Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops

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Summary
Arbuscular mycorrhizal fungi (AMF) are ubiquitous in agroecosystems and often stated to be critical for crop yield and agroecosystem sustainability. But, should farmers modify management to enhance abundance and diversity of AMF? We address this question with a focus on field experiments that manipulated colonisation by indigenous AMF and report crop yield, or investigated community structure and diversity of AMF. We find the literature presents an overly optimistic view of the importance of AMF in crop yield due, in part, to flawed methodology in field experiments. A small body of rigorous research only sometimes reports a positive impact of high colonisation on crop yield, even under phosphorus limitation. We suggest that studies vary due to the interaction of environment and genotype (crop and mycorrhizal fungal). We also find that the literature can be overly pessimistic about the impact of some common agricultural practices on mycorrhizal fungal communities and that interactions between AMF and soil microbes are complex and poorly understood. We provide a template for future field experiments and a list of research priorities, including phosphorus-efficient agroecosystems. However, we conclude that management of AMF by farmers will not be warranted until benefits are demonstrated at the field scale under prescribed agronomic management.

Key words: agronomy, crop sequences, experimental methodology, phosphorus-efficient agroecosystems, trade-balance model, soil microbes, yield
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I. Introduction
Arbuscular mycorrhizal fungi (AMF) provide benefits to host plants (Smith & Read 2008) and show functional diversity assumed to have implications for agroecosystem management (Verbruggen & Kiers, 2010). Their ability to enhance plant uptake of relatively immobile nutrients, particularly phosphorus (P), and several micronutrients, is their most-recognised benefit. However, AMF may also potentially improve soil health through their external hyphae that sustain the soil food web (Newsham et al., 1995), aid soil structure maintenance (Miller & Jastrow, 1990), and provide a large number of other benefits to host plants such as pathogen and herbivory protection, alleviation of water stress, enhanced tolerance of salinity, low pH and heavy metals, and biofortification of grain with micronutrients.

AMF have received increasing attention as part of a popular paradigm that considers an abundant, active and diverse community of AMF as essential for agroecosystem sustainability (see overview of this approach in Verbruggen et al., 2012). However, when we previously reviewed the literature on the role of AMF in commercial agroecosystems (Ryan & Graham, 2002) we concluded that AMF did not play a vital role in the nutrition and growth of crops. Here, 16 years later, we review the subsequent literature. To ensure relevance to commercial agroecosystems, we focus on field experiments that manipulate indigenous AMF and report crop yield; the outcome of most immediate relevance to farmers. We also explore the impact of variation in mycorrhizal fungal community structure and diversity in agroecosystems. We rarely refer to the field inoculation literature as it was recently reviewed (Hart et al., 2018) and inoculation is little used in commercial agroecosystems in spite of some reports of yield benefits (e.g. Pellegrino & Bedini, 2014). We primarily consider broadacre field crops in the high-yielding systems in Europe, North America, China and Australia. However, many of our examples and conclusions will be relevant to other systems. Many studies we review involve wheat (Triticum aestivum); this reflects its dominance in the field-based literature on AMF and it being the only crop subject to meta-analysis of field data (Pellegrino et al., 2015). Overall, we use this review to ask if there is sufficient evidence to recommend farmers consider impact on AMF when making management decisions and to suggest high priority areas for future research.
II. Investigating activity of AMF in agroecosystems

Quantifying abundance of AMF

Measuring the activity of AMF in agroecosystems, particularly impact on host plant yield, is complicated by the difficulties with producing non-colonised control plants. Comparisons are therefore mostly made amongst treatments differing in the abundance of AMF. This approach cannot quantify the overall contribution of AMF to crop yield, but it does allow us to ask whether farmers should consider AMF in their farm management. Abundance is most often quantified as percentage of root length colonised and this is the measurement most often used in meta-analyses of the impact of AMF on plant growth (e.g. Lekburg & Koide, 2005). However, differences among treatments or genotypes in percentage of root length colonised may not reflect provision of nutrients by AMF to the host plant (e.g. Kaeppler et al., 2000) due to variation in: root growth; the proportion of colonisation that consists of arbuscules; the density of colonisation across the root; the density of external hyphae, and; other variables that impact the activity of AMF such as microbial community. Other measures could better reflect mycorrhizal fungal activity including percentage of root length containing arbuscules, total length of colonised root and density of external hyphae (Kabir et al., 1998; Sawers et al., 2017). However, the studies we review generally report only percentage of root length colonised, except Ren et al. (2018) and Mai et al. (2018) who report only external hyphal density. It is important that future studies report both, if feasible. Note that measuring external hyphal density with confidence depends upon an ability to distinguish living hyphae of AMF from dead hyphae and hyphae of other soil fungi (e.g. Gavito et al. 2003) and that the time required for this work may be prohibitive. More knowledge is also likely required on the optimal sample size and sampling location (i.e., soil depth and placement relative to crop rows) for collecting external hyphae in the field.

Can results of glasshouse experiments be extrapolated to the field?

In comparison with field experiments, it is far simpler to measure activity of AMF in glasshouse experiments where non-colonised controls are easily established, total root length quantifiable and management costs low. Many useful insights can be gained from glasshouse experiments, particularly into the physiology of the symbiosis (Smith & Read, 2008) and many aspects of physiology may be impossible, with current experimental techniques, to investigate under field conditions. However, we believe that any conclusions about the benefits of AMF for crop growth and yield, that is, the mycorrhizal growth response (MGR), require verification under field conditions across a range of environments, as is routine in
other areas of agronomic research such as crop breeding (Dreccer et al., 2018) and crop rotation (Angus et al., 2015). Note that MGR is defined here as the difference in growth between colonised plants and non-colonised plants - or plants with high and low colonisation - at a defined level of resource supply to the plant (Janos, 2007; Johnson et al., 2015). The reasons why results from glasshouse experiments may not be applicable to the field involve: environmental extremes and variation being minimised or absent (e.g. no very cold night temperatures, no occasional periods of high or low water availability, homogenous sieved soil and absence of a soil profile); the impact of soil sterilisation treatments on soil properties and soil microbes; nutrients being manipulated to ensure only P is limiting plant growth; absence of sward conditions (Jeffery et al., 2017) or unrealistic plant densities; the impact of constraining roots within pots on rooting depth, root density and root system architecture; the frequent watering required for plants in pots, and; the impact of pots on soil temperature gradients (see Poorter et al., 2012). As we are interested in agronomic relevance, in this review we focus whenever possible on field experiments.

III. Crop benefit from AMF: agronomic and mycorrhizal literature differ

Mycorrhizal literature suggests a need to manage AMF to increase crop yield

A crop yield benefit from high colonisation by AMF is a core claim of researchers of AMF. Smith & Read (2008) state “there are many instances where crop productivity is influenced by AM symbiosis”, Martin-Robles et al. (2018) note the “global importance of AM in agriculture”, Bakhshandeh et al. (2017) say that AMF “may play a key role in crop rotation systems decreasing the dependency on fertilizers” and Srivastava et al. (2017) claim “AMF could reduce up to 50% usage of chemical fertilizers for optimal agriculture production”. An optimistic view is nearly universally maintained, even when Thirkell et al. (2017) note that colonisation does not necessarily translate to enhanced yield, their paper title asks “are mycorrhizal fungi our sustainable saviours?”

Crop agronomy literature suggests no need to manage AMF

The crop agronomy literature rarely is focussed on AMF. However, this large literature can provide insight into the need to consider AMF when making management decisions. For instance, both short (< 12 months) and long (> 12 months) bare plant-free fallows and non-mycorrhizal crops may often reduce the level of colonisation by AMF in following crops (Thompson, 1987; Lekburg & Koide, 2005; Bowles et al., 2016). Thus, if high colonisation provides a yield benefit, yield penalties should occur for crops following fallow or non-
mycorrhizal crops. This issue can be explored through the meta-analysis of Angus et al. (2015) which was based on > 900 comparisons from around the world and quantified the impact of non-cereal “break crops” on the yield of following wheat. They found no difference between the yield benefit from mycorrhizal legumes - such as field peas (Pisum sativum) and faba beans (Vicia faba) – and the yield benefit of non-mycorrhizal lupins (Lupinus angustifolius). There was also no difference between non-mycorrhizal brassicas - such as canola (Brasica napus) - and mycorrhizal flax (Linum usitatissimum). Angus et al. (2015) suggest that reduced colonisation following non-mycorrhizal break crops may actually benefit yield of following wheat. Similarly, in Western Australia, the results of 167 field experiments showed the highest yield benefit for wheat after a break crop was for non-mycorrhizal lupins (0.60 t ha⁻¹), followed by field pea, canola, oats (Avena sativa) and long bare fallow (0.30 t ha⁻¹) (Seymour et al., 2012). Thus, these two meta-analyses contrast with the mycorrhizal literature as they do not suggest that wheat routinely suffers a yield penalty if colonisation by AMF is reduced. However, crop rotation effects involve complex changes in many factors (soil nutrients, soil structure, root and shoot pathogens, soil microbes, etc.; Angus et al. 2015) and the majority of studies included in these two meta-analyses were likely of crops with no P limitation, and hence little chance of a large mycorrhizal benefit. We therefore present case studies of wheat, and other crops, where these other factors were carefully controlled (or at least considered) and where crop P status was known and, sometimes, deficient (Section V). However, we first consider whether the mycorrhizal literature projects a realistic expectation of mycorrhizal benefit.

IV. Flawed methodology leads to benefits of mycorrhizas being overstated

Meta-analyses

As meta-analyses identify broad trends they can strongly influence the literature, it is imperative that they be rigorously conducted and reviewed. We critique two meta-analyses. Lekberg & Koide (2005) compiled 290 field and glasshouse experiments that reported the impact of agricultural practices, including inoculation, on plant growth. They reported that increased colonisation increased average yields in the field by 23%. They stated that their aim was to determine the effects of various agricultural practices on colonisation and yield. Yet, only a small number of field studies were included (~24) and some of these were conducted in soils of little general agricultural relevance, for instance, reclaimed mined soils in Australia (Noyd et al., 1996) and eroded soil in China (Wu et al., 2002). Also, some studies involved
perennial plants and pre-inoculated transplants: treatments of little relevance to broadacre agriculture.

Pellegrino et al. (2015) found a “strong significant” relationship between the level of colonisation by AMF and wheat (*Triticum aestivum*) yields in the field and concluded that “a high mycorrhizal infection potential of agricultural soil should be ensured” through appropriate management. However, they considered only 16 studies where colonisation by indigenous AMF was manipulated. Four of these did not provide grain yields and four compared treatments that confounded the impact of changes in colonisation level with changes in other factors likely to greatly impact yield including P fertiliser addition and agroecosystems differing significantly in P availability (where increasing P is related to increasing yield and decreasing colonisation) (Ryan et al., 2004; Covacevich et al., 2007; Teng et al., 2013) and cultivar (Kirk et al., 2011). Of the remaining studies, one had treatments that did not differ in colonisation (Germida & Walley, 1996). The remaining seven studies involved a mixture of tillage and residue retention treatments (Duan et al., 2010) (no correlation between AMF and yield), four farming system treatments (Hildermann et al., 2010), crop rotation treatments (Karasawa & Takebe, 2012) (positive correlation), tillage and crop rotation treatments (Gao et al., 2010) (no correlation), and three crop rotation studies in southern Australia (Ryan et al., 2002; Ryan & Angus, 2003; Ryan et al., 2008) (negative or no correlation). We note several concerns with this meta-analysis including: the paucity of studies with valid methodology for determining the consequences of differing levels of colonisation by AMF for crop yield; the failure to question whether the co-occurrence of high colonisation and high yield always resulted from a strong empirical relationship, and; failure to acknowledge that a significant proportion of studies showed no, or a negative, relationship between colonisation level and yield.

**Field experiments**

Review papers and meta-analyses rely on the quality of source information. As noted by Thirkell et al. (2017) and Lekberg & Helgason (2018), data from field experimentation on AMF are notably lacking. Lekberg & Helgason (2018) attribute this to the difficulties with inducing variation in abundance of AMF, without changing other variables, and the logistics of field research. Furthermore, we suggest that field studies in agroecosystems have often lacked sufficient rigour for the co-occurrence of high colonisation and increased yields to be confidently assigned as causative. In part, this may reflect sampling of experiments not
designed to assess activity of AMF. However, it also reflects a lack of consideration for the many other factors that can vary among treatments in agronomic field experiments, especially crop sequence treatments.

These problems are illustrated by McGonigle et al. (2011) who concluded that “rotation of flax after canola should be avoided” based on poor early season flax growth after canola, compared with after wheat, and its association with a small decrease in colonisation (from ~45% to 35% of root length containing arbuscules) and reduced uptake of copper, P and zinc (Zn) (Manitoba, Canada). They did not report yield. They also did not consider other factors that could negatively affect flax growth following canola including nutrient removal by canola from the nutrient-poor soil. Canola is nutrient-rich, and may have higher nutrient removal in grain than wheat, even when yields are lower (Ryan & Kirkegaard, 2012).

Similar problems are highlighted by Bakhshandeh et al. (2017) who sampled wheat following chickpea (Cicer arietinum) and canola (northern New South Wales – NSW, Australia). Soil bicarbonate-extractable P was variable, but no lower than 28 mg kg$^{-1}$; addition of P fertilizer did not increase yield. Bakhshandeh et al. (2017) state an expectation of finding colonisation by AMF positively related to wheat grain yield, but do not consider or cite any of the relevant local literature (e.g. Ryan & Angus 2003 or Owen et al., 2010). They report high wheat yield following chickpea and ascribe it to higher colonisation based on a weak positive correlation between grain yield and the percentage of root length containing arbuscules ($R^2$=0.18-0.20). However, the study did not report the standard set of parameters that agronomists use to determine the key variables influencing crop yield in rotation experiments. For instance, the most likely reason for a yield benefit when wheat follows chickpea rather than canola is greater soil mineral nitrogen (N) and water in the soil profile (Felton et al., 1997) due to biological N-fixation by chickpea and its shallower roots and a shorter growing season. Bakhshandeh et al. (2017) only report soil mineral N ~3 months after sowing the wheat and did not identify that the extremely low values (< 4.2 mg kg$^{-1}$, KCl-extracted) indicated most mineral N had been taken up by the wheat and hence differences at sowing had been translated into crop biomass and N content (not reported). They did not report soil water or consider a major crop pathogen in the region, crown rot (Fusarium pseudogrminearum) (Felton et al., 1997; Kirkegaard et al., 2004); greater Zn removal by the canola was also not considered. As Bakhshandeh et al. (2017) did not measure key explanatory variables or place their research in the context of the local literature, the most plausible explanations for
enhanced yield of wheat following chickpea were not explored and it is likely that their conclusions about the role of AMF in crop yields are incorrect.

A need to critically examine co-occurrence of low colonisation and poor growth/yield

When high colonisation by AMF coincides with high yield, it is generally assumed that the high colonisation was necessary for the high yield. However, there are examples where this has been shown to not be the case. For instance, a cotton (Gossypium hirsutum) growth disorder was characterised by stunted growth, low plant P content, symptoms of Zn deficiency and low colonisation by AMF (Nehl et al., 1996) (northern NSW). However, the disorder was also associated with root browning, and bioassays showed low levels of mycorrhizal fungal inoculum were not the cause of the low colonisation (Nehl et al., 1998). Thus, low colonisation was a consequence of the growth disorder: the cause was not determined. Similarly, on a soil with “optimum” availability of P, maize (Zea mays) growth was up to 3-fold greater following soybean (Glycine max) than following canola while colonisation by AMF was around 65% of root length following canola and 85% following soybean (Koide & Peoples, 2012) (Pennsylvania USA). However, the higher yields following soybean were not ascribed to the higher colonisation due to no evidence of yield being driven by crop P or N nutrition. For instance, for a given colonisation level, maize growth following soybean varied nearly 4-fold suggesting other patchy growth-limiting factors. These could not be identified, but may have included allelopathic effects from unusually abundant canola residues due to pod shattering.

In summary, we suggest that some researchers have not been sufficiently critical in interpreting field experiment results and this, along with failure to apply agronomic context and methodology, has often resulted in overstating of the importance of high colonisation by AMF for crop yield.

V. Rigorous methodology suggests low colonisation by AMF can sometimes reduce crop yield

Grant et al. (2009) examined the impact of AMF on flax in 2-year cropping sequences, established in three consecutive years, at two sites with low soil P (< 13 mg kg\(^{-1}\) bicarbonate extractable P) (Manitoba, Canada). The sequences had canola and wheat in the first year and flax in the second (and also included P fertiliser and tillage treatments). While P fertiliser had little impact on flax growth or yield, flax establishment, early season growth, and yield were
all greater after wheat; by an average of 14% for yield. Colonisation by AMF, five weeks after seeding, was greater following wheat by an average of 3.5% points, but this would have been magnified in total colonised root length as flax root biomass to 30 cm soil depth was at least 2-fold greater following wheat (Monreal et al., 2011). Grant et al. (2009) suggest lower flax yield following canola could have resulted from decreased colonisation, allelopathy from residues or early season competition from canola volunteers. Overall, in spite of this being an extensive study with a comprehensive sampling regime, the role of AMF in crop yield remains unclear. The absence of a P fertiliser response suggests P uptake by AMF was not the mechanism of any mycorrhizal benefit. The reason for the large flax root systems following wheat is unknown and alone could account for increased yield through greater soil exploration.

An agronomic study specifically designed to investigate the role of AMF in wheat growth was undertaken on a low P soil (11 mg kg\(^{-1}\) bicarbonate – Colwell – extractable P) by Ryan & Angus (2003) (southern NSW). Five first year treatments manipulated the level of mycorrhizal fungal inoculum (Figure 1a). In the second year, wheat, field peas and non-mycorrhizal canola were grown with and without P fertiliser (Figure 1b): canola was a control to identify crop sequence effects not due to AMF and field peas were assumed to have high MGR as reported for other grain legumes (e.g. Pellegrino & Bedini 2014). The design was intended to maximise the chances of showing a positive impact of high colonisation by AMF on crop yield, but none was evident even under P limitation (Figure 1c). Other studies in this region are consistent with this result (Kirkegaard & Ryan 2014) including studies which show a positive impact of canola on following wheat (Kirkegaard et al., 1997). Interestingly, roots at the site shown in Figure 1a were colonised by both AMF and the arbuscule-forming symbiont ‘fine root endophyte’ (Ryan & Kirkegaard, 2012).

In contrast to the findings of Ryan & Angus (2003), a field experiment on a very low P soil (7.1 mg kg\(^{-1}\) bicarbonate extractable P) by Owen et al. (2010) found a positive impact of high colonisation on crop growth (southern Queensland, Australia). Wheat was sown following long bare fallow and canola, as well as a range of mycorrhizal crops. There was no impact of first year treatment on soil water, soil nitrate-N, P, or Zn (0-30 cm) or the pathogen Pratylenchus thornei. Colonisation was higher following mycorrhizal crops (up to 80% root length) than following fallow and most canola varieties (15–39%). Low colonisation was associated with shoot biomass after ear emergence being 2–3-fold lower and consequently
there was a strong positive linear correlation between biomass and colonisation ($R^2=0.75$, $n=15$). Colonisation also correlated strongly with heads per $m^2$ ($R^2=0.71$), but less strongly with yield ($R^2=0.40$), likely due to water limiting yield. These findings are consistent with reports in this region of crops following long bare fallows sometimes showing poor growth and symptoms of P and Zn deficiency, as well as low colonisation by AMF (‘long fallow disorder’) (Thompson, 1987).

The above case studies demonstrate the challenges in agronomic experiments with determining the factors responsible for yield variation among treatments and with apportioning any impact to the level of colonisation by AMF. Application of standard agronomic methodology would ensure high-quality results, allow rigorous comparison among studies and, ultimately, enable identification of the factors that cause the impact of colonisation level on yield to vary among studies. We therefore provide a template for this purpose (Table 1).

VI. Predicting when mycorhizas matter for crop yield

Mycorrhizal literature: trade-balance model

In the mycorhizal literature, based on studies largely undertaken in the glasshouse, it has been proposed that the conditions under which AMF overcome resource limitations may be key to understanding variations in MGR (Hoeksema et al., 2010; Johnson et al., 2015). In this context, there may be a need to consider nutrients other than P as while enhanced P uptake is the most reported plant nutritional benefit from AMF, uptake of other nutrients may also be aided especially under varying field conditions (Lekberg & Koide, 2014; van der Heijden et al., 2008). For instance, N uptake through external hyphae can sometimes be significant (Johansen et al., 1992; Hodge & Fitter, 2010; Fellbaum et al., 2012); although whether it can be sufficient to be of agronomic relevance remains to be proven. In addition, the availability of plant carbon (C) to support the symbiosis and the strength of the fungal C drain will also impact MGR (Graham and Abbott 2000). Thus, the relative supply of P, N, other nutrients (perhaps), rates of C supply from photosynthesis, and diversion of C to AMF, and factors that affect crop growth rate such as the levels of light and water availability likely together determine MGR and, thereby, result in a range of “mycorrhizal phenotypes” from mutualism to commensalism and parasitism (Johnson et al., 1997, 2015; Johnson & Graham, 2013). This concept has been termed the “trade-balance model” (Johnson et al., 2010).
Johnson et al. (2010) identified the most significant benefits for MGR under P limitation with N and light not limiting. However, competition for N between AMF and host plants may also be important (see Hodge & Fitter, 2010; Hodge & Storer, 2015; Johnson et al., 2015). For instance, Thirkell et al. (2016) demonstrated that organic N could elicit “strong mutualism” whereby both plants and AMF benefit from the addition of N in a P-limited system. Similarly, Püschel et al. (2016) tested the impact of inoculation with an indigenous mycorrhizal fungal community and a laboratory culture of *Rhizophagus irregularis* on growth, C allocation and nutrition of *Andropogon gerardii* under varying N and P supply. The plants competed with AMF for N when supply was low, resulting in no or a negative MGR and N-uptake response. The mycorrhizal fungal community inoculant only increased plant P nutrition under N fertilisation, whereas *R. irregularis* slightly increased P uptake without N supply. Both inoculants consistently increased nutritional and growth benefits to the host with increasing N supply coincident with increasing root colonisation.

The trade-balance model is based on research under glasshouse conditions and has not been agronomically tested; glasshouse experiments may not well mimic the resource availability under field conditions for the reasons discussed in Section II. Applying the trade-balance model to field crops will be complicated by temporal change as, for instance, warmer drier weather dries the topsoil and reduces P availability or greater early crop growth due to high colonisation reduces soil water availability later in the season and, if N is abundant, induces “haying-off” (van Herwaarden et al., 1998; Kirkegaard & Ryan, 2014).

**Agronomy literature: crop growth models**

Crop growth models are becoming increasingly sophisticated to include crop genotype (G) and crop management (M) (e.g. stubble retention, sowing date, plant density) in addition to soil and climate information (E, environment) (Kirkegaard & Hunt, 2010; Zhang et al., 2012; Teixeira et al., 2017). Whilst initially developed in countries with high input agricultural systems, such as Australia and the USA, these models are now being used in many countries and agricultural systems (e.g., Seyoum et al., 2018). A better understanding of the mechanisms underlying the relationship between level of colonisation by AMF and crop yield could enable colonisation level, or other measures of mycorrhizal activity to be included in crop growth models using the G × E × M framework. Prediction of the impact of colonisation level on crop yield could then be made without large, logistically-challenging, expensive, field experiments.
A G × E × M approach was used by Ryan & Kirkegaard (2012) to investigate why wheat and other crops have been reported to suffer a yield penalty when colonisation by AMF is low, as may occur following long bare fallow or non-mycorrhizal crops, in the subtropical northern wheatbelt of Australia (Thompson, 1987; Owen et al., 2010), but not in the southern temperate wheatbelt (Figure 1; Ryan and Kirkegaard, 2012 and references therein). Differences in soil type (E) and P availability (E) were discounted due to southern wheatbelt experiments on vertisols typical of the northern wheatbelt yielding no relationship between colonisation and yield for wheat and flax even under P limitation (Ryan & Kirkegaard, 2012). However, they used the crop growth model APSIM to demonstrate that autumn-sown crops in the southern wheatbelt have an initial slow growth rate due to winter conditions (E), and hence low P demand and, perhaps, little need for AMF to supply P. They also speculated that low winter soil temperatures (< 10°C at 0–10 cm depth) could reduce the spread of, and P flow through, the external hyphae of AMF (Cooper & Tinker, 1981; Gavito et al., 2003). For instance, when Gavito et al. (2003) grew field pea in growth chambers with soil maintained at either 10°C or 15°C, they found no development of external hyphae at 10°C, in spite of an average of 40% of root length being colonised, while external hyphae were present at 15°C (71% of root length colonised). Interestingly, root mass ratio was 31% lower at 15°C than at 10°C, suggesting greater importance for roots in soil exploration for P at 10°C. Hence, Ryan and Kirkegaard (2012) hypothesised that E may dictate crop response to variation in the colonisation level of AMF both directly (crop P demand) and indirectly (mycorrhizal fungal activity). Ryan & Kirkegaard (2012) also suggested that genotype (crop and mycorrhizal fungal community) could contribute to a higher MGR in the northern wheatbelt.

VII. Crop genotype
Which crop genotypes benefit most from AMF?
Crop genotype is undoubtedly important for understanding variation in MGR (Francis & Read, 1995; Graham et al., 1991; Hetrick et al., 1993; Pringle & Bever, 2008). Plants with root systems poorly able to explore soil for nutrients have traditionally been considered to likely have a high MGR with short root hair length proposed as a key trait. For instance, in a glasshouse experiment, Schweiger et al. (1995) found annual pasture species with shorter, less frequent root hairs responded most to inoculation (Figure 2) and Jakobsen et al. (2005) found AMF substituted for lack of root hairs on mutant barley (Hordeum vulgare). However, a meta-analysis by Maherali (2014) detected no impact on MGR of root system architecture,
including root hair length and density, and Martín-Robles et al. (2018) found no relationship between MGR and fine root traits. Moreover, the meta-analysis of Hoeksema et al. (2010) of 306 greenhouse and growth chamber studies found that plant functional group, rather than root morphology, determined MGR, with C4 grasses more positively responsive to inoculation than C3 grasses; a finding consistent with a high P demand by C4 grasses due to high growth rates with high temperatures and irradiance (Ludlow, 1985).

We suggest that the outcomes of Maherali (2014) and Hoeksema et al. (2010) may be problematic as their data likely originated from studies that included only two levels of P. As shown in Figure 2, MGR can vary greatly among P levels, and in a manner that differs among genotypes, due to different shaped P response curves for colonised and non-colonised plants (Schweiger et al. 1995). Differences among studies in resource availability and growth conditions (section VI), and mycorrhizal fungal and soil microbial communities (sections VIII, IX) also likely obscure the role of root morphology. In addition, the mechanisms of root morphological adaptation to low P, which may influence MGR, may be variable and complex. For instance, subterranean clover cultivars differ in external critical P requirement (i.e. the P level sufficient to achieve 90% of maximum yield) due to variation in ability to acclimate to low P conditions through maintenance of dry matter allocation to roots in soil layers relatively high in P (e.g. topsoil) and high specific root lengths which together confer a high root length density (Haling et al., 2018). To improve our ability to predict the MGR of crop genotypes we suggest that investigations of MGR should generate a P response curve to allow external critical P requirements of non-colonised and colonised plants to be compared (Schweiger et al., 1995; Kelly et al., 2001; Figure 2): a thorough assessment of root morphology traits and their response to low P conditions should also occur. Field experiments are required.

Should crop breeders focus on crop MGR or crop phosphorus-use efficiency?

The potential of crop domestication to reduce MGR is often raised as a concern (e.g. Martín-Robles et al., 2018). At the same time, improvement of P-acquisition efficiency and P-utilization efficiency, i.e., P-use efficiency (PUE), is increasingly being suggested as a goal for crop breeders (Lynch, 2011; Leiser et al., 2016; Mart et al., in press). So, should crop breeders be considering MGR and PUE simultaneously?
Lehmann et al. (2012) conducted a meta-analysis of 39 publications on 320 crop plant genotypes and the importance of year of release on MGR, colonisation and PUE. Cultivars released after 1950 were less colonised, but more responsive, than ancestral genotypes; although, recently, Martín-Robles et al. (2018) found that domesticated crops only benefited from AMF under P-limitation. Lehmann et al. (2012) found colonisation was a significant explanatory variable for the MGR trend with year of cultivar release, but PUE was not. This suggests that breeding for optimised plant–mycorrhizal interactions may be as or more important than selecting for PUE. Kaeppler et al. (2000) discussed variation in MGR attributable to differences in plant–mycorrhizal interaction versus that attributable to the host genotype PUE at a given soil P supply. They suggested, based on their glasshouse experiment with in-bred lines of maize, that if the genetic potential to accumulate biomass at low P in the absence of AMF is of greater consequence in determining MGR than genetic potential to benefit from the symbiosis, then selection for PUE would become the more important crop improvement target. Mycorrhizal interaction with host genotype and PUE was also studied by Sawers et al. (2017) who evaluated the relative growth of 30 highly diverse maize lines with and without inoculation and found that host genetic factors influenced fungal growth strategy with P uptake by the most responsive line linked to higher abundance of external hyphae.

Overall, it seems that the interactions of host genotype, MGR and PUE are complex. Little data are available from field studies and MGR data in the literature most often come from studies with only two levels of P application. Moreover, whilst the level of colonisation by AMF may vary greatly among crop genotypes (e.g. Leiser et al., 2016; Ryan et al., 2016), breeding for high colonisation may be difficult due to low heritability (Leiser et al., 2016). Furthermore, breeding for improved root soil exploration for P could result in selection for a lower MGR and such plants may limit colonisation (Graham et al., 1991). In view of the above, and the uncertainties over the importance of AMF for crop yield in the field (Section VI), it currently is not possible to advise crop breeders to consider AMF when selecting for PUE.

VIII. Fungal genotype

It is frequently stated that the mycorrhizal fungal community in agroecosystems functions sub-optimally such that higher applications of P fertiliser and other inputs are required (Srivastava et al., 2017). This view is based on the idea that industrial agricultural practices select for genotypes less beneficial for crop hosts, for example, AMF with increased host C
cost because they rapidly colonise and sporulate. Verbruggen & Kiers (2010) suggest that agricultural practices disfavour development of a more complex and functional (i.e. more beneficial) mycorrhizal fungal community. Selection pressures most often cited and researched that may reduce mycorrhizal fungal diversity and functionality in agroecosystems are (i) tillage, (ii) high fertiliser input and (iii) crop rotations with non-hosts or low plant diversity. Below, we consider whether these practices consistently reduce diversity and functionality of the mycorrhizal fungal community, as well as whether higher diversity is desirable and whether diversity can be usefully manipulated with inoculants.

Tillage alters the mycorrhizal fungal community, but may not reduce abundance and diversity of AMF

Shifts in mycorrhizal fungal community composition and genetic diversity have repeatedly been documented when high-intensity tillage (e.g. mouldboard or chisel-disk ploughing) and low-intensity tillage systems are compared (Börstler et al., 2010; Miller et al., 1995; Boddington & Dodd, 2000; Jansa et al., 2002, 2003; Alguacil et al., 2008; Verbruggen et al., 2010). In a recent meta-analysis of 54 field studies, Bowles et al. (2016) reveal a large (~30%) increase in colonisation level, but only a slight (11%) increase in species richness of AMF in response to less intensive tillage. However, some studies find no impact of tillage intensity on mycorrhizal fungal abundance and diversity. For instance, in Switzerland, Jansa et al. (2002, 2003) found an increased incidence of certain AMF following long-term (13 years) tillage treatments of differing intensity (conventional, chisel plough, no-tillage). However, while the mycorrhizal fungal community structure was greatly affected by tillage, no difference in diversity was detected among tillage treatments.

A shift in the community structure of AMF under less intensive tillage may, however, affect crop growth. For instance, Köhl et al. (2014) used microcosms to study whether tillage-induced changes in the mycorrhizal fungal community altered plant productivity and nutrient uptake. They found no-tillage communities increased P supply, but not plant productivity, compared with conventional tillage communities, likely due to a two-fold increase in length of external hyphae. Similarly, Miller et al. (1995) demonstrated, from multiple field studies of maize evaluating effects of tillage on colonisation and external hyphal development in the rhizosphere, that P uptake, not yield, is greater with no-tillage.
The decline in mycorrhizal fungal abundance with high intensity tillage may be relatively short-term. Rasmann et al. (2009) evaluated the impact of 3-4 years of management system (bahiagrass [Paspalum notatum] pasture, weed fallow, organic, disk fallow, and conventional) on subsequent tomato (Solanum lycopersicum) cropping in a long-term commercial tomato field (Florida, USA). Although colonisation of tomato was dramatically decreased in soil actively undergoing intensive tillage, there was no long-term suppressive effect on mycorrhizal fungal infectivity. Indeed in the disk fallow treatment following 14 years of intensive agricultural management - including four consecutive years of continuous soil disturbance - inoculum density and infectivity of AMF was not significantly impacted compared to the other farming practices investigated after tomato roots were present for a single growing season. Thus, it seems the mycorrhizal fungal community may rapidly recover from periods of intense disturbance and the absence of host tissue.

In a more extreme comparison, Lekberg et al. (2012) contrasted non-disturbed grassland soil with intensely disturbed soil, finding 32 operational taxonomic units (OTUs) distributed across five families of AMF (Zeeland, Denmark). Soil disturbance did not significantly alter community composition and OTU-richness. OTUs from undisturbed soil were also common after severe disturbance. Approximately 40% of all sequences within a sample were a single OTU. They concluded that the assembly and abundance of the grassland mycorrhizal fungal community indicated a high level of resilience and disturbance tolerance.

Fertilisation selects for tolerant AMF, but are they less functional? Studies of long-term fertiliser trials report N fertiliser alters diversity of AMF, depending on their sensitivity (Oehl et al., 2004), and may stimulate colonisation by certain species (Toljander et al., 2008). Most commonly cited is the apparent tolerance of R. irregularis to nutrient enrichment, as its abundance increased following eight years of N and P additions compared to non-fertilised controls (Johnson, 1993). In maize fields in Central Italy, Boriello et al. (2012) found that Glomeraceae were the main colonisers in fertilised plots, but they co-occurred with Gigasporaceae and Paraglomus regardless of the management practices applied. Members of Diversisporaceae and Entrophosporaceae were detected in N fertilised and the untreated plots, respectively. In general, mycorrhizal fungal communities were primarily influenced by N fertilisation and, to a lesser extent, by tillage. However, the relationship between AMF selected under high fertility and their symbiotic functioning has
not been adequately evaluated; thus it remains unconfirmed if this vital agronomic practice selects for a less functional mycorrhizal fungal community.

Crop rotation and other crop management practices promote diversity of AMF

Crop rotation has been demonstrated to promote mycorrhizal fungal communities (Oehl et al., 2004; Hijri et al., 2006) that may closely resemble those from natural sites (Verbruggen et al., 2010). Oehl et al. (2010) found that crop rotation, including a perennial grass–clover mixture, increased diversity of AMF compared to continuous monoculture, and promoted even more diversity than found in natural grasslands. Moreover, species of AMF not recovered from continuous monoculture fields were detected after eight months in microcosms constituted from these fields. Apparently, slower-sporulating genotypes increased in abundance with host plants when propagated longer than an annual crop season. Somewhat analogous to Oehl et al. (2010), Jansa et al. (2002, 2003) used trap cultures to show that mycorrhizal fungal community composition is affected more by the species of trap plant than by the tillage treatment of the field soil. Thus, agroecosystems with a diversity of host species and host functional groups (annual/perennial, legume/grass etc) may promote diversity of AMF.

Do more abundant or diverse communities of AMF promote agroecosystem productivity and sustainability?

Hoeksema et al. (2010) found that MGR was lower when plants were inoculated with one species of AMF, compared to multiple species. However, colonisation by multiple species can result in competitive, synergistic or antagonist interactions (Maherali & Klironomos, 2007) depending on the species of AMF, time of harvest, and environmental conditions (Daft & Hogarth, 1983; Alkan et al., 2006; Jansa et al., 2008). For instance, Argüello et al. (2016) found that diversity of AMF promotes cooperation between plants and AMF. However, promoting diversity and inoculum density of AMF may trade-off their ability to stimulate plant productivity in some instances. Janoušková et al. (2013) tested in potted field soil the effects of isolates of *Claroideoglomus claroideum*, *Glomus intraradices* and *G. mosseae*, each inoculated into a background synthetic mycorrhizal fungal community of the three isolates, on colonisation and plant growth. They found a transient positive response to an increase in root colonisation for the least competitive isolate. The two other more competitive isolates responded negatively to intra- and interspecific inoculations, and in some cases, reduced plant growth. Similarly, Thonar et al. (2014) found trade-offs that depended on the
species of AMF when they co-inoculated barrel medic (*Medicago truncatula*) with *Claroideoglomus* and *Rhizophagus*. Even though the species competed for root colonisation sites, co-inoculated plants were larger and higher in P than plants inoculated with each species alone. In contrast, inoculation with *Gigaspora* and *Rhizophagus*, which facilitated each other’s root colonisation, produced plants that were smaller than those with each species inoculated separately. In the field, prior root occupancy may also impact the assembly and functionality of AMF in soil microbial communities (Dumbrell *et al.*, 2010).

For natural mycorrhizal fungal communities, high diversity is not always linked to greater productivity. Verbruggen *et al.* (2012) inoculated maize with soil and roots from three organic and three conventional maize fields and measured productivity, and nutrient loss during leaching events induced by simulated rain (Netherlands). Maize growth responded negatively to inoculation, especially for inoculum from organic fields which harboured the greater diversity of AMF (Verbruggen *et al.*, 2010). Productivity was inversely correlated with colonisation level, suggesting C allocation to AMF was responsible for plant growth reduction. Soil inoculation did reduce P leaching after simulated rain in response to increased hyphal density related to the abundance of particular mycorrhizal fungal types. These results demonstrate the potential for AMF to provide agroecosystem benefits other than crop yield, but that they may not be optimised together.

Thus it seems there are complex interactions in diverse mycorrhizal fungal communities which may induce significant changes in plant host growth and/or P acquisition and, therefore, increasing diversity is no guarantee of increased MGR and crop productivity.

**Augmentation with indigenous AMF may benefit crops**

The view that low diversity of AMF causes a lack of mycorrhizal benefit in agroecosystems has driven the development of commercial inoculants (Vosátka *et al.*, 2012). The performance of commercial products has been variable (e.g., Lojan *et al.*, 2017) and they are thus little used (Tarbell & Koske, 2007; Faye *et al.*, 2013). More broadly, although inoculation has the potential to increase growth and P uptake, particularly under low soil P availability (e.g. Pellegrino & Bedini, 2014), numerous studies report little or no positive, and even negative MGRs (Klironomos, 2003; Smith & Smith, 2011; Tawaraya, 2003).

Use of indigenous inoculant would mitigate bulk and expense, and avoid questions about the desirability of exotic inoculants (Hart *et al.*, 2018). Several field studies demonstrate that
indigenous AMF, produced on-farm with mycotrophic hosts, may be an economical and sustainable practice. In Italy, Pellegrino et al. (2011) assessed shoot dry weight and nutrient uptake of berseem clover (*Trifolium alexandrinum*) inoculated with four isolates of AMF either alone or mixed, and compared to indigenous inoculum. Inoculation increased productivity of berseem clover and a following maize crop, with indigenous inoculum as, or more, effective than the introduced AMF. However, a risk with on-farm inoculum production is propagation of soil-borne pathogens and parasitic nematodes, so measures should be taken to ensure quality control of inoculum quality with soil bioassays using the target crop as the host.

Use of an indigenous soil community of AMF may not always enhance plant growth when the plant has not previously been grown in the soil. For instance, in a glasshouse experiment, Řezáčová et al. (2017) tested the effect of soil microbial inocula with and without indigenous mycorrhizal fungal communities, with and without P addition, on plant biomass and C fluxes from plant to soil of a C₃ and C₄ Panicum grass (not native to the field soil used). When the inoculum contained AMF, all plants had lower P and N status, and less biomass, due to greater allocation of C belowground. Řezáčová et al. (2017) interpreted this negative impact from AMF as perhaps being due to symbiotic incompatibility due to the fungal and plant partners being of different geographic origins: see also Hart & Reader (2002) and Maherali & Klironomos (2007). Řezáčová et al. (2017) also recognised that the negative impacts could have resulted from slow development of colonisation or differences in microbial communities between inoculants. However, there are also reports of plant growth enhancement when a non-indigenous inoculant is added to soil containing indigenous AMF. For instance, Köhl et al. (2016) inoculated microcosms of a grass–clover mixture (*Lolium multiflorum* and *Trifolium pratense*) grown in a glasshouse with non-indigenous *Rhizoglomus irregulare*. The microcosms contained eight nonsterilised field soils varying in soil type, chemical characteristics and indigenous mycorrhizal fungal community. Inoculation increased the abundance of *R. irregulare* in all soils, irrespective of soil P availability (which varied from 53–210 mg kg⁻¹, ammonium acetate EDTA extraction), the initial abundance of *R. irregulare* and the abundance of indigenous mycorrhizal fungal communities. Inoculation with AMF did not affect the grass but significantly enhanced clover yield in five of eight field soils. The results demonstrate that inoculation can be successful, even when the inoculant is not indigenous, soil P is high, and indigenous communities are abundant. However, Köhl et al.
(2016) noted that the amount of inoculum applied was large (corresponding to $1.4 \times 10^5$ L ha$^{-1}$), expensive and possibly commercially unrealistic.

IX. Complex interactions between the mycorrhizal fungal and soil microbial communities

In their meta-analysis, Hoeksema et al. (2010) reported plant response to inoculation with AMF was greater with the use of whole soil inoculum presumed to contain multiple species of AMF as well as other soil microbes, or if other soil microbes were added back to the background growing media. These findings suggest AMF are more beneficial when a diverse soil microbial and mycorrhizal fungal community is also present, that is, in a realistic biotic context.

Pizano et al. (2017) examined, in the glasshouse, the effects of AMF and soil filtrates on the growth of 11 host species from three habitats in a tropical montane landscape in Colombia using reciprocal habitat inoculation. Most plant species received a similar benefit from AMF but differed in their response to soil filtrates from the three habitats. Soil filtrate from pastures had a net negative effect, while filtrates from coffee (Coffea arabica) plantations and forests had a positive effect, on plant growth. Pasture grass, coffee, and five pioneer tree species performed better with the filtrate from soils in which they rarely occurred, while four shade-tolerant tree species grew similarly with all filtrates. These results suggest that some habitats accumulate species-specific soil pathogens, while others accumulate soil microbes that benefit indigenous plants and crops. Moreover, non-mycorrhizal soil microbes acting as mediators of plant–soil feedbacks may exert even greater habitat and host-specific effects on plants than AMF (Bever et al., 1997).

In summary, interactions among species of AMF in diverse communities and the soil microbiota are complex and poorly understood. Little research has been conducted under field conditions. Additional complexity is added by the recent discovery that fine root endophyte, which can be abundant in agroecosystems and co-inhabit roots with AMF, is likely not phylogenetically aligned with the AMF (Orchard et al., 2017a, b).

X. Phosphorus-efficient agroecosystems

We propose that higher abundance and diversity of AMF may be best promoted not by the methodologically-challenging task of quantifying their role in crop yields, but as a secondary consequence of the development of P-efficient agroecosystems. Such agroecosystems are
desirable due to rock phosphate being a non-renewable resource (Cordell & White, 2015) and the environmental damage caused worldwide by movement of P originating from excessive application of P fertilisers off farm into waterways and estuaries (Sharpley et al., 2015).

It has been recently proposed that, for pastures, reducing the critical external P requirement of the least P-efficient species through its replacement with a species better able to explore soil for P allows the target soil extractable P level to be lowered without yield penalty (Simpson et al., 2014, 2015; Haling et al., 2016). This then confers savings in P fertiliser use due to less P leaching (see Khan et al., 2018) and, in pasture soils, less conversion of P into poorly plant-available organic forms (Simpson et al., 2015). The MGR of a pasture or crop species with P as the primary limitation is also reflected in a decrease in external critical P requirement due to greater plant growth at moderate to low P availability when colonised (Figure 2). Hence, high colonisation could aid development of P-efficient agroecosystems if the fungi enhance crop P uptake.

Even if AMF are not required for optimal crop P nutrition, if the pasture or crop external critical P requirement comes close to coinciding with maximum colonisation and/or length of external hyphae, then yield and any benefits of AMF for soil health might be desirably balanced. This concept was explored by Mai et al. (2018) in a field experiment where cotton was grown at four P application rates over two years (Xinjiang region, northwest China). A moderate P rate favoured density of external hyphae, soil exploration by roots and efficient uptake of fertiliser P, and corresponded to around 90% of maximum yield. The authors therefore recommended that the target bicarbonate-extractable soil P could be halved from the 30 mg kg\(^{-1}\) currently recommended to 15-20 mg kg\(^{-1}\). For more diverse agroecosystems, with crop rotation, intercropping or mixed-species pastures, identifying the target soil P level will be more complicated. For instance, when Deng et al. (2017) grew a wheat-maize rotation over two years at six P rates (Hebei Province, China), yield and colonisation by AMF of wheat, but not maize, responded strongly to P addition (Figure 3). However, at the target bicarbonate-extractable soil P concentration, determined by the authors to be 22 mg P kg\(^{-1}\) (Olsen), yields and colonisation levels over the rotation were reasonable well balanced. Note that for both these studies, the crop MGR and hence the yield impact of reduced colonisation at a given P application rate due to, for instance, a preceding non-mycorrhizal crop or intense tillage, is unknown.
XI. Conclusions

Our review shows the yield benefits of maintaining high abundance and diversity of AMF in agroecosystems are often overstated due to an underlying optimism about the need for high colonisation and a diverse community to maximise crop yield and failure to apply rigorous agronomic methodology. We also show that the mycorrhizal fungal community may be more resilient to many agricultural practices than often assumed. However, there is a paucity of field studies and our ability to manage AMF to favour crop yield is compromised by rudimentary knowledge of many important aspects of the symbiosis. These include: the determinants of MGR for crop genotypes; the impact of resource balance on MGR in the field; and the complexity of interactions among species of AMF in diverse communities and between AMF and soil microbes. This lack of knowledge means that mycorrhizal fungal abundance and diversity cannot be currently included in crop growth models. To address this situation, we recommend field experiments be conducted in collaboration with local agronomists and use a standard rigorous agronomic framework (Table 1). Researchers with an agricultural focus who operate experimentally in the glasshouse should take care to ensure treatments and environmental conditions as relevant to the field as possible. We also suggest eight research priorities (Table 2).

We conclude that adjusting farm management to favour abundance or diversity of AMF cannot be widely recommended based on current knowledge and that gains in agricultural yields and sustainability (e.g., efficient use of inputs, reduced nutrient losses) are likely more easily achievable through rectifying soil nutrient deficiencies, diversification through crop rotation and intercropping (especially with legumes), crop selection and breeding, and improved understanding of $G \times E \times M$ (Leiser et al., 2016; Wani et al., 2017; Kirkegaard & Hunt, 2010; Kermah et al., 2017; Plaza-Bonilla et al., 2017). We suggest research on AMF in agroecosystems be routinely placed into the context of the broader literature on crop agronomy, breeding and agroecosystem sustainability.

Acknowledgements

Thank you to Tony Stewart and the UWA pasture science team for stimulating discussion and to Larry Duncan and three anonymous reviewers for helpful comments. MHR was funded by ARC Future Fellowship FT140100103.
Author contributions
MHR and JHG shared equally the research and writing of this manuscript.

References


Verbruggen E, Röling WFM, Gamper HA, Kowalchuk GA, Verhoef HA, van der Heijden MGA. 2010. Positive effects of organic farming on below-ground mutualists: large-


Fig. 1 An agronomic crop sequence experiment specifically designed to investigate if crop growth and yield differed among treatments with either low or high levels of colonisation by arbuscular mycorrhizal fungi in a low phosphorus (P) soil (11 mg kg\(^{-1}\) bicarbonate – Colwell – extractable P) (Ryan & Angus, 2003). (a) Initially high inoculum levels were manipulated in Year 1 with five contrasting treatments: fallow maintained with tillage (TF), canola (Brassica napus) (C), fallow maintained with herbicides (CF), linola (flax; Linum usitatissimum) (L) and subterranean clover (Trifolium subterraneum) pasture (Pa); there were four replicates (Reps). (b) In Year 2, three crops were grown, both with (+P) and without (–P) P-fertiliser: canola (non-host), wheat (Triticum aestivum, assumed to have a moderate mycorrhizal growth response - MGR) and field pea (Pisum sativum, assumed to have a high MGR). Crop growth was much increased by addition of P fertiliser. (c) Yield of the –P Year
2 mycorrhizal crops did not vary with the level of colonisation despite being strongly limited by P. Year 1 treatments had no significant impact on other measured factors with the potential to greatly influence Year 2 crop growth (crop emergence, weeds, fungal root pathogens, pre-season soil moisture and mineral nitrogen). Photograph in (a) courtesy of John Angus.

**Figure 2**

Fig. 2 Shoot dry weight response, and fitted curves, of three annual pasture species – (a) subterranean clover (*Trifolium subterraneum*), (b) yellow serradella (*Ornithopus compressus*) and (c) annual ryegrass (*Lolium rigidum*) – grown in a glasshouse with 12 levels of phosphorus (P) application, with and without inoculation with an arbuscular mycorrhizal fungus (AMF) (*Glomus* sp.) (adapted from Schweiger et al., 1995; note different scale of axes in (c)). Also shown is the average root hair length at 60% of maximum shoot growth for non-colonised plants and the approximate critical P application level for 90% of maximum growth (stars). Critical P application levels required for 90% of maximum growth in a field experiment (Yass, New South Wales, Australia), where roots were colonised by indigenous AMF, were also higher for subterranean clover (55-83 kg P ha\(^{-1}\)) than for yellow serradella (22-49 kg P ha\(^{-1}\)) (Sandral *et al.*, 2015).
Fig. 3 Grain yield and the percentage of root length colonised by arbuscular mycorrhizal fungi for irrigated (a) wheat (*Triticum aestivum*) and (b) maize (*Zea mays*) in a double-cropped rotation system grown with six levels of phosphorus (P) application over two years in Hebei Province, China, in a low P soil (7 mg P kg\(^{-1}\) of bicarbonate extractable – Olsen – P) (adapted from Deng *et al.*, 2017). The critical level of soil extractable P was determined to be 22 mg kg\(^{-1}\) to optimise yields of wheat and maize, and it was calculated that this could be maintained through application of 45-50 kg P ha\(^{-1}\) in each wheat-maize rotation (see stars on graph), with application to the wheat only.
Table 1. Experimental design template for assessment of the role of arbuscular mycorrhizal fungi (AMF) in crop sequence experiments and to produce rigorous results applicable to commercial agroecosystems and comparable with results from experiments from other crops and regions. Prepared for wheat (*Triticum aestivum*) in a 2-year crop sequence in Australia: modify for other crops and regions or if testing inoculants.

<table>
<thead>
<tr>
<th>Planning stage</th>
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<tbody>
<tr>
<td>Identify key mycorrhizal and agronomic literature for the crop(s) of interest for the region of interest and develop an hypothesis with regard to the role of AMF</td>
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<td>Identify a suitable site and record its management history</td>
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<td>Talk to local agronomists, farmers and others to determine the crop management practices most commonly used for commercial crops</td>
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<td>Decide on the treatments to be used to manipulate colonisation levels (e.g. pre-crop, tillage, inoculation) avoiding those likely to have a strong independent effect on yield (e.g. some crop species, watering regime, phosphorus (P) fertiliser rate). If possible, use multiple means to achieve contrasting levels of colonisation (Figure 1). Consider including a non-agronomically relevant treatment to maximise the chances of finding an impact of AMF on crop growth (e.g. no P fertiliser and a crop considered to have a high mycorrhizal growth response) and a control non-mycorrhizal crop in Year 2 (Figure 1b)</td>
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<tr>
<td>Identify the factors most likely to influence yield that may vary among treatments after year 1 and decide how to ameliorate (e.g. weeds, disease, soil water, soil nutrients)</td>
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<tr>
<td>Decide how to characterise mycorrhizal fungal abundance and the mycorrhizal fungal community</td>
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<tr>
<td>Determine on a sampling strategy that will ensure differences among Year 2 treatments in nutrition, growth and yield can be confidently ascribed to a cause (see below)</td>
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<tr>
<th>Measurements</th>
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<tr>
<td>Record all major operations on site (tillage, sowing, application of chemicals and fertilisers, grazing etc.)</td>
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<tr>
<td>Continuously record key weather data (e.g. daily air and soil temperature, rainfall and soil moisture) and note any key events (e.g. frosts, floods)</td>
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<tr>
<td>Note dates of key crop development stages (emergence, flowering, maturity)</td>
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<tr>
<td>Note any other factors that may affect yields (e.g. Year 1 crop stubble loads, Year 2 crop lodging, pest outbreaks, grain loss though shattering)</td>
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<tr>
<th>Pre-sowing</th>
<th>Frequency</th>
<th>Why required</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>Soil type, macro- and micronutrients, pH, organic matter (top 10 cm)</td>
<td>Year 1 – for site</td>
<td>To identify limitations to growth</td>
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<tr>
<td>Soil mineral N and soil water (top 1.5 m)</td>
<td>Year 2 – for each treatment</td>
<td>Ideally should not differ as can greatly impact yield</td>
<td>Mineral N likely to be higher following legumes Soil water likely to be higher following crops that senesced earlier Soil water and N may be stored at depth below shallow-rooted species</td>
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<tr>
<td>In-crop (noting crop phenological stage) for Year 1 and Year 2 (may only require a subset in Year 1)</td>
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<tr>
<td>Characterisation of mycorrhizal fungal community</td>
<td>At least three times</td>
<td>Assess impact of agronomic treatments</td>
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<td></td>
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<td>Differences among treatments may change as the season progresses</td>
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<tr>
<td>Plant density</td>
<td>Seedling stage</td>
<td>Seedling density may affect yield</td>
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<tr>
<td>Crop shoot biomass (and weeds and volunteers of previous crop, if present)</td>
<td>At least three times</td>
<td>Shoot biomass indicates yield potential prior to events that may differentially impact yield of treatments</td>
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<tr>
<td>Root and shoot pest and disease rating</td>
<td>As appropriate</td>
<td>May cause differences among treatments</td>
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<tr>
<td>Soil microbiome</td>
<td>If possible</td>
<td>Likely to change with treatments</td>
<td></td>
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<tr>
<td>Grain yield</td>
<td>Final harvest</td>
<td>Most agronomically relevant measure of crop success</td>
<td></td>
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<tr>
<td>Yield components (e.g. straw dry weight, individual grain weight)</td>
<td>Final harvest</td>
<td>Total crop biomass, harvest index and individual grain weight all provide insight into the factors that affected crop yield</td>
<td></td>
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<tr>
<td>Shoot, straw and grain nutrient concentrations</td>
<td>As required</td>
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High soil water and N may reduce yield in a dry finish due to “haying-off”

Assess abundance of AMF using percentage of root length colonised and, if possible, morphology (e.g. frequency of arbuscules). Check whether fine root endophyte is present. If resources allow, measure external hyphal density. If possible, assess mycorrhizal fungal community structure using molecular techniques.

Crop stubble type and density may impact emergence and establishment

Three sampling points allows P uptake rates to be calculated throughout the growing season

Measure at anthesis as large treatment effects can occur later due to, for example, frost if slight differences in flowering time, or drought if initial differences in profile soil water, or “haying-off”.

Measure root biomass at one time point if possible

Disease may be lower if the pre-crop is a different type to the crop (e.g. cereal, pulse, brassica)

Can be assessed using molecular techniques, but, expensive and likely to be difficult to interpret results in terms of relevance to crop growth and yield.

Look for shedding due to small grain or uneven ripening

Frost at anthesis may cause a low harvest index

Drought during grain filling may reduce grain size

Table 2. Research priorities for arbuscular mycorrhizal fungi (AMF) in agroecosystems.

1) Determine if percentage of root length colonised is an adequate proxy for abundance of AMF in field trials or if more time-consuming alternatives should be used (e.g., percentage of root length containing arbuscules, colonisation density, colonised root length in topsoil, or density of external hyphae in topsoil).

2) Determine the plant genotype factors that influence the mycorrhizal growth response under field conditions.

3) Test the relationship between abundance of AMF and crop yield across a range of crop management systems and locations with rigorous standardised methodology (Table 1).

4) Determine if mycorrhizal fungal community composition or diversity, or interactions with the soil microbial community including fine root endophyte, are limiting the mycorrhizal growth response of crops in the field.

5) Test resource balance concepts in the field including whether the mycorrhizal growth response is greatest when phosphorus is limiting and crop growth rate high due to high light, optimal temperature, sufficient water and no other growth-limiting nutrients or environmental factors.

6) Modify a crop growth model with results of 1) to 5) and determine if it can predict under which circumstances (crops, regions, soil extractable phosphorus level, etc.) management unfavourable to abundance of AMF, e.g. high-intensity tillage, long bare fallows or non-mycorrhizal crops in the rotation, results in a yield penalty.

7) Investigate under field conditions whether impacts of AMF on agroecosystem sustainability can be quantified and are significant in an agronomic context (e.g. soil structural maintenance, reduction in phosphorus leaching etc).

8) Further test whether the external critical phosphorus requirement framework can be used to identify a soil extractable phosphorus level at which the benefits of mycorrhizas to crop yield and agroecosystem sustainability, and crop yields, are desirably balanced.