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REGULAR ARTICLE

Field inoculation with arbuscular-mycorrhizal fungi overcomes phosphorus and zinc deficiencies of linseed (*Linum usitatissimum*) in a vertisol subject to long-fallow disorder

J. P. Thompson · T. G. Clewett · M. L. Fiske

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Abstract
Background and aims Long-fallow disorder is expressed as exacerbated deficiencies of phosphorus (P) and/or zinc (Zn) in field crops growing after long periods of weed-free fallow. The hypothesis that arbuscular-mycorrhizal fungi (AMF) improve the P and Zn nutrition, and thereby biomass production and seed yield of linseed (*Linum usitatissimum*) was tested in a field experiment.
Methods A factorial combination of treatments consisting of ± fumigation, ±AMF inoculation with *Glomus* spp., ±P and ±Zn fertilisers was used on a long-fallowed vertisol. The use of such methods allowed an absolute comparison of plants growing with and without AMF in the field for the first time in a soil disposed to long-fallow disorder.
Results Plant biomass, height, P and Zn concentrations and contents, boll number and final seed yield were (a) least in fumigated soil with negligible AMF colonisation of the roots, (b) low initially in long-fallow soil but increased with time as AMF colonisation of the roots developed, and (c) greatest in soil inoculated with AMF cultures. The results showed for the first time in the field that inflows of both P and Zn into linseed roots were highly dependent on %AMF-colonisation (R^2=0.95 for P and 0.85 for Zn, $P<$ 0.001) in a soil disposed to long-fallow disorder. Relative field mycorrhizal dependencies without and with P+Zn fertiliser were 85 % and 86 % for biomass and 68 % and 52 % for seed yield respectively.
Conclusions This research showed in the field that AMF greatly improved the P and Zn nutrition, biomass production and seed yield of linseed growing in a soil disposed to long-fallow disorder. The level of mycorrhizal colonisation of plants suffering from long-fallow disorder can increase during the growing season resulting in improved plant growth and residual AMF inoculum in the soil, and thus it is important for growers to recognise the cause and not terminate a poor crop prematurely in order to sow another. Other positive management options to reduce long fallows and foster AMF include adoption of conservation tillage and opportunity cropping.

Keywords Long-fallow disorder · Plant P and Zn nutrition · Arbuscular mycorrhizal fungi · Relative field mycorrhizal dependency · Plant P and Zn inflows · Linseed (*Linum usitatissimum*) · Soil fumigation

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Abbreviations

AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
DAS	Days after sowing
RFMD	Relative field mycorrhizal dependency

Introduction

Vertisols are dark cracking clay soils occurring over 350 million ha world-wide (Ahmad 1996), mainly in semi-arid tropical and subtropical regions subject to wet and dry seasons. In the 600–800 mm median rainfall zone of subtropical eastern Australia, vertisols are favoured for grain production because of their deep soil profiles providing high plant-available water capacities (Webb et al. 1997). Although long-term rainfall is summer dominant in the north of this region (~22°S) and equi-seasonal in the south (~32°S), it is variable and unreliable. Consequently, rain-fed cropping in this region is dependent on both stored soil moisture and in-crop rainfall. The temperature regime, modified by elevation, allows winter and summer crops to be grown, with principally wheat (*Triticum aestivum* L.) in the winter season and sorghum (*Sorghum bicolor* Moench.) in summer, but other cereal, grain legume and oilseed crops are also produced (Webb et al. 1997; Unkovich et al. 2009). Two crops per year are possible (termed 'double-cropping') if sufficient rain falls just before or after harvest of the first crop. On average, however, one crop per year is obtained with intervening fallow of ~6 months, termed 'short' or 'normal' fallow. 'Long' fallows can arise through (a) switching from a winter-crop sequence to a summer-crop sequence (~11 months) or vice versa (~14 months), (b) missing a crop in a winter- or summer-crop sequence through failure of sowing rains (~18 months), and (c) prolonged drought (even longer periods).

Although these soils in Australia were initially fertile, crop responses to nitrogen (N), phosphorus (P) and zinc (Zn) fertilisers are obtained (Strong and Holford 1997) as with vertisols in other parts of the world (Le Mare 1987). Growers attempt to keep fallow land weed-free to maximise soil water storage, and the accumulation of nutrients (particularly nitrate) from mineralisation of soil organic matter during the fallow is also beneficial for the next crop. However, some crops after long periods of fallow are stunted and have leaf chlorosis resulting from exacerbated deficiencies of phosphorus (P) and zinc (Zn), a phenomenon known as 'long-fallow disorder' (Leslie and Whitehouse 1965; Thompson 1987). In less severe cases the problem is expressed as slow early growth of the crop without clear deficiency symptoms. Long-fallow disorder was shown to be associated with poorer root colonisation by arbuscular-mycorrhizal fungi (AMF) for a range of crop species growing after long fallows in farmers' fields compared with paired healthier crops growing after short fallows or as double-crops (Thompson 1987). The appearance and severity of the problem depend on the mycorrhizal dependency of the crop species, the amount of natural AMF inoculum in the soil as influenced by prior crops and the duration and frequency of cultivation of the fallow, and the availability of P and Zn in the soil (Thompson 1991; 1994b).

There are ~150 species of AMF in seven genera of the phylum Glomeromycota (Schüßler and Walker 2010) that form symbioses with the roots of most species in 80 % of plant families (Gianinazzi et al. 2010). Species of the genus *Glomus* are the most common AMF in agricultural soils worldwide, e.g. in New South Wales (Hayman and Stovold 1979) and Western Australia (Tibbett et al. 2008) and in Europe (Oehl et al. 2005). The arbuscular mycorrhizal association is mainly mutualistic with the plant supplying sugar to the obligately biotrophic fungus and the fungus supplying inorganic nutrients to the plant, particularly P and Zn, which are poorly mobile in soil. The AMF hyphae can extend >10 cm from the root surface well beyond the P depletion zone surrounding roots and root-hairs, thereby accessing a large volume of soil for non-mobile nutrients (Jakobsen et al. 1992). The hyphae also produce fine feeder extensions (~2 μm diameter) which can enter narrow soil pores unavailable to roots and take up P from drier soils than roots (Smith and Read 2008). Phosphorus taken into the extra-radical hyphae of AMF is converted into polyphosphate granules, which are rapidly transported by cytoplasmic streaming through the aseptate hyphae into the root cortical cells (Cox et al. 1980), where orthophosphate is released through the fungal membrane of arbuscules (tree-shaped structures with a large surface:volume ratio) into an apoplastic compartment for uptake through the plant cytoplasmic membrane surrounding the arbuscule (Cox and Sanders 1974; Dexheimer et al. 1985; Smith and Smith

2011a). It is possible that Zn^{2+} acts as one counter ion to polyphosphate and is rapidly transported with the granules (Cavagnaro 2008). Because AMF are obligately biotrophic, viable propagules (chlamydospores, colonised root pieces, and hyphae) decline during extended periods of weed-free fallow resulting in inadequate colonisation of the next crop (Thompson 1987). Bare fallowing as practised in the Australian northern grain region, with emphasis on weed control to maximise soil water storage, is similarly used on heavy-textured soils in semi-arid environmnents of Canada, USA and the former Soviet Union (Neider and Benbi 2008), but is quite different from 'bush' fallow practised in Africa and similar 'green' fallows in other parts of the world, where land is allowed to naturally revegetate and AMF fungi may increase (Duponnois et al. 2001; Neider and Benbi 2008).

Linseed (*Linum usitatissimum* L.), also known as oilseed flax, is one of the most sensitive winter crops to long-fallow disorder and has been used as a model plant for agronomic and soil chemical investigations into the phenomenon (Leslie and Whitehouse 1965, 1968). In glasshouse experiments with vertisols from fields disposed to long-fallow disorder on the Darling Downs (an area extending inland from Toowoomba (27°33′S, 151°58′E) Australia), linseed was shown to be highly dependent on AMF for uptake of both P and Zn, and thus biomass production and seed yield (Thompson 1994a, 1996). Treatments used to demonstrate the effects of AMF on plant nutrition and growth in these glasshouse experiments were factorial combinations of (a) gamma irradiation or pasteurisation of soil to eliminate all natural AMF, and (b) inoculation with AMF spores recovered from cropped soil or from cultures of local isolates of AMF (Thompson 1994a, 1996). Application of both P and Zn fertilisers to the soil containing no live AMF was required to obtain similar growth in linseed to that obtained from AMF without P and Zn fertilisers (Thompson 1996).

Because AMF are obligately biotrophic, large-scale production of inoculum is expensive and most often based on AMF cultures on the roots of whole plants in sterilised soil or other media (Ijdo et al. 2011). Despite this limitation, inoculation has been considered for horticultural vegetable crops and transplanted perennial fruit crops, mainly with plants that have been pre-inoculated and mycorrhizas established during a period of glasshouse growth before transplantation to the field. There have been successful outcomes in terms of growth responses and/or yield of harvestable product, for example, with garlic (*Allium sativum* L.) in Jordan (Al-Karaki 2002), leek (*Allium porrum* L.) in Denmark (Sorensen et al. 2008), lettuce (*Lactuca sativa* L.) in Turkey (Cimen et al. 2010), taro (*Colocasia esculenta* L.) in China (Li et al. 2005), green pepper (*Capsicum annuum* L.), parsley *Petroselinum crispum* (Mill.) Fuss, carrot (*Daucus carota* L.) and tomato (*Lycopersiconn esculentum* L.) in Slovenia (Regvar et al. 2003), tomato in south India (Subramanian et al. 2006), vine rootstocks (*Vitis berlandieri* Planch x *Vitis ruprestris* Scheele) in Spain (Camprubi et al. 2008) and peach seedlings (*Prunus persica* L.) in southwest Japan (Rutto and Mizutani 2006). Inoculation with AMF has also been used to establish mungbean (*Vigna radiata* (L.) R. Wilczek) and the leguminous shrub *Lespedeza formosa* (Vogel) Koehne in a severely eroded field site in southern China (Wu et al. 2002). Despite such successes it is unlikely that direct AMF inoculation using present technology for inoculum production would be commercially viable for the broadacre field crops grown in the Australian grain belt. Nonetheless, direct inoculation can be a useful research tool to understand the value of indigenous AMF for broadscale field crops.

Research and on-farm experiences in the northern grain region indicate that naturally-occurring AMF contribute to the production of a wide range of field crops (Thompson 1987), whereas it is considered that AMF are of little value to wheat production in the southern, more temperate regions of the Australian grain belt (Ryan and Kirkegaard 2012). The occurrence of long-fallow disorder in the northern grain region under conditions where AMF inoculum is severely depleted indicates the value of AMF to crop production in this region under the more normal condition of adequate levels of natural AMF.

In recent reviews, Smith and Smith (2011b) stated that there is a strong need for a research continuum between laboratory- and field-oriented research into the significance of AMF for economic crop plants, while Cavagnaro (2008) indicated more studies are required on the role of AMF in Zn nutrition under field conditions. This paper reports an investigation into the role of AMF in the P and Zn nutrition, biomass production and seed yield of linseed under field conditions at a site subject to long-fallow disorder. A field experiment

was conducted with similar treatments to previous glasshouse experiments, namely, soil fumigation to kill all naturally occurring AMF, inoculation with AMF cultures, and application of P and Zn fertilisers. The results demonstrated the importance of AMF in overcoming dual P and Zn deficiencies for linseed growth in the field and the role of AMF in preventing long-fallow disorder.

Materials and methods

Field site

A site was selected in a field of the Queensland Department of Agriculture Fisheries and Forestry Experimental Farm at Wellcamp (Lat 27° 33′ S, Long 151° 52′ E; Elevation ~500 m above sea level), Australia, where long-fallow disorder had been observed in previous crops. This site on the eastern Darling Downs is typical of the northern grain region in locaton, soil type and climate. The soil is a self-mulching black cracking clay classified as Vertisol (FAO 1998), Vertosol (Australian Soil Classification, Isbell 1996), or Ustic Pellustert (US Soil Taxonomy, Soil Survey Staff, 1999). It belongs to the Irving Series (Thompson and Beckmann 1959) which has developed from basaltic colluvium and contains 78 % of mainly smectite clay (Powell and Christianos 1985). The area had been kept in stubble-mulch fallow for 4 years with weed control by herbicides and some tillage. Some growth of wild oat (*Avena* spp. L.) and volunteer barley (*Hordeum vulgare* L.) had occurred during periods when management was delayed by weather conditions.

Experimental design

The experimental design was a randomised split-plot with three replications in blocks. The two main plot treatments were (a) soil fumigation to kill naturally occurring AMF and (b) un-fumigated long-fallow soil. The subplots were eight treatments consisting of a full factorial combination of AMF inoculation (nil and two rates of AMF inoculum), ±P fertiliser and ±Zn fertiliser.

Production of AMF inoculum

For the AMF treatments, local strains isolated from vertisols in grain fields in Queensland (Thompson 1996) were used. These were *Glomus mosseae* (Nicolson and Gerdemann) Gerdemann and Trappe strain Hart 5; *G. mosseae* strains Emerald 4 and 8; *Glomus macrocarpum* Tulasne and Tulasne, strain Schmelzer 42; and *Glomus etunicatum* Becker and Gerdemann, strain Emerald 7. These cultures were initiated from single spores and maintained on the roots of maize grown in pasteurised soil-grit mixes placed on sand trays for capillary watering. Inoculum of AMF for the field experiment was produced in pasteurised soil, a widely used and cost-effective method for inoculum production (Ijdo et al. 2011). Spores from these strains were collected in the 63–250 μm fraction by wet-sieving the soil from pot cultures of mature maize plants (Gerdemann and Nicolson 1963). A suspension to supply 25 AMF spores/g soil was mixed into 3 kg soil (oven dry equivalent) supplied with basal nutrients (Thompson 1987) in 20-cm diameter plastic pots with polythene bag liners to prevent drainage. Wheat cv. Kite (10 plants/pot) was grown in a glasshouse with temperature control of 15–25 °C, and regularly brought to 0.56 g/g soil water (equivalent to pF2) until maturity. Wheat was also grown in pots of pasteurised soil which were not inoculated with any AMF spores.

All pots of soil of each AMF strain were composited, thoroughly mixed together, and the numbers of spores were determined on four subsamples of 100 g. The soil subsample was dispersed in 1 L of 0.5 % sodium pyrophosphate solution in an end-over-end shaker then wet-sieved (Gerdemann and Nicolson 1963) to recover spores on 63 and 106 μm sieves. Intact, protoplasmic spores were counted in a 1-mL Hawksley slide under a compound microscope at 100x magnification. The soil of all AMF strains was then composited as a mixed inoculum containing 32 spores/g soil (oven dry equivalent) with the percentage of spores of the three AMF species being *G. mosseae* 80 %, *G. macrocarpum* 8 %, and *G. etunicatum* 12 %.

Field experiment

The experimental area was fertilised in late autumn with 137 kgN/ha as Nitram® (NH_4NO_3) applied at 10 cm depth though a combine seed drill with 20 cm spaces between tynes. One of two parallel bays of 38 x 1.8 m in each of three replicates was randomly chosen for fumigation. These were treated for 4 days with methyl bromide (98 g/m^2) applied as 98 % methyl

bromide: 2 % chloropicrin from cans (George Wills and Co.) under heavy duty (200 μm) polyethylene sheets with edges sunk 20 cm into the soil (Van Berkum and Hoestra 1979). After fumigation and aeration, a single furrow ~25 cm wide and ~15 cm deep was opened the length of each main plot. The twelve fertiliser x AMF inoculation treatments were applied as subplots each 2 m long with ends of neighbouring plots separated by a gap of 1 m. The required P and/or Zn fertiliser for each treatment was applied evenly in the bottom of the furrow to provide 40 kg P/ha as triple superphosphate [$Ca(H_2PO_4)_2$] and 15 kg Zn/ha as ZnO powder mixed with quartz sand to facilitate even spreading. The soil inoculum of AMF spores was applied at two rates, 3.5 and 5.8 kg/m row. The nil treatment plots received a mixture of 1.1 kg of pasteurised soil from pots that had grown wheat without AMF plus 2.4 kg of pasteurised soil.

The required AMF inoculum was sprinkled evenly along the furrow, then wet with 1 L of water per m row and covered with soil. A weighed quantity (equivalent to 200 seeds) of linseed cv. Glenelg was spread evenly along each 2 m row and covered with 2 cm soil. Substantial rain fell 2 days after sowing resulting in poor emergence and therefore existing plants were sprayed out with glyphosate 19 days later and the plots were re-sown. A trench was manually opened 5 cm deep along each plot row; 1 L of water/2 m plot was applied, and then 200 linseed seeds were distributed evenly and covered with soil to provide a single 2-m row of linseed for each treatment in each replication.

Soil water, pH, available N, P and Zn, and AMF spores

Twelve days before the second sowing, the soil profile was sampled to 150 cm depth with an over-plot hydraulic corer by taking two cores of 45 mm diameter at ~45 cm either side of the treated row at three positions along the length of each replication of the fumigated and non-fumigated main plots. Each pair of soil cores was composited in depth intervals 0–15, 15–30, 30–45, 45–60, 60–90, 90–120, 120–150 cm. Field-moist subsamples were taken for extraction of ammonium (NH_4) and nitrate (NO_3) and for determination of AMF spores. Soil moisture was determined by drying subsamples in a forced-draught oven at 105 °C for 2 days. Other subsamples for chemical analyses were air-dried in a forced-draught oven at 40 °C for 4 days, and then ground to pass a 2-mm sieve. Chemical analyses were conducted as described by Rayment and Higginson (1992) under the following alphanumeric test codes: 7C2=NH_4–N and NO_3–N (determined by autoanalysis of 2 N KCl extracts of field-moist soil); 9B2=bicarbonate-extractable P (Colwell method); 9 G2=acid-extractable P (Kerr and von Stieglitz method); 12A1=DTPA-extractable Zn (method of Lindsay and Norvell, with analysis by atomic absorption spectrometry), and 4A1=pH (1:5 soil:water suspension).

The soil profile had stored moisture close to the drained upper limit and was well supplied with nitrate nitrogen (Table 1). It was alkaline throughout with moderate levels of bicarbonate-extractable P and Zn in the 0–15 cm layer decreasing markedly to the 15–30 cm layer and remaining low further down the soil profile. The soil also had a high level of acid-extractable P to 90 cm depth in the profile. Fumigation increased NH_4–N and extractable Zn in the 0–15 cm soil layer (Table 1).

Numbers of naturally-occurring AMF spores in the long-fallow soil were determined on 100-g subsamples of undried soil from the 0–15 and 15–30 depth intervals using the method described in section 'Production of AMF inoculum'. There were 1.7±0.4 intact spores/g soil at 0–15 cm and 2.7±0.5 spores/g soil at 15–30 cm depth. The species *Glomus mosseae* and *G. etunicatum* were identified from spore morphology (Morton 1988; INVAM 2011).

Plant harvests and chemical analysis

Plants were sampled from 20 cm lengths of row at 37, 56, 76, 97 and 132 days after sowing (DAS). The height of the plants was measured, and plants were then cut at ground level and collected in paper bags for analyses. The soil with roots beneath the cut plants of the unfertilised treatments was excavated from the 20 cm of row in a section 20-cm wide×20-cm deep. Plants were dried in a forced-draught oven at 70 °C for 3 days for determination of dry biomass. The dried plant material from each of the first four sampling times was ground, and a subsample was digested with nitric acid/perchloric acid mixture (Johnson and Ulrich 1959), and analysed for P by an automated colorimetric procedure (Murphy and Riley 1962) and for Zn by atomic absorption spectrophotometry. These analyses provided P and Zn concentrations per unit weight of plant tissue; P and Zn contents (also termed uptake by some authors) were calculated by multiplying

Table 1 Properties of the soil profile at the site of the experiment on the Wellcamp Experimental Farm, Queensland. Values are means of analyses of nine samples from each of fumigated and non-fumigated soil with significance of the *t* test given for the 0–15 cm layer; the overall mean is given for deeper layers where there was no significant difference between fumigated and non-fumigated soil

Soil depth (cm)	Soil water (g/g)	pH	NH_4-N (mg/kg)	NO_3-N (mg/kg)	Bicarb P (mg/kg)	Acid P (mg/kg)	DTPA Zn (mg/kg)
0–15 (fumigated)	0.55	8.1	16.4	40.9	36.8	406	0.60
0–15 (non-fumigated)	0.55	8.0	5.0	51.8	31.4	398	0.47
t test probability	ns	ns[a]	0.01	ns	ns	ns	0.02
15–30	0.55	7.9	5.5	33.5	11.8	435	0.30
30–45	0.57	8.0	3.6	27.0	11.1	467	0.26
45–60	0.57	8.0	3.8	26.4	9.5	482	0.23
60–90	0.55	8.3	4.2	19.9	8.3	493	0.23
90–120	0.48	8.5	2.7	13.3	4.1	367	0.17
120–150	0.39	8.8	2.5	8.2	2.1	183	0.16
Method[b]		4A1	7C2	7C2	9B2	9 G2	12A1

[a] *ns* non-significant at $P=0.05$

[b] Method: codes from Rayment and Higginson (1992). 7C2=NH_4–N and NO_3–N (determined by autoanalysis of 2 N KCl extracts); 9B2 bicarbonate-extractable P (Colwell method); 9 G2=acid-extractable P (Kerr and von Stieglitz method); 12A1=DTPA-extractable Zn (method of Lindsay and Norvell, with analysis by atomic absorption spectrophotometry), and 4A1=pH (1:5 soil:water suspension)

concentrations by plant weights. At the final sampling time (132 DAS), linseed bolls (seed capsules) were counted, and then threshed to determine seed yield.

AMF colonisation of linseed roots

The soil and roots sampled were soaked in a solution of sodium pyrophosphate to disperse the clay soil adhering to the roots which were then washed repeatedly and recovered on a 425-μm sieve. Roots were blotted dry and fresh weights were determined. A root subsample of 0.5 g was stained for AMF with trypan blue after clearing in KOH (Phillips and Hayman 1970). Total root length, %AMF colonisation, and length of AMF-colonised root were determined by the grid intersect method (Giovannetti and Mosse 1980).

Rainfall and soil moisture

Rainfall for intervals between harvests was 22 mm for 0–37 DAS, 22 mm for 37–56 DAS, 42 mm for 56–76 DAS, 69 mm for 76–97 DAS, and 36 mm for 97–132 DAS, for a total of 191 mm, compared with a mean long-term rainfall for this period of 262 mm (mean of two neighbouring Bureau of Meteorology weather stations at Westbrook and Moyola). Soil moistures (g/g) in the 0–20 cm interval for nil and AMF-inoculated treatments at the various sampling times were respectively 0.47 and 0.45 at 37 DAS, 0.48 and 0.41 (significant difference at $P<0.05$) at 57 DAS, 0.50 and 0.48 at 76 DAS, 0.49 and 0.44 at 97 DAS and 0.35 and 0.32 at 132 DAS. Moisture characteristics of this clay soil are drained upper limit (field capacity)=0.56 g/g and lower limit (wilting point)=0.33 g/g.

Statistical analysis

The data for each parameter were analysed by a split-plot analysis of variance (ANOVA) with harvest and fumigation treatments as main plots and a balanced factorial array of AMF inoculation, and P and Zn fertiliser as subplots. The %AMF colonisation of roots was transformed by arcsine, and total root-length and AMF-colonised root length were transformed by ln(x+1) to normalise the data for ANOVA. Regression analysis in Genstat (VSN International 2012) was used to relate mean P and Zn inflows as response variates to mean %AMF colonisation (arcsin transformed) as explanatory variate for unfertilised treatments over the first three harvest intervals.

P and Zn inflows into roots

Mean P inflows into linseed roots of plants growing in fumigation and AMF inoculation treatments in unfertilised soil were calculated for the first three harvest intervals using the equation:

$$Ip = (P2 - P1) \div [0.5(L1 + L2)(t2 - t1)]$$

where I_P is P inflow in mol/m/s, P is P content (mol) of the shoots (all above-ground parts), t is time (s), L is total root length (m), and 1 and 2 indicate successive harvests (Jakobsen 1986).

Mean Zn inflows (I_Z) were calculated similarly using the equation:

$$Iz = (Zn2 - Zn1) \div [0.5(L1 + L2)(t2 + t1)]$$

where I_Z is Zn inflow in mol/m/s, Zn is the Zn content (mol) of the shoots, t is time (s), L is total root length (m), and 1 and 2 indicate successive harvests.

No account is taken of P and Zn contents of roots in these equations (Jakobsen 1986).

Relative field mycorrhizal dependency

Relative field mycorrhizal dependency (RFMD) (%) was calculated from mean values for plant biomass, plant P content and plant Zn content at 97 DAS, and for seed yield using the following equation (Plenchette et al. 1983):

$$RFMD = 100(MP - NMP) \div MP$$

where MP is value for mycorrhizal plants and NMP is value for non-mycorrhizal plants. RFMD was determined separately at the four combinations of P and Zn fertiliser treatments. The use of mycorrhizal plants as the denominator in this formula constrains values to + 100 %, whereas use of non-mycorrhizal plants as denominator in a similar equation measuring 'mycorrhizal growth response' allows values to range to + infinity (Smith and Smith 2011b).

Results

There were no significant differences ($P<0.05$) between the two rates of AMF inoculation and therefore mean values are presented as the AMF inoculation treatment in the results. There were significant effects of harvest time and significant interactions between treatments and time. Therefore, means for the highest order interactions that had significant F tests ($P<0.05$) at most harvest times are presented with the appropriate least significant differences (l.s.d.) at each harvest. Results for transformed values of AMF colonisation are presented in figures with appropriate l.s.d., and numbers mentioned in the text are back-transformed means.

AMF colonisation of the linseed roots

Soil fumigation produced control treatments in which the linseed roots remained virtually un-colonised with AMF (<0.2 % AMF colonisation) for the duration of the experiment (Fig. 1a). Linseed roots in long-fallow soil were poorly colonised with AMF fungi at 37 DAS (9 % AMF colonisation), but increased to 54 % AMF colonisation by 132 DAS (Fig. 1a). Plants in both fumigated and long-fallow soil inoculated with AMF already had ~60 % AMF-colonisation of the root length by 37 DAS and reached ~70 % AMF colonisation at 132 DAS.

The AMF-colonised root length of plants in fumigated soil never exceeded 1.5 m/m row, but with AMF inoculation reached ~80 m/m row at 37 DAS which increased to ~1,250 m/m row at 132 DAS (Fig. 1b). In long-fallow soil, the AMF-colonised root length was only 3.6 m/m row at 37 DAS but increased to 450 m/m row at 132 DAS. Inoculation of long-fallow soil with AMF resulted in AMF-colonised root length of 52 m/m row at 37 DAS which increased to 675 m/m row at 132 DAS (Fig. 1b).

Biomass and height of the linseed plants

Biomass of the linseed shoots paralleled the order for AMF colonisation of the roots with fumigated soil < long-fallow soil < long-fallow soil with AMF inoculation < fumigated soil with AMF inoculation (Fig. 2a). At maturity (132 DAS), the biomass of linseed in fumigated soil with AMF inoculation was 2.5 times that in fumigated soil. The addition of Zn fertiliser increased plant biomass resulting in a 53 % increase at 97 DAS (Fig 2b).

At 57 and 76 DAS, the height of plants grown in fumigated soil was less than that in long-fallow soil which was less than that in both AMF-inoculated soils

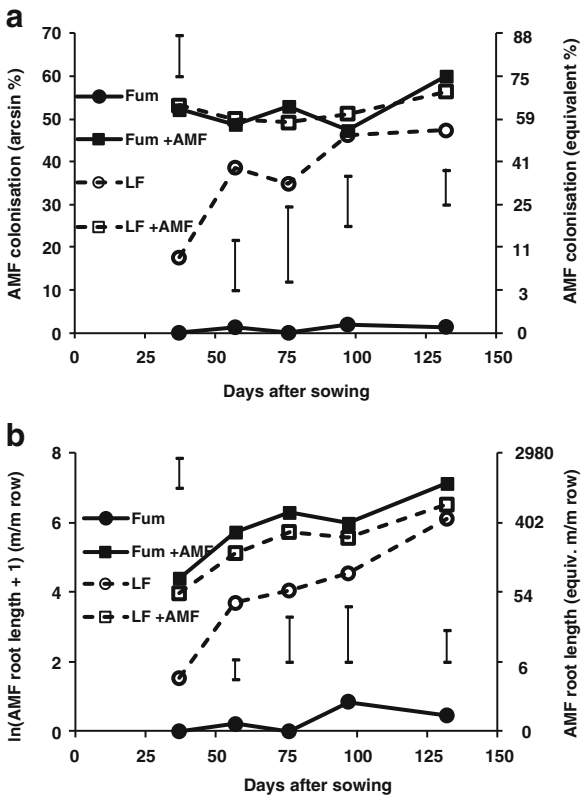

Fig. 1 AMF colonisation of linseed roots as determined by **a** % AMF-colonised root, and **b** AMF-colonised root length (m/m row) from a cross-section of soil and roots 20 cm wide×20 cm deep) was negligible in fumigated soil (Fum) and delayed in long-fallow soil (LF), but was markedly increased by inoculation with AMF (+AMF). Left vertical axis is in transformed units and right vertical axis gives equivalent units. Results presented are for the highest order interactions of factorial treatments that were statistically significant at most sampling times. Bar markers=l.s.d (P=0.05) in transformed units

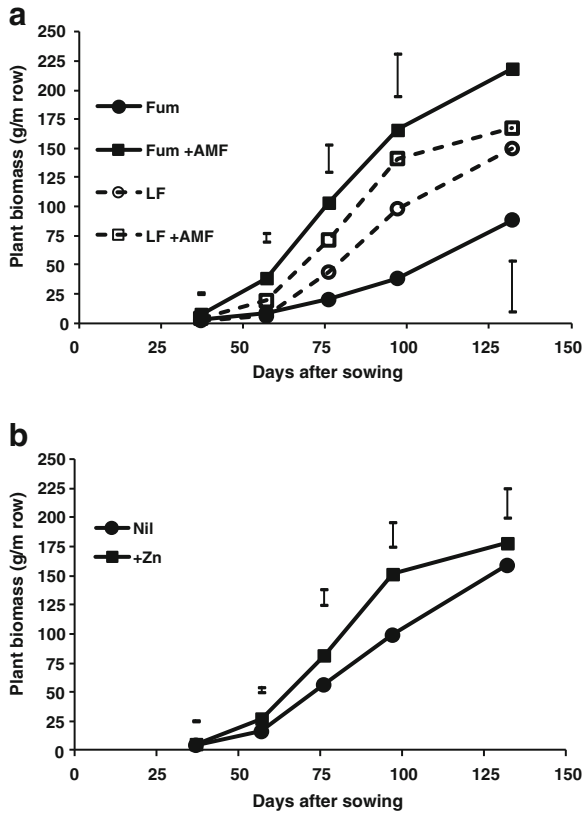

Fig. 2 Plant biomass was **a** least in fumigated soil (Fum) and long-fallow soil (LF), but was increased by inoculation with AMF (+AMF), and **b** was increased by Zn fertiliser. Results presented are for the highest order interactions of factorial treatments that were statistically significant at most sampling times. Bar markers=l.s.d (P=0.05)

(Fig. 3a). Subsequently, plants in the fumigated soil with AMF inoculation plateaued at a greater height (42.3 cm) than the other three treatments (mean 36.3 cm). In an interactive effect between P and Zn fertilisers, Zn increased plant height, and P with Zn increased it more, but P without Zn decreased plant height (Fig. 3b).

P concentration and content of linseed shoots

Phosphorus concentration of linseed plants at 37 DAS was low in both fumigated soil (1.7 mg P/g tissue) and long-fallow soil (2.0 mg/g), but was higher with AMF inoculation of both long-fallow soil (3.3 mg/g) and fumigated soil (3.5 mg/g) (Fig 4a). By 57 DAS, the P concentration of plants in long-fallow soil (3.7 mg/g) had increased to be similar to that in long-fallow soil with AMF inoculation (3.6 mg/g), but that in fumigated soil remained low (1.9 mg/g) in comparison with fumigated soil with AMF inoculation (4.1 mg/g) and long-fallow soil with or without AMF inoculation. At 76 DAS, P concentration of the fumigated soil (2.9 mg/g) was closer to that of the other treatments but at 97 DAS (3.0 mg/g) was still significantly less than the other three treatments (mean 3.6 mg/g) (Fig 4a). Application of P fertiliser in the absence of Zn fertiliser resulted in the highest plant P concentrations at all sampling times (Fig. 4b). Application of Zn fertiliser either alone or with P fertiliser resulted in lower P concentrations than nil fertiliser or P alone, for example at 97 DAS, P concentration was 4.2 mg/g for P fertiliser, 3.6 mg/g for nil fertiliser and 3.1 mg/g for Zn fertiliser (mean of both with and without P).

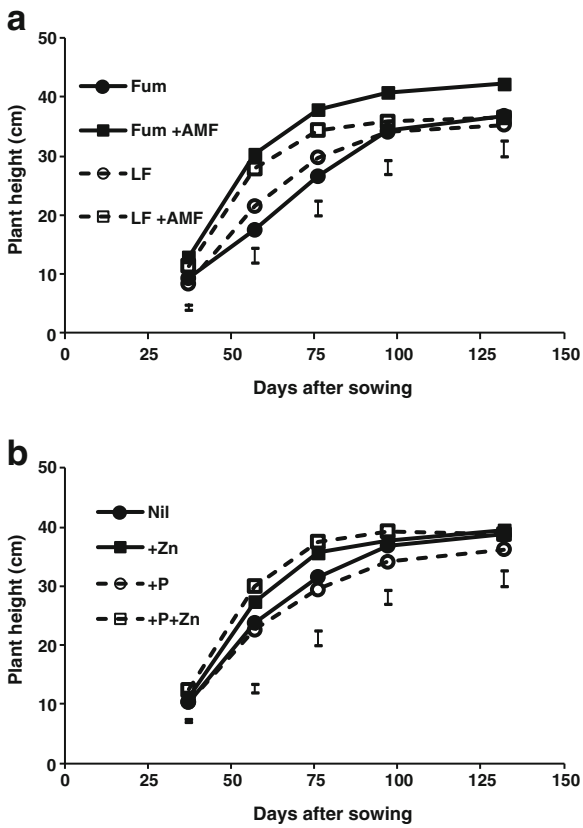

Fig. 3 Plant height **a** was least in fumigated soil (Fum) and long-fallow soil (LF), but was increased by inoculation with AMF (+AMF) particularly in fumigated soil, and **b** was least with P fertiliser but greatest with P+Zn fertiliser. Results presented are for the highest order interactions of factorial treatments that were statistically significant at most sampling times. Bar markers=l.s.d (P=0.05)

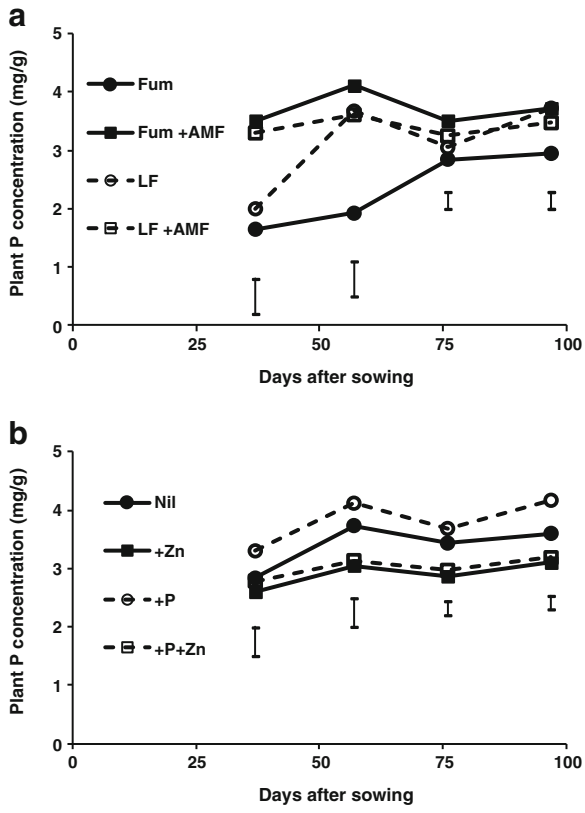

Fig. 4 Plant P concentration **a** was least in fumigated soil (Fum) and long-fallow soil (LF), but was increased by inoculation with AMF (+AMF), and **b** was increased by P fertiliser in the absence of Zn fertiliser, and was decreased by Zn fertiliser (both in the presence and absence of P fertiliser). Results presented are for the highest order interactions of factorial treatments that were statistically significant at most sampling times. Bar markers=l.s.d (P=0.05)

At 37 DAS, the plant P contents from fumigated and long-fallow soil were similar (mean 0.10 mg P/plant) and substantially less than long-fallow soil with AMF inoculation (0.29 mg/plant) and fumigated soil with AMF inoculation (0.38 mg/plant). At 57 DAS, plant P content in fumigated soil was lower (0.26 mg/plant) than in long-fallow soil (0.68 mg/plant) which was substantially lower than in long-fallow soil with AMF inoculation (2.1 mg/plant) and fumigated soil with AMF inoculation (2.6 mg/plant) (Fig. 5a). Plant P content increased slowly in fumigated soil over time to 2.3 mg/plant at 97 DAS, but increased faster in long-fallow soil to 9.2 mg/plant to approach long-fallow soil with AMF inoculation (11.3 mg/plant) and fumigated soil with AMF inoculation (13.9 mg/plant) (Fig. 5a). For P content at 76 DAS, there was a significant interaction among fumigation, AMF inoculation and P fertiliser treatments. Inoculation with AMF had a far greater effect at increasing P content than did P fertiliser, a difference which was more pronounced in fumigated soil than in long-fallow soil (Fig. 5b).

Zn concentration and content of linseed shoots

Plant Zn concentration at 37 DAS was least in fumigated soil (19 mg Zn/kg plant tissue), next lowest in long-fallow soil (23 mg/kg) and greatest with AMF inoculation both in long-fallow (31 mg/kg) and fumigated soil (30 mg/kg) (Fig. 6a). The Zn concentration increased up to 76 DAS in plants from the fumigated soil but declined with time in the three other treatments. Plant Zn concentration at 37 DAS was greater

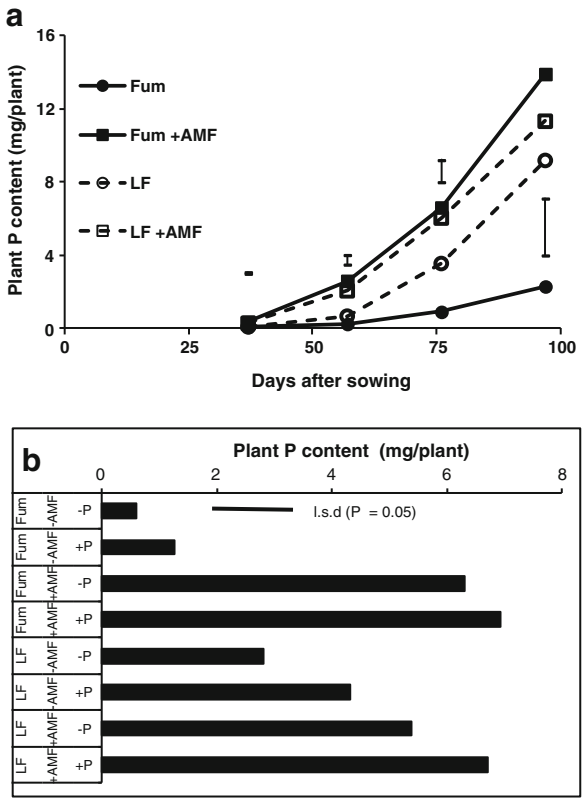

Fig. 5 Plant P content **a** remained low in fumigated soil (Fum), increased in long-fallow soil (LF) and was greatest in soil inoculated with AMF (+AMF), and **b** P content of linseed in fumigated soil at 76 DAS was increased somewhat by P fertiliser, but far more by AMF inoculation, while P content of linseed in long-fallow soil was greater than in fumigated soil and was increased more by P fertiliser and even more by AMF inoculation. Results presented are for the highest order interactions of factorial treatments that were statistically significant at most sampling times. Bar markers=l.s.d (P=0.05)

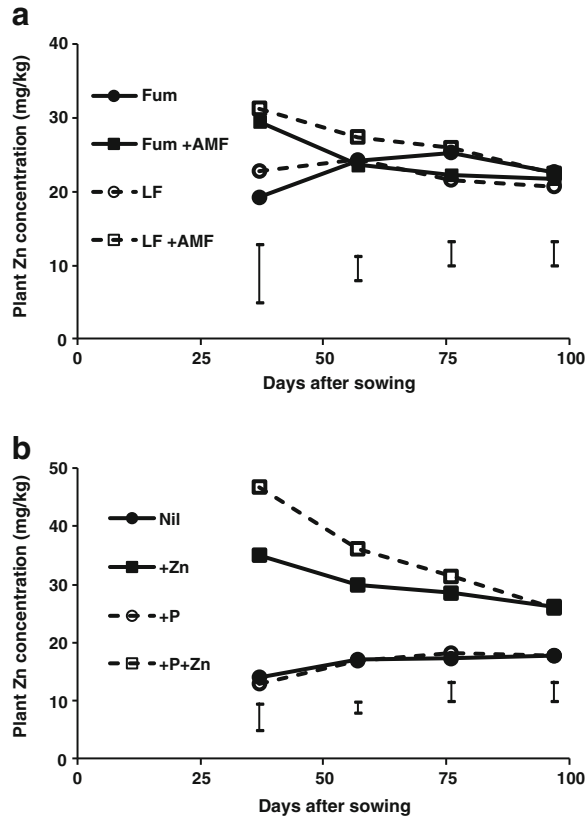

Fig. 6 Plant Zn concentration **a** was least in fumigated soil (Fum) and long-fallow soil (LF), but was increased by inoculation with AMF (+AMF), and **b** was increased by Zn fertiliser particularly in the presence of P fertiliser. Results presented are for the highest order interactions of factorial treatments that were statistically significant at most sampling times. Bar markers=l.s.d (P=0.05)

with the combination of P plus Zn fertiliser (47 mg/kg) than with Zn alone (35 mg/kg) which was considerably greater than both nil fertiliser (14 mg/kg) or P alone (13 mg/kg) (Fig. 6b). The plant Zn concentration declined with time in soil fertilised with Zn and the difference between plus and nil P treatments in Zn fertilised soil disappeared by 97 DAS (mean 26 mg/kg), but was still greater than in the soils without Zn fertiliser (mean 18 mg/kg).

Plant Zn content at 37 DAS was lower in fumigated soil (1.1 μg Zn/plant) and long-fallow soil 1.2 μg/plant) than in long-fallow soil with AMF inoculation (2.7 μg/plant) and fumigated soil with AMF inoculation (3.3 μg/plant) (Fig. 7a). However, with time Zn content increased more in the long-fallow soil (54 μg/plant at 97 DAS) than in the fumigated soil (19 μg/plant) (Fig. 7a). Plant Zn content was increased by AMF inoculation of both fumigated soil (83 μg/plant) and long-fallow soil (77 μg/plant) compared with respective un-inoculated treatments throughout the experiment (Fig. 7a). Plant Zn content at 37 DAS was low with nil treatment (0.4 μg/plant), but was increased by Zn fertiliser (1.9 μg/plant) or AMF inoculation (1.4 μg/plant) and particularly by the combination of AMF and Zn treatments (4.6 μg/plant). Although plant Zn content increased considerably with time this pattern of response to treatments was maintained to 97 DAS when fumigated soil (23 μg/plant) had less Zn content than either Zn fertiliser (51 μg/plant) or AMF inoculation alone (56 μg/plant), which were both less than the

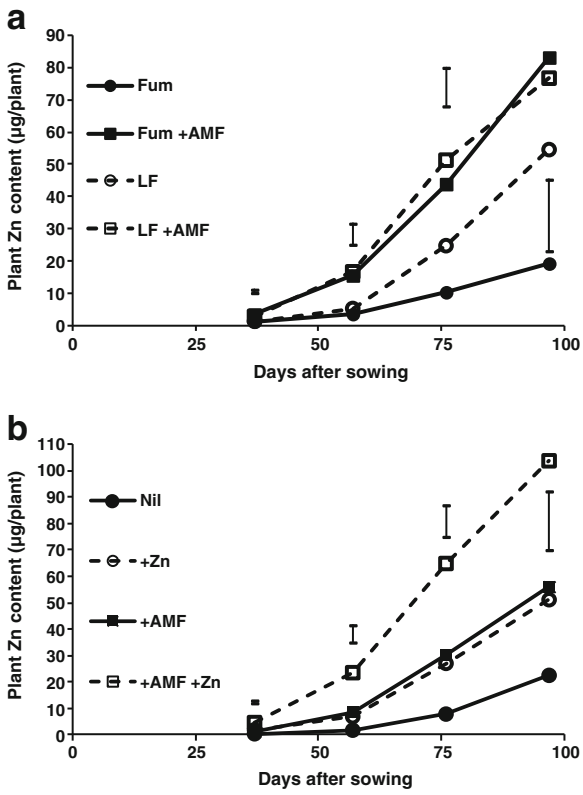

Fig. 7 Plant Zn content **a** remained lower in fumigated soil (Fum) than in long-fallow soil (LF) which was lower than in either soil with AMF inoculation (+AMF), and **b** was lowest without both AMF inoculation and Zn fertiliser (Nil) and highest with both (+AMF +Zn). Results presented are for the highest order interactions of factorial treatments that were statistically significant at most sampling times. Bar markers=l.s.d (P=0.05)

combination of Zn fertiliser with AMF inoculation (104 µg/plant).

P and Zn inflows into roots

The inflows into roots of both P and Zn were considerably lower in both fumigated soil and long-fallow soil up to 37 DAS than in either soil inoculated with AMF (Table 2). Inflows of P and Zn in fumigated soil remained relatively low in the next two harvest intervals (up to 76 DAS), but increased in the long-fallow soil to approach the higher inflows of the AMF-inoculated treatments. Both P inflow (Fig. 8a) and Zn inflow (Fig. 8b) were highly dependent on the %AMF colonisation of the roots over the three harvest intervals. For every 1 % increase in AMF colonisation

Table 2 Phosphorus and zinc inflows into linseed roots as affected by fumigation and AMF inoculation of unfertilised long-fallow soil in a field experiment at Wellcamp

Fumigated or Long-fallow soil	AMF Inoculation	Harvest interval (days)		
		0–37	37–57	57–76
		P inflow (mol P/m/s x 10^{-13}) [a]		
Fumigated	−AMF	6.7	2.1	13.8
Fumigated	+AMF	38.4	59.0	67.4
Long-fallow	−AMF	9.8	33.7	54.9
Long-fallow	+AMF	28.9	60.8	54.5
		Zn inflow (mol Zn/m/s×10^{-15}) [b]		
Fumigated	−AMF	2.0	1.7	3.9
Fumigated	+AMF	7.7	10.4	16.9
Long-fallow	−AMF	2.3	7.7	12.5
Long-fallow	+AMF	6.8	11.6	14.9

[a] P inflow, $I_P=(P_2-P_1)/[0.5(L_1+L_2)(t_2-t_1)]$, where I_P is P inflow in mol/m/s, P is P content (mol) of the shoots (all above-ground parts), t is time (s), L is total root length (m), and subscript numerals indicate successive harvests (Jakobsen 1986)

[b] Zn inflow $I_{Zn}=(Zn_2-Zn_1)/[0.5(L_1+L_2)(t_2-t_1)]$, where Zn is the Zn content of the shoots and other symbols are as given for P inflow

over the range 0–51.5 % arcsin % AMF units (equivalent to 0–61.4 %AMF), P inflow increased by1.1 mol P/m/s×10^{-13}, and Zn inflow increased by 0.23 mol Zn/m/s×10^{-15}.

Boll number and seed yield

Boll number (Fig. 9a) and seed yield (Fig. 9b) were least in fumigated soil (1,113/m row and 28 g/m row) and second lowest in long-fallow soil (1,772/m row and 49 g/m row). Both boll number and seed yield responded to AMF inoculation more in fumigated soil (2,666 bolls/m row and 72 g seed/m row) than in long-fallow soil ((2,142 bolls/m row and 54 g seed/m row) (Fig. 9a and b). Without AMF inoculation, the combination of P and Zn fertilisers resulted in the most number of bolls (1,791/m row) while P fertiliser alone resulted in the least number (1,148 bolls/m row) (Fig. 9c). Inoculation with AMF resulted in considerable increases in the number of bolls in all fertiliser treatments with the increase being greatest with nil fertiliser (1,190 bolls/m row increase) and least with the combination of P and Zn (627 bolls/m row

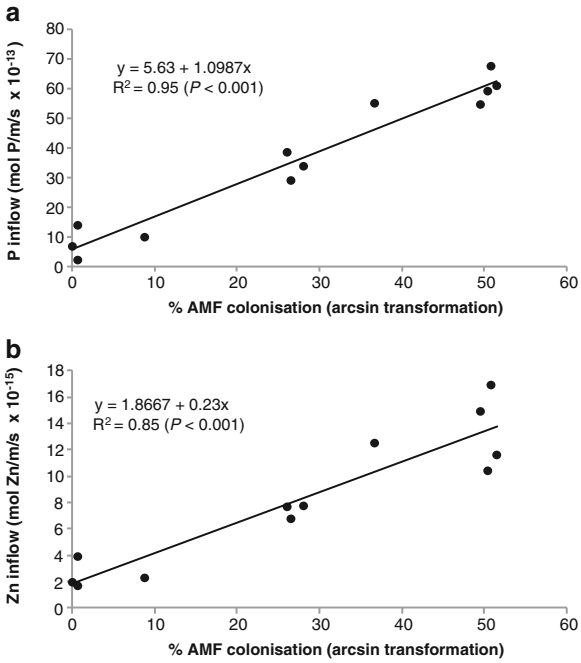

Fig. 8 Inflows of **a** P and **b** Zn into linseed roots were linearly related to the mean %AMF colonisation of the roots (arcsin transformation) for four unfertilised soil treatments over three harvest intervals

difference) (Fig. 9c). Seed yield was 16 % lower with the application of P fertiliser without Zn than for the other fertiliser treatments (data not shown).

Relative field mycorrhizal dependency

The linseed plants at 97 DAS had high RFMD values (Table 3) for biomass production and P and Zn contents for all combinations of AMF inoculation treatments and P and Zn fertiliser treatments in both fumigated and long-fallow soil. The values for RFMD based on biomass production ranged from 67 % in long-fallow soil with P fertiliser to 91 % in long-fallow soil with Zn fertiliser. The values for RFMD based on P content ranged from 87 % in long-fallow soil both without fertiliser and with Zn fertiliser to 93 % in fumigated soil with AMF inoculation. The values for RFMD based on Zn content ranged from 83 % in long-fallow soil with P fertiliser to 94 % in fumigated soil with AMF inoculation and Zn fertiliser either with and without P fertiliser, and in long-fallow soil with AMF inoculation and Zn fertiliser. The RFMD values for seed yield ranged from 29 % in long-fallow soil with P and Zn fertilisers to 74 % in fumigated soil with AMF inoculation and P fertiliser, and although these values are somewhat lower than for biomass and P and Zn contents, they are still substantial. These RFMDs are based on values for the non-mycorrhizal plants in fumigated soil where there was a large synergistic response to P and Zn fertilisers in biomass and smaller ones in P content, Zn content and seed yield (Table 3).

Discussion

The field experiment reported here clearly demonstrated the value of AMF for P and Zn nutrition, biomass production and seed yield of linseed growing in a vertisol subject to long-fallow disorder. By using treatments of soil fumigation combined with inoculation with AMF cultures it was possible to quantify in the field the value of AMF for crop growth in this environment for the first time. Linseed without P or Zn fertilisers had RFMD values of 85 % for biomass, 91 % for P content, 90 % for Zn content and 68 % for seed yield. This result confirms the high relative mycorrhizal dependency of linseed established in previous glasshouse experiments with other vertisols from the Darling Downs, in which values up to 85 % for biomass and 95 % for both P content and Zn content were obtained when long-fallow soil was irradiated and re-inoculated with AMF spores from a cropped soil (Thompson 1994a), and 97 % for biomass and 99 % for seed yield when steamed soil was inoculated with *G. mosseae* (Thompson 1996). These results provide a research continuum between glasshouse and field experiments as sought by Smith and Smith (2011b) and establish linseed as a highly mycorrhizal dependent species when growing in vertisols of the Darling Downs. Even where P and Zn fertilisers were applied in this experiment, root colonisation with AMF resulted in greater contents of both P and Zn in the plants with the extra amounts coming from both soil and fertiliser sources. The very close regression relationships between P and Zn inflows and %AMF colonisation of the root systems further emphasised the dependence of linseed on AMF for P and Zn nutrition under field conditions. Previously, highly significant linear regression relationships between %AMF at early harvest times as the explanatory variate and the biomass of linseed plants at later harvest times as the

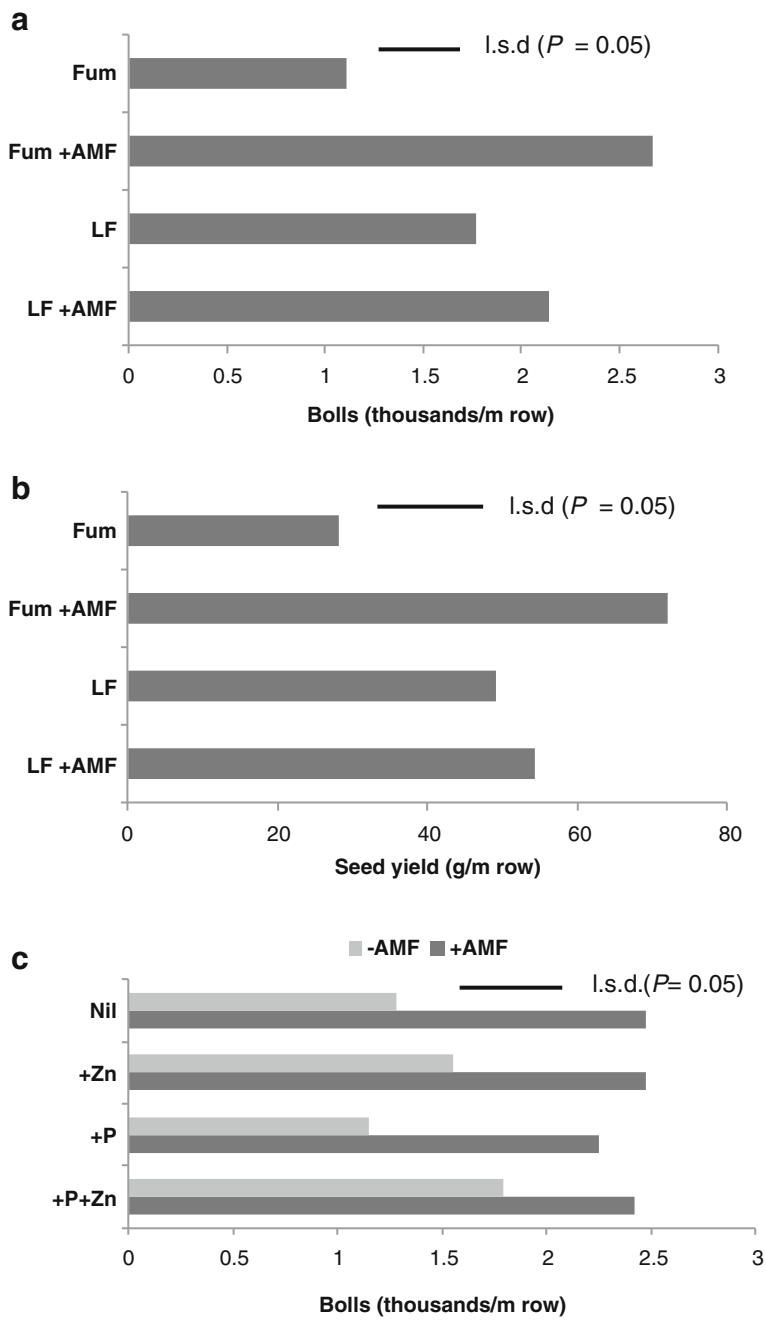

Fig. 9 **a** Boll number and **b** seed yield of linseed were least in fumigated soil (Fum), but were increased markedly by AMF inoculation (+AMF) to exceed long-fallow soil (LF, or LF +AMF), and **c** Boll number was increased by Zn fertiliser in the presence of P in fumigated soil. Results presented are for the highest order interactions of factorial treatments that were statistically significant at most sampling times. Bar markers=l.s.d (P=0.05)

response variate were recorded in pot experiments (Thompson 1994a, 1996).

The improvement in P and Zn nutrition and growth of linseed in long-fallow and fumigated soil from AMF inoculation shown here is similar to the correction of long-fallow disorder in linseed obtained by double-cropping after foxtail millet (*Setaria italica* (L.) P. Beauvois) or cowpea (*Vigna unguiculata* L. (Walp.) on similar vertisols of the Darling Downs (Leslie and Whitehouse 1965), and in the synergistic response to P and Zn fertilsers of linseed in long-fallow soil (Leslie and Whitehouse 1968). Although the levels of bicarbonate-extractable P and DTPA-extractable Zn in the soil at our field site were moderate, the high pH and high clay content both reduce the amount of Zn in soil solution and lessen the ability of

Table 3 Relative field mycorrhizal dependencies (RFMD) of linseed based on plant biomass or P and Zn contents at 97 days after sowing (DAS), and on seed yield at 132 DAS, in fumigated or long-fallow soil inoculated with AMF and with or without P and Zn fertilisers in a field experiment at Wellcamp

Treatments[a]				97 DAS			132 DAS
Fum or LF	AMF	P	Zn	Biomass	P content	Zn content	Seed yield
				Values for non-mycorrhizal plants in original units			
				(g/m row)	(mg/plant)	(μg/plant)	(g/m row)
Fum	−AMF	−P	−Zn	21.8	1.09	6.28	23.0
Fum	−AMF	−P	+Zn	33.7	2.63	26.6	33.1
Fum	−AMF	+P	−Zn	23.0	1.90	8.5	18.4
Fum	−AMF	+P	+Zn	74.6	3.60	35.3	38.0
				Relative field mycorrhizal dependency[b] (%)			
Fum	+AMF	−P	−Zn	85	91	90	68
Fum	+AMF	−P	+Zn	91	92	94	50
Fum	+AMF	+P	−Zn	82	93	89	74
Fum	+AMF	+P	+Zn	86	92	94	52
LF	−AMF	−P	−Zn	73	87	84	55
LF	−AMF	−P	+Zn	82	87	89	39
LF	−AMF	+P	−Zn	67	88	83	52
LF	−AMF	+P	+Zn	82	90	93	29
LF	+AMF	−P	−Zn	80	90	89	60
LF	+AMF	−P	+Zn	85	90	94	47
LF	+AMF	+P	−Zn	82	89	87	62
LF	+AMF	+P	+Zn	88	91	94	30

[a] Treatments: *Fum* fumigated soil, *LF* long-fallow soil, *+AMF* AMF inoculation, +P=40 kg P/ha, +Zn=15 kg Zn/ha

[b] Relative field mycorrhizal dependency=100×(value for mycorrhizal plants – value for non-mycorrhizal plants)/(value for mycorrhizal plants), (Plenchette et al.1983)

plants to extract Zn from the soil (Brennan et al. 1993; Marschner 1993). Out of 65 soils from the northern grain region tested in glasshouse experiments, linseed responded to Zn fertiliser in 68 % of soils that had both pH>7.0 and DTPA-Zn<0.8 mg/kg, but in no soil outside these parameters (Whitehouse 1973).

Reuter et al. (1997) listed concentrations for P deficiency in whole shoots of linseed plants grown in soil as 2.5 mg P/g tissue at 37 DAS and 1.9–2.1 mg/g at 63 DAS in one study, while concentrations for adequate P nutrition were listed as 3.7–6.9 mg/g at 53 DAS, 2.7–10.0 mg/g at 63 DAS and 4.9–8.9 mg/g at 70 DAS in another study (Reuter et al. 1997). Thus taking 2.5–3.0 mg P/kg tissue as a critical nutrient concentration for our study, the P concentration of linseed at 37 DAS was below the critical level in both fumigated and long-fallow soil, and at 56 DAS remained below the critical level in fumigated soil. Inoculation with AMF raised the P concentration of the linseed above the critical level in both fumigated and long-fallow soil by 37 DAS.

For Zn concentration of whole shoots of linseed grown in soil, deficient ranges were listed in one study as ≤10 mg Zn/kg tissue at 21 DAS and ≤16 mg/kg at 63 DAS, and in another study as 15–18 mg/kg at 63 DAS, 18–20 mg/kg at 70 DAS and 12.7–19 mg/kg at 70 DAS (Reuter et al. 1997). Ranges of Zn concentration for adequate Zn nutrition were listed as 19–37 mg Zn/kg tissue at 53 DAS, 24–31 mg/kg at 63 DAS, 17–35 mg/kg at 63 DAS, 32–83 mg/kg at 70 DAS and 19.3–38 mg/kg also at 70 DAS (Reuter et al. 1997). Thus, taking ~25 mg Zn/kg tissue as a critical level for our study, AMF inoculation raised plant Zn concentration above this level in both fumigated and long-

fallow soil. Addition of Zn fertiliser also raised the Zn concentration well above the critical level.

The most important interaction between P and Zn in plant nutrition occurs in soils where both elements are deficient or marginally deficient (Loneragan and Webb 1993). When fertiliser P is added and plants grow larger, tissue concentration of Zn decreases below the critical level and limits further growth. Adding both nutrients can help overcome this effect as found in a previous pot experiment (Thompson 1996). However, in our field experiment, linseed without AMF still could not respond to the combination of both P and Zn fertilisers to the full extent it could when colonised with AMF.

Crops affected by long-fallow disorder may improve over time and this has been considered due to increasing availability of soil Zn as temperature increases in late spring (Duncan 1967) from mineralisation of organic matter and increased root development (Marschner 1993). Supporting this notion are Moraghan's (1980) results from a pot experiment in which linseed grew better at higher soil temperatures (7, 15 and 24 °C were tested), and in which greater rates of P and Zn fertilisers were required at lower soil temperatures to achieve near maximum growth (Moraghan 1980). All treatments were subject to the same temperatures in our field experiment which provides un-confounded evidence that the improvement of linseed growth in long-fallow soil with time is due to an increasing %AMF colonisation of the roots. Our research further showed in the field that if effectively all AMF are killed the plants do not recover with time, but field inoculation with AMF cultures can correct P and Zn deficiencies and improve biomass production and seed yield. Growers have sometimes ploughed or sprayed out poor crops suffering from undiagnosed long-fallow disorder in early vegetative stages, to prepare the land and sow another crop. When early growth is poor due to inadequate AMF colonisation, it is important to recognise the cause and allow the crop to continue to grow and develop late AMF colonisation, which will aid partial or full crop recovery and leave increased AMF propagules in the soil for a subsequent crop.

Linseed has been used as a model crop for investigations into long-fallow disorder because of its sensitivity to the phenomenon, which is now recognised as due to its very high mycorrhizal dependency. Linseed was an important alternative winter crop to wheat in the northern grain region when its oil was widely used in paint, and it is now grown as a minor crop for use in multi-grain breads. Other crops with very high mycorrhizal dependency grown in the northern grain region are the summer crops cotton (*Gossypim hirsutum* L.) (grown in rotations with grain crops), maize (*Zea mays* L.), pigeonpea (*Cajanus cajan* (L.) Millsp.) and lablab (*Lablab purpureus* L. (Sweet), and the winter crop faba bean (*Vicia faba* L.) (Thompson et al. 1997). Yet other crops with high mycorrhizal dependency grown in the northern grain region are the summer crops sunflower (*Helianthus annuus* L.), soybean (*Glycine max* (L.) Merr.), navybean (*Phaseolus vulgaris* L.), mungbean and sorghum, and the winter crop chickpea (*Cicer arietinum* L.). Sorghum and cotton are major economic crops for the region while mungbean and chickpea are the most important summer and winter grain legumes respectively (Webb et al. 1997; Unkovich et al. 2009). Other crops grown that are colonised by AMF but have low mycorrhizal dependency are the winter crops wheat, oats (*Avena sataiva* L.), triticale (x *Triticosecale* Wittm. ex A. Camus) and fieldpea (*Pisum sativum* L.), while barley (*Hordeum vulgare* L.) has very low dependency (Thompson et al. 1997). The winter oilseed crop canola (*Brassica napus* L.) is non-mycorrhizal. Long-fallow disorder has occurred in farmers' fields most frequently in crop species of very high and high mycorrhizal dependency, but also in wheat (Hart 1962; Owen et al. 2010). Instances of long-fallow disorder affecting a crop in a single field can remain undiagnosed (P. McIntosh pers. comm.), but are better recognised when paired comparsions are available (Thompson 1987), such as when growers reconfigure strip cropping for soil erosion control (Titmarsh and Stone 1997) and the one crop is sown across land of two or more durations of fallow.

Traditionally, fallows in the northern grain region were managed by burning the stubble soon after harvest, ploughing, and using secondary tillage after each fall of rain to control weeds and develop a fine seedbed for the next crop. Most growers deliberately kept one third of their land in long fallow to accumulate soil water as insurance against future crop failure (Hart 1962). Hart (1962) stated, 'Despite the advantages of additional moisture and nitrogen reserves, plant growth and grain yields from these long fallows have been inferior to those from the short fallow. Such results, though not general, are certainly not rare. In

fact, some farms are known where the long fallow consistently gives the lowest return.'

Because the normal state for most crop plants is mycorrhizal (Smith and Smith 2011b), it is important to manage cropping systems in order not to destroy AMF propagules for good early colonisation and vigorous crop growth. Thompson (1994b) listed agricultural practices that can reduce AMF inoculum potential such as plant-free fallows, cropping with non-hosts, tillage, waterlogging, topsoil removal, fires, and use of certain fungicides and fertilisers. Practices listed to maximise value from AMF were to sow crops of mycorrhizal dependency appropriate to the AMF inoculum potential of the soil, build AMF inoculum potential in rotations by growing appropriate host crops that are not also hosts of pathogens of major economic crops, and use P and Zn fertilisers judiciously (Thompson 1994b).

There has been increasing adoption of conservation tillage practices in the northern grain region, with stubbles retained instead of burnt and herbicide applications replacing tillage operations for weed control (Thomas et al. 2007). These conservation practices reduce soil erosion and increase soil moisture storage allowing better crop growth. A further advantage has been an increase in opportunities for sowing a crop at the correct time (Thomas et al. 2007) permitting increased cropping intensities. In 2005, about 50 % of the grain cropping area in the southern and central Queensland regions was managed by no-tillage with up to 85 % among some groups of growers (Thomas et al. 2007). Growers who have adopted no-till report reduction in the incidence of long-fallow disorder, probably through better retention of viable AMF inoculum between crops.

Soil disturbance, as caused by tillage, can disrupt AMF hyphal networks and reduce the AMF colonisation and P and Zn uptake of following maize plants (Evans and Miller 1988, 1989; Kabir et al. 1998). The AMF infectivity of vertisols from cotton fields in the northern grain region declined during 24-months laboratory storage and was reduced by severe disturbance (McGee et al. 1997). Infectivity did not decline in dry soil stored for 18 months, but did so with periodic wetting and drying (Pattinson and McGee 1997), indicating periodic rainfall during a long fallow would hasten the decline of viable AMF propagules. These laboratory results also suggest there might be little loss of AMF viability in a dry soil during prolonged drought. However, AMF in the dry topsoil of these dark vertisols under subtropical field conditions could suffer prolonged heating and loss of viability, and long fallow disorder can be prominent after prolonged drought in the northern grain region (A. Farquharson pers. comm.).

The adoption of no-tillage facilitates double-cropping to change from summer to winter crop sequences and vice versa when soil moisture permits thus avoiding long fallow. One favoured double-crop is chickpea following sorghum where no application of nitrogen fertiliser is required (Doughton et al. 1993). The highly mycorrhizal-dependent chickpea gains from early AMF colonisation by inoculum residual from the sorghum crop, quickly supplying P to the chickpea roots and nodules to drive N fixation (Munns and Mosse 1980, Abbott and Robson 1984).

One recommendation for growers to avoid long-fallow disorder is to avoid having long fallows where possible leading to 'opportunity cropping', i.e. sowing a winter or summer crop whenever rainfall has recharged the soil profile sufficiently. In other situations, use of quick-growing millet to break the long fallow has been advocated. Use of foxtail millet was shown to reduce symptoms of long-fallow disorder in a following linseed crop (Leslie and Whitehouse 1965). Growth of white French millet (*Panicum miliaceum* L.) as a cover crop in spring then removed before December was shown experimentally and by crop growth modelling of long-term weather data to have little effect on the store of soil water at the end of the fallow before wheat was sown (Whish et al. 2009). This practice also increased mycorrhizal colonisation and in some instances wheat yield (Seymour et al. 2006; PA Castor pers. comm.), similar to benefits found from cover cropping of increased mycorrhizal colonisation, P uptake and yield of sweet corn in Pennsylvania (Kabir and Koide 2002).

However, despite growers' plans to avoid long fallows, the variable climatic environment of the northern grain region can enforce long fallows through drought or floods that prevent sowing or destroy seedling crops, resulting in extended fallows and poor mycorrhizal colonisation of the next crop sown as described in the USA as post-flood syndrome (Wetterauer and Killorn 1996; Ellis 1998). Crops can also be destroyed at an early stage by hail similarly causing an extended fallow before the next crop is sown (G. McDouall pers. comm.). In parts of the

northern grain region, dryland summer crops may be grown on wide row spacings to extend the supply of soil water during crop growth. Long-fallow disorder has occurred in cotton when switching from a double-skip-row configuration (alternate 1 m and 3 m row spacings) back to uniform 1 m row spacings and the new rows on the former 3 m gap have been stunted from long-fallow disorder (G. McDouall pers.comm).

Long-fallow disorder occurs less frequently in wheat, the major winter crop of the northern grain region, than in crops of higher mycorrhizal dependency. In the authors' experience where long-fallow disorder has been noted in wheat, soil P levels were <15 mg P/kg soil (Colwell P), and/or Zn was low. A clear example of long-fallow disorder of wheat was in a crop rotation experiment (Owen et al. 2010) on a Darling Downs vertisol with 7.1 mg P/kg soil (Colwell P), 0.68 mg Zn/kg (DTPA-extractable) and pH8.8 (1:5 water). In this experiment, wheat produced less biomass and yield after a long fallow of 18 months or after a crop of canola, than it did after various mycorrhizal winter crops including wheat, durum (*Triticum turgidum* subsp. *durum* (Desf.) Husn.), barley, triticale, chickpea, faba bean and canaryseed (*Phalaris canariensis* L.). There was little effective rain from sowing to heading and the crop grew mainly on stored soil moisture, probably making the wheat more dependent on AMF for nutrient acquisition, biomass production and yield than under conditions of better in-crop rainfall where successive production of nodal roots would help the wheat with poorer AMF colonisation to more effectively access topsoil P. Thomas et al. (2007) indicated fallow dependency of winter crops to be 36 % to 82 % of transpiration water depending on location in the northern grain region.. Chenu et al. (2011) found from crop growth modelling, using >100 years of climatic data, that wheat suffers water deficits in mid to late growth stages (termed flowering stress) in 34 % of production environments, and in the grain filling stage (termed terminal stress) in 50 % of production environments in the northern grain region. Applying this model with parameters from the experiment of Owen et al. (2010), showed that the wheat that responded in biomass production and grain yield to AMF, underwent a drought stress similar to the flowering stress environment (K. Chenu pers. comm.) and therefore relevant to ~34 % of wheat production environments in the northern grain region.

The wheat in the experiment of Owen et al. (2010) was unaffected by differential populations of the root-lesion nematode (*Pratylenchus thornei* Sher and Allen), a major pathogen of wheat, but which requires adequate water films for movement, allowing the benefits of AMF to be exhibited in this dry year. *P. thornei* invades the cortex of wheat roots resulting in poor response to nutrients (N, P and Zn in Darling Downs vertisols) and nutrient deficiency symptoms in unfertilised wheat plants (Thompson et al. 2012). The detrimental effects of *P. thornei* in wheat have sometimes been confused with the detrimental effects of inadequate colonisation with AMF. For example, where the second wheat crop in sequence after long fallow from sorghum grew poorly and exhibited nutrient deficiency symptoms, growers considered this also to be an expression of long-fallow disorder which took one extra season to express in wheat than in other crops. However, this second-year wheat problem was really due to *P. thornei* building up on the first wheat crop to population densities sufficient to damage the second wheat (Thompson et al. 2012).

The responsiveness of crops to AMF in the northern grain region has been contrasted with lack of response of crops in experiments in the southern grain region of Australia (Ryan and Kirkegaard 2012). The southern grain region has winter dominant rainfall and no dryland summer cropping. The major crop is wheat with significant areas of canola used as a break crop for soil-borne diseases such as take-all (caused by the fungus (*Gaeumannomyces graminis* var *tritici* J. Walker) (Angus et al. 1994) and root-lesion nematode (*P. neglectus* (Rensch) Filipjev and Schuurmans) (Potter et al. 1998). Cropping in the southern grain region is based on a diversity of soil types with proportionally smaller areas of vertisols than in the northern grain region. Ryan et al. (2002) compared wheat crops grown after fallow, canola and wheat on grey vertisols at two locations in the southern grain region and found lesser AMF colonisation of the wheat crop after the canola and fallow than after a first wheat crop. The higher AMF colonisation following the wheat was not associated with better wheat growth, leading the authors to contrast the lack of response to AMF in the southern grain region to AMF responses in the northern grain region. While that may be the case, it should be noted that in the six experiments of Ryan et al. (2002) on two agricultural sites, the soil P levels (range 17–104 mg P/kg soil) and soil Zn levels (1.1–6 mg Zn/kg soil) were greater than those where a response in wheat to different AMF levels might be expected in the northern grain region. There were major

soil-borne wheat pathogens present at the sites including take-all, another root-rot fungus (*Rhizoctonia solani* J.G. Kuhn), cereal-cyst nematode (*Heterodera avenae* Wollenweber), and root-lesion nematodes (unidentified *Pratylenchus* spp.). Symptoms of these diseases were decreased and yield of the following wheat was increased by fallow or cropping with canola compared with wheat in the previous year. Thus Ryan et al. (2002) concluded that use of canola in cropping systems for control of root pathogens was more important for wheat production in the southern grain region than any potential loss from decreased AMF colonisation. Nonetheless, where population densities of both soil-borne pathogens and AMF vary in crop rotation experiments, inferences about the role of AMF can be confounded.

In the southern grain region, fertiliser P applications are greater than removal in grain resulting in accumulation of P in soil and concern how to reduce current fertiliser inputs and better utilise soil residues for economic reasons (Cornish 2009; Vu et al. 2011). There is a global concern that P fertilisers are a finite resource that should be used conservatively (Stewart et al. 2005), and that their over-use in high-input agriculture has contributed to eutrophication of water bodies (Foy 2005). In the northern grain region, fertilising with P below the removal rate (National Land and Water Resources Audit 2001; Lester et al. 2010) has probably been possible because of the efficiency of the mycorrhizal systems of many of the crop species grown in recovering P from soil and fertiliser sources. In this regard, soils like the one in this study with high levels of acid-extractable P have reserves of recalcitrant Ca phosphates that may be unavailable to crop roots but slowly available to AMF and an important source of P in the long-term (Wang et al. 2007; Lester et al. 2010). A number of authors have called for a greater understanding of the role that AMF can play in moving towards more sustainable agricultural systems with less reliance on high inputs of fertilisers, particularly P but also Zn (Plenchette et al. 2005; Cavagnaro 2008; Gianinazzi et al. 2010; Smith and Smith 2011b). Therefore, it is important that the benefits that cropping systems of the northern grain region receive from the ecosystem services provided by AMF be fully recognised and valued as suggested by Miller et al. (1994). Further research is required to quantify this value not only in terms of crop growth and yield but also in terms of the reduced amounts of P and Zn fertilisers required for profitable agricultural production (Abbott and Robson 1984).

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