Plant Soil (2022) 472:421–449
https://doi.org/10.1007/s11104-021-05254-8

REGULAR ARTICLE

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*)

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Received: 21 July 2021 / Accepted: 30 November 2021 / Published online: 23 January 2022

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.
Conclusions 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.

Keywords Arbuscular mycorrhizal fungi · Rhizobia · Biological nitrogen fixation · *Vigna radiata* · Plant nutrition · *Pratylenchus thornei*

Responsible Editor: Katharina Pawlowski.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-021-05254-8.

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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 soilborne 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 broadleaf 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 3×10^6 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) (Thompson 1994a). 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). Mung bean was inoculated using an initial inoculation density of 10 *P. thornei*/g soil which is an order of population density that 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 (2 M KCl extraction), ammonium-N 16 mg/kg soil (2 M 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 h 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 efficiency of 88% at 48 h (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 and 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

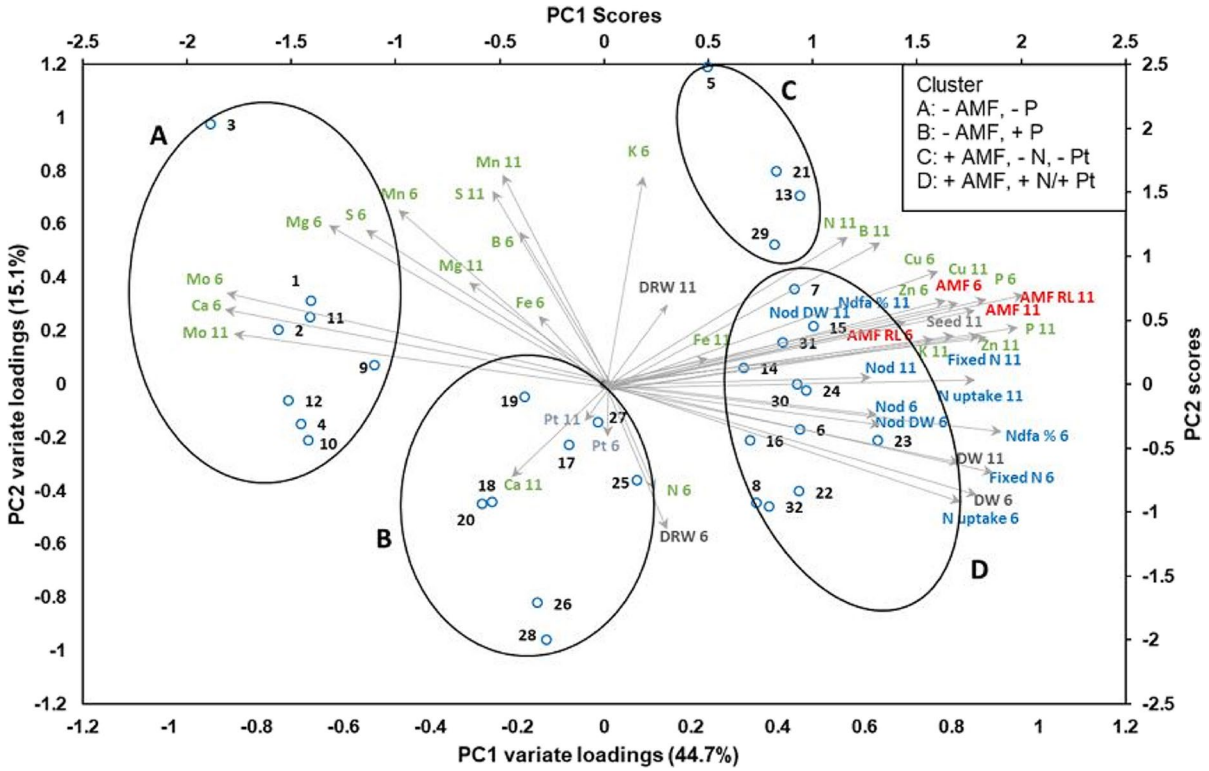

Fig. 1 Biplot from principal components analysis of the rhizobia-inoculated treatments for mung bean after 6 and 11 weeks growth. Small blue open circles represent treatment scores, with the code numbers referring to treatment designations listed in Supplementary Table S1, while numerical values of treatment 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

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.

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).

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 all other ANOVAs are given in Supplementary Tables S6–S9. 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

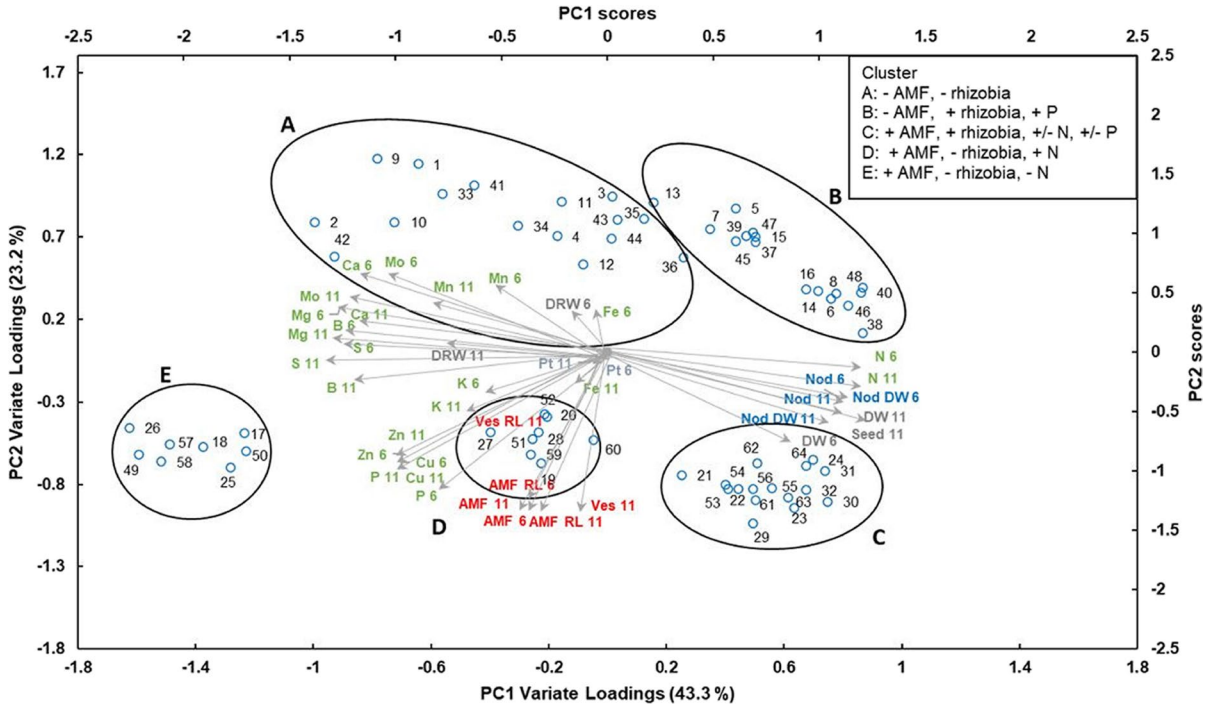

Fig. 2 Biplot from principal components analysis of the treatments for mung bean after 6 and 11 weeks growth. Small blue open circles represent treatment scores, with the code numbers referring to treatment designations listed in Supplementary Table S2, while numerical values of treatment scores and 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 (*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 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

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

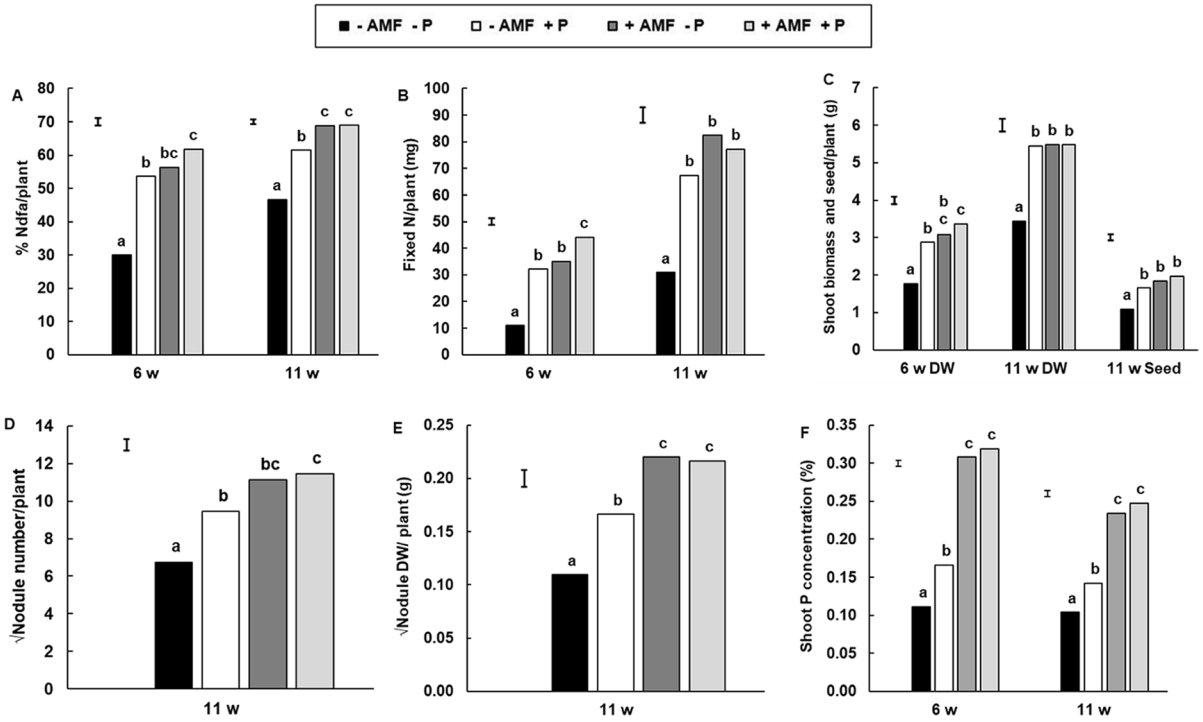

Fig. 3 The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and P in rhizobia-inoculated plants at 6 and/or 11 weeks (w) on (**A**) % nitrogen derived from the atmosphere (% Ndfa), (**B**) fixed nitrogen (N) (mg/kg) in the plant, (**C**) shoot biomass/plant (DW) and seed weight/plant, (**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 at 6 or 11 weeks 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.)

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).

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, 5 and 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

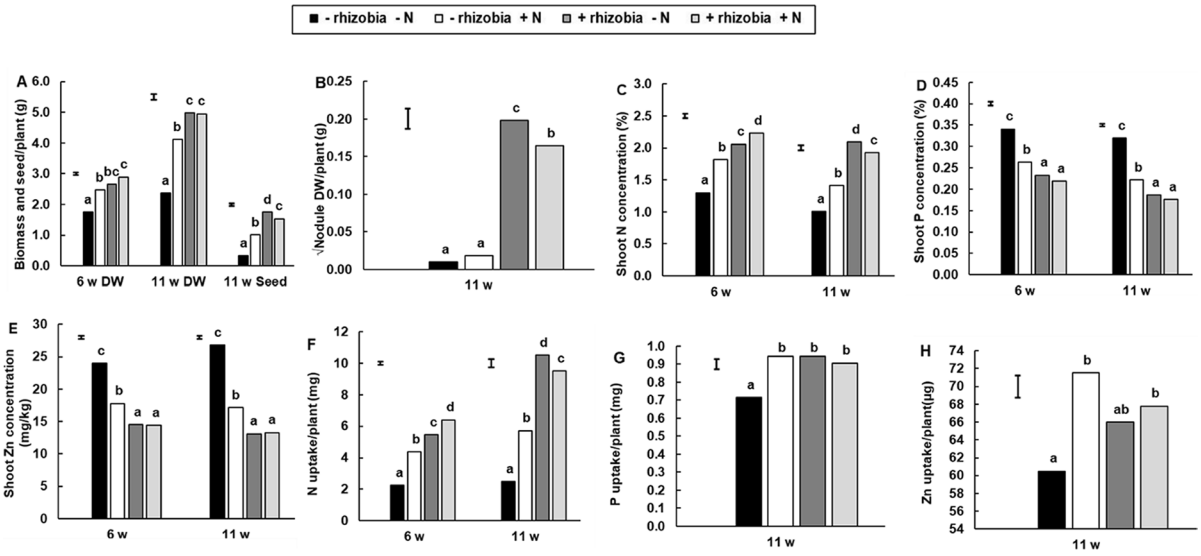

Fig. 4 The interactive effects of rhizobia and nitrogen (N) at 6 and/or 11 weeks (w) on (**A**) shoot biomass (DW) and seed yield, (**B**) nodule dry weight/plant, (**C**) shoot N concentration (%), (**D**) shoot P concentration (%), (**E**) shoot Zn concentration (mg/kg), (**F**) N uptake/plant (mg), (**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 at 6 or 11 weeks 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.)

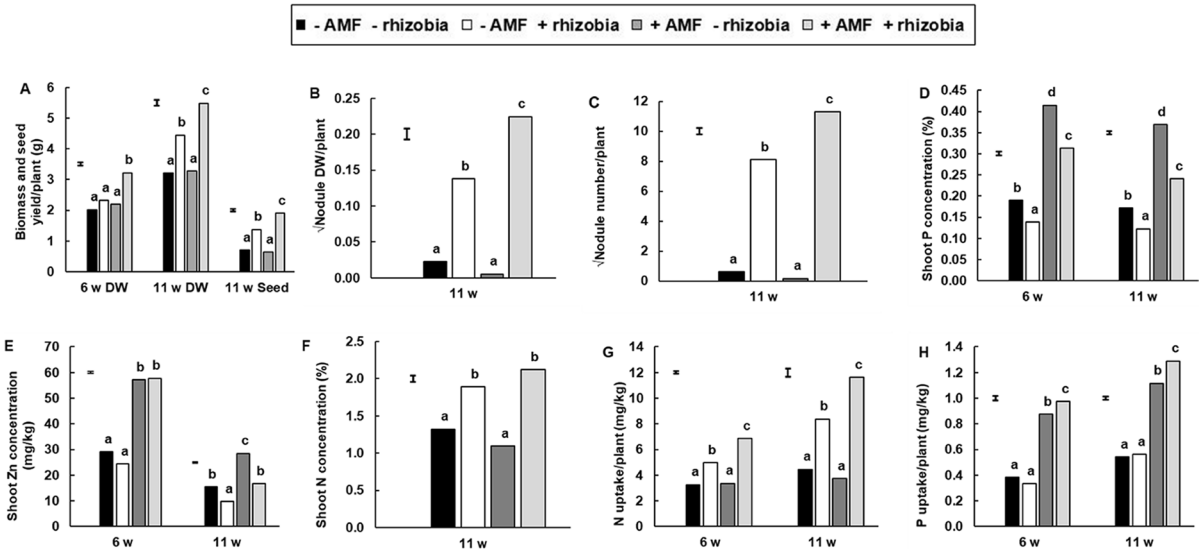

Fig. 5 The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and rhizobia at 6 and/or 11 weeks (w) on (**A**) shoot biomass and seed yield, (**B**) nodule dry weight/plant, (**C**) nodule number/plant, (**D**) shoot P concentration (%), (**E**) shoot Zn concentration (mg/kg)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

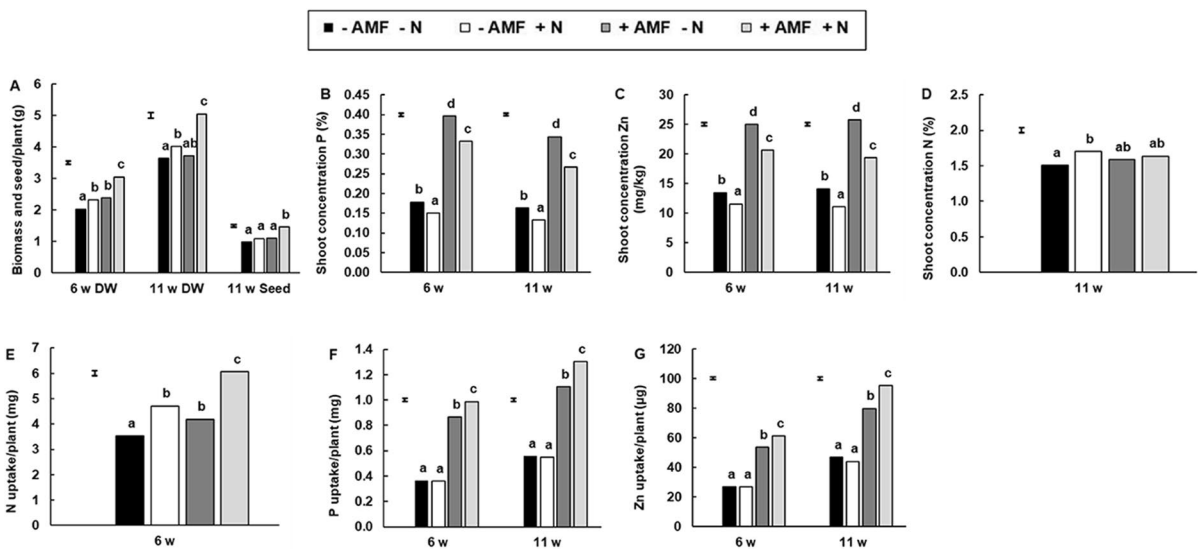

Fig. 6 The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and N at 6 and/or 11 weeks (w) on (**A**) plant biomass and seed yield, (**B**) shoot P concentration (%), (**C**) shoot Zn concentration (mg/kg) and, (**D**) shoot N concentration (%), (**E**) N uptake/plant (mg), (**F**) P uptake/plant (mg) and, (**G**) Zn uptake/plant (µg). Different letters above each bar graph at 6 or 11 weeks 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.)

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 E 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 H).

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 D 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).

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 and 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 G).

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.

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).

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 resulted in the greatest increase in shoot biomass compared to the uninoculated control, and 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

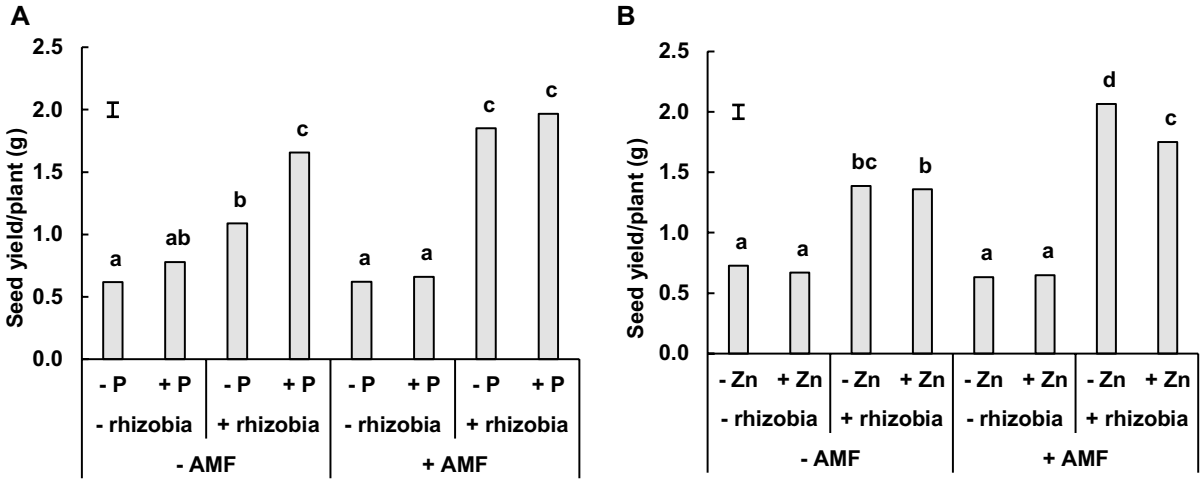

Fig. 7 The interactive effects ofco-inoculation of (**A**) arbuscular mycorrhizal fungi (AMF) x rhizobia x P, (**B**) arbuscular mycorrhizal fungi (AMF) x rhizobia x Zn on seed yield per plant of mung bean at 11 weeks after sowing. Different letters above each bar graph indicate significant differences 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.)

and rhizobia together compared to AMF and rhizobia together (Fig. 8B).

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.*

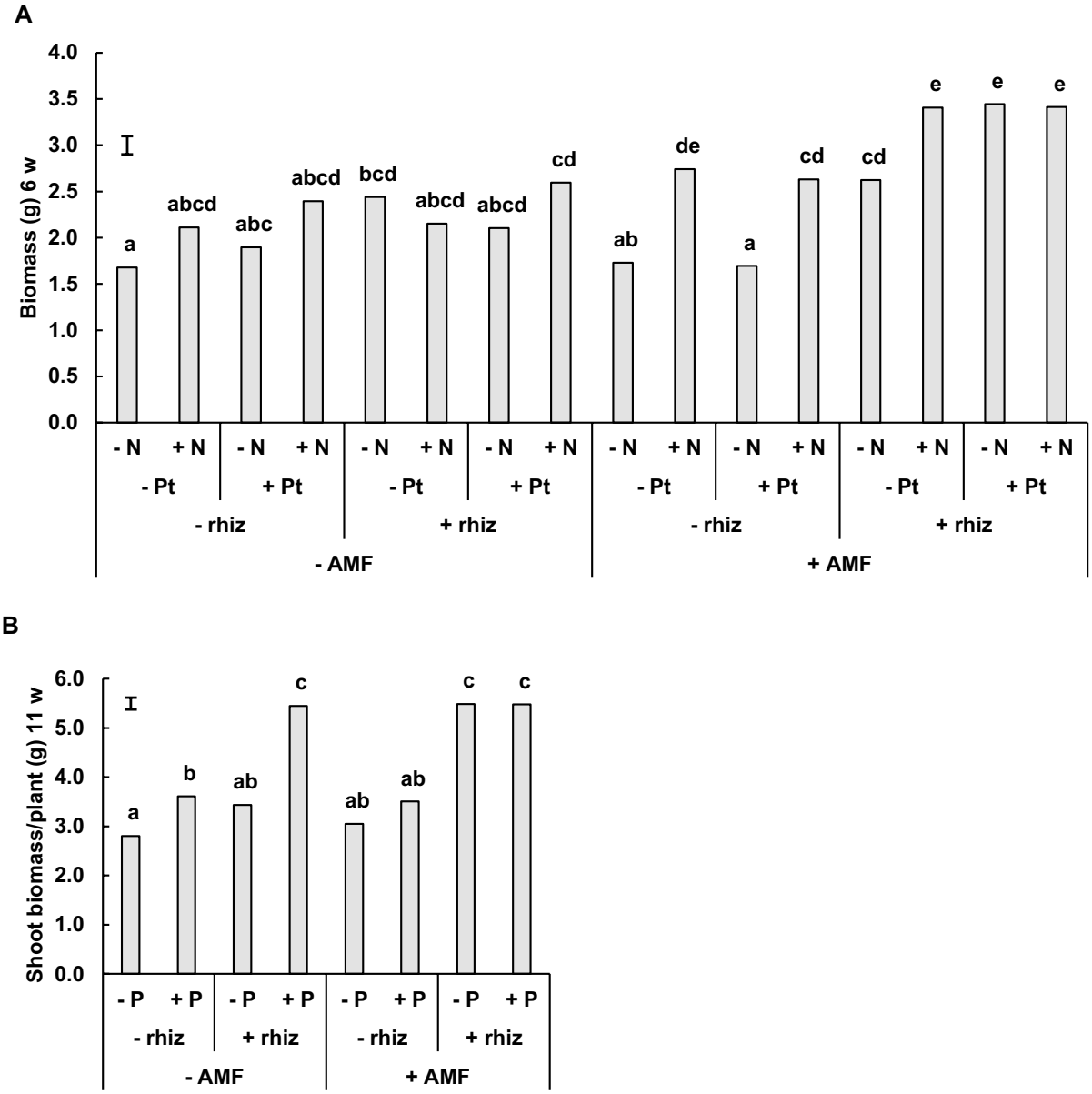

Fig. 8 The interactive effects of co-inoculation of (**A**) arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (Pt) x N on shoot biomass per plant of mung bean at 6 weeks (w) after sowing, (**B**) arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P on shoot biomass of 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)

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).

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).

Shoot N:P ratio

There was no relationship between dry weight and N:P ratio at 6 weeks of plant growth (Supplementary Table S10). At 11 weeks, the optimal N:P ratio

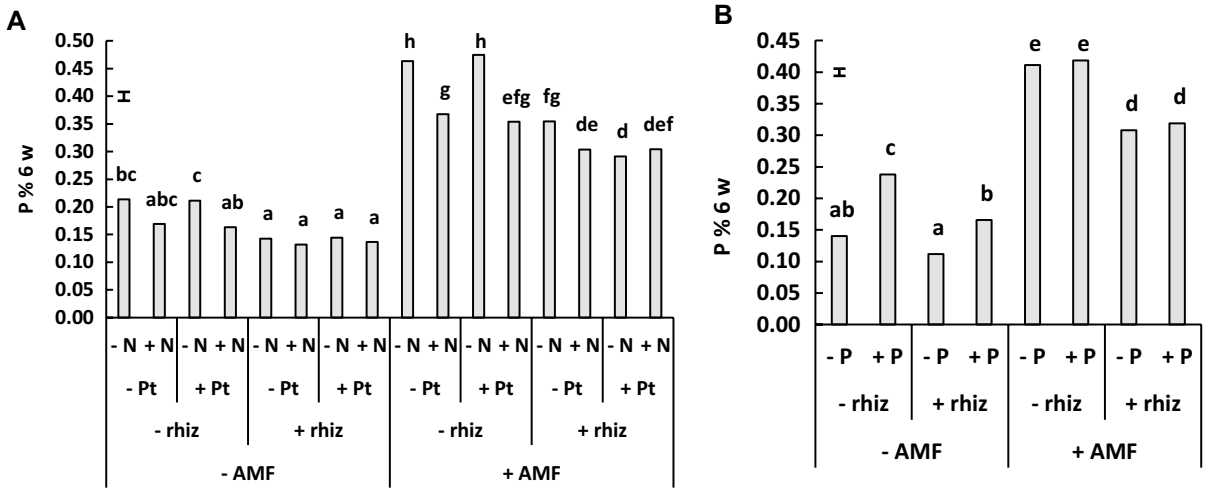

Fig. 9 The interactive effects of co-inoculation of (**A**) arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (Pt) x N and (**B**) arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x P on P concentration (%) in the shoots of mung bean at 6 weeks (w) after sowing. Different 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.)

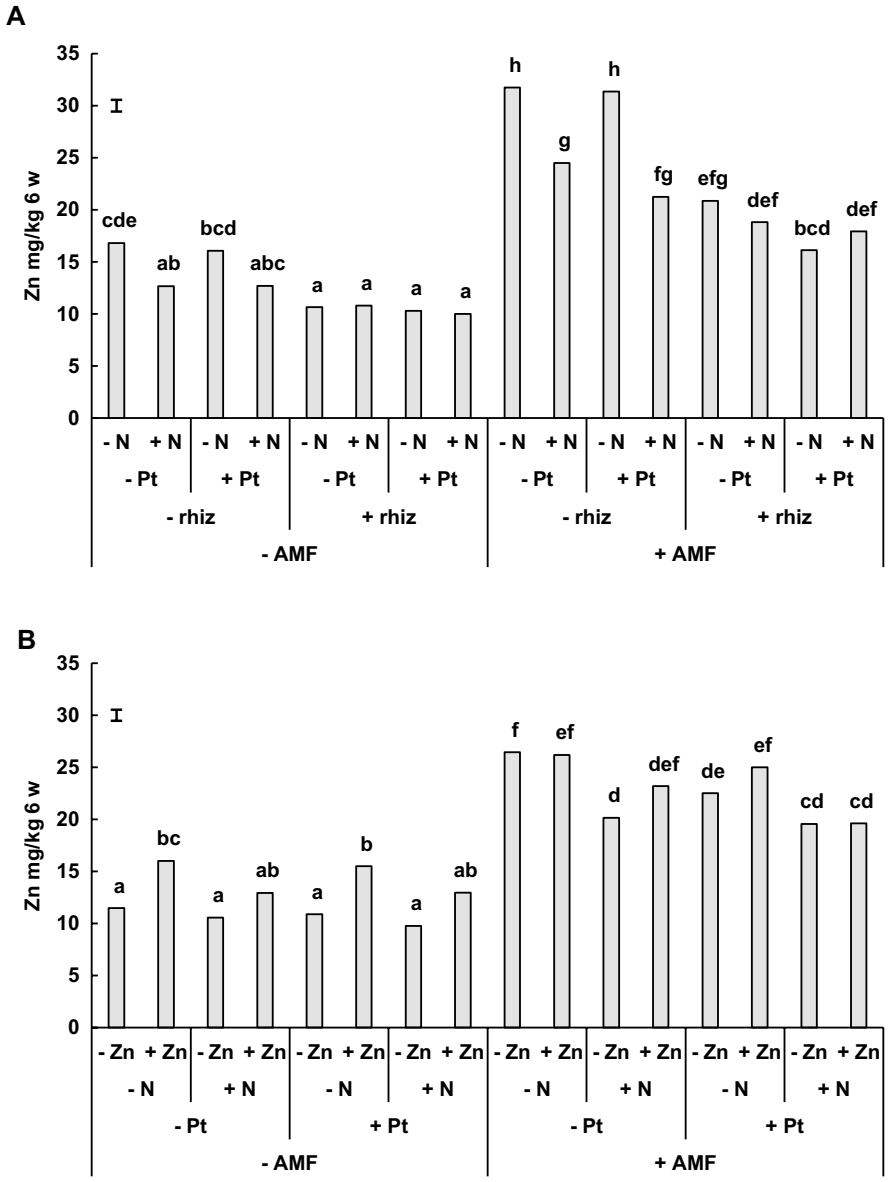

Fig. 10 The interactive effects of co-inoculation of (**A**) arbuscular mycorrhizal fungi (AMF) x rhizobia (rhiz) x *Pratylenchus thornei* (Pt) x N and (**B**) arbuscular mycorrhizal fungi (AMF) x *Pratylenchus thornei* (Pt) x N x Zn on Zn concentration (mg/kg) in the shoots of mung bean at 6 weeks (w)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

for dry weight across all treatment combinations was determined as 11.67 from Gaussian regression (R^2= 0.43, P<0.001, n=64).

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

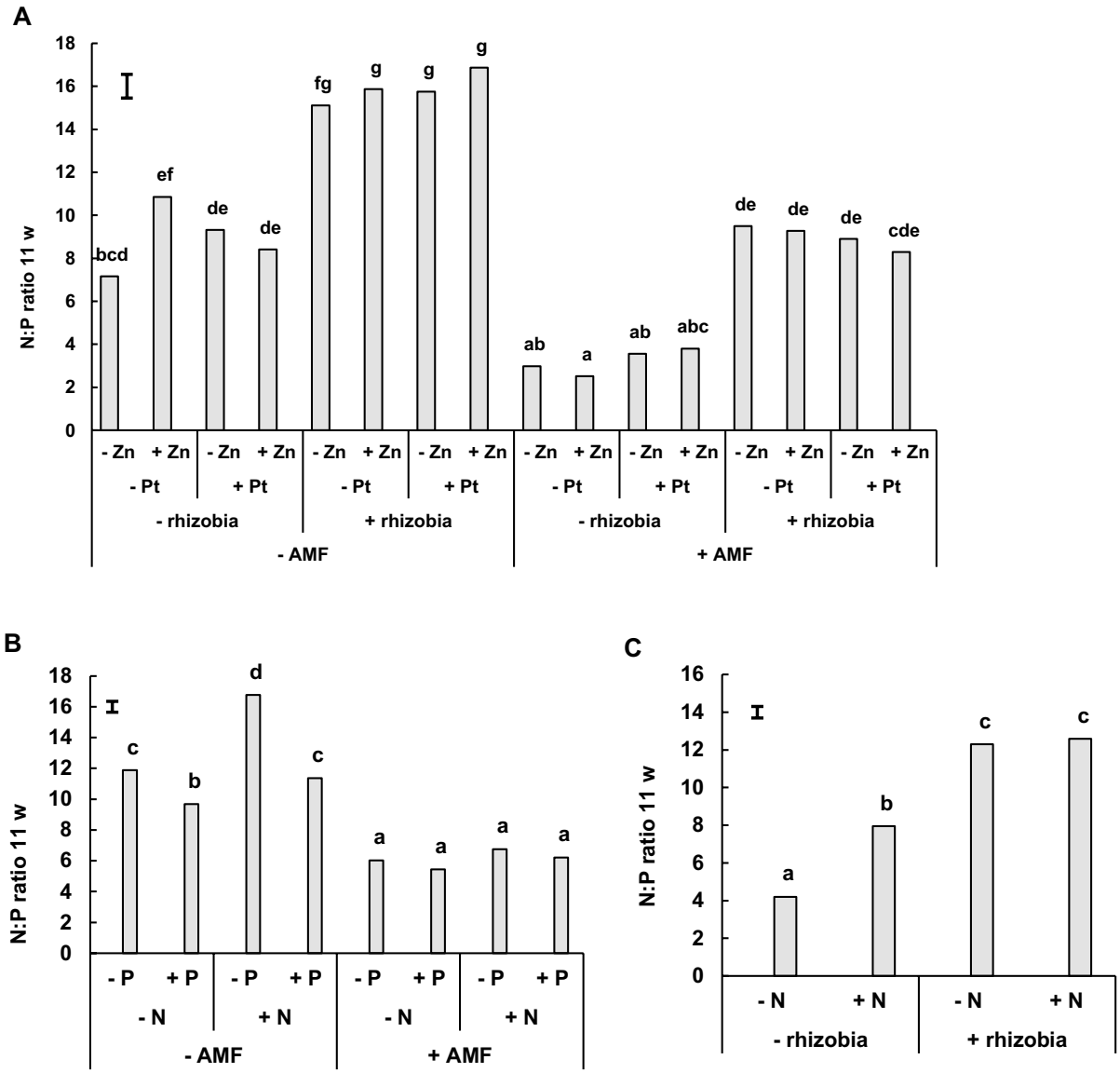

Fig. 11 The interactive effects on the shoot N:P ratio at 11 weeks of (**A**) arbuscular mycorrhizal fungi (AMF) x rhizobia x *P. thornei* (Pt) x Zn, (**B**) arbuscular mycorrhizal fungi (AMF) x N x P, (**C**) rhizobia x N at 11 weeks (w). Different letters above each bar graph indicate significant differences 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.)

addition of AMF and N had an N:P ratio 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).

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 with some plant species (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). It is established that AMF increases the concentration and uptake of the nutrients Zn and Cu in other plants (Clark and Zeto 2000). 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. To our knowledge this is the first report on AMF increasing Cu in mung bean in a vertisol. 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 2014), 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.

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. 2006). 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 for whole plants at 11 weeks growth) compared to the ratio in plants fertilised with N. On the other hand, plants inoculated with AMF had a greatly reduced N:P ratio 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 maximal 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 (i) increases in 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. Management of AMF in the cropping systems, alongside inoculation of legumes with rhizobia, should lead to increases (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. Differences between the level of *P. thornei* reproduction

were noted between this current research and those in Gough et al. (2020). Variation in nematode reproduction can be explained by differences in abiotic and biotic environmental conditions (de Waele and Elsen 2002; Thompson et al. 2020). Our previous research was undertaken in spring, while this current research was carried out in late summer. Variation in light intensity between experiments could have influenced nematode reproduction by altering biomass and rates of photosynthesis in mung bean (Karim et al. 2003).

The observed reductions in population densities of *Pratylenchus* sp. following the application of N fertilisers at 11 weeks, or inoculation with rhizobia at 6 weeks, 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 2005; 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 1994b; Owen et al. 2014).

Conclusions

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*.

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.

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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