0.039	0.547	0.777	0.106	0.797	0.031	0.124	0.269	0.003	0.633
Rhiz.N	<.001	<.001	0.101	0.012	0.915	0.317	0.468	0.309	0.448	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.879	<.001	<.001	<.001	<.001	<.001	<.001	0.002	<.001
AMF.P	<.001	<.001	<.001	<.001	0.058	0.008	0.410	0.005	0.169	0.108	<.001	0.059	0.131	0.737	0.830	0.172	0.972	0.062	0.253	0.027	<.001	<.001	<.001	<.001	<.001
PT.P	0.714	0.939	0.072	0.574	0.697	0.983	0.200	0.333	0.960	0.220	0.328	0.079	0.521	0.259	0.148	0.034	0.808	0.684	0.409	0.027	0.669	0.938	0.412	0.156	0.303
Rhiz.P	0.066	0.002	0.148	0.920	0.075	0.788	0.252	0.352	0.630	0.353	<.001	0.422	0.296	0.194	<.001	0.044	0.887	0.005	0.109	0.812	0.001	0.009	0.532	0.486	0.595
N.P	<.001	0.501	0.305	0.591	0.521	0.515	0.542	0.790	0.304	0.016	0.003	0.884	0.134	0.399	0.002	0.065	0.872	0.375	0.038	0.008	0.018	0.367	0.002	0.001	0.002
AMF.Zn	0.485	0.165	0.076	0.502	0.33	0.985	0.891	0.498	0.672	0.062	0.021	0.268	0.003	0.006	0.825	0.145	0.692	0.047	0.045	<.001	0.898	0.109	0.471	0.137	0.006
PT.Zn	0.940	0.798	0.013	0.054	0.86	0.638	0.290	0.644	0.691	0.620	0.176	0.382	0.422	0.579	0.580	0.672	0.135	0.307	0.312	0.234	0.800	0.98	0.394	0.729	0.056
Rhiz.Zn	0.757	0.051	0.507	0.310	0.763	0.369	0.557	0.294	0.225	0.284	0.946	0.917	0.713	0.766	0.991	0.006	0.472	0.980	0.282	0.629	0.675	0.386	0.684	0.511	0.456
N.Zn	0.644	0.386	0.657	0.900	0.4	0.511	0.317	0.315	0.737	0.822	0.194	0.371	0.950	0.850	0.368	0.310	0.566	0.980	0.548	0.049	0.225	0.959	0.857	0.167	0.641
P.Zn	0.346	0.906	0.694	0.520	0.657	0.682	0.446	0.957	0.577	0.177	0.062	0.334	0.392	0.635	0.758	0.086	0.779	0.209	0.681	0.300	0.144	0.954	0.192	0.459	0.33
AMF.PT.N	0.723	0.127	0.295	0.963	0.054	0.620	0.559	0.859	0.628	0.063	0.208	0.868	0.423	0.291	0.854	0.555	0.711	0.739	0.445	0.230	0.283	0.088	0.634	0.185	0.052
AMF.Rhiz.N	0.441	0.473	0.945	0.531	0.536	0.115	0.904	0.157	0.393	0.960	<.001	0.122	0.443	0.249	<.001	<.001	0.100	0.980	<.001	0.809	0.053	0.818	0.082	0.871	0.233
PT.Rhiz.N	0.275	0.340	0.363	0.736	0.946	0.762	0.496	0.206	0.527	0.706	0.537	0.147	0.140	0.081	0.675	0.274	0.544	0.071	0.183	0.461	0.035	0.438	0.551	0.331	0.632
AMF.PT.P	0.448	0.077	0.153	0.350	0.892	0.670	0.163	0.291	0.651	0.171	0.369	0.866	0.189	0.860	0.595	0.763	0.904	0.092	0.192	0.690	0.706	0.21	0.52	0.742	0.204
AMF.Rhiz.P	<.001	0.034	0.158	0.433	0.113	0.294	0.332	0.878	0.652	0.983	0.047	0.131	0.271	0.174	0.107	0.437	0.980	0.375	0.865	0.034	0.009	<.001	0.014	0.003	0.963
PT.Rhiz.P	0.737	0.520	0.719	0.121	0.270	0.222	0.975	0.434	0.490	0.941	0.145	0.076	0.949	0.282	0.058	0.274	0.824	0.873	0.527	0.632	0.129	0.786	0.787	0.705	0.566
AMF.N.P	0.589	0.081	0.992	0.803	0.631	0.953	0.898	0.510	0.644	0.375	0.341	0.434	0.434	0.946	0.977	0.794	0.004	0.824	0.679	0.181	0.964	0.359	0.311	0.045	0.002
PT.N.P	0.379	0.574	0.795	0.460	0.164	0.075	0.029	0.467	0.602	0.161	0.429	0.608	0.027	0.087	0.858	0.220	0.049	0.739	0.143	0.617	0.096	0.924	0.414	0.436	0.348
Rhiz.N.P	0.096	0.673	0.859	0.163	0.237	0.302	0.070	1.000	0.580	0.941	0.047	0.612	0.658	0.360	0.010	0.112	0.109	0.133	0.102	0.804	0.347	0.246	0.023	0.356	0.212
AMF.PT.Zn	0.603	0.173	0.577	0.471	0.401	0.914	0.823	0.913	0.646	0.093	0.076	0.204	0.402	0.126	0.420	0.308	0.471	0.739	0.757	0.208	0.396	0.344	0.612	0.924	0.026
AMF.Rhiz.Zn	0.091	0.020	0.396	0.589	0.469	0.573	0.734	0.736	0.511	0.204	0.883	0.206	0.209	0.682	0.825	0.626	0.999	0.824	0.909	0.782	0.858	0.237	0.196	0.373	0.887
PT.Rhiz.Zn	0.288	0.259	0.096	0.643	0.999	0.163	0.493	0.204	0.641	0.610	0.203	0.438	0.522	0.788	0.145	0.809	0.826	0.482	0.843	0.190	0.235	0.231	0.649	0.193	0.058
AMF.N.Zn	0.728	0.728	0.008	0.018	0.357	0.304	0.691	0.117	0.242	0.621	0.620	0.563	0.193	0.095	0.655	0.599	0.565	0.730	0.918	0.588	0.781	0.602	0.109	0.178	0.817
PT.N.Zn	0.465	0.380	0.097	0.644	0.873	0.217	0.579	0.834	0.620	0.721	0.223	0.754	0.568	0.667	0.214	0.862	0.458	0.437	0.149	0.643	0.101	0.509	0.712	0.374	0.984
Rhiz.N.Zn	0.600	0.758	0.902	0.920	0.876	0.436	0.861	0.353	0.439	0.641	0.255	0.891	0.842	0.474	0.628	0.079	0.977	0.922	0.112	0.775	0.499	0.455	0.563	0.348	0.395
AMF.P.Zn	0.434	0.761	0.566	0.135	0.624	0.814	0.694	0.886	0.570	0.836	0.156	0.216	0.004	0.044	0.587	0.473	0.013	0.168	0.429	0.110	0.063	0.377	0.496	0.938	0.18
PT.P.Zn	0.665	0.148	0.769	0.954	0.745	0.044	0.421	0.876	0.981	0.095	0.893	0.208	0.039	0.068	0.371	0.889	0.469	0.164	0.262	0.734	0.396	0.646	0.652	0.609	0.57
Rhiz.P.Zn	0.892	0.888	0.187	0.065	0.118	0.729	0.781	0.397	0.328	0.276	0.453	0.724	0.142	0.319	0.219	0.012	0.776	0.444	0.583	0.982	0.088	0.526	0.999	0.178	0.239
N.P.Zn	0.400	0.525	0.062	0.023	0.929	0.875	0.899	0.944	0.419	0.149	0.035	0.954	0.098	0.102	0.541	0.266	0.544	0.922	0.090	0.429	0.712	0.862	0.107	0.19	0.279
AMF.PT.Rhiz.N	0.968	0.360	0.143	0.869	0.355	0.695	0.216	0.213	0.491	0.515	0.391	0.207	0.116	0.220	0.269	0.763	0.176	0.342	0.461	0.094	0.438	0.589	0.644	0.57	0.533
AMF.PT.Rhiz.P	0.240	0.421	0.544	0.873	0.421	0.751	0.176	0.925	0.802	0.447	0.255	0.257	0.987	0.622	0.931	0.801	0.938	0.648	0.134	0.559	0.570	0.836	0.513	0.26	0.725
AMF.PT.N.P	0.547	0.748	0.139	0.416	0.476	0.561	0.156	0.931	0.969	0.699	0.799	0.833	0.730	0.797	0.593	0.749	0.903	0.252	0.408	0.335	0.158	0.39	0.423	0.603	0.581
AMF.Rhiz.N.P	0.223	0.286	0.565	0.199	0.963	0.496	0.261	0.705	0.541	0.385	0.758	0.387	0.254	0.726	0.374	0.097	0.555	0.019	0.282	0.117	0.343	0.046	0.111	0.016	0.527
PT.Rhiz.N.P	0.181	0.562	0.495	0.598	0.040	0.788	0.906	0.863	0.998	0.414	0.056	0.081	0.123	0.044	0.229	0.050	0.491	0.342	0.021	0.184	0.781	0.638	0.904	0.864	0.783
AMF.PT.Rhiz.Zn	0.217	0.104	0.267	0.019	0.319	0.604	0.912	0.712	0.937	0.103	0.007	0.147	0.050	0.043	0.030	0.195	0.798	0.060	0.579	0.440	0.009	0.064	0.257	0.905	0.003
AMF.PT.N.Zn	0.500	0.169	0.617	0.684	0.077	0.895	0.550	0.187	0.152	0.581	0.737	0.560	0.879	0.806	0.298	0.771	0.653	0.205	0.466	0.267	0.373	0.315	0.935	0.56	0.472
AMF.Rhiz.N.Zn	0.123	0.344	0.319	0.206	0.057	0.562	0.825	0.366	0.273	0.145	0.168	0.008	0.761	0.474	0.358	0.510	0.950	0.337	0.400	0.053	0.820	0.94	0.154	0.111	0.518
PT.Rhiz.N.Zn	0.595	0.393	0.927	0.691	0.661	0.284	0.329	0.298	0.172	0.240	0.737	0.651	0.569	0.735	0.432	0.290	0.369	0.513	0.056	0.417	0.318	0.295	0.798	0.202	0.833

Table S6 cont.	Plant parameters									%						mg/kg						mg/plant		µg/plant	
Treatment	DW	Seed	# Nod	Nod DW	DR W	AMF prop	AMF RL cm	Ves Prop	Ves RL cm	N	P	K	Mg	Ca	S	Zn	B	Cu	Fe	Mn	Mo	N uptake	P uptake	Zn uptake	N:P ratio
AMF.PT.P.Zn	0.109	0.571	0.950	0.547	0.209	0.102	0.919	0.902	0.978	0.911	0.249	0.423	0.780	0.890	0.173	0.395	0.474	0.188	0.286	0.165	0.216	0.197	0.343	0.178	0.648
AMF.Rhiz.P.Zn	0.321	0.682	0.941	0.912	0.791	0.122	0.299	0.835	0.624	0.781	0.914	0.132	0.016	0.053	0.717	0.608	0.711	0.019	0.662	0.023	0.633	0.303	0.257	0.27	0.252
PT.Rhiz.P.Zn	0.672	0.433	0.795	0.113	0.672	0.983	0.580	0.488	0.894	0.025	0.238	0.213	0.223	0.131	0.171	0.669	0.078	0.513	0.495	0.427	0.520	0.094	0.754	0.841	0.121
AMF.N.P.Zn	0.483	0.817	0.830	0.294	0.258	0.182	0.252	0.564	0.088	0.050	0.097	0.079	0.535	0.116	0.089	0.110	0.833	0.184	0.667	0.340	0.410	0.546	0.389	0.446	0.496
PT.N.P.Zn	0.210	0.105	0.439	0.691	0.156	0.111	0.197	0.029	0.113	0.177	0.103	0.236	0.885	0.845	0.115	0.268	0.886	0.648	0.625	0.978	0.181	0.813	0.98	0.834	0.663
Rhiz.N.P.Zn	0.462	0.393	0.833	0.245	0.688	0.485	0.402	0.601	0.393	0.727	0.502	0.324	0.111	0.032	0.091	0.299	0.492	0.147	0.375	0.132	0.204	0.751	0.163	0.672	0.963
AMF.PT.Rhiz.N.P	0.387	0.805	0.106	0.230	0.460	0.701	0.590	0.863	0.944	0.760	0.429	0.831	0.887	0.616	0.593	0.682	0.330	0.079	0.565	0.882	0.570	0.835	0.329	0.446	0.457
AMF.PT.Rhiz.N.Zn	0.136	0.239	0.931	0.373	0.613	0.128	0.603	0.500	0.903	0.757	0.511	0.302	0.266	0.730	0.733	0.383	0.171	0.164	0.936	0.756	0.235	0.352	0.009	0.018	0.31
AMF.PT.Rhiz.P.Zn	0.534	0.903	0.369	0.385	0.219	0.485	0.911	0.816	0.488	0.393	0.502	0.377	0.309	0.323	0.992	0.797	0.325	0.022	0.217	0.042	0.553	0.791	0.461	0.768	0.555
AMF.PT.N.P.Zn	0.476	0.301	0.186	0.473	0.698	0.772	0.265	0.247	0.488	0.343	0.348	0.175	0.577	0.668	0.649	0.032	0.203	0.739	0.559	0.492	0.029	0.252	0.928	0.244	0.182
AMF.Rhiz.N.P.Zn	0.785	0.850	0.916	0.410	0.931	0.259	0.466	0.630	0.627	0.295	0.893	0.295	0.722	0.371	0.887	0.835	0.241	0.337	0.956	0.096	0.865	0.421	0.981	0.757	0.611
PT.Rhiz.N.P.Zn	0.369	0.348	0.931	0.030	0.450	0.380	0.305	0.989	0.822	0.362	0.572	0.868	0.814	0.845	0.605	0.182	0.513	0.434	0.224	0.181	0.181	0.36	0.334	0.374	0.343
AMF.PT.Rhiz.N.P.Zn	0.654	0.710	0.887	0.805	0.108	0.123	0.540	0.151	0.299	0.648	0.173	0.767	0.610	0.171	0.064	0.494	0.482	0.839	0.321	0.042	0.042	0.486	0.77	0.378	0.228

Table S7. *P* values from the *F* statistic of the main effects of ANOVA for nutrient concentrations in mung bean shoots at 6 and 11 weeks after sowing. AMF=Arbuscular mycorrhizal fungi, Rhiz=rhizobia, PT=*Pratylenchus thornei*; N=nitrogen; P=phosphorus; Z=zinc

6 weeks												
	%						mg/kg					
TRT	N	P	K	Ca	Mg	S	Zn	B	Cu	Fe	Mo	Mn
AMF	0.365	<.001	0.068	<.001	0.095	0.324	<.001	0.751	<.001	0.177	<.001	<.001
Rhiz	<.001	<.001	0.425	<.001	<.001	<.001	<.001	<.001	<.001	0.373	<.001	0.034
PT	0.349	0.313	0.867	0.237	0.289	0.567	0.043	0.346	0.973	0.847	0.879	0.036
N	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.532	<.001	<.001
P	0.146	<.001	0.279	0.134	0.16	0.092	0.231	0.016	0.118	0.48	<.001	<.001
Zn	0.17	0.005	0.082	0.003	<.001	0.006	<.001	<.001	0.003	0.467	0.364	<.001
	11 weeks											
	N	P	K#	Ca #	Mg #	S #	Zn	B	Cu	Fe#	Mo	Mn#
AMF	0.994	<.001	<.001	0.696	0.352	0.002	<.001	<.001	<.001	0.475	0.005	0.041
Rhiz	<.001	<.001	0.126	<.001	<.001	<.001	<.001	<.001	<.001	0.617	<.001	<.001
PT	0.356	0.604	0.124	0.86	0.537	0.491	0.1	0.074	0.135	0.738	0.183	0.893
N	0.003	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.993	<.001	<.001
P	0.789	<.001	0.017	0.406	0.858	0.64	0.084	0.008	0.532	0.055	0.004	<.001
Zn	0.523	0.998	0.424	0.448	0.166	0.391	<.001	0.012	0.199	0.814	0.548	<.001

# indicates transformed data

Indicates increase in nutrient concentration

Indicates decrease in nutrient concentration

Table S8. *P* values from the *F* statistic in the ANOVA for mycorrhization parameters at 6 and 11 weeks (w) after sowing in the added AMF dataset. AMF=Arbuscular mycorrhizal fungi, Rhiz=rhizobia, PT=*Pratylenchus thornei*; N=nitrogen; P=phosphorus; Z=zinc

Treatment	Arcsine AMF prop 6 w	RL colonised AMF cm 6 w	Arcsine AMF prop 11 w	RL colonised AMF cm 11 w	Arcsine Vesicles prop 11 w	RL vesicles cm 11 w
Rhiz	0.002	0.117	0.015	0.616	0.040	0.112
PT	0.052	0.239	0.441	0.742	0.845	0.494
Rhiz.PT	0.742	0.176	0.584	0.517	0.856	0.583
N	0.102	0.295	0.047	0.034	0.097	0.032
P	0.085	0.511	0.063	0.887	0.032	0.488
Zn	0.417	0.972	0.995	0.898	0.437	0.661
Rhiz.N	0.849	0.498	0.105	0.739	0.185	0.391
PT.N	0.080	0.224	0.778	0.891	0.861	0.811
Rhiz.P	0.743	0.649	0.621	0.246	0.670	0.987
PT.P	0.857	0.842	0.798	0.143	0.269	0.789
N.P	0.796	0.742	0.708	0.685	0.829	0.428
Rhiz.Zn	0.745	0.805	0.832	0.892	0.448	0.320
PT.Zn	0.769	0.548	0.819	0.481	0.754	0.648
N.Zn	0.624	0.981	0.814	0.740	0.160	0.422
P.Zn	0.979	0.433	0.683	0.526	0.914	0.549
Rhiz.PT.N	0.760	0.405	0.660	0.292	0.170	0.482
Rhiz.PT.P	0.793	0.273	0.567	0.446	0.632	0.814
Rhiz.N.P	0.642	0.635	0.280	0.108	0.840	0.535
PT.N.P	0.802	0.456	0.137	0.049	0.656	0.765
Rhiz.PT.Zn	0.081	0.508	0.228	0.662	0.369	0.836
Rhiz.N.Zn	0.088	0.277	0.391	0.827	0.317	0.320
PT.N.Zn	0.707	0.088	0.388	0.527	0.403	0.305
Rhiz.P.Zn	0.779	0.691	0.232	0.469	0.563	0.434
PT.P.Zn	0.330	0.254	0.023	0.618	0.985	0.978
N.P.Zn	0.444	0.106	0.456	0.575	0.781	0.181
Rhiz.PT.N.P	0.364	0.739	0.680	0.718	0.850	0.971
Rhiz.PT.N.Zn	0.211	0.467	0.775	0.801	0.841	0.506
Rhiz.PT.P.Zn	0.405	0.529	0.649	0.714	0.801	0.659
Rhiz.N.P.Zn	0.880	0.415	0.249	0.390	0.582	0.475
PT.N.P.Zn	0.814	0.121	0.234	0.188	0.068	0.226
Rhiz.PT.N.P.Zn	0.890	0.559	0.589	0.811	0.821	0.439

Table S9. *P* values from the *F* statistic in the ANOVA for *P. thornei* population densities at 6 and 11 weeks (w) after sowing in the added *P. thornei* dataset. *P. thornei* data are log transformed. AMF=Arbuscular mycorrhizal fungi, Rhiz=rhizobia, PT=*Pratylenchus thornei*; N=nitrogen; P=phosphorus; Z=zinc

Treatment	lnPTKG(x+1) 6 w	lnPTKG(x+1) 11 w
AMF	0.579	0.018
Rhiz	0.111	0.919
AMF.Rhiz	0.603	0.200
N	0.484	0.001
P	0.874	0.007
Zn	0.881	0.001
AMF.N	0.403	0.677
Rhiz.N	0.009	0.457
AMF.P	0.840	0.485
Rhiz.P	0.045	0.247
N.P	0.520	0.675
AMF.Zn	0.002	0.146
Rhiz.Zn	0.766	0.507
N.Zn	0.369	0.689
P.Zn	0.721	0.932
AMF.Rhiz.N	0.770	0.556
AMF.Rhiz.P	0.082	0.467
AMF.N.P	0.722	0.948
Rhiz.N.P	0.318	0.185
AMF.Rhiz.Zn	0.700	0.207
AMF.N.Zn	0.627	0.851
Rhiz.N.Zn	0.816	0.814
AMF.P.Zn	0.699	0.344
Rhiz.P.Zn	0.678	0.727
N.P.Zn	0.457	0.532
AMF.Rhiz.N.P	0.903	0.869
AMF.Rhiz.N.Zn	0.209	0.712
AMF.Rhiz.P.Zn	0.258	0.514
AMF.N.P.Zn	0.746	0.704
Rhiz.N.P.Zn	0.058	0.994
AMF.Rhiz.N.P.Zn	0.208	0.807

Table S10 *P* values from the *F* statistic in the ANOVA for parameters of nodulation at 6 and 11 weeks (w) after sowing in the added rhizobia dataset. Nodule numbers and nodule dry weights are square root transformed. AMF=Arbuscular mycorrhizal fungi, Rhiz=rhizobia, PT=P*ratylenchus thornei*; N=nitrogen; P=phosphorus; Z=zinc.

Treatment	Nodule numbers/plant 6 w	Nodule DW/ plant 6 w	Nodule numbers/plant 11 w	Nodule DW/plant 11 w
AMF	0.044	0.098	<.001	<.001
PT	0.443	0.786	0.137	0.897
AMF.PT	0.305	0.743	0.05	0.153
N	0.195	0.021	0.089	<.001
P	<.001	0.004	0.004	0.003
Zn	0.773	0.734	0.669	0.171
AMF.N	0.035	0.394	0.535	0.713
PT.N	0.822	0.886	0.292	0.638
AMF.P	0.14	0.15	<.001	<.001
PT.P	0.069	0.317	0.363	0.741
N.P	0.524	0.582	0.45	0.558
AMF.Zn	0.846	0.253	0.101	0.381
PT.Zn	0.333	0.731	0.01	0.116
N.Zn	0.233	0.727	0.721	0.47
P.Zn	0.155	0.931	0.282	0.258
AMF.PT.N	0.002	0.356	0.116	0.388
AMF.PT.P	0.675	0.807	0.202	0.816
AMF.N.P	0.782	0.495	0.712	0.006
PT.N.P	0.503	0.139	0.554	0.947
AMF.PT.Zn	0.679	0.729	0.296	0.026
AMF.N.Zn	0.773	0.864	0.023	0.006
PT.N.Zn	0.279	0.246	0.324	0.417
AMF.P.Zn	0.057	0.112	0.753	0.788
PT.P.Zn	0.283	0.873	0.983	0.848
N.P.Zn	0.705	0.235	0.192	0.042
AMF.PT.N.P	0.833	0.622	0.053	0.506
AMF.PT.N.Zn	0.298	0.711	0.712	0.607
AMF.PT.P.Zn	0.996	0.493	0.546	0.122
AMF.N.P.Zn	0.895	0.14	0.946	0.598
PT.N.P.Zn	0.847	0.636	0.476	0.112
AMF.PT.N.P.Zn	0.014	0.207	0.575	0.066

Table S11 Gaussian Regression Relationships between DW and N:P ratio at different times of assessment

Gaussian Regression Relationships between DW and time of assessment						
	m	s	a	b	R2	P- value
N:P ratio 6 and DW 6	11.56	25.54	-0.8552	216.5	NA	ns
N:P ratio 11 and DW 11	11.17	10.3	-3.1	211	0.43	P<0.001
N:P ratio 6 and DW 11	11.46	22.09	-23.42	1575	0.29	P<0.001
Gaussian Regression Relationships between DW and N:P ratio at 11 weeks and grouping						
Treatment	m	s	a	b	R2	P- value
Rhizobia grouped					0.63	*P*<0.001
- rhizobia	9.38	18	-12	9.377		
+ rhizobia	10.59	11.5	-4.7	10.59		
AMF grouped					0.64	P<0.001
- AMF	12.91	5	1.71	12.91		
+ AMF	8.47	11.77	-17.33	8.474		
P. thornei grouped					0.42	P<0.001
- *P. thornei*	11.55	3.876	2.733	11.55		
+ *P. thornei*	11.27	20.1	-25.46	11.27		
N grouped					0.71	P<0.001
- N	12.24	3.304	2.051	12.24		
+ N	8.63	4.11	3.326	8.629		
P grouped					0.57	P<0.001
- P	9.96	1.13	3.121	9.955		
+ P	12.57	18.32	-13.29	12.57		
Zn grouped					0.42	P<0.001
- Zn	10.49	2.91	2.884	10.49		
+ Zn	11.15	21.51	-24.42	11.15		

APPENDIX D

Conference Citations (Poster & Oral)

Poster

Tabah EC, Owen KJ, Zwart RS, Thompson JP (2018) Interactions of nematode, AM fungi and rhizobia in mung bean, 5th Feb 2018, Centre for Crop Health opening day, USQ

Tabah EC, Owen KJ, Zwart RS, Marchuk A, Thompson JP (2019) Arbuscular mycorrhizal fungi drive nodulation by rhizobia and yield of mung bean despite infestation with *Pratylenchus thornei*. Proceedings, Australasian Plant Pathology Conference, 25-28th Nov 2019, Melbourne, Victoria, Poster Board 67, p 238 (*Awarded second prize for Student poster*)

Oral

Tabah EC, Owen KJ, Thompson JP (2019) Arbuscular mycorrhizal fungi drive nodulation by rhizobia and yield of mung bean. Proceedings, Australian Summer Grains Conference, 8-10th July 2019, Gold Coast, Queensland.

Gough EC, Owen KJ, Zwart RS, Marchuk A, Thompson JP (2020) Multipartite interactions between *Pratylenchus thornei*, arbuscular mycorrhizal fungi, rhizobia and nutrients in mung bean. Proceedings, 7th International Congress of Nematology, Antibes-le-Pins, France (accepted for oral presentation). **Original date May 2020. Postponed due to Covid-19.*

Gough EC, Owen KJ, Zwart RS, Marchuk A, Thompson JP (2020) The good, the bad and the mung bean; Complex interactions between mycorrhizal fungi, rhizobia and root-lesion nematodes and their effects on nitrogen fixation and yield in mung bean. SunFix 2020, Sydney University Nitrogen Fixation Online Symposium, Nov 2020. (*Alan Gibson Memorial Prize for the best student presentation*)

Gough EC, Owen KJ, Zwart RS, Marchuk A, Thompson JP (2021) Mycorrhizal fungi improve yield, biomass and nitrogen fixation by rhizobia but increase population densities of the root-lesion nematode *Pratylenchus thornei* in mung bean. APPS QLD Seminar Series-online, July 2021.

Gough EC, Owen KJ, Zwart RS, Thompson JP (2021) Comparing interactive effects of microbial symbionts and nutrients on biological nitrogen fixation, nutrition and yield of mung bean (*Vigna radiata*). Association of Applied Biologists, Legume Science and Practice 2, Sep 2021. Online Conference.

Farmer Newsletters/ Articles

Owen KJ, **Tabah EC**, Thompson JP (2018) New PreDictaB function helps sidestep nutrient disorder. Ground Cover Issue 136 (Northern) Sept-Oct, pp22-23 (Liz Wells, reporter). http://groundcover.realviewdigital.com/?xml=GCN&iid=161090#folio=22

General presentations

Thompson JP, **Tabah EC** (2018) Research on root-lesion nematodes and arbuscular mycorrhizal fungi of mung bean. Australian Mung bean Association Members research review, October 2018, Toowoomba.

Owen KJ, Sheedy JG, **Tabah EC** (2019) Nematodes, arbuscular mycorrhizal fungi and rhizobia. Australian Mung bean Association, Hermitage Field Walk, April 2019, Warwick, QLD.

Thompson, JP, Owen KJ, **Tabah, EC** (2019) Mung beans, nematodes and mycorrhizal fungi. Mung bean Agronomy annual team meeting, June 2019, Toowoomba.

Gough EC (2020) Exploring interactions of AMF, rhizobia and *P. thornei* in mung beans - could lack of AMF be a cause of nodulation failure in mung beans? GRDC Updates, Online Seminar, August 2020.

Gough EC (2020). The interactions between arbuscular mycorrhizal fungi, root-lesion nematodes and rhizobia in mung bean. Australian Mung bean Association technical day, Jondaryan, September 2020.

Gough EC (2021) AMF and P interactions improving N fixation in mung bean. Mung bean extension and communication meeting, May 2021, Toowoomba.