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RESEARCH ARTICLE

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Arbuscular mycorrhizal fungal-induced tolerance is determined by fungal identity and pathogen density

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Societal Impact Statement

Plant-parasitic nematodes are a major concern for global food security, and many existing control options are being phased out due to adverse impacts on the environment. Here, we show that although application of arbuscular mycorrhizal fungi (AMF) increases host tolerance to these parasites, these benefits decrease as the parasite burden increases, limiting long-term benefits. This effect was consistent between experiments in the glasshouse and in the field environment, demonstrating the relevance of research into usable technologies. Our findings have potential to aid decision making regarding application of AMF inocula for optimum results in agricultural systems.

Summary

- Plant-parasitic nematodes are a leading global threat to crop production and food security aims. Control strategies based on nematicides and fertilisers are increasingly undesirable due to economic and environmental impacts. Arbuscular mycorrhizal fungi (AMF) may induce host tolerance against pests such as the potato cyst nematode (PCN).
- Here, we determined the impact of PCN density on the tolerance induced by AMF-host interactions. Additionally, we evaluated the effects of five AMF inocula on PCN fitness though glasshouse and field trials.
- Greater PCN densities reduce the increased tolerance that AMF may confer on their hosts. This may be due to reduced mycorrhizal colonisation of hosts under higher PCN infection and potentially a threshold at which the presence of PCN severely impacts fungal growth. When tested in the field, the outcomes of AMF inoculation on crop yields were still positive. Inoculation of soil in the field also increased PCN multiplication, suggesting that AMF-induced tolerance may become reduced in the near future when the threshold PCN density is reached.
- Addition of AMF to agricultural soils may provide a short-term benefit yet lead to a long-term detriment by increasing PCN populations. The effects observed were driven by only one out of the five introduced AMF species, indicating that the remaining species were redundant for this application. This raises important considerations for future application of AMF inocula in agricultural systems and aids

our understanding of how widely used 'beneficial' soil amendments impact the agricultural ecosystem.

KEYWORDS
arbuscular mycorrhizal fungi, food security, plant-parasitic nematode, potato, potato cyst nematode, soil community, symbiosis, tolerance

1 | INTRODUCTION

Plant-parasitic nematodes are an important threat to global food production, responsible for crop losses in excess of USD$80 billion per annum (Nicol et al., 2011). The white potato cyst nematode, *Globodera pallida*, is a highly damaging sedentary endoparasitic nematode that invades the root of solanaceous hosts and establishes a feeding site that sustains development and reproduction (Moens et al., 2018). *G. pallida* is widespread across potato (*Solanum tuberosum*) producing regions (CABI, 2020) and along with the closely related *Globodera rostochiensis*, accounts for almost 10% of potato crop losses worldwide (Kantor et al., 2022). Recently, *G. pallida* (referred to hereafter as PCN) cysts were detected in 43% of UK potato fields (Dybal, 2019) and were noted as an emerging concern in Africa (Mburu et al., 2020). The multiplication rate of PCN is largely determined by the resistance status of the cropped potato variety (Dybal, 2019), which can have long-term implications due to the persistence of PCN in agricultural soils in the absence of a host (Gartner et al., 2021). The density of PCN within the field can be affected by abiotic conditions, such as soil temperature and rainfall, and cropping of susceptible or resistant/non-host species (Koehler et al., 2021). Greater densities of PCN increase yield losses (Seinhorst, 1966) and restrict the selection of varieties available to growers to those that can tolerate higher PCN burdens and produce sufficient yields (Bayer Crop Science, 2018; Gartner et al., 2021).

In PCN infected fields, potato roots are usually simultaneously colonised by multiple soil-borne symbionts, including arbuscular mycorrhizal fungi (AMF) (Strom et al., 2020). AMF are near-ubiquitous in soils and can colonise the vast majority of land plant roots (Deliopoulos et al., 2010; Soudzilovskaia et al., 2020). AMF are generally considered to be mutualistic plant symbionts, assimilating and transferring inorganic nutrients from the soil to the host and, in return, assimilating organic carbon-based compounds fixed via photosynthesis by the host plant (Johnson et al., 1997). Thanks to their small diameter and exudation of organic acid phosphatases, AMF hyphae access soil nutrients such as phosphorus and nitrogen that would otherwise be inaccessible to roots (Sato et al., 2015; Smith & Read, 2008). Given that production and application of fertilisers to agricultural fields is expensive and unsustainable in terms of energy usage, soil degradation and depletion of the natural resources (e.g., rock phosphate), application of AMF to agricultural soils might help to enhance uptake of soil nutrients in crops in a more sustainable manner, thereby reducing reliance on chemical fertilisers (Leake et al., 2004).

It has recently been shown that co-colonisation of potato roots by PCN and AMF causes restriction of host carbon allocation towards AMF partners whilst the transfer of mycorrhizal-acquired nutrients to the host is largely maintained (Bell et al., 2022). By maintaining the flow of nutrients to the host plant, the presence of AMF in these co-colonised plants may be responsible for driving the increased PCN fitness compared to those on asymbiotic plants, as well as increased host plant tolerance towards PCN (Bell et al., 2022). The pattern of disruption in resource allocation between symbionts that was reported by Bell et al. (2022) was determined using only a single density of PCN on the plant roots. In the field, variation in the density of PCN infection of host plant roots is common, relating to the success of the previous generation of PCN and resultant number of eggs in the soil. Differences in the number of PCN infecting plant roots may drive different responses in terms of resource allocation between symbionts. Host plant tolerance towards PCN can be heavily impacted by the density of the pest, with some resistant varieties not performing well (in terms of yield), whereas some tolerant varieties may cope in part with a greater parasitic burden. As these varieties offer no restriction to PCN reproduction, even greater densities can be expected for the next season (Gartner et al., 2021). However, the impact of PCN density on AMF-induced tolerance in potato is unknown. By enhancing nutrient supply to the host plant, AMF appear to increase PCN populations whilst mitigating their impact on the host by improving plant vigour (Bell et al., 2022). However, AMF-induced PCN tolerance may not hold when the host plant is exposed to high densities of PCN, potentially resulting in crop failure and persistence of elevated PCN populations for years to come. Moreover, AMF colonisation of the host can increase populations of other plant-parasitic nematodes (Frew et al., 2018; Gough et al., 2020), widening this potential issue.

Based on encouraging results of glasshouse trials, AMF are increasingly being considered and applied as soil amendments or bioinoculants in agricultural systems (Cely et al., 2016; Verbruggen et al., 2013) to obtain increased yields (Ryan & Graham, 2018), enhanced biosynthesis of desirable phytochemicals (Rouphael et al., 2015; e.g., antioxidants and essential oils) and improved plant tolerance towards pests and abiotic stresses (Frew et al., 2021; Sbrana et al., 2014). Application of mycorrhizal amendments in the field is an attractive proposition for crop protection strategies as a valuable alternative to heavy reliance on pesticides that are increasingly restricted for environmental reasons (Nyczepir & Thomas, 2009). AMF inocula have successfully increased yields of several crops in the

field, including potato (Hijri, 2016); however, such studies can be overly optimistic with regard to the benefits of AMF and outcomes can vary greatly according to plant and fungal genotypes (Ryan & Graham, 2018). Compatibility issues may potentially also underpin the lack of efficacy of certain commercial inocula in glasshouse trials (Elliott et al., 2021). Potential competition between foreign and native species is a concern and needs to be fully understood at a community level to determine the survival of inoculants and their functionality compared to culturing natural populations (Rodriguez & Sanders, 2014). Rather than introducing AMF species that are foreign to the area, it may be beneficial to utilise native species in agricultural systems as they have evolved in situ, acclimating and adapting to the specific environment and are therefore likely to have a higher chance of success and persistence (Séry et al., 2016). Additional factors such as the field capacity for AMF (Verbruggen et al., 2013) and farming practice (Ryan & Graham, 2018) can impact AMF function and require assessment (Cely et al., 2016), whilst their efficacy and costs must compete with chemical fertilisers to gain the favour of growers. To date, there have been only a limited number of field trials investigating the application of AMF inoculum to soils to enhance plant tolerance to plant parasitic nematodes. These have shown inoculation with AMF to reduce infection and enhance host plant tolerance towards root-knot nematodes (Calvet et al., 2001; Séry et al., 2016). Field experiments using cyst nematodes showed that AMF promote PCN hatching and so it was proposed that they could function synergistically with nematicides that target hatched juveniles to reduce the PCN population in the soil (Deliopoulos et al., 2010). Whether or not AMF are able to ameliorate the effects of cyst nematode pests in the field environment remains under-investigated (Hol & Cook, 2005; Pawlowski & Hartman, 2020; Schouteden et al., 2015).

Here, we used a combination of glasshouse and field-based experiments to determine the impact of PCN density on AMF-induced tolerance and PCN fitness. Furthermore, we determined the impact of a commercial AMF inoculant on the native AMF community.

2 | METHODS

2.1 | Growth conditions and application of AMF and PCN on potato plants in the glasshouse

Potato tubers (*S. tuberosum* cv. Désirée) were planted in 21 cm diameter pots containing sterilised sand:topsoil (50:50), and treatment pots were inoculated with AMF and/or PCN at different densities. Treatments included PCN free controls, PCN at 15, 35, and 125 eggs/g soil. All treatments were conducted both with and without the addition of AMF inoculum (details below). Plants were randomised for layout and grown in a containment glasshouse with a controlled environment (18–20°C/16 h day length) and watered every other day. PCN inoculum was created by inoculating plants with *Globodera pallida* cysts (population Lindley) and then grown for 12 weeks with regular applications of general fertiliser (1:1:1 NPK), until plant death, to enable reproduction and increased PCN populations for use as stocks. The PCN content of these inoculum soils was determined through Fenwick's (1940) method and then mixed with sterilised sand:loam topsoil to get the desired PCN densities of 15, 35 and 125 eggs/g. These densities were selected based on general thresholds for PCN management actions and to simulate increasing conditions: <15 eggs/g soil (low, grow resistant/tolerant varieties) and >15 eggs/g soil (high; do not grow potatoes) (Bayer Crop Science UK, 2020). Two aliquots of the PCN inoculum and fresh soil mixes were sent to NRM Laboratories, UK, for soil analysis (specifically the Micronutrient Report). pH, available Phosphorus, Potassium, Magnesium, Copper, Boron, Sodium, Zinc, Calcium, Molybdenum, Iron, Sulphate, Manganese and Organic Matter (%), were quantified to assess the impact of larger concentrations of fresh soil in the lower PCN density pots, and the larger concentration of PCN cysts in the greater density pots, on soil nutrition. This determined no considerable difference throughout the range of soil characteristics (Table S1), indicating no impact for subsequent experiments. For the AMF inoculum, 30 g of a commercially available inoculum of the AM fungus *Rhizophagus irregularis* (PlantWorks Limited, UK; approximately 7,000 spores plus hyphae, manufacturer's recommended dose) was added to each pot. The control treatment also received a blank inoculum in order to control for any nutrients present. Treatments had six biological replicates.

2.2 | Sampling and harvesting glasshouse trials

One week after planting, plant height and chlorophyll content (Hansatech, CL-01 Chlorophyll Content Meter) were recorded at weekly intervals. Leaves of similar size from the canopy of each plant were sampled. After 12 weeks of growth, plants were harvested, excised into shoots, roots and tubers, and weight measurements were taken for each plant component before and after freeze-drying for approximately 3 days (CoolSafe 55-4, LaboGene, Allerød, Denmark) to collect dry weights. Roots were cleaned with tap water and a subsample taken for quantification of AM root length colonisation and stored in 50% EtOH (v/v) at 4°C until further use.

2.3 | Quantification of PCN infection

The pot soil was thoroughly mixed and then nematode cysts were extracted from a 100 g aliquot using Fenwick's (1940) method to estimate the number of cysts. Ten extracted cysts from each were then opened, and the number of unhatched second-stage juveniles was counted to determine the number of eggs per cyst, which reflects the reproductive capacity of the nematodes under each treatment.

2.4 | Fungal colonisation of roots and bulk substrates

Plant roots were stained using the 'ink and vinegar' staining method (Vierheilig et al., 1998). Root samples were first washed in water and then cleared in 10% KOH (w/v) in a 90°C water bath for 20 min, and AM fungal structures were stained with ink and vinegar (5% Pelikan

Brilliant Black, 5% acetic acid, 90% dH_2O) (Vierheilig et al., 1998) in a 90°C water bath for 15 min. Roots were de-stained in 1% acetic acid and mounted on microscope slides using polyvinyl lacto-glycerol (16.6 g polyvinyl alcohol powder, 10 ml glycerol, 100 ml lactic acid, 100 ml dH_2O). Assessment of % root length colonisation was made using the magnified intersection methodology (minimum of 150 intersects per pot, 200X magnification) (McGonigle et al., 1990). AM fungal hyphae were extracted from 4 to 5 g of bulk soil in 500 ml H_2O, from which 10 ml was filtered through a cellulose nitrate membrane filter (47 mm diameter, 0.45 µm pore size; Sartorius, Göttingen, Germany) and stained with ink and vinegar. Filter papers were then cut in half and mounted on microscope slides using polyvinyl lacto-glycerol and oven-dried at 65°C for an hour. AM hyphal lengths per pot were calculated using the gridline-intersection method (50 fields of view per half filter paper, 200X magnification) (Tennant, 1975).

2.5 | Field trial setup and harvest

To test the consistency of glasshouse data in agroecosystems, we established a field trial. This was located in Bensgate, Spalding, UK (52.788, 0.045). Previous cropping on the site consisted of wheat (*Triticum* sp) 2016, kale (*Brassica oleracea*) in 2017, leek (*Allium ampeloprasum*) in 2018, kale in 2019 and pea (*Pisum sativum*) in 2020. Potatoes were planted 30 cm apart in plots with 30 g inoculum of a commercially available fungal inoculum containing *Clarioeoglomus*, *Glomus*, *Diversispora*, *Rhizophagus irregularis* and *Funneliformis* (PlantWorks Limited, UK). The mixed inoculum was used for field experiments as it more closely matches that which is applied to fields by growers. Non-AMF plots received the blank inoculum as described previously. Nemathorin (Syngenta) was applied to half of the plots at 30 kg/ha to provide soil with reduced PCN populations (Figure S1). Fallow plots contained AMF inoculum but no potatoes. All plots were randomised. Within each plot, 13 tubers were planted per row resulting in a total of 26 tubers. There were eight plots per treatment, apart from follow plots ($n = 5$). The trial was set up on 12 April 2021, without irrigation, and haulms were removed on 6 September 2021 prior to harvest the following month. Rainfall and temperature data were obtained from ClearAg, DTN, UK. Pre-planting and post-harvest, a 1 kg soil sample was taken from each plot from which PCN cyst and egg counts were quantified by Richard Austin Agricultural Ltd, UK. Upon harvest, root samples were collected from six, randomly selected plants within each plot to determine AMF colonisation (as previously in Methods) and the composition of root-associated fungal communities through DNA barcoding. Eight tubers were harvested from the middle of each plot to avoid edge effects. Tubers were size graded by hand through potato riddles and weighed to provide yield data.

2.6 | Fungal barcoding of the field trial

A random subsample of potato roots collected from the field prior to harvest (approximately 20 g per sample). Three samples were collected from potato roots that had AMF inoculum and three from non-inoculated roots, all in the absence of nematicide (i.e., greater PCN densities). Samples consisted of a mix of all root sizes and were briefly washed in water to remove soil before grinding to a fine powder in liquid nitrogen. Extraction buffer (100 mM Tris pH 8.0, 50 mM EDTA pH 8.0, 500 mM NaCl) was added to 500 mg of each sample, and a homogenous suspension was obtained. Samples were prepared in technical triplicate. DNA was then extracted and purified by ×2 phenol: chloroform, precipitated with isopropanol and re-suspended in nuclease-free water. A total of 400 ng of DNA pooled from technical replicates was sent for fungal barcoding via Next-Generation Sequencing of the Internal Transcribed Spacer II by Genewiz, UK. Sequencing was performed on Miseq 2 × 250 bp reads with forward (GTGAATCATCGARTC) and reverse (TCCTCCGCTTATTGAT) primers. Raw data are held under submission SUB11603494 (www.ncbi.nlm.nih.gov/sra). The taxonomic classification of fungi within the root samples was delivered by the sequencing provider, along with diversity indices. All following analysis was performed in QIIME (Bolyen et al., 2018). In brief, the two sequences of each read pair were merged according to overlapping sequences and primer/adapter sequences were removed along with ay resulting sequences <200 bp. Chimeric sequences were removed via UCHIME 'Gold', and operational taxonomic unit (OTU) was assigned to sequences (read count >1; VSEARCH 1.9.6) based on a similarity of >97%. To obtain the classification of the OTU, a representative sequence was selected and annotated using the RDP Bayesian classifier algorithm using the UNITE ITS database (unite.ut.ee/) at a confidence threshold of 0.8. For each sample, the relative percentage of each species at different taxonomic levels was obtained.

The alpha diversity was determined via the Chao index to estimate the number of OTU in the samples. The Shannon index was then determined to reflect the diversity index of the samples.

2.7 | Statistical analysis

Tests for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's Test) of the residuals were carried out using OriginPro (OriginLab Corporation, 2021). Data did not require normalising or transforming before the following tests were applied. For yield, chlorophyll content, pant height, AMF root colonisation and PCN cyst count, data were analysed by One-way ANOVA. Upon $p < .05$ post-hoc Student–Newman–Keuls (SNK) tests were run to identify significant differences between the treatments.

3 | RESULTS

3.1 | Glasshouse trial

3.1.1 | PCN density impacted host growth and AMF-induced host tolerance

Upon harvest of the glasshouse trial, the tuber yield of potato plants was quantified to assess the impact of the different PCN densities in the presence of AMF. Greater tuber yields (total weight) were

obtained on plants with AMF and in the absence of PCN, whilst PCN infection reduced the yields in a density-dependent manner, with higher PCN densities resulting in lower potato yields (Figure 1a). AMF colonisation provided a benefit to yields even in the presence of PCN, if PCN densities were ≤15 eggs/g soil. Similar trends were recorded for shoot biomass, where mycorrhizal inoculation increased shoot biomass at all densities apart from 35 PCN eggs/g soil (Figure 1b).

Plant height was reduced when inoculated with 125 PCN eggs/g soil but unaffected by densities below this value (Figure S2a). AMF colonised potatoes had increased shoot height compared to non-AMF plants at the same PCN density over the growth period. The time point at which AMF plants grew taller than PCN-only plants was PCN density dependent, with increasing PCN densities delaying the AMF-induced increase to shoot height (Figure S2a). The shoot height at 8 weeks was positively correlated with tuber yields (Pearson's $r = .514$, $p = .00425$; Figure S3b).

Chlorophyll content was measured throughout plant development as key physiological parameters for plant productivity and potentially indicators of stress (Mafakheri et al., 2010). There was a general trend of decreased chlorophyll content in the leaves of plants as they aged (Figure S3). At the end of the experiment (week 8), AMF-inoculated plants displayed increased chlorophyll content of leaves compared to non-AMF plants at the same PCN density, when PCN density was ≤35 PCN eggs/g soil. This appears to be largely driven by changes at week 6.

3.1.2 | AMF-induced increases in PCN fitness diminished as PCN density increased

Next, we were able to determine the impact of different initial PCN densities on the previously reported AMF-induced increase in PCN infection and reproduction (Bell et al., 2022). AMF colonisation increased the number of PCN cysts recovered at harvest, from plants with initial PCN densities of ≤35 eggs/g soil (Figure 2a). The increase in cysts was stronger at lower initial PCN densities (15 eggs/g soil = 2.2×, 35 eggs/g soil = 1.5×, 125 eggs/g soil = 1.1×; Figure 2). AMF colonisation also increased the number of eggs per cyst at initial PCN densities ≤35 eggs/g soil (Figure 2b). At a PCN density of 125 eggs/g soil more nematodes developed to cysts (Figure 2a) but with fewer eggs per cyst (Figure 2b) compared to lower density treatments, with the presence of AMF not impacting either.

The presence of PCN at lower densities increased AMF colonisation of roots compared to treatments without PCN (Figure 3a). However, with increasing soil density and root infection of PCN (Figure 3a), AMF colonisation was reduced. Conversely, PCN density of 125 eggs/g soil resulted in a greater amount of AMF hyphae in the soil, compared to controls without PCN (Figure 3b).

3.2 | Field trial

3.2.1 | AMF-colonisation of potato increased PCN fitness and enhanced tuber production in the field

A field trial was established to determine whether the glasshouse results are consistent in agro-ecosystems. In general, it was a dry season (Figure S4), however, shortly after planting in April, increased rainfall and temperatures established the crop. Mycorrhizal colonisation of the potato crop was quantified upon harvest to determine the efficacy of mycorrhizal inoculation of field soil. Potato roots from both inoculated (AMF+) and non-inoculated (AMF-) plots were colonised by AMF; however, the inoculated plots showed greater root colonisation by AMF (Figure 4a). The use of the nematicide nemathorin

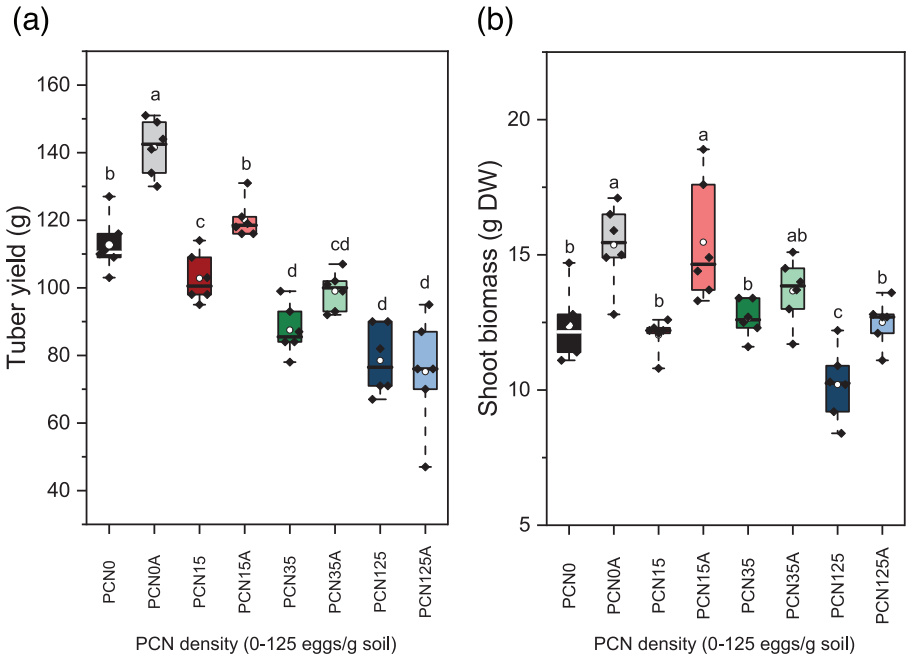

FIGURE 1 AMF-induced PCN tolerance of potato at lower initial PCN densities. (a) Tuber yield and (b) shoot biomass (dry weight [DW]) were quantified for plants under potato cyst nematode (PCN) densities of 0 (grey), 15 (red), 35 (green) or 125 eggs/g soil (blue) with arbuscular mycorrhizal fungi (AMF) inocula (lighter) or without (darker). *X* axis labels with 'A' denote AMF inocula (e.g., PCN0A). Boxes represent six biological replicates and extend from the first to the third quartile with the middle bold line representing the median value, white circle representing the mean and whiskers extending to min/max. Different letters denote significance ($p < .05$; One-way ANOVA, SNK).

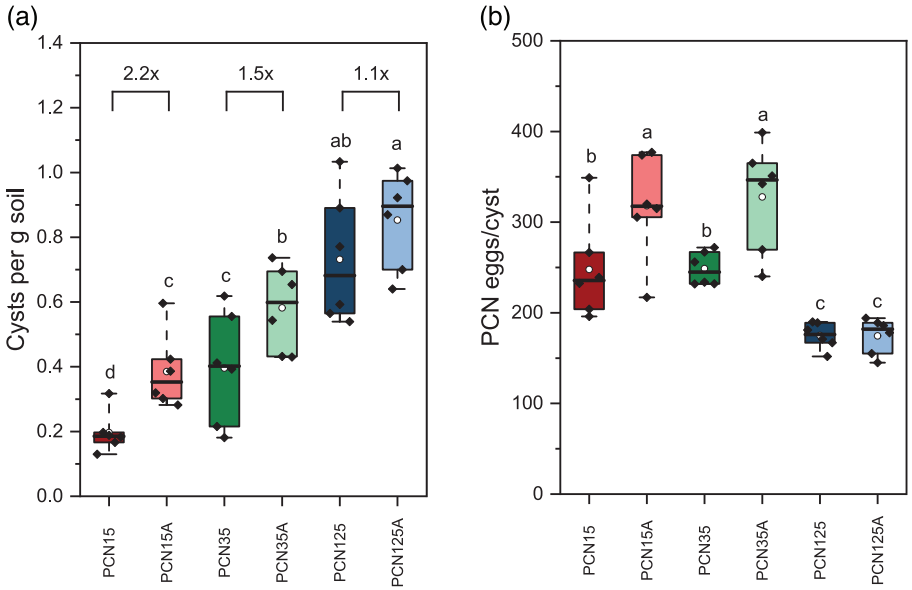

FIGURE 2 AMF increased PCN fitness on potato at lower initial PCN densities. (a) The number of potato cyst nematode (PCN) cysts per g soil and (b) eggs per cyst on plants under PCN densities of 15 (red), 35 (green) or 125 eggs/g soil (blue) with arbuscular mycorrhizal fungi (AMF) inocula (lighter) or without (darker). *X* axis labels with 'A' denoting AMF inocula (e.g., PCN0A). The fold change effect of AMF on the mean number of cysts per g soil (a) is noted above the boxes. Boxes represent six biological replicates and extend from the first to the third quartile with the middle bold line representing the median value, white circle representing the mean and whiskers extending to min/max. Different letters denote significance ($p < .05$; One-way ANOVA, SNK).

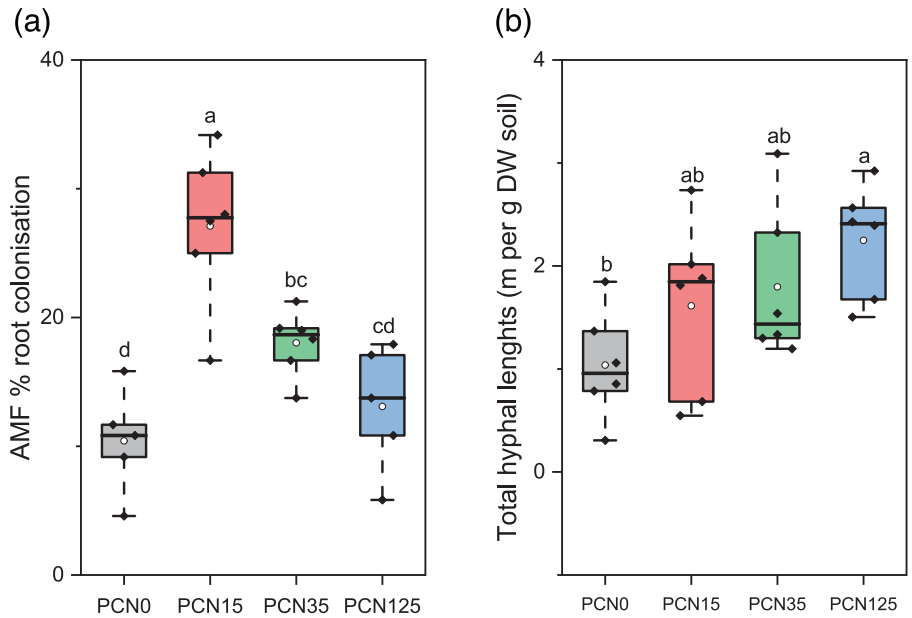

FIGURE 3 Increasing initial PCN densities reduced AMF root colonisation but increased hyphal lengths in the soil. The percentage of root colonised by arbuscular mycorrhizal fungi (AMF) (a) and total hyphal lengths (b) of plants under potato cyst nematode (PCN) densities of 0 (grey), 15 (red), 35 (green) or 125 eggs/g soil (blue). The *x*-axis labels indicate the PCN density. Hyphal lengths represented as m per g dry weight (DW) of soil. Boxes represent six biological replicates and extend from the first to the third quartile with the middle bold line representing the median value, white circle representing the mean and whiskers extending to min/max. Different letters denote significance ($p < .05$; One-way ANOVA, SNK).

(Syngenta) and subsequent lower densities of PCN did not impact AMF colonisation of roots in the field.

Fungal barcoding of root tissue samples prior to harvest (Table S2) inferred that the addition of AMF inoculum did not impact the total number of fungal OTU associated with the root (Chao1 index), but did increase their diversity (Shannon index; Figure 4b). The AMF detected in non-inoculated plots was identified as *Funneliformis* sp., indicating that this genus is native to the field. Overall, we found a greater relative abundance of Glomeromycetes from samples that had received the AMF inocula (Figure S5). Although *Clarioeoglomus* sp., *Glomus* sp. and *Diversispora* sp. were present in the commercial inoculum applied to the soil, they were not identified as associated with potato roots, with only *Rhizophagus irregularis* and *Funneliformis* sp. detected in the roots. Inclusion of *Funneliformis* sp. within the inoculum did not increase its relative abundance in the root, compared to non-inoculated plots containing the native soil fungi. Fungal barcoding indicates that the increased AMF content of inoculated roots was from the presence of *R. irregularis*.

The PCN cyst and egg counts were lower in nematicide treated plots (Nemat+), compared to non-nematicide plots (Nemat−) both pre-planting (Nemat+, average 3 eggs/g; Nemat−, average 9 eggs/g soil) and post-harvest (Nemat+, average 86 eggs/g; Nemat−, average 299 eggs/g soil) (Figure 5a,c). AMF inoculation did not impact cyst counts but increased PCN fitness in terms of egg production (Figure 5b,d). The AMF-induced increase in PCN egg content was greater in soils treated with nematicide, that is, lower PCN densities,

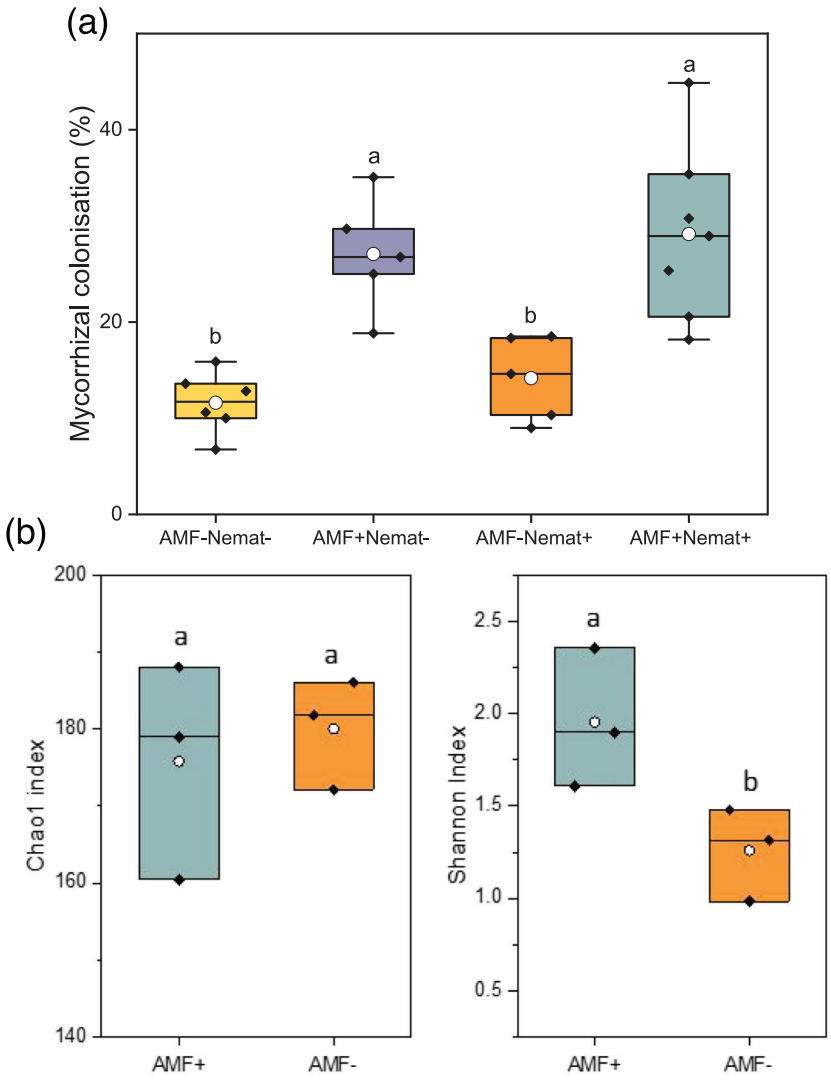

FIGURE 4 Inoculation of field trial plots pre-planting increased mycorrhizal colonisation of potato roots and the diversity of root-associated fungi. Colonisation of potato roots by arbuscular mycorrhizal fungi (AMF) collected from the field trial (a) ($n = 6$). Nemathorin nematicide was applied to half of the field plot soils before potato planting to reduce the potato cyst nematode population (Nemat+). Half of the plots also had a commercial AMF inoculum added to boost mycorrhizal content (AMF+). Fungal DNA was barcoded to indicate Chao1 and Shannon indexes (b) ($n = 3$). Boxplots extend from the first to the third quartile with the middle bold line representing the median value, the white circle representing the mean and whiskers extending to the minimum and maximum data points. Different letters denote significance ($p < .05$; One-way ANOVA, SNK [a]; *t* test [b]).

compared to non-nematicide controls, that is, higher PCN densities (Figure 5d).

Upon harvest, nematicide treated plots (Nemat+) produced a greater yield (average 16% increase) than non-nematicide plots (Nemat−) (Figure 6a). AMF inoculation (AMF+) did not impact total yield tonnage; however, it increased harvested tuber size compared to non-inoculated plots (AMF−) (Figure 6b).

4 | DISCUSSION

Results from our pot-based experiments suggest that AMF-induced tolerance towards PCN is reduced at greater PCN densities. Inoculation with AMF also increased PCN fitness, however this was diminished at greater initial PCN densities. AMF had no impact on PCN infection at the highest PCN density. This may be due to the reduced mycorrhizal colonisation of hosts under higher PCN infection, resulting in a weakened contribution by the AMF to the host plant. There appears to be a PCN density threshold at which AMF colonisation ceases to benefit the host. In the field, there was a reduced positive impact of AMF inoculation on crop yields compared to in the glasshouse, whilst PCN fitness was consistently increased in the presence of AMF symbionts. Our results appear to have been driven by only one out of the five introduced AMF species, raising important considerations for the potential application of AMF inocula in agricultural systems and potential long-term detriments.

4.1 | High PCN densities overcome AMF-induced tolerance

We applied PCN at a range of densities to potato hosts to determine their effect on AMF-induced pest tolerance in potato (Bell et al., 2022). We found that AMF colonisation of potato roots induced tolerance towards PCN, in terms of increased biomass (Figure 1), plant height (Figure S3) and chlorophyll content (Figure S4). The tolerance was diminished at higher PCN densities, with plants treated with ≥35 PCN eggs/g soil exhibiting no tolerance to the PCN in terms of tuber yields and shoot biomass. Increasing PCN infection densities appeared to delay the impact of AMF colonisation on crop height. As crop

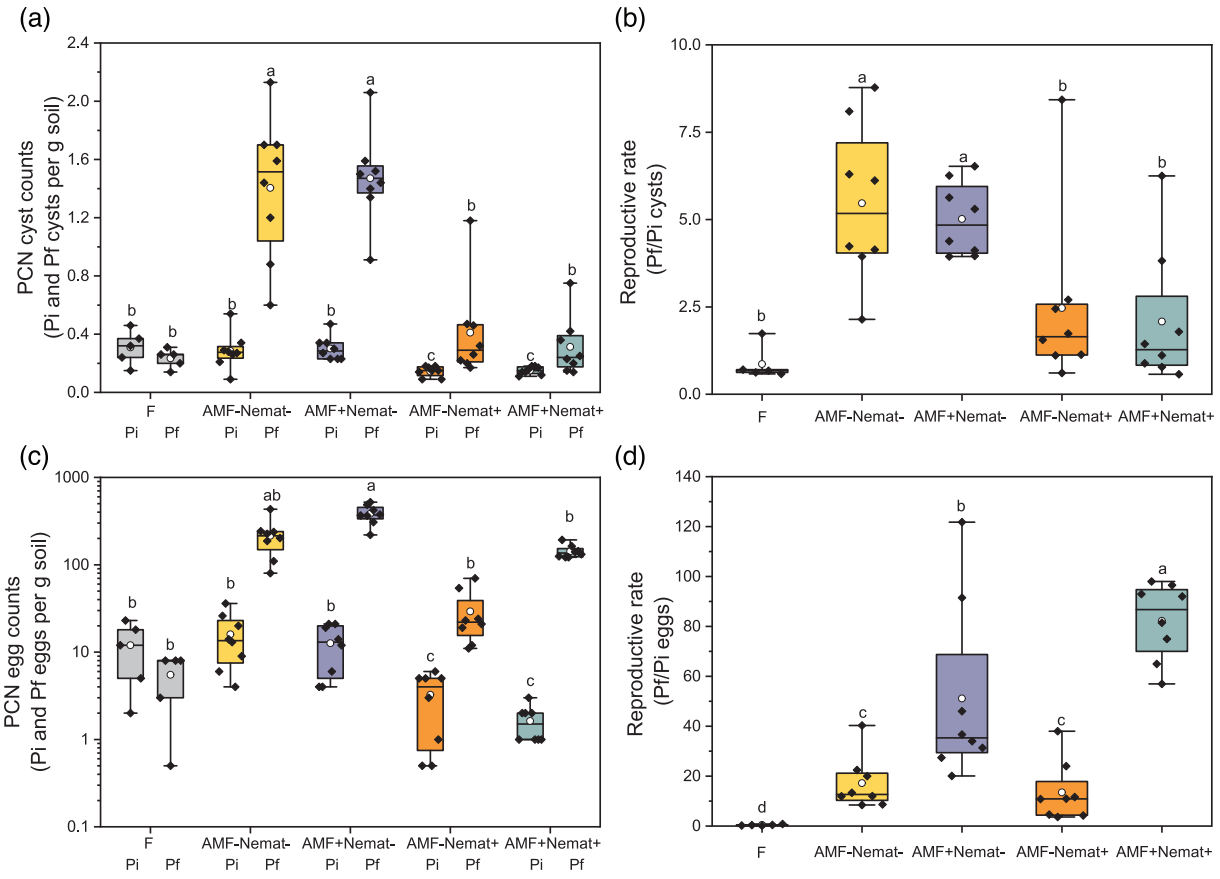

FIGURE 5 AMF inoculation of field trial plots increased PCN egg counts, but not the number of cysts, post-harvest. Potato cyst nematode (PCN) cyst (a) and egg counts (c; $\log_{10}$ scaled *y* axis) pre-planting (Pi) and post-harvest (Pf) from plots treated with nematicide and/or arbuscular mycorrhizal fungi (AMF) inoculum. These data were then described as reproductive rate (Pf/Pi) for both measures (b, d). Nemathorin nematicide was applied to half of the field plot soils before potato planting (Nemat+) Half of the plots also had a commercial AMF inoculum added to boost mycorrhizal content (AMF+). Boxplots extend from the first to the third quartile with the middle bold line representing the median value, the white circle representing the mean and whiskers extending to the minimum and maximum data points. F indicates data from fallow plots. Different letters denote significance ($p < .05$; One-way ANOVA, SNK).

height was positively correlated with yield (Figure S3b) in the glasshouse, this may collectively indicate a delay in the impact of AMF colonisation on tuber formation, as well as above-ground height, at higher PCN densities. These responses are akin to 'natural' potato tolerance which is severely reduced at greater PCN densities (Gartner et al., 2021), potentially due to competition between the symbionts for space within the host plant's root system (Vos et al., 2012), possibly limiting the capacity for AMF to interact with the host. Alternatively, the presence of PCN may disrupt carbon for nutrient exchange between host plant and the AMF. Our previous research (Bell et al., 2022) has shown that PCN infection reduces host carbon transfer to AMF despite maintenance of AMF-acquired nutrient (P/N) assimilation. In those experiments, a substantial proportion of AMF-acquired nutrients was assimilated by co-colonising PCN; as such, increased numbers of PCN in the present experiments may have caused a greater reduction in AMF-derived nutrients acquisitioned by the host plant, resulting in reduced or delayed AMF effects on host physiology. In the present experiments, we observed reduced root colonisation by AMF in pots with greater PCN concentrations, suggestive that reduced transfer of AMF-acquired nutrients may have occurred. However, direct links between mycorrhizal colonisation and function are often equivocal (e.g., Bell et al., 2022; Thirkell et al., 2017), with root colonisation counts by staining being unreliable indicators of activity, or even viability, of AMF structures (Vierheilig et al., 2005). Despite these equivocal reports, links between reduced AMF function and root colonisation may be strengthened by the observation of greater hyphal lengths in the soil of higher PCN densities (Figure 3). This is indicative of development of a larger extraradical mycelial network which may be a fungal mechanism for AMF to expand the range over which they forage for resources, including alternative hosts which they could form symbioses, potentially mitigating loss of carbon resource from a heavily PCN-infected host. Host 'quality' (i.e., ability to supply required resources) is a dynamic characteristic and future research should seek to determine the perception of host 'quality' over time and if this influences colonisation by AMF. It would make little sense for AMF to colonise a host that is low quality from the outset unless there are little-to-no alternatives available (Lekberg et al., 2010), as simulated in our glasshouse trial.

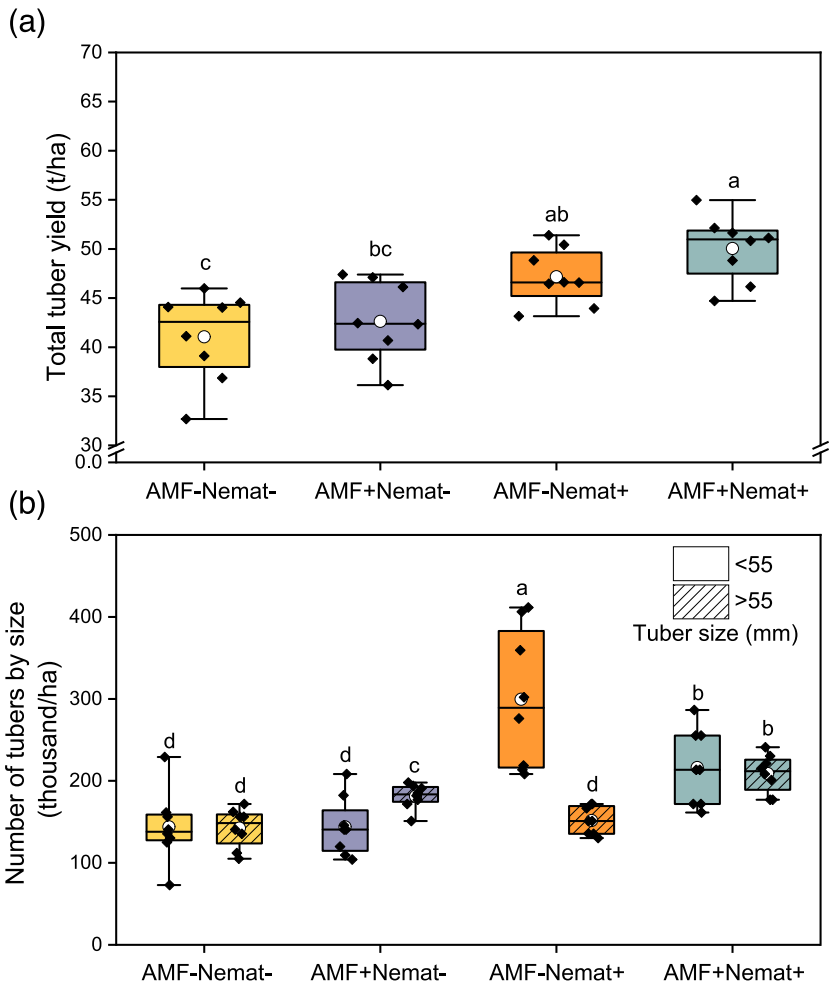

FIGURE 6 Nematicide treated plots produced greater yields (t/ha) whereas AMF inoculum increased tuber sizes. Total tuber yield (t/ha) of plots treated with Nemathorin nematicide and/or arbuscular mycorrhizal fungi (AMF) inoculum (a), and the yield then separated into tubers >55 mm and <55 mm (b). Nemathorin was applied to half of the field plot soils before potato planting as a nematicide (Nemat+) to reduce potato cyst nematode populations. Half of the plots also had a commercial AMF inoculum (AMF+). Boxplots extend from the first to the third quartile with the middle bold line representing the median value, the white circle representing the mean and whiskers extending to the minimum and maximum data points. Different letters denote significance ($p < .05$; One-way ANOVA, SNK). $n = 8$

4.2 | AMF-induced increases in PCN fitness weaken with increasing PCN densities

At PCN densities ≤35 eggs/g soil, AMF colonisation increased both the number of PCN developing on the host as well as the number of eggs produced per cyst (Figure 2). This may be a result of increased nutrient status of an AMF-colonised potato plant (Bell et al., 2022), effectively increasing the nutrients accessible to the nematodes. It could also be due to AMF-colonisation of potatoes causing increased hatching of PCN eggs, resulting in a greater number of infective juveniles in the rhizosphere (Deliopoulos et al., 2007). As PCN rely upon root exudates to hatch and begin the infective process (Moens et al., 2018) the addition of symbionts that can impact this is potentially important for control strategies. The exact factors in root exudates that are affected by AMF colonisation remain unknown.

At the greatest PCN density treatment in our experiments (125 eggs/g soil), there was no effect by AMF on post-harvest PCN cyst counts. A decrease in reproduction factor of PCN as infection densities increase is a reported phenomenon (Hearne et al., 2017; Joshi & Kumar, 2020) and could be due to limited root space, enhancing competition for infection sites (Fourie et al., 2010). At this PCN density, there was also a reduction of eggs produced per cyst, regardless of AMF presence, indicating that the host cannot support this level of PCN infection and that the nematodes potentially suffer from lack of nutrition. At this density we also observed reduced colonisation by AMF. Increasing the inoculum concentration may potentially boost root colonisation and provide enhanced nutrition to the host, however as mentioned previously the relation between function and colonisation is often unreliable (Thirkell et al., 2017). Additionally, increasing the density of inoculum does not necessarily increase root colonisation, and there may (or may not) be a threshold of AMF that the root can support (Garrido et al., 2010; Sylvia et al., 1993), which may be due to species-host identities as well as root morphologies (Thirkell et al., 2017).

4.3 | In the field, AMF increased tuber sizes and PCN egg production

It may seem promising that AMF can induce tolerance to the effects of PCN at 15 and 35 PCN eggs/g soil; however, using established decline rates (Koehler et al., 2021), multiplication rates and rotations (Gartner et al., 2021), an initial density of 15 eggs/g soil could reach 125 eggs/g soil within two potato rotations with a susceptible variety (pcncalculator.ahdb.org.uk/). The enhanced reproduction of PCN on AMF-colonised hosts implies that AMF applications in agricultural

systems could potentially quicken the rate at which the nematode field population grows and AMF-induced tolerance may be broken by only the second rotation. The ability of PCN to persist for decades in the soil without a host (Evans & Stone, 2009) indicates long-term consequences of such actions. Additionally, the susceptibility status of the crop is of great importance and combining resistance with tolerance is now seen as a necessity for breeders; however, it is difficult to engineer (Gartner et al., 2021). Utilising tolerance given by AMF interactions with a resistant variety may be a viable option and produce an effective management strategy; however, field-based data are distinctly lacking. Additionally, this will depend on whether AMF-induced tolerance functions in hosts that provide a resistance response to PCN, or if the response negatively impacts the host's interaction with the AMF.

Due to the disparate conditions between lab and field in general (Poorter et al., 2016), we established a field-based experiment using a commercial inoculum containing five AMF species applied to field plots of potatoes. Pre-planting nematicide treatment in half of the plots reduced the soil population of PCN cysts (average twofold). The nematicide chosen was one of only a few remaining options due to increasing restrictions based on environmental toxicity (Nyczepir & Thomas, 2009). This has directed research into alternative biocontrol options, including application of AMF inoculants (Schouteden et al., 2015). Upon harvest, we found that inoculation, and subsequent increased colonisation, by AMF did not impact cyst counts at harvest but increased the number of eggs produced per cyst (Figure 5). This finding corroborates our pot-based experiments, indicating that AMF increase PCN fitness in both glasshouse and field environments, potentially through greater availability of root nutrients (Bell et al., 2022). A lack of nutrients to the developing nematodes can increase the proportion of males to females (Price et al., 2021), and as only females produce eggs, AMF colonisation of the host may increase PCN egg production by increasing the ratio of females to males, via enhanced host nutrition (Bell et al., 2022). This was apparent in the glasshouse but was not in the field, possibly affected by the larger root system in field-grown plants that may generally allow for increased nutrient uptake and allow for spatially separated symbionts. The AMF-induced increase in PCN egg production was enhanced in nematicide treated plots, indicating that the AMF-induced increase in PCN fitness is stronger at reduced densities, consistent with the earlier glasshouse studies. Nematicide-treated plots produced a greater tuber yield, as expected (Norshie et al., 2016). Application of AMF did not impact total yields but did increase the production of larger tubers (>55 mm), compared to non-inoculated plots (Figure 6) potentially through the delivery of AMF-acquired nitrogen to the host (Bell et al., 2022), which positively correlates with tuber size (Zebarth & Rosen, 2007).

4.4 | Fungal identity drove the impact of AMF on PCN

The fungal community associated with the roots was analysed to indicate the species inducing the observed effects. Application of AMF increased the diversity of fungal taxa associated with potato roots but did not impact overall quantity (Figure 4b). Increasing the diversity of soil communities, including fungi, through long-term agricultural practices can promote the growth of predators of plant-parasitic nematodes and potentially reduce crop losses to these parasites (Masson et al., 2022), indicating potential long-term impacts of AMF inoculation. The inoculum used in our study consisted of five species of fungus: *Clarioeoglomus* sp., *Glomus* sp., *Diversispora* sp., *Rhizophagus irregularis* and *Funneliformis* sp. Of these, only *R. irregularis* and *Funneliformis* sp. were detected within the roots, indicating that the remaining three fungi were incompatible with potato or fungal-fungal competition led to their ineffectiveness (Werner & Kiers, 2015). This indicates that a universal inoculum is likely not an efficient way to apply AMF in the field, and a directed approach may yield better results (Sanders, 2010). Although additional data are required to provide tailored inocula for different scenarios, such an approach may improve the cost effectiveness of AMF soil amendments by only applying the required species. Of the two AMF found colonising potato, *Funneliformis* sp. was also present and surprisingly at the same quantity in non-inoculated plots compared to inoculated, indicating that this genus is native to the field and enhanced initial density does not impact the degree to which it is able to colonise potato roots. This has important implications as it implies that the apparent ineffectiveness of AMF inoculum on total yields in our experiments may be due to *Funneliformis* sp. already providing benefits to the crop, and adding *R. irregularis* did not have an additive effect. Furthermore, this indicates that the greater presence of AMF species in inoculated plots is due to the addition of *R. irregularis* to these soils, rather than increased *Funneliformis* sp. inocula. This suggests that *R. irregularis* was the differential treatment between inoculated and non-inoculated plots, therefore responsible for the increased tuber sizes and PCN fitness on these hosts. This indicates that a specifically tailored inoculum per field approach may be required, which is a daunting task, or a diverse mixture of fungal inocula that may cover a wide range of scenarios, in the hope that no species are detrimental to the system. Altering the cropping rotation may be a more appropriate method of managing the native AMF community (Sandoz et al., 2020). Although both *Rhizophagus* and *Funneliformis* are considered generalists (Öpik et al., 2006; Smith & Read, 2008), *Funneliformis* may be better suited for the crops preceding potato on this land (wheat, kale, leeks, kale and pea), hence its presence in the field. Additionally, host status is not as simple as previously thought as the strain of fungal species can impact its generalist nature (Serghi et al., 2021). This can be driven by the diversity of cropping, with polycultures promoting the largest diversity of AMF (Guzman et al., 2021) and possibly establishing a richer community for future crops. These studies are important due to the increasing usage of AMF soil amendments (Cely et al., 2016; Verbruggen et al., 2013) and require further understanding to avoid applications that may increase the populations of damaging and highly persistent pests.

In conclusion, we found that inoculation of soil with AMF provided benefits for the host both in the glasshouse and the field; however, it also increased PCN multiplication. At greater PCN infection densities, AMF-induced tolerance was reduced, suggesting that AMF-induced

tolerance may not be sustainable in the long term due to the resultant increases in pathogen populations. This is unlikely to be unique to the pathogen studied here; however, field-based data for the impact of AMF inocula on other organisms are lacking. Combining host resistance with AMF-induced tolerance is an exciting prospect for stacking defences against detrimental symbionts; however, this remains to be explored. Furthermore, our data indicate that inoculating with a native AMF species did not increase its relative abundance over native soil and that introducing a foreign AMF species appeared to have a greater effect. Better understanding which AMF species may form beneficial relationships with specific hosts under known conditions will assist in their deployment to assist and secure food production.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any potential conflict of interest.

AUTHOR CONTRIBUTIONS

CAB and EM conducted the experiments. AB and HB conducted field trials. CAB analysed and wrote the paper. KJF and PEU obtained funding, assisted with experimental design and commented on paper drafts. All authors acknowledge submission of this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. The DNA barcoding data are publicly available under submission SUB11603494 (www.ncbi.nlm.nih.gov/sra).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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