Effect of Agronomic Practices on Arbuscular Mycorrhizal Colonisation, Spore Density and Phosphorus Uptake in Spelt (*Triticum spelta*)

A thesis submitted for the degree of Doctor of Philosophy (PhD) by

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Declaration

I declare that this thesis is my own work and that I have correctly acknowledged the work of others. This thesis has not been previously submitted for assessment at Newcastle University or elsewhere and is in accordance with University and School guidance on good academic conduct

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Abstract

Arbuscular mycorrhizae (AM) fungi form symbiotic, normally mutualistic, associations with roots and can translocate nutrients, especially phosphorus (P) to the host plant. These fungi can play an important role in agroecosystems, which is why it is important to understand how agricultural practices and genotypes affect their existence and function.

There were three main components to this project. First, a systematic review of the literature was carried out to document differences in AM fungal populations, diversity and colonisation in crops grown in organic and conventional production systems; where possible, this included meta-analyses of data extracted from the literature. In addition, two field experiments were conducted. Experiment 1 ("fertiliser trial") was conducted for two years (2014/15 and 2015/16) to find out the effect of spelt variety (Oberkulmer Rotkorn, ZOR, Rubiota and Filderstolz) and fertiliser type (compost and mineral N) and rate (50 and 100 kg N ha^{-1}) on AM fungal colonisation in spelt roots, spore density in the soil and grain yield, P (concentration, uptake and total) in straw and grain. Experiment 2 ("tillage trial", 2015/16 and 2016/17) was designed to study the effect of crop protection management (conventional and organic), fertiliser type (compost and mineral N), tillage system (minimum and conventional), and spelt variety (Oberkulmer Rotkorn and Filderstolz) on the same mycorrhizal and crop parameters. Both trials were conducted under field conditions at Nafferton farm in northeast England.

Twenty studies were identified in the meta-analysis, with soil spore density, AM fungi diversity, and root colonisation reported as indicators of AM fungal diversity and function. Results from the fertiliser trial indicated that lower levels of fertiliser input promote vesicle formation, while highest numbers of spores in 2015/16 were measured at high levels of compost input. The tillage trial showed that crop protection had a significant effect on spore density and was higher where organic approaches were used compared to conventional. Both minimum tillage and compost fertilisation increased spore density, whereas, both conventional tillage, and compost fertilisation enhanced AM fungal colonisation. Highest spore densities were measured where the spelt variety was Oberkulmer, while AM fungal colonisation was highest for Filderstolz.

The adoption of organic approaches could be a good strategy to encourage AM fungal symbiosis especially using compost fertiliser. Selective breeding could also enhance the ability of spelt to form symbioses with AM fungi, as shown for the variety Filderstolz.

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Chapter 1. Introduction

1.1. Context of the Study

1.1.1. Food security and the need for sustainable intensification

The demand for food within the next few decades is expected to increase dramatically due to projected increases in human population to as high as 9.5 billion by 2050 (Godfray *et al.*, 2010; Bharucha and Pretty, 2014). Recent studies suggest that the world will need to increase crop production by 25% to 70% to meet 2050 food demands (Godfray *et al.*, 2010; Hunter *et al.*, 2017). During the last century, conventional farming was able to meet increasing demands for food through the application of pesticides and mineral nitrogen (N) and phosphorous (P) fertilisers (Cooper *et al.*, 2011a; Kirchmann *et al.*, 2016). The introduction of high-yielding varieties during the Green Revolution resulted in increases in global food production between 1970 and 1995 of 70% in response to inputs of fertilisers combined with advances in pest control (Kirchmann *et al.*, 2016; Shennan *et al.*, 2017). But while Green Revolution technologies increased food production and crop yields there were also negative environmental consequences such as increases in surface and groundwater contamination (e.g. eutrophication), soil erosion, human health risks, greenhouse gas emissions and reduced biodiversity (Bender *et al.*, 2016; Fuhrimann *et al.*, 2019).

Sustainable intensification is one alternative method that has been proposed as an agricultural system or process where crop yields are increased with minimum or even positive impacts on the environment (Godfray *et al.*, 2010; Bharucha and Pretty, 2014). Various management strategies have been suggested that could be used for sustainable intensification in agroecosystems. These include agricultural practices that enhance biological processes such as plant-microbial interactions with symbiotic microorganisms including arbuscular mycorrhizal (AM) fungi which promote plant growth (de Vries and Wallenstein, 2017; Mdee *et al.*, 2019), reduced or no tillage e.g. conservation agriculture (Säle *et al.*, 2015; Blanco-Canqui and Ruis, 2018), organic farming (Peigne *et al.*, 2016) and diversification of cropping systems such as the use of cover crops instead of longer bare fallow periods (Wittwer *et al.*, 2017). Organic farming is proposed for sustainable intensification because it has a lower environmental impact and enhances biodiversity and soil fertility (Dimitrios *et al.*, 2017). Conservation agriculture, which includes systems with no tillage, residue cover and diversified crop rotations (Wittwer *et al.*, 2017) can sustain soil quality, protect the soil and result in a better

exploitation of natural resources such as plant growth promoting soil organisms and AM fungi (Powlson *et al.*, 2016; Wittwer *et al.*, 2017).

Although these systems present clear environmental benefits, organic yields (Seufert *et al.*, 2012) and yields under conservation agriculture (Pittelkow *et al.*, 2014) are often lower than conventional farming which means that more land is required to produce equivalent amounts of food. Currently less than 10% of arable land is under organic and no till agriculture in Europe (Zikeli and Gruber, 2017). Therefore, it is important to understand how natural processes in these systems such as associations with AM fungi can be optimised to compensate for this yield gap and increase areas under these sustainable systems of agriculture.

The systems of sustainable intensification listed above are all reliant on enhancing soil health. Soil health can be defined as "the continued capacity of soil to function as a vital living system, within land-use boundaries, to maintain biological productivity, enhance the quality of water and air environments and sustain human health, plant and animal" (Doran and Zeiss, 2000). Maintenance of healthy soil microbial communities such as AM fungi and rhizobacteria are considered important factors for soil health (Trivedi *et al.*, 2016). These communities play an important role in nutrient cycling, decomposition of soil organic matter and impacting the soil physical and chemical properties which lead to direct effects on soil fertility and sustainability. Organic fertilisers such as compost are used in organic farming to enhance soil health. Increased organic matter can regulate biological activity by providing a source of nutrients, C, and energy (Cooper *et al.*, 2011b). Reducing tillage intensity is another strategy that can build soil health and reduce the negative effects of conventional tillage which include reduced soil organic matter, loss of microbial diversity and activity, increased risk of erosion and loss of soil structure (Hevia *et al.*, 2007; Blanco-Canqui and Ruis, 2018).

1.1.2. The role of arbuscular mycorrhizal fungi in sustainable intensification

Current interest in soil health has increased the focus on the exploitation of soil biology for agricultural sustainability including crop associations with symbiotic soil organisms such as AM fungi (Kaminsky *et al.*, 2019). AM fungi are a type of endomycorrhizae: fungi that penetrate the root cell walls and become enclosed in the cell membrane of plants. AM fungi are the most common form of mycorrhizae and are associated with approximately 65 % of land plant species (Wang and Qiu, 2006; van der Heijden *et al.*, 2015) representing a vital bridge

between the plant and soil. AM fungi can translocate nutrients, particularly phosphorus, which has led to significant interest in their potential to minimise the requirement for chemical fertilisers without reducing crop yields (Bender *et al.*, 2016; Berruti *et al.*, 2016). Simultaneously, AM fungi take up and utilize carbon compounds from plants as an energy source: up to 20% in the form of carbohydrates (Andrino *et al.*, 2019). Mycorrhizae may also receive lipids from plant-made fatty acids as an important C source (Bravo *et al.*, 2017).

The benefits of AM fungal colonisation for agricultural crops have still not been demonstrated (Ryan and Graham, 2018). Even though AM fungi can improve nutrient acquisition, soil structure and resistance to pests and pathogens, high colonisation by AM fungi does not always translate to increases in crop yield (Thirkell *et al.*, 2017). In a recent debate about the role of AM fungi in enhancing crop yield Ryan and Graham (2018) concluded that "management of AM fungi by farmers will not be warranted until benefits are demonstrated at the field scale under prescribed agronomic management". This review focused on cereal (mostly wheat) field experiments that involve indigenous AM fungi; however, meta-analyses including studies with AM fungal field inoculation (Pellegrino *et al.*, 2015; Zhang *et al.*, 2018b) showed increased crop yield with inoculation. Others have emphasised the importance of long-term benefits for sustainability and yield stability from AM fungal colonisation (Rillig *et al.*, 2019) and the need to identify crop management practices favouring AM fungi. Because of the multiple benefits that may be realised from enhanced AM fungal colonisation, it is therefore important to understand the effect of crop management practices on AM fungal populations in agricultural soil.

There are very few studies about AM fungal functions and their impacts on crop yields in field trials which include critical biotic and abiotic factors; most studies are conducted using short-term controlled environment systems (e.g. greenhouse experiments) (Ryan and Graham, 2018). Furthermore, there is a critical knowledge gap regarding how the function of AM fungi in many major crop species varies among specific genotypes. Improved understanding of this could result in targeted breeding programmes for varieties that are more compatible with AM fungi (Thirkell *et al.*, 2017; Rillig *et al.*, 2019).

1.1.3. Strategies for sustainable intensification

Organic farming has emerged as a strategy to address the long-term sustainability of our food system and to meet the growing demand for healthy and safe food that minimises environmental

contamination (Tuomisto *et al.*, 2012; Schröder *et al.*, 2019). Organic farming is a 'whole system' approach to farming and food production that recognises the close interrelationships between all parts of the production system from the soil to the consumer (Soil Association, 2014). It is characterised as farming with strict limitations on the use of synthetic pesticides (fungicides, herbicides and insecticides) and mineral fertilisers and relies on reuse and recycling of natural resources such as compost and animal manure (Mazzoncini *et al.*, 2010; Timsina, 2018). Diverse crop rotations including green manure and cover crops are used for control of diseases, weeds and pests (Reganold and Wachter, 2016; Timsina, 2018).

Another system proposed to address the challenges of sustainable intensification is conservation agriculture. Conservation agriculture is based on three components including reduced tillage or no-tillage, maximum soil cover and diversified crop rotations (Petersen and Snapp, 2015; Wittwer *et al.*, 2017). Conservation agriculture is often associated with greater microbial activity and biomass and reduced soil degradation (Mbuthia *et al.*, 2015; Rehman *et al.*, 2015); however, crop yield can be lower under reduced tillage than inversion tillage due to low rates of nutrient mineralisation and high weed pressure (Säle *et al.*, 2015; Cooper *et al.*, 2016; Zikeli and Gruber, 2017).

Reducing tillage in organic systems is particularly challenging because of the need to mix crop residues into the soil to increase nutrient mineralisation. Tillage can also reduce the pressure of soil-borne pathogens and weeds in organic systems even though it can have negative effects on the environment (Cooper *et al.*, 2016). But excessive tillage can increase water and wind erosion and accelerate mineralisation of organic matter, negatively affecting soil structure, biological processes and soil health (Hevia *et al.*, 2007; Blanco-Canqui and Ruis, 2018). Therefore, there are challenges to combining reduced tillage with organic farming where no synthetic pesticides can be used.

An additional strategy proposed to address the challenge of sustainable intensification has been the diversification of cropping systems and the introduction of "minor" cereals including spelt, a close relative of wheat. Spelt is an ancient crop that has attracted increased attention in recent years, particularly in central Europe (Andruszczak, 2018). The demand for food made from spelt (*Triticum spelta*) is particularly high among organic food consumers, because spelt has higher concentrations of beneficial phytochemicals (e.g. phenolics) than common wheat (Kohajdová *et al.*, 2008; Kraska *et al.*, 2019). It also is adapted to harsh/varied climatic conditions (Arzani and Ashraf, 2017) and the hull of spelt can protect the grain from insects,

pollutants, and diseases, and improves seed germination (Dumalasová *et al.*, 2017). These properties mean that spelt is widely considered a crop well suited to low-input or organic management systems.

Diversification of cropping systems can also mean the use of a range of varieties or genotypes that are suited to a specific cropping system. These may be bred for traditional targets (e.g. disease resistance, high yield) but in the context of sustainable intensification may be selected for adaptations to low-input or organic systems (Gawęda *et al.*, 2019). AM fungal associations may differ between crop cultivars (Ellouze *et al.*, 2016; Bazghaleh *et al.*, 2018; Davidson *et al.*, 2019) and some crop cultivars could develop AM fungal associations under low soil fertility levels better than under high fertiliser levels (Ellouze *et al.*, 2016). This suggests that there could be potential to develop varieties that are adapted to form associations with AM fungi; the potential for this in spelt is not yet well understood. In particular, the differences between landraces and modern spelt varieties has not been investigated.

1.1.4. The phosphorus challenge

A key element of the Green Revolution was the reliance on soluble fertilisers to supply high yielding varieties with the major plant nutrients (N, P and K). Sources of P in conventional agriculture predominantly originate from mined rock phosphate. The availability and accessibility of this global resource has become increasingly deficient and expensive as supplies have declined in the past few decades (Cordell and White, 2015). About 80% of the finite global reserves of rock phosphate is used every year to make fertiliser P and demand is expected to increase at a rate of 2% per year in the next five years (Heffer and Prud'homme, 2014). Therefore, strategies that reduce reliance on mined P sources are increasingly important for the sustainability of food production.

Phosphorus is particularly difficult to manage because it is highly reactive and can become fixed (unavailable to plants) by reacting with soil minerals soon after application (Holford, 1997). The release of P from fixed forms is very slow and cannot be compensated due to rapid uptake by plant roots (Richardson, 2001). P fixation leads to poor efficiencies of P fertiliser use with studies reporting that P uptake from applied fertilisers in the year of application rarely exceeds 25% and more often is only 10-15% of the total applied (Sanyal and De Datta, 1991; Johnston *et al.*, 2014).

Excessive applications of P to address low fertiliser P efficiency can have negative effects on the environment (Rinot *et al.*, 2019). High rates of P fertilisation can lead to increases in soluble P levels in soil and eventual leaching of P to groundwater. P is also lost from the system when surface water runoff carries sediments that are high in P to watercourses. Both of these processes result in eutrophication, one of the key negative impacts of P use in agriculture (Ulén *et al.*, 2007). The need to manage P efficiently to address future limitations in supply and minimise environmental impacts is one of the key challenges that must be met to achieve sustainable intensification of the agricultural system.

1.2. The Role of Arbuscular Mycorrhizal Fungi in Agro-Ecosystems

1.2.1. Enhanced crop nutrition

AM fungi have the potential to enhance the sustainability of agricultural production systems. AM fungal symbioses can make a living relationship between soil and plant roots and play an important role in assisting the uptake of nutrients, particularly when the nutrients are rare (Lalitha *et al.*, 2017; Chen *et al.*, 2018b). For example, uptake of P and Zn was improved in wheat in a greenhouse experiment where the plants were inoculated with *Scutellospora calospora* by 46% for P and 33% for Zn while inoculation with *Glomus macrocarpum*, increased P and N uptake by 32% and 127% respectively compared to uninoculated treatments. In addition to P nutrition, AM fungi can enhance nitrogen uptake in a partner plant in many situations which in turn can decrease N losses from the soil (Hodge and Fitter, 2010; Cavagnaro *et al.*, 2015).

1.2.2. Mechanisms of improved plant P nutrition

Plants can only take up available P as free phosphate anions in the forms of $H_2PO_4^-$ or HPO_4^{2-} (Becquer *et al.*, 2014) which exist in very low concentrations in the soil solution (~10 μM or less (Smith and Smith, 2012). Most P in the soil occurs as unavailable phosphorous either as fixed inorganic phosphate in Fe, Ca and Al precipitates, or bound to Al or Fe hydroxides and oxides, or in organic forms including phosphomonoesters and phosphodiesters such as DNA, RNA, organic polyphosphates and phospholipid fatty acid (PLFA) (George *et al.*, 2018).

There are two P uptake pathways in the mycorrhizal plant: direct uptake through the plant's epidermis and root hairs and indirect uptake via the extra-radical hyphae of mycorrhizae and

transfer to the plant roots (Smith *et al.*, 2011; Ferrol *et al.*, 2019). Direct uptake of P by the plant causes a lowering in P concentrations in the rhizosphere around the root (the depletion zone) due to slow replenishment from the bulk soil (Smith *et al.*, 2011). AM fungi enhance P uptake primarily by allowing the plant to explore a greater soil area/volume via small-diameter hyphae which extend the depletion zone of the rhizosphere (Bücking *et al.*, 2012; Campos *et al.*, 2018). The reach of AM fungal hyphae is a hundred times longer than that of roots allowing mycorrhizal plants to access nutrients through soil pores not accessible to roots (Smith and Read, 2008; Walder *et al.*, 2012). Concentrations of P in the depletion zone around AM fungal hyphae is lower than around the plant (Schachtman *et al.*, 1998) which significantly increases the influx of P to the plant compared to non-mycorrhizal roots (Clark and Zeto, 2000). The small diameter of the hyphae also helps them to penetrate into smaller soil pores than roots in search of P thus increasing P influx rates per surface unit (Drew *et al.*, 2003; Cavagnaro *et al.*, 2005). Furthermore, AM fungi store P in the form of polyphosphate, which keeps P concentrations inside the fungus low relative to the soil, thus creating a concentration gradient that increases influx of P to the roots (Bücking *et al.*, 2012).

Enhanced uptake and translocation of nutrients from soil organic sources to plants has also been observed in plants with AM fungal associations (Sato *et al.*, 2015; Andrino *et al.*, 2019). This may be due to the capacity of AM fungi to take up P in the organic form (phytic acid) as well as orthophosphate. Extra-radical hyphae of AM fungi can secrete enzymes such as acid phosphatases that hydrolyse organic P forms in the soil, supplying plants with P commonly unavailable to non-mycorrhizal plants (Sato *et al.*, 2015; Andrino *et al.*, 2019).

Stimulation of microbial communities by AM fungi in the mycorrhizosphere (the volume of soil that includes plant roots, roots colonised by AM fungi and extra-radical hyphae of AM fungi) may also play an important role in nutrient mobilisation. The extra-radical hyphae can act as a conduit for plant-derived C between the host plant and decomposing microbial communities both supplying decomposers with C inputs and transporting nutrient by-products of decomposition back to the plant (Bücking and Kafle, 2015).

Beneficial microorganisms including plant growth promoting rhizobacteria (e.g. phosphorous solubilizing bacteria) can produce organic acids for mobilisation of P from sparingly soluble precipitates as well as acid phosphatase for mineralisation of P from unavailable organic P complexes (Ordoñez *et al.*, 2016; Zhang *et al.*, 2018a).

Once inorganic P is taken up by extra-radical hyphae of AM fungi it is quickly converted into linear polymers of phosphates linked by energy-rich phospho-anhydride bonds (PolyP) inside vacuoles (Ezawa and Saito, 2018). This maintains a concentration gradient between the fungal cytoplasm which has high concentrations of inorganic P and the tubular vacuole, thus allowing continued uptake of inorganic P (Bücking *et al.*, 2012). PolyP is then transferred to the intra-radical hyphae through cytoplasmic streaming and/or along a motile tubular vacuolar system which keeps it separate from the cytoplasm allowing the fungus to regulate its local cytoplasmic P concentration (Hijikata *et al.*, 2010). PolyP breaks down in the intra-radical hyphae producing a large amount of negative charges which are balanced by uptake of near-equivalent positively charged cations such as Na^+, K^+, Ca^+ and Mg^+ and possibly the amino acid Arg^+ (Smith and Smith, 2011; Bücking and Kafle, 2015). The inorganic phosphate molecule is delivered to the periarbuscular space inside the plant root (Lanfranco *et al.*, 2018) where nutrients exchange, located between the fungal plasma membrane and the plant periarbuscular membrane which surrounds the arbuscule. It is then imported into plant cortical cells by AM fungal-inducible P transporter genes.

1.2.3. Impacts on plant N nutrition

The positive effects of AM fungal associations on plant P nutrition have been extensively reported, but studies about the contribution of AM fungi to the N nutrition of their host plant are often contradictory. N is highly mobile in the soil, especially in the nitrate form, therefore benefits in N uptake may not be realised due to AM fungal colonisation, in contrast to P nutrition. However, the view that high mobility of N ions in the soil prevents AM fungi improving N acquisition is not supported by cases of positive effects of mycorrhizal colonisation on N uptake in plants provided with NO_3^- but not NH_4^+, suggesting AM fungi have ability to enhance uptake of N, even in the nitrate form (Vaast and Zasoski, 1992; Cuenca and Azcón, 1994).

Some researchers have argued that increased plant growth and P uptake in mycorrhizal plants indirectly results in higher N uptake than non-mycorrhizal plants (Reynolds *et al.*, 2005; Corrêa *et al.*, 2015). This view was supported by some studies, for example Ibijbijen *et al.* (1996) found that the uptake of N and P in mycorrhizal plants depended on the P concentration in the plant tissue, not N levels. Lehman *et al.* (2019) confirmed that AM fungi can contribute to early N uptake and they found a positive relationship between plant N concentration and arbuscule colonisation at the v6 growth stage of corn plants.

Furthermore, AM fungi may enhance uptake of N from organic sources through releasing exoenzymes such as proteases and peptidases that dissolve complex organically bound N and then release N as NH_4^+ for uptake by the fungus and transfer to the host plant (Nygren *et al.*, 2007; Whiteside *et al.*, 2012). AM fungi may also indirectly affect uptake of N from organic N sources through stimulation of the microbial community of decomposers in the litter which mineralise organic N (Saia *et al.*, 2014; Bukovská *et al.*, 2018) as described in section 1.2.1 above.

In addition to P assimilation, AM fungi can also assimilate inorganic N. The extra-radical hyphae takes up N from the soil a long distance from the roots and converts it to the amino acid arginine (Bücking and Kafle, 2015; Chen *et al.*, 2018a). Arginine is translocated from extra-radical hyphae via vacuoles into the intra-radical hyphae. Arginine could serve together with cations such as K^+, Ca^+ and Mg^+ as positively charged ions that contribute to the required charge balance in fungal vacuoles that often contains negative polyP (Bücking and Kafle, 2015; Dreyer *et al.*, 2019). Once internal migration is completed, the N is released from the stored arginine and translocated to the mycorrhizal interface as NH_4^+ and the plant takes up N from the mycorrhizal interface through mycorrhiza-inducible transporters (Bücking and Kafle, 2015).

Therefore, while AM fungal benefits are mostly discussed only in terms of enhanced P nutrition, this is not always the case as sometimes the mycorrhizal growth effect is the result of the sum of both P and N nutritional benefits (Nouri *et al.*, 2014; Mensah *et al.*, 2015).

1.2.4. Crop yield

AM fungi may also play a role in improving growth and crop yield by improving crop nutrition. Several meta-analyses have investigated the effect of AM fungal colonisation on crop yield for a range of crops and experiment types (e.g. greenhouse and field experiment) (Lekberg and Koide, 2005; Pellegrino *et al.*, 2015; Zhang *et al.*, 2018b). In general authors found a positive relationship between AM fungal colonisation and crop yield.

On the other hand, many studies have reported that high colonisation by AM fungi did not result in increased grain yield and plant growth in field crops such as maize (Galvez *et al.*, 2001), wheat (Ryan *et al.*, 2002; Ryan and Angus, 2003; Gao *et al.*, 2010) and pea (Ryan and Angus, 2003) or in greenhouse experiments with *Cucumis sativus* (cucumber, *Cucurbitaceae*) (Barber *et al.*, 2013) and bell pepper (*Capsicum annuum* var. California Wonder) (Tanwar *et al.*, 2013).

This could be due to environmental conditions for example, when plant available nutrients such as N and P are high in the soil, there may be no benefit to mycorrhizal associations (Gao *et al.*, 2010; Yang *et al.*, 2018b). There has also been an effect of temperature on mycorrhizal functions with associations formed at low temperatures resulting in insufficient transfer of photosynthates to the fungi and low transfer of nutrients to the partner plant (Gavito *et al.*, 2003; Hawkes *et al.*, 2008). Other field factors may limit the yield, preventing increased shoot P concentration from contributing to significant yield benefit (Miller, 2000).

In some cases colonisation by AM fungi has been shown to have a negative effect on crop growth (Smith *et al.*, 2003; Jin *et al.*, 2017). When there is sufficient soil available P for the plant, the direct uptake pathway for P will be favoured and there will be no benefit from the extra-radical hyphae network. In this case, the association with AM fungi may be parasitic (Smith and Smith, 2012; Ryan and Graham, 2018). Parasitic relationships may also develop when both partners come from different geographical origins or when the roots become colonised by low efficacy AM fungal species which develop very slowly in the plant roots (Jin *et al.*, 2017; Řezáčová *et al.*, 2017). Parasitic associations may be indicated by a higher proportion of vesicle formation compared to other AM fungal structures, highlighting the value of differentiating these structures in root colonisation studies (Johnson, 1993).

1.2.5. Additional non-nutritional benefits of AM fungi

Biotic factors

AM fungi can confer other non-nutritional benefits such as increasing crop resistance to pests and diseases and suppression of soil-borne pathogens, providing an effective alternative strategy to chemical pesticides (French, 2017; Kothe and Turnau, 2018). Mustafa *et al.* (2016) found that different wheat genotypes were protected against powdery mildew infection by *F.mossease* inoculation. Damage from plant parasitic nematodes, a common soil-borne pest, may also be reduced when plants are colonised with AM fungi (Schouteden *et al.*, 2015).

AM fungi effects on plant resistance to pathogens may result from different mechanisms operating together. For example, enhanced plant growth due to AM fungal associations may effectively compensate for the detrimental effects caused by pathogenic microorganisms (Kula *et al.*, 2005; Jung *et al.*, 2012). However, the enhancement in plant disease resistance is not always due to nutritional benefits of AM fungal colonisation. AM fungi may also suppress

pathogens and pests via modulation of the host plant defense response that accompanies AM fungal inoculation: known as mycorrhiza-induced resistance (MIR) (Jung *et al.*, 2012; Cameron *et al.*, 2013). Plant phytohormones related to defense such as salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA) and ethylene (ET) play important roles in establishing and regulating MIR in plants (Bastías *et al.*, 2018; Meier and Hunter, 2018).

Mycorrhiza-induced resistance is essentially an enhancement of the plant's defense mechanisms triggered by mycorrhizal infection. This includes transient expression of microbe-associated molecular patterns (MAMP) immunity at the early stages of infection (Zhang and Zhou, 2010). Infection also causes long-lasting priming of SA-dependent defenses and systemic acquired resistance (SAR) (Luna *et al.*, 2012). Since SA has a negative effect on AM fungal colonisation (de Román *et al.*, 2011), the fungus secretes effector proteins to suppress the SA-dependent defenses reaction and successfully colonise the host roots, as a third step (Soto *et al.*, 2009; Cameron *et al.*, 2013). The amount of SA starts to decrease and JA begins to increase in plant cells as the AM fungal symbiosis is well established (Frew and Price, 2019).

The production of the plant hormone abscisic acid (ABA) which can contribute to priming of cell wall defences to protect the shoots of host plants against pathogen attack (Adolfsson *et al.*, 2017; He *et al.*, 2017). Plant defences can also be primed prior to pest or pathogen attack by AM fungi through modulation of JA pathways similar to induced systemic resistance (ISR) which can be elicited by beneficial organisms including plant growth promoting rhizobacteria (PGPR) (Jung *et al.*, 2012; Cameron *et al.*, 2013).

Mycorrhiza-induced resistance (MIR) may also be linked to enhanced release of volatile organic compounds (VOCs) that attract parasitoid insects that are natural enemies to pests (Cabral *et al.*, 2018; Turlings and Erb, 2018). Additionally, AM fungi can transfer signals through its common hyphae network and change VOCs in neighbouring plants which leads to repelling of herbivores (Johnson and Gilbert, 2015; Bücking *et al.*, 2016; Meier and Hunter, 2019).

AM fungi may suppress some weed species which could reduce the need for herbicides (Li *et al.*, 2016; Thirkell *et al.*, 2017). AM fungi and their network of hyphae (also known as a common mycorrhizal network or CMN) may regulate plant-plant interactions in potentially suppressing weeds (Hu *et al.*, 2019; Wang *et al.*, 2019). For example, foxtail suppression by CMN and root interactions with maize, wheat and faba beans has been observed by Qiao *et al.*

(2016). This may be a simple competitive effect with the crop species in the presence of the CMN outcompeting the foxtail for resources (Li *et al.*, 2016; Qiao *et al.*, 2016). But the suppression of weeds by AM fungi could also be related to transfer of allelochemicials from donor plants to target plants such as weeds via the CMN (Jakobsen and Hammer, 2015; Qiao *et al.*, 2016). Allelochemicals are detrimental compounds produced by a particular plant that limit the growth of surrounding plants (Barto *et al.*, 2011). The rates of diffusion of allelochemicals in soil are often low due to limiting factors such as soil moisture, organic matter and existing microorganisms, but the CMN can effectively transfer allelochemicals a long-distance to target plants in high enough doses to be bioactive (Barto *et al.*, 2011; Jakobsen and Hammer, 2015).

Abiotic factors

As well as biotic stresses like pathogens and pests, AM fungal symbiosis can have a positive influence on tolerance to abiotic stresses such as drought, salinity and heavy metal toxicity. AM fungi improve plant growth, water use efficiency and nutrient uptake (indirectly) through extending more roots in the soil under drought conditions (Bowles *et al.*, 2016; Wang *et al.*, 2019). As described for nutrient acquisition, AM fungi increase the soil volume accessed, allowing extra-radical hyphae to reach small and distant water-filled pores which cannot be accessed by roots (Sun *et al.*, 2017; Leyva-Morales *et al.*, 2019; Wang *et al.*, 2019). AM fungal symbiosis also contributes to plant drought tolerance through the accumulation of specific osmolytes such as proline, and soluble sugars in plant tissues as a stress defense mechanism (Gill *et al.*, 2016; Santander *et al.*, 2017). This osmoprotectant may help to maintain osmotic balance through reducing cell osmotic potential which results in increased water uptake under drought conditions (Ortiz *et al.*, 2015; Yooyongwech *et al.*, 2016). AM fungi may also be involved in the regulation of aquaporin gene expression which facilitates the translocation of water and small solutes (e.g. ammonia, urea and gases) across biological membranes of plants under drought stress (Gill *et al.*, 2016; Recchia *et al.*, 2018). AM fungi may increase antioxidant enzymes such as catalase and peroxidase which act against damaging reactive oxygen species (ROS) generated by drought stress conditions such as singlet oxygen (O_2^{1-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\circ}OH$), thus protecting mycorrhizal plants from oxidative damage (Santander *et al.*, 2017; Saxena *et al.*, 2017).

Associations with AM fungi can also alleviate stress due to salinity (Fileccia *et al.*, 2017; Saxena *et al.*, 2017). AM fungi contribute to improved tolerance to salinity through improving

plant uptake of major nutrients (N, P) and cations (e.g. K^+, Mg^{2+} and Ca^{2+}) over harmful Na^+ ions (Abdelhamid *et al.*, 2019; Santander *et al.*, 2019), enhancing hydraulic conductivity of host plant roots to maintain higher water contents in mycorrhizal plants under salt stress conditions (Kumar *et al.*, 2015; Saxena *et al.*, 2017), retaining Na and Cl in intra-radical hyphae (Mardukhi *et al.*, 2015; Santander *et al.*, 2019) and, altering levels of phytohormones such as salicyclic acid in host plants (Kumar *et al.*, 2015; Garg and Bharti, 2018).

AM fungi can provide protection against excesses in trace minerals as well as toxic heavy metals such as mercury (Hg), arsenic (As), lead (Pb) and cadmium (Cd) using several mechanisms (Silva *et al.*, 2013; Abdelhameed and Metwally, 2019). AM fungi can lead to dilution of the metal levels in plant tissue by increasing the uptake of water and nutrients (e.g. P and N), and enhancing the growth of plants (Garg *et al.*, 2017; Basu *et al.*, 2018). Glomalin produced by AM fungi may play a role in decontaminating the soil through extraction and chelation of the heavy metals from polluted soil, thus reducing metal availability and toxicity (Cornejo *et al.*, 2017; Miransari, 2017). Immobilization of heavy metals by AM fungi can be achieved through sequestration of the metals in extra-radical hyphae, retention inside the cortical cells of plant roots, or on the surface of the spore's cell wall of the AM fungus, thus reducing their allocation to the plant's aerial parts (Huang *et al.*, 2018; Spagnoletti *et al.*, 2018). AM fungi can also change the selectivity of the plasma membrane in the desorption or absorption of heavy metals (Miransari, 2017). Finally, AM fungus can increase production of metallothioneins which form chelates with heavy metals in the cytosol of both fungi and the host plant thus reducing heavy metal stress (Miransari, 2017; Talaat and Shawky, 2017).

Finally, healthy populations of AM fungi in cropping systems may enhance soil structure in the long-term. AM fungi can directly affect soil aggregation and improve soil structure through production of extra-radical hyphae (Kohler *et al.*, 2017) that bind soil particles and aggregates together (Kohler *et al.*, 2017; Ji *et al.*, 2019) improving porosity, water holding capacity, infiltration and resistance to soil erosion. AM fungi also enhance soil aggregation through depositing organic compounds such as insoluble proteins known as glomalin and chitin that act like a "glue" between soil particles (Wu *et al.*, 2016; Lehmann *et al.*, 2017). These effects result in a resilient soil with the potential to deliver sustainable crop yields.

1.3. Arbuscular Mycorrhizal (AM) Fungal Taxonomy and Development

1.3.1. Taxonomy and classification of AM fungi

The widespread nature of associations between mycorrhizal fungi and plants roots was first recognized by Frank in 1885 (Smith and Read, 2008). Four major mycorrhizal types have been described based on their structure, extent of penetration into the host roots and function, namely arbuscular mycorrhiza (AM), ectomycorrhiza (EM), ericoid mycorrhiza and orchid mycorrhiza (Brundrett, 2004; van der Heijden *et al.*, 2015). Arbuscular mycorrhizal (AM) fungi are the most ubiquitous and widespread root-fungus association. Formerly called vesicular arbuscular mycorrhiza (VAM) or Glomeromycotina mycorrhizas (Spatafora *et al.*, 2016). This name was replaced because some AM fungi do not generate vesicles in roots, although the term VAM fungi is still often used (Brundrett and Abbott, 2002).

AM fungi were initially placed in the phylum Zygomycota, however, molecular studies revealed that they are phylogenetically different from other members of the Zygomycota, so a new phylum, Glomeromycota, was created by Schüßler et al. (2001). More recently, phylogenetic analysis-based genome-wide sequencing reorganised Zygomycota and Glomeromycota as Zoopagomycota and Mucoromycota (Spatafora et al., 2016). Mucoromycota is sub-divided into three sub-phyla, including Glomeromycotina, Mortierellomycotina, and Mucoromycotina. AM fungi are placed in Glomeromycotina which consists of a single class of Glomeromycetes (Bruns et al., 2018). Within this class AM fungi are distributed among order, family, and then genera and genera type, as shown in (Fig 1.1) which follows the classification by Spatafora et al. (2016).

Glomeromycetes consists of four orders: Paraglomerales, Archaeosporales, Glomerales and Diversisporales, and 11 families and 25 genera (Fig 1.1*)* (Schüßler *et al.*, 2001; Redecker *et al.*, 2013). The most ecologically and economically important AM fungi belong to the order Glomerales which has two families, *Glomeraceae* and *Claroideoglomeraceae* and 6 genera: *Claroideodeoglomus*, *Glomus*, *Funneliformis*, *Septoglomus*, *Sclerocystis* and *Rhizophagus* (Redecker *et al.*, 2013).

Paraglomerales

Archaeosporale

Glomeromycotina → Glomeromycetes

Glomerales

Diversisporaceae

Diversisporaceae

Paraglomeraceae → Paraglomus → P.occultum
Ambissporaceae → Ambispora → A.fennica
Geosiphonaceae → Geosiphon → G.pyriformis
Archaeosporaceae → Archaeospaor → A.trappei
Claroideoglomeraceae → Claroideoglomus → C.claroideum
Glomeraceae → Glomus → G.macrocarpum
Funneliformis → F.mosseae
Septoglomus → S.constrictum
Sclerocystis → S.coremioides
Rhizophagus → R.populinus

Diversisporaceae → Tricispora
Otospora
Diversispora → D.spurca
Corymbiglomus
Redeckera → R.megalocarpa
Acaulosporaceae → Acaulospora → A.laevis
Sacculosporaceae → Sacculospora
Paciisporaceae → Pacispora → P.scintillans
Gigasporaceae → Scutellospora → Scutellospora
Gigaspora → Gigaspora
Intraomatospora
paradentiscutata
Dentiscutata → Dentiscutata
Cetraspora → Cetraspora
Racocetra → Racocetra

Fig 1.1. Classification of AM fungi (Redecker *et al.*, 2013; Redecker and Schüßler, 2014; Spatafora *et al.*, 2016).

1.3.2. Relationships and structure of AM fungi associations with plants

Mycorrhizal fungi have a large number of functions and most mycorrhizal associations are 'mutualisms because both partners (AM fungi and host plant) benefit from the association. AM fungi exist in obligate symbiosis, meaning that they are dependent on their host plant for carbon supply while providing resources (nutrients, water) to the host plant. In some cases, AM fungi may show parasitic behaviour, with consumption of carbon by mycorrhizae exceeding the resources delivered to the plant (Smith and Smith, 2012).

Extra-radical hyphae

AM fungi exist as a number of different structures in soil and roots (Fig 1.2). The soil structures of AM fungi include extra-radical hyphae in the soil, which are filamentous structures, branching in soil as channels with a finer diameter than plant roots. Extra-radical hyphae can be thin hyphae known as absorptive hyphae and thick hyphae known as distributive hyphae (Brundrett, 1991). The CMN which allows communication between AM fungi and plants (Bücking *et al.*, 2016; Bonneau *et al.*, 2019). It contributes to the long-distance transfer and distribution of nutrients (e.g. P, N, carbon or micronutrients) among neighbouring plants (Bücking *et al.*, 2016; Weremijewicz *et al.*, 2018). Moreover, CMN can indirectly contribute to transfer of nutrients to host plants through recruiting microorganisms in the mycorrhizophere and facilitating mobilisation and mineralisation of nutrients that are taken up by CMN to transfer to host plants (Ezawa and Saito, 2018; Bunn *et al.*, 2019).

AM fungi also have the ability to reallocate water via shared CMN between plants to enhance water use efficiency and preferentially translocate more water to mycorrhizal plants (Ji *et al.*, 2019; Wang *et al.*, 2019). Direct transfer of allelochemicals from particular plants to target plants is another benefit from the CMN which can facilitate competition between plants in intercropping systems and suppress the growth of plant competitors such as weeds (Barto *et al.*, 2011; Jakobsen and Hammer, 2015). Furthermore, CMN can contribute to the plant's defense system and facilitate the transport of warning signals between plants within one CMN as described in section 1.2.5 (Cabral *et al.*, 2018; Meier and Hunter, 2019). Additionally, they are also considered an important AM fungal propagule for the next crop season and inoculum resource in the soil (Varela-Cervero *et al.*, 2016).

Spores

AM fungi also exist as spores which are reproductive structures (propagules; 20 and 1000 μm diameter) that form in soil and in roots and are more resistant to unfavourable environmental conditions than other AM fungi propagules (Brundrett, 1991). AM fungi spores are swollen structures that sometimes are free in soil or may be connected to hyphae (Brundrett, 2008). AM fungal spores can remain viable for many years because they are preserved by a multi-layered cell wall, and this characteristic is important for the establishment of new colonies (Brundrett, 1991).

Intra-radical hyphae

Intra-radical hyphae, which form in roots and branch within the cortex, act as a conduit between the extra-radical hyphae in the bulk soil and arbuscules inside the cortical cells of the root system (Bücking and Kafle, 2015). These hyphae can release nutrients and water into the arbuscule and exchange them with C from the host plant (Jacott *et al.*, 2017).

Arbuscules

Arbuscules are produced by AM fungal hyphae in cortex cells and are an essential site of exchange for C, P, N, water and other nutrients (Luginbuehl and Oldroyd, 2017; Ferrol *et al.*, 2019). Arbuscules may only last for a short time – unlike vesicle and intra-radical hyphae - and are often absent in roots. They may be difficult to see in roots depending on the root's age and the quality of chemical pigments used in the staining method (Brundrett *et al.*, 1996).

Vesicles

Vesicles are swollen structures containing lipids, and cytoplasm, and generally are considered to be resting or storage organs (Kubota *et al.*, 2001; Kobae *et al.*, 2016). Vesicles contain a high number of vacuoles and thus have potential to absorb elements when mycorrhizal plants are subject to stress including excess heavy metals, drought and salinity (Johnson *et al.*, 2016; Miransari, 2017). Vesicles can be activated after conditions improve which could promote new AM fungal structures to be regenerated (Jin *et al.*, 2017). Vesicles can also function as propagules as root fractions colonised by vesicles and intra-radical hyphae of AM fungi can propagate AM fungi in soil (Staddon and Fitter, 2001).

AM fungal structures in soil
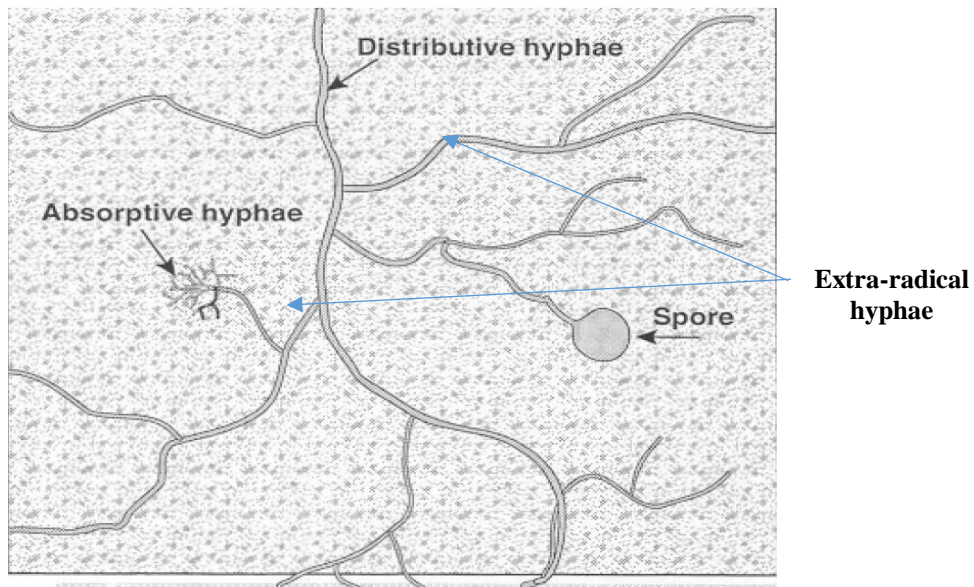
AM fungal structures in roots
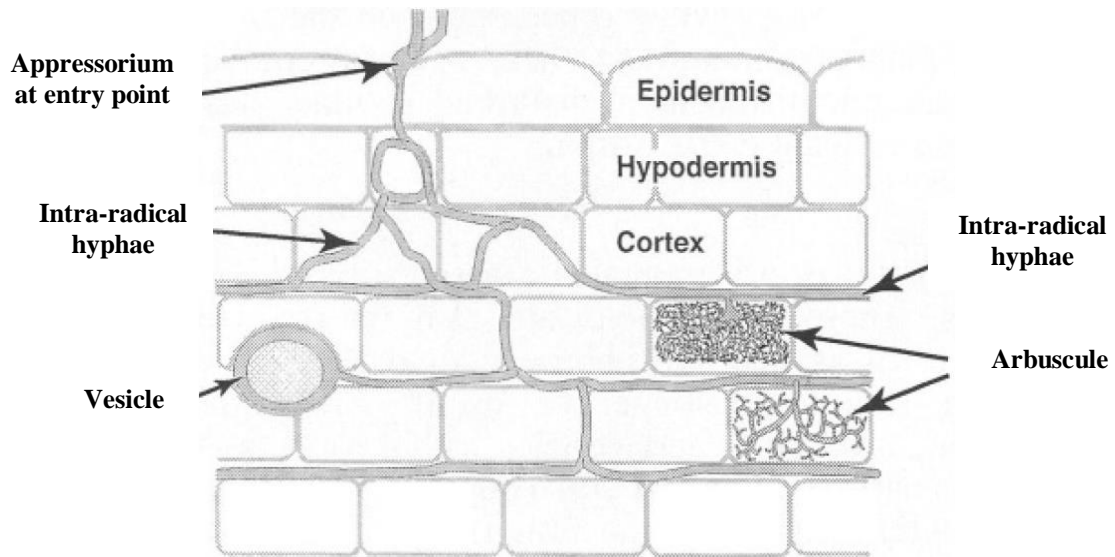
Fig 1.2. AM fungal structures in soil and in plant roots (Brundrett and Abbott, 2002).

1.3.3. The life-cycle of AM fungi

AM fungal associations with plants can be established from different propagules which act as inoculum such as soil hyphae, spores, and root fractions that are colonised by AM fungal structures (intra-radical fungal structures), including vesicles and intra-radical hyphae.

Root colonisation by AM fungi takes place in successive steps. Before physical contact between the plant and fungus, plant roots release hormones known as strigolactones (Fig 1.3) which stimulate AM fungal propagules in soil (e.g. spores) to germinate, metabolise stored lipids and use these reserves to begin hyphal branching (Chen *et al.*, 2018a; Lanfranco *et al.*, 2018). Simultaneously, AM fungi produce diffusible signals known as "Myc factors" (e.g. lipo-chito-oligosaccharide) that are recognized by plant receptors through activating a so-called calcium oscillations and common symbiotic signalling pathway (Jin *et al.*, 2016). When fungal hyphae touch the host root surface, they form an adhesion structure called a hyphopodium (Jacott *et al.*, 2017; Kobae, 2019). Subsequently, intra-radical hyphae initiate from this hyphopodium and enter the root through the pre-penetration apparatus (PPA) on the root epidermis of the host plant (Bücking *et al.*, 2012). The intra-radical hyphae spread through the epidermal cells of the root, and then travel between the root cells to enter the inner cortical cells (Jacott *et al.*, 2017; Kobae, 2019). After branching repeatedly, the intra-radical hyphae form arbuscules within the cortical cells where nutrient and carbon exchange takes place (Pimprikar and Gutjahr, 2018; Voß *et al.*, 2018). In addition to intraradical hyphae, extra-radical hyphae start to develop outside the host plant's roots in the surrounding soil (Bücking *et al.*, 2016). Spores are generally produced on the extra-radical hyphae, completing the life-cycle of AM fungi (Bücking *et al.*, 2012).
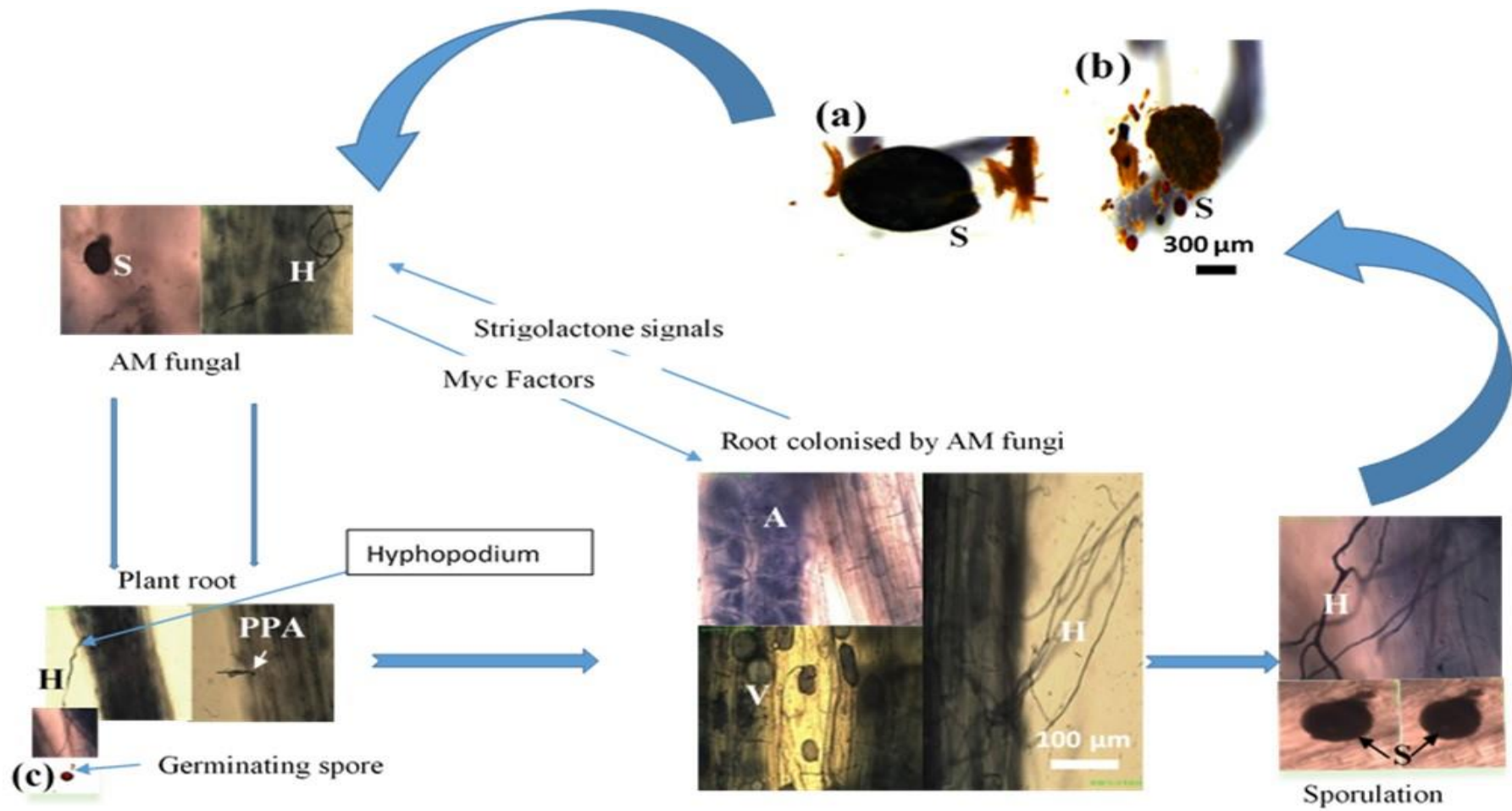
Fig 1.3. The life-cycle of AM fungi (all images taken by the author using a compound Leica DMLB microscope for intra-radical structures of AM fungi in spelt roots including intra-radical hyphae (H), arbuscule (A), vesicle (V) and Point of entry in plant apparatus (PPA). Spore (S) images taken by using a microscope (MEIJI 13066). All images have the same scale bar (100 μm) except spore images in panel a, b, c have the same scale bar (300 μm).

1.3.4. Effect of soil properties and environmental conditions on AM fungal associations

AM fungi-plant associations are affected by a range of soil and environmental conditions such as pH, nutrient levels, temperature and moisture (Entry *et al.*, 2002). AM fungal colonisation can occur at both low and high moisture conditions (Augé, 2001), including some reports from wetlands or flooded soil (Dhillion and Ampornpan, 1992). However, the greatest AM fungal colonisation appears to occur under moist conditions (Miller, 2000).

The effect of soil pH on AM fungi is species-specific. For example, in a greenhouse experiment using subterranean clover, inoculation with *Glomus fasciculatum* increased plant growth at pH levels ranging from 5.3 to 7.5, whereas inoculation with *Glomus* sp. only increased plant growth when soil pH was 7.0 or higher (Abbott and Robson, 1985). Furthermore, the response of AM fungal structures is also different under varying levels of soil pH. For instance, both AM fungal species *Glomus intraradices* and *Scutellospora calospora* formed more extra-radical hyphae at the higher pH (around 6) compared to low level pH (around 5) (Van Aarle *et al.*, 2002). In contrast, AM fungal total root colonisation was reduced for both fungal species at the higher pH as well as arbuscule and vesicle formation of *Glomus intraradices* were also reduced at the higher pH. Some AM fungal species are quickly established where the soils are acidic, whereas other species could be more active where the soils are alkaline (Mosse, 1972). In general, Green *et al.* (1976) found that although *Glomus mosseae* spores germinated easily at a pH above 7, the best spore germination occurred at pH 7, while spore germination was low at pH 5 and tended to be unsuccessful at pH 4. This is further evidence of variations in AM fungal response to pH in general and that it may vary among species.

Soil type can be considered as another factor affecting AM fungal activity. For example, Oehl *et al.* (2010) found that the genus diversity (Shannon-weaver index) and genus richness were significantly lower in Leptosols compared to Cambisols in a study in Central Europe. Lower AM spore richness was found for a Chernozem soil compared to Vertisolic soils (Bainard *et al.*, 2015). Differences in soil physical and chemical characteristics (e.g. soil pH, organic matter content and natural nutrients) of soil groups may be the main reason for this variation in AM fungal community structures (Oehl and Sieverding, 2004).

Soil temperature can impact AM fungal association and their functions. Low temperatures may reduce P uptake by extra-radical hyphae of AM fungi. For example, low winter soil

temperatures (< 10°C at 0–10 cm depth) could decrease the spread of and P flow through, the extra-radical hyphae of AM fungi (Gavito and Olsson, 2003; Ryan and Angus, 2003). Low soil temperature can inhibit photosynthate transfer from plants to AM fungi and AM fungal growth can become carbon-limited, resulting in reducing AM fungal colonisation (Hawkes *et al.*, 2008; Zhang *et al.*, 2019).

1.4. Impact of Agronomic Practices on Arbuscular Mycorrhizal Fungi Development

Agricultural crop management has a profound effect on the activities of soil microorganisms such as AM fungi (Boddington and Dodd, 2000; Verzeaux *et al.*, 2017a) and understanding how crop management practices affect native mycorrhizal fungal populations will provide valuable information for the design of future sustainable agricultural systems.

1.4.1. Fertilisation

The amount of fertiliser used can affect AM fungal colonization (Johnson, 2010). For example, over-usage of P fertiliser may lead to increased levels of available P in soil which can lead to a decrease in AM fungal colonisation (Ryan and Tibbett, 2008; Tavarini *et al.*, 2018). In a long-term P-N fertilisation experiment, it was found that increasing fertilisers from 0 to 180 kg ha^{-1} year^{-1} for both N and P_2O_5 reduced AM fungal spore density by 70% (47 to 1 spores g^{-1}) under field conditions in a maize cropping system (Bhadalung *et al.*, 2005). High nutrient status (e.g. P and N) in plant tissues may act as a negative feedback mechanism that limits C transfer to the AM fungus under such conditions to avoid parasitism (Kobae *et al.*, 2016; Ferrol *et al.*, 2019). This results in suppression of symbiosis under conditions of high nutrient availability (e.g, P and N) (Lanfranco *et al.*, 2017). In contrast under low P conditions, the plant may send specific signals (e.g. strigalactones) to active AM fungi to colonise plant roots (Lanfranco *et al.*, 2018; Kobae, 2019). In contrast, at low levels of soil P, especially early in the season, AM fungi development can be enhanced and increase nutrient uptake. Taffouo *et al.* (2014) found that root colonisation was significantly higher under low P fertilisation compared to medium and high P fertilisation during both the vegetative and pod-filling stages for field grown Cowpea plants.

The type of fertiliser may influence AM fungal functions in the soil, due to its effects on soil available P levels. A long-term (19 year) experiment found that mineral fertiliser NPK had a

negative effect on AM fungal colonisation possibly due to increased availability of soil P (Hu *et al.*, 2010). Organic sources of nutrients such as manure and compost supply less immediately available P and therefore AM fungi activities may be enhanced where these sources of nutrients are used as reported by Gryndler *et al.* (2006).

Fertiliser inputs can alter the structure of AM fungi communities. Wang *et al.* (2011) found that both mineral fertilisers and organic manure led to reductions in AM fungal species richness and species diversity and the highest reduction occurred under organic fertiliser. This was attributed to accumulation of nutrients in soil, especially P, from high rates of manure (Wang *et al.*, 2011). In other cases, long-term fertiliser did not affect AM fungi species' composition. For example, long-term field studies (20 years) in Northern Europe comparing two levels of P (no P fertiliser versus 45 kg P ha^{-1}) found that regular applications of P fertiliser led to decreased spore density and colonisation by AM fungi, but did not change AM fungal species composition (Kahiluoto *et al.*, 2001).

1.4.2. Crop variety

Genotypes of the same crop species have been shown to differ in AM fungal colonisation (Zhou *et al.*, 2015; Leiser *et al.*, 2016; Martín-Robles *et al.*, 2018). Some genotypes are highly compatible with AM fungi and these genotypes can show higher colonisation by native populations of AM fungi than other genotypes (Lehnert *et al.*, 2017). AM fungus improves P uptake in cultivars with relatively coarse root systems and this supports the hypothesis that the primary benefit to crop growth from AM fungal colonisation is improved P uptake, particularly under limited P conditions, where plants may rely totally on mycorrhizal associations to meet P requirements (Smith *et al.*, 2003). Cultivars with highly branched root hairs may derive little benefit from AM fungal symbiosis because they are already well adapted to acquire nutrients from soil (Smith *et al.*, 2011).

There is considerable genetic variation among modern durum wheat cultivars in compatibility with AM fungi at different levels of soil fertility. A greenhouse experiment comparing five modern cultivars of durum wheat found that there was genetic variation in AM fungal colonisation under both medium and low soil fertility levels, with some cultivars developing greater levels of colonisation than others (Singh *et al.*, 2012). For example, 'Mongibello' had similar AM fungal colonisation levels at both high and low soil fertility levels, while

'Commander' showed a low level of AM fungal colonisation at medium fertility, but a high level at low soil fertility (Singh *et al.*, 2012). This indicates that there is variation in the response of cultivars to AM fungal colonisation dependent on initial levels of available nutrients in soil (e.g. P and N).

AM fungal root colonisation may not always vary among different crop cultivars. For example, AM fungal root colonisation did not significantly differ between different modern wheat genotypes (hybrids), although the agronomic traits were largely different among these genotypes (Mao *et al.*, 2014). The authors explained that N and P content of both soil and shoot were similar among cultivars and this could explain why there were no differences among AM fungal root colonisation (Mao *et al.*, 2014). Other field studies have also reported no variation in AM fungal colonisation among wheat cultivars (Hildermann *et al.*, 2010).

1.4.3. Crop protection

Although the use of pesticides in crop protection is useful in agriculture to control detrimental weeds, diseases, and harmful insects, it may have negative effects on soil microorganisms such as AM fungi.

Herbicides

Herbicides can affect AM fungi directly through inhibitory (toxic) effects on AM fungi (Graham *et al.*, 1986) or indirectly through disrupting the supply of fixed carbon to AM fungi due to inhibition of photosynthesis by weeds (Baumgartner *et al.*, 2005). This can reduce the populations of mycorrhizal weeds which act as hosts for AM fungi (Baumgartner *et al.*, 2005). For example, RH-2915 (oxyfluorfen) herbicide can negatively affect AM fungal diversity which can be attributed to reductions in populations of alternate hosts e.g. weeds. Similarly, fomesafen (active ingredient) reduced AM fungal colonisation by 31% compared to control (non-treated) bean roots (Santos *et al.*, 2006).

Different herbicides may differ in their effect on AM fungi. An experiment comparing pre-plant soil applications of Lasso® Monsanto Sdn. Bhd (alachlor) and Roundup® Monsanto (glyphosate) on spore density and inoculation of peanut plants by *G. mosseae* found that the spore densities were significantly decreased with increasing rates of alachlor application, while

they were unaffected by glyphosate (Pasaribu *et al.*, 2013b). In fact glyphosate treatment can increase shoot P concentration with no negative effects on plant growth (Pasaribu *et al.*, 2013a). This could be due to differences in herbicide modes of action since alachlor is a soil- acting herbicide, which is absorbed through root uptake while glyphosate is absorbed through the plant foliage (Pasaribu *et al.*, 2013a). Reductions in plant growth with alachlor application may be due to enhanced uptake and transfer of the herbicide from soil to the plant tissues when mycorrhizae were present which decreases photosynthesis (Pasaribu *et al.*, 2013a). In glyphosate treatments the increased P in plant tissues may be due to uptake of P released from microbial degradation of glyphosate which contains P. Another mechanism for enhanced colonisation following herbicide use may be early contact by AM fungal hyphae between weed roots and crop seeds that promotes early colonisation by AM fungi prior to weed death (Brito *et al.*, 2013).

Fungicides

The use of systemic fungicides is a common practice to control or prevent cereal diseases, but simultaneously this process may eliminate beneficial fungi including AM fungi. A systemic fungicide is translocated throughout the plant including the roots and therefore may inhibit fungal growth on roots (Jin *et al.*, 2013). In contrast contact fungicides kill the fungal organisms on the plant parts they contact, leaving AM fungi on the roots unaffected (Jin *et al.*, 2013). For instance, in a greenhouse experiment on cultivated pea and chickpea plants AM fungal colonisation was reduced by systemic fungicide application, including Apron Maxx® RTA® (fludioxonil and metalaxyl), Crown® (carbathiin and thiabendazole), AllegianceTM FL (metalaxyl), Vitaflo® 280 (carbathiin and thiram) and Trilex® (trifloxystrobin and metalaxyl) (Jin *et al.*, 2013). In the same experiment the contact fungicides Thiram 75WP (thiram) and Agrox® FL 75WP (captan) had less impact on AM fungal colonisation (Jin *et al.*, 2013).

However, the use of systemic fungicide applications does not always negatively affect AM fungal symbiosis. The impact of twenty-five systemic and non-systemic fungicides applied to the soil and leaves at recommended rates was tested in leek plants inoculated with two AM fungal species (*Glomus mosseae* and *Glomus intraradices*) (Hernandez-Dorrego and Mestre Pares (2010). This study found that the non-systemic fungicides applied to the soil: Metaram (tetramethylthiuram-disulfide 80%), Ditiver (mancozeb 80%), Octagon (prochloraz 45%) and Parmex (iprodione 50%), as well as three systemic fungicides recommended for foliar

application: Sinthane (miclobutanil 24%), Rubigan (fenarimole 12%) and Frupica (mepanipyrim 50%), significantly reduced AM fungal colonisation. However, mycorrhizal colonisation was not impacted by treatment with the systemic fungicides Beltanol (Chinosol 50%) and Previcur (propamocarb 60.5%) as well as the non-systemic fungicide INACOP (Copper oxychloride 50%) and other systemic fungicides Ortiva (Azoxystrobin 25%), Aliette (fosethyl-aluminium 80%) and Forum (dimetomorph 11.3 +Folpet 60%) did not seem to reduce it strongly. The fungicides that do not affect AM fungal colonisation irrespective of their application method (foliar or soil) may be those with only a brief period of activity in the plant or soil which is not long enough to cause inhibition of AM fungal symbiosis (Hernandez-Dorrego and Mestre Pares, 2010).

The effect of fungicides on AM fungi may also be related to whether fungicides are broad spectrum (target range of pathogenic fungi) or specific to a particular disease (target specific pathogenic fungi) (Buysens *et al.*, 2015). For example, the active ingredient of both systemic fungicides normally used for *Botrytis* control: Teldor (fenhexamide 50%) and Switch (ciprodinyl 37.5 +Fludioxonyl 25) are recorded as environmentally friendly because they target specific fungi and are not toxic to non-target fungi such as AM fungi (Hernandez-Dorrego and Mestre Pares, 2010). In contrast inoculation of all three *Glomus* species was greatly decreased by foliar applications of the fungicide Bavistin (carbendazim), a broad spectrum fungicide no longer widely used by cereal growers in the UK due to high levels of resistance (Dodd and Jeffries, 1989). Similarly, Gill *et al.* (2013) found that AM fungi infections were totally suppressed by Benlate (benomyl) and recommended avoiding using it in any management strategy because of its harmful effect on AM fungal functions. This fungicide is also no longer widely used in the UK due to widespread fungal resistance use to cereal growers in the UK.

1.4.4. Tillage

Tillage is an important agricultural practice and includes primary and secondary tillage prior to crop planting, as well as inter-row cultivation for weed control during crop growth. Even though using soil tillage can lead to improved soil conditions, which in turn improves the productivity of the crop, it may suppress AM fungal colonisation through disrupting AM fungal networks within the soil. The direct impacts of the different types of tillage, especially conventional and reduced intensity (no-till and minimum) tillage, are attributed to mixing of surface residues (organic matter, microorganisms and nutrients) within the soil layers and to the physical

disruption of the soil hyphae network (Kabir, 2005). This could negatively affect AM fungal symbiosis with plants, especially the next generation of AM fungal colonisation of the following crop (Santos *et al.*, 2006; Alguacil *et al.*, 2008; Duan *et al.*, 2010).

There is considerable evidence to demonstrate that reducing tillage intensity enhances AM fungal colonisation. AM fungal colonisation was lower with conventional tillage than with no-till in field-cultivated wheat in the Argentinean Pampas (Schalamuk *et al.*, 2011). Galvez *et al.* (2001) found that AM fungal spores in soil and AM fungal colonisation in roots were higher under no-tillage compared to conventional tillage using moldboard plough or chisel-discs in maize. Finally, a recent meta-analysis demonstrated that AM fungal colonisation was increased by about 30% by less intensive tillage, while AM fungal species richness was increased by 11% in low intensity versus conventional tillage systems (Bowles *et al.* (2017).

When the soil is not tilled or tillage is reduced and host crops are cultivated, AM fungal propagules, including active soil hyphae and colonised roots, are the main source of AM fungal inoculation (Klironomos and Hart, 2002). They are more efficient and quicker in achieving colonisation than spores (Brundrett, 1991) and this could be the main reason for enhanced AM fungal colonisation under reduced tillage intensity compared to conventional tillage.

1.4.5. Rotation

Crop rotation is the cultivation of a sequence of crops on a piece of land to maintain soil fertility and control pests, weeds and diseases. Including mycorrhizal plants in a rotation (e.g. wheat or maize) may promote the formation of AM fungal symbioses in subsequent crops (Lekberg and Koide, 2005; Higo *et al.*, 2019). AM fungal colonisation increased from 10.4% to 38.8% in rice plants cultivated after maize/ horse gram plants compared to three other previous crops (green gram/rice, black gram/rice and radish/horse gram/rice) (Maiti *et al.*, 2012). In some cases, legume cover crops can maintain a high level of AM fungal inoculum due to a strong relationship between the AM fungi and legumes, that affects the subsequent crop. This was demonstrated in peach (*Prunus persica*) seedlings that had higher AM fungal inoculation when planted in soil which was previously cultivated with five legume cover crops compared to controls (Rutto *et al.*, 2003).

A number of studies have reported that including non-mycorrhizal plants as a previous crop can negatively affect AM fungal formation in the following crops. For example, preceding crops with non-mycorrhizal plants such as canola reduced AM fungal abundance in maize plants more than a preceding crop of alfalfa or maize plants in fields in southern Ontario, Canada (Gao *et al.*, 2010). In the same region, delays in AM fungal colonisation and reduced early-season P uptake followed by reduced biomass and grain yield in maize were found when the previous crop was canola (*Brassica napus* L.) (Miller, 2000). AM fungi colonisation of flax crops was about 3.5% greater when it followed wheat compared to canola in a study in Manitoba, Canada (Monreal *et al.*, 2011).

Impacts of crop rotation on the beneficial effects of AM fungi, such as enhancement of growth and P uptake improvement, have also been reported. For example, the growth, P uptake, and grain yield were enhanced in maize crops that succeeded mycorrhizal plants such as sunflower, soybean and potato to a greater extent than in maize plants cultivated after a fallow period or non-mycorrhizal plants such as rape and sugar beet (Arihara and Karasawa, 2000).

1.4.6. Organic vs conventional management

In the commercial agricultural sector, a combination of practices are implemented to achieve the goal of economically sustainable crop production. Conventional agricultural systems rely on the use of inputs such as fertilisers and pesticides to achieve crop yields, particularly from modern high yielding varieties. In contrast, organic systems exploit the natural agro-ecosystem through the use of organic matter inputs, carefully integrated pest management and diverse crop rotations; the use of inputs like mineral fertilisers and synthetic pesticides is not permitted in organic systems (Dimitrios *et al.*, 2017; Templer *et al.*, 2018). While organic systems may have a lower impact on the environment, yields in organic cereal production systems are significantly lower than those achieved in conventional production (Seufert *et al.*, 2012). In a recent study by Bilsborrow *et al.* (2013) this was linked to less efficient crop protection and fertilisation regimes used in organic production systems. While nitrogen supply is considered to be the main yield limiting factor, other nutrients (e.g. P and K) may also contribute to the yield gap between organic and conventional systems. Since organic systems rely on organic fertilisers and finely ground rock phosphate as P-inputs, there is considerable interest in optimising conditions for AM fungi with respect to availability and uptake of nutrients by plants (Suri *et al.*, 2011).

Organic agricultural systems may rely more on AM fungi than conventional management systems. Organic practices may improve the diversity and richness of the AM fungal community more than conventional management (Verbruggen *et al.*, 2010). For example, the impact of two different agricultural practices – organic farming with a 5-year crop rotation, and a conventional agricultural system with continuous high-input rice monocropping – on the biodiversity of AM fungi in the rhizosphere of rice was investigated by Lumini *et al.* (2011). This study found that the organic cropping system supported the preservation of a greater diversity of AM fungal communities in soil compared to the conventional system.

Contrasting agricultural management on farms may also affect the type of AM fungal association with host plants. Mutualistic associations of AM fungi can occur under organic farms where low levels of soil available P can increase benefits to host plants compared to conventional farms. However, poor mutualists from some strains of AM fungi may be selected by the high nutrient levels in conventional farm. This could result in weak AM fungal colonisation, with reduced nutrient uptake and increased consumption of carbon from the host plant, which leads AM fungi in the direction of parasitism (Johnson and Graham, 2013).

In conclusion, to meet the growing global demand for food, sustainable intensification is needed that will enhance crop yield and minimise adverse impacts on the environment. Production of P fertiliser from rock phosphate relies on a non-renewabel resource and causes severe environmental pollution. Crop management strategies including organic farming, reduced tillage and diversification of cropping systems have been suggested as strategies for sustainable intensification of agroecosystems. But there remain challenges with a yield gap when organic farming practices including both organic fertiliser and reduced tillage are implemented compared to conventional practices. AM fungi can contribute to several ecosystem services including enhanced plant nutrition, stabilization of soil structure and tolerance to abiotic and biotic stresses but AM fungal colonisation does not always correlate with crop yield. Therefore, it is important to quantify the effect of agricultural practices on AM fungal colonisation in crop roots and their populations in soil and functions in terms of P uptake and grain yield. With this knowledge, system approaches that combine organic farming and reduced tillage with other AM fungal promoting agricultural practices including crop diversification can be designed to enhance AM symbiosis and their functions in agricultural systems.

1.5. Aims and objectives

To design improved cropping systems it is essential to understand the impact of cropping systems as a whole, and the specific agronomic practices used in these systems (e.g. variety choice, fertilisation, tillage, and crop protection) on **(a)** viable AM-fungal spore density and **(b)** mycorrhizal colonisation of spelt roots. This information can then be used to design cropping systems that improve/optimise:

- inoculum density of indigenous AM-fungi in agricultural soils
- nutrient uptake/ crop yield via functional AM fungal association under sustainable agricultural practices.

The overall aim of this PhD project is to investigate the effects of cropping systems (organic versus conventional) and specific agronomic practices (fertiliser source, tillage intensity, varietal choice and crop protection practices) in spelt production systems on AM fungal parameters in the soil and roots and associated impacts on crop yields and P nutrition. The specific objectives of the project were to:

- Understand the role of AM fungi in agro-ecosystems and effects of different agricultural management practices on natural populations of AM fungi.
- Identify effect of cropping systems (organic versus conventional) on AM fungal root colonisation in a range of crop species, AM fungal spore density and diversity in the soil.
- Quantify the effects of fertilisation regimes (fertiliser input type and rate) and spelt variety on AM fungal colonisation of spelt roots, AM fungal spore density in the soil, crop yield and P nutrition.
- Quantify the effects of fertiliser type, spelt variety, tillage system and crop protection practices on AM fungal colonisation of spelt roots, AM fungal spore density in the soil, crop yield and P nutrition.

Chapter 2. Differences in Colonisation and Soil Spore Density for Arbuscular Mycorrhizal (AM) Fungi Between Crops Grown in Organic and Conventional Production Systems: A Meta-analysis.

2.1. Introduction

A meta-analysis is an approach involving numerical analysis of data extracted from studies that have been previously published. It is defined as "a set of statistical methods for combining effect sizes (summaries of the information in each study) across different data sets addressing the same research question" (Curtis and Wang, 1998; Allison and Goldberg, 2002). A meta-analysis approach can provide more powerful and precise estimates of overall effect across studies on the same topic. It can also examine heterogeneity among results of studies generated from different factors and contexts in which studies were undertaken (Koricheva and Gurevitch, 2013). Meta-analysis also allows exploration of publication bias which may distort scientific evidence (Koricheva and Gurevitch, 2013). Publication bias describes the tendency to submit or accept studies for publication dependent on strength or direction (significant or non-significant) of the study results (Jennions *et al.*, 2013). It can misrepresent the view of scientific evidence and it can cause overestimation of the overall effect (Jennions *et al.*, 2013). Patterns consistent with publication bias can be identified in meta-analyses by looking at the symmetry of relationships between the size and direction of study effects and the precision of the effects (funnel plots). Meta-analysis is therefore a powerful technique to understand how different crop management practices (organic versus conventional) affect AM fungal parameters such as root colonisation, soil spore density and species diversity of AM fungi when designing new cropping systems to meet the challenge of sustainable intensification. Therefore the aim of this chapter is to carry out a systematic review of the literature which describes differences in AM fungal parameters between crops grown in organic and conventional production systems; this will be used to develop an overview at the cropping system level of impacts of practices on AM fungi in a range of crops. The following research question was addressed:

What are the effects of organic versus conventional management systems on colonisation, spore density and diversity of AM fungi in different crop species, vegetables and perennial pastures?

2.2. Methodology

Systematic reviews incorporating meta-analysis have an explicit methodology designed to minimise bias and increase transparency in comparison to standard narrative reviews or meta-analyses without systematic searches, both of which are frequently biased (Stewart *et al.*, 2009).

Systematic reviews describe the methodology including details of systematic search and eligibility criteria (Koricheva and Gurevitch, 2013). Without a systematic approach to defining, obtaining and collecting data, the results obtained from meta-analysis may be imprecise or biased. Missing studies out of an analysis can lead to reduced precision of any statistical methods (Koricheva and Gurevitch, 2013). Choosing to select or analyse specific subgroups of studies can lead to biases if inclusion is dependent on the size or direction of effects (Koricheva and Gurevitch, 2013).

2.2.1. Eligibility criteria

2.2.1.1. Type of study included

Relevant for inclusion in the meta-analysis were peer-reviewed studies written in English and with data from two different types of study: (1) controlled field experiments in which samples were collected from experimental plots, and (2) farm surveys in which samples were collected from separate farms in the same country or region. They had to include the populations and outcomes defined below.

2.2.1.2. Types of participants

Results from experiments that included both organic and conventional treatments, or surveys comparing organic and conventional systems, were compared. For treatments and systems to be included as organic in this meta-analysis management must have been conducted according to the organic principles described (Sec. 1.1.3). In some studies, biodynamic practices were followed (BDYN). Biodynamic farming is a system of organic farming which follows the strict practices outlined by Rudolf Steiner, including planting crops according to lunar cycles and using life enhancing preparations (e.g. BDYN 500, BDYN 507) (Reganold, 1995; Turinek *et al.*, 2009; Uzunova and Atanasov, 2017). Certified biodynamic systems meet all the organic

system requirements, and were included under the organic category in this meta-analysis (Reganold, 1995).

Conventional systems included systems using exclusively mineral fertilisers and integrated conventional systems that used farmyard manures with mineral fertiliser as nutrient sources.

2.2.1.3. Types of outcome measures

The data for AM fungal parameters were collected from studies which measured (Table 2.3) (1) spore density in the soil, such as the number of spores and mycorrhizal inoculum potential (MIP) (2) species diversity, such as the species diversity index (Shannon index, H') and species richness, and (3) colonisation by AM fungi which included arbuscule colonisation and total root colonisation of AM fungi reported in different terms as either length of root colonised (%) or root colonisation (%). In cases where presence of all three AM fungal structures (hyphae, vesicles, arbuscules) were presented, (e.g. Galvan et al., 2009) only arbuscule colonisation was included in the meta-analysis. This is because arbuscule colonisation has been considered as an important indicator for AM fungal colonisation (Galvan *et al.*, 2009; Lehman *et al.*, 2019; Ren *et al.*, 2019) and it is the structure responsible for exchange of nutrients and carbon between both partners (Luginbuehl and Oldroyd, 2017).

AM fungal soil spore density, species diversity and root colonisation were selected in the weighted meta-analysis study because they are important AM fungal response variables which show a strong response to agriculture management practices (Martinez and Johnson, 2010; Lehman *et al.*, 2019). They also reflect the standard techniques of AM fungal measurements to determine the effects of these practices on AM fungi in agricultural soil (Martinez and Johnson, 2010; Knerr *et al.*, 2018). For example, AM fungal soil spore and soil inoculum potential (AM fungal propagules e.g. spores, soil hyphae fragments and infected root pieces in soil) are important AM fungal parameters because they are effective response variables for distinguishing agricultural management treatments. Although, the methodological details of AM fungal root colonisation differed considerably among studies, it is the most frequently measured AM fungal response variable that may be related to crop performance (e.g. yield, biomass and nutrient uptake) (Treseder, 2013; Pellegrino *et al.*, 2015). Furthermore, species diversity index (Shannon index, H') and species richness were included in the meta-analysis

because they are highly recommended and commonly used when analysing microbial species diversity (Purin *et al.*, 2006; Trivedi *et al.*, 2016).

Other measures of AM fungal presence and diversity such as AM fungal soil hyphae length, AM fungal biomass in soil and species diversity of AM fungi in roots are also important standard techniques of AM fungal measurements (Martinez and Johnson, 2010; Knerr *et al.*, 2018; Lehman *et al.*, 2019). Even though, these AM-fungal parameters were extracted from studies, they were excluded later from the analysis due to insufficient data to conduct a statistical analysis.

2.2.2. Search strategy for the identification of studies

2.2.2.1. Search strategy

The literature search strategy and meta-analysis protocol were conducted according to published methods from Baranski *et al.* (2014). The literature search was conducted using three different online databases, namely Web of Science, Google Scholar and Scopus. The following search terms were used: "arbuscular mycorrhiza*" AND ("organic*" OR "biologic*" OR "biodynamic") AND "conventional*". The star sign (*) is a boolean truncation used to include different variations of the initial term of interest. Publications in different languages, years, terms for AM fungi (vascular or arbuscular AM fungi) and published in peer-reviewed journals were collected.

2.2.2.2. Search screening

All papers considered for meta-analysis were collected in an EndNote library. All duplicates were removed and the remaining publications were examined according to the eligibility criteria. The search and screening process (number of papers found, and included and excluded papers) was illustrated (Fig 2.1) on a PRISMA flow chart (Moher *et al.*, 2009).

The paper screening was conducted in two stages:

a) The relevance of the paper was assessed by reading the full title and abstract. The studies having no abstract available were included in stage two. These searches yielded n=145

published studies. After title and abstract reading of these references, many studies were rejected (n=90) because they did not meet certain criteria to be included. For instance, they did not report keyword terms (e.g. both systems organic or conventional and mycorrhizal fungi). Some studies were excluded because they did not compare the whole crop management systems, as those studies compared organic vs conventional fertilisers impacts on AM fungal parameters. The study list was refined to (n=55) eligible studies based on these criteria and all these references in English language.

b) The full texts of all the studies were read and checked with regard to whether they reported data within the text, as graphs or in tables. The decisions about final inclusion in the meta-analysis were made on the basis of the eligibility criteria mentioned above. The reasons for each exclusion of a study were provided.

For example, after full text of studies checking still more studies were excluded from the meta-analysis depending on certain criteria mentioned above. As all studies should present data that showed the effect of different crop management systems (organic & conventional) on AM fungal response variables including soil spore density, soil inoculum potential, species diversity and root colonisation. Some studies were excluded because they did not include suitable data. Furthermore, some studies were excluded from the meta-analysis because they focused on comparing effects of organic vs conventional management on AM fungal parameters which were not included in the meta-analysis list of response variables. As another example, Lee and Kim (2011) measured one AM fungal parameter which was AM fungal biomass (16:1n-5cis) and this parameter was excluded from the analysis because the data was insufficient to conduct a statistical analysis. Additional studies were excluded from the meta-analysis because they compared different systems and they did not report about organic vs conventional systems. For example, some studies compared low-input vs conventional systems or conventional vs grassland or no-fertiliser; low-input is a different management system from organic as its aim is to reduce the use of synthetic chemicals such as mineral fertilisers and pesticides for improving sustainable agriculture (Hartmann *et al.*, 2014).
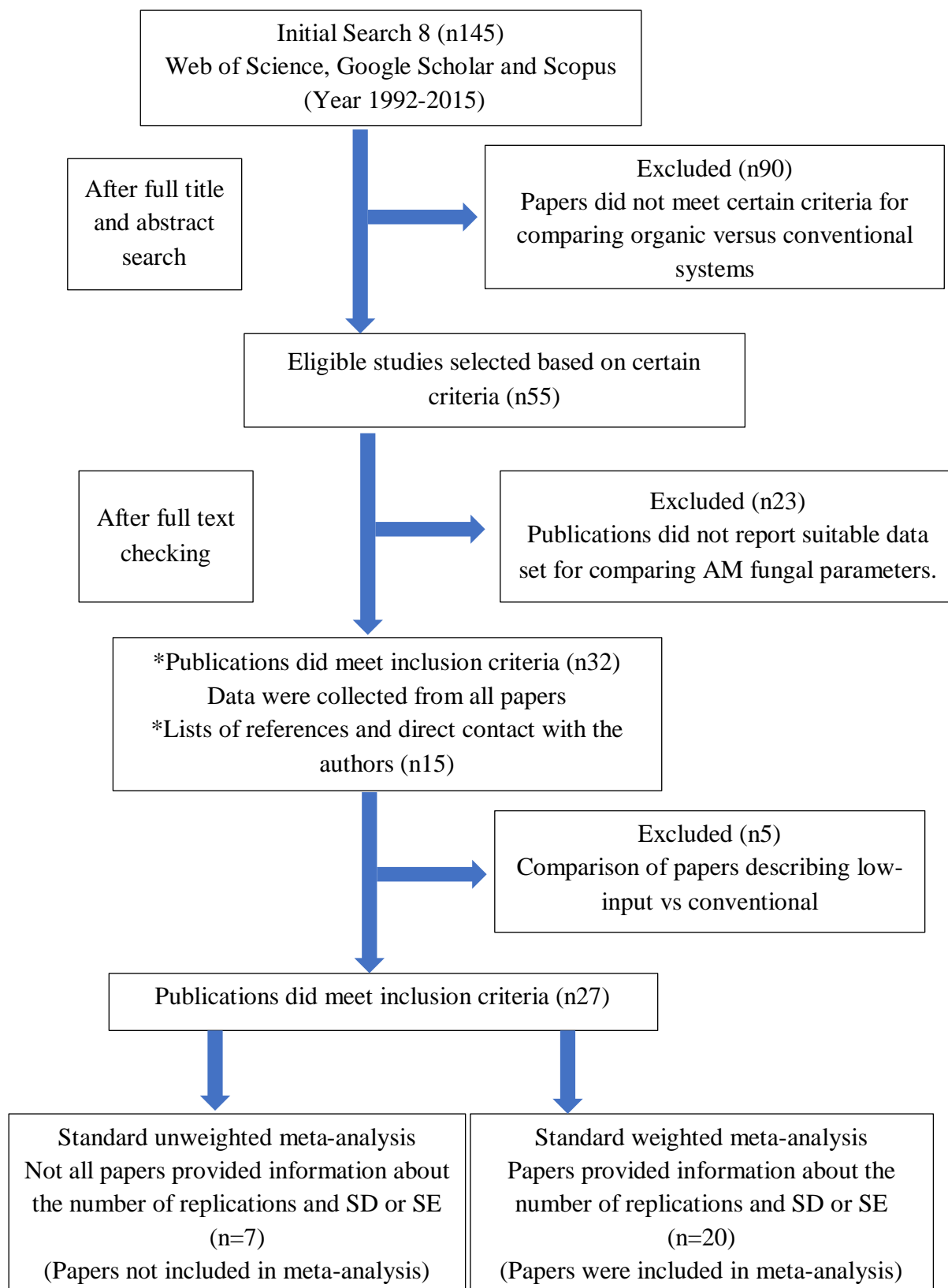
Fig 2.1. A PRISMA flow chart for summarising the search and selection protocols used to identify papers included in the meta-analysis. Review carried out by one reviewer and data extraction carried out by two reviewers.

The systems which were included in the meta-analysis may be described using different terms. For example, conventional and organic management with different levels of intensity were included in the meta-analysis with respect to the level of fertilisers (high or low). In addition, organic was termed as organic or biodynamic, whereas treatments with mineral fertilisers were termed as conventional or integrated farm (farmyard manures FYM plus exclusive mineral fertilisers).

Finally, the study list was refined to (n=27) eligible studies as they divided into type of meta-analysis. The first was standard weighted meta-analysis that included (n=20) publications which provided information about the number of replications and measures of variability (standard deviation and standard error). The second standard unweighted meta-analysis which included (n=7) publications that did not provide this information. The standard weighted meta-analysis (20 publications) was only used in this meta-analysis study.

2.2.3. Data extraction and management

Extraction of the data and additional information including crop, country, experimental year, location, weed control and fertilisers from collected publications were related to the main objectives, study types, participant types and outcome measurements. The numerical data used in the statistical analysis included, for each agricultural management system, the values of (1) the AM fungal parameter means, (2) measures of variability (standard error and standard deviation) and (3) sample size.

Whenever missing data were detected in a paper, such as for the number of replications or variability measures, the authors were contacted by email in order to provide those data (Higgins and Green, 2011). Data from text and tables were collected directly, while numerical values were extracted from figures from a printout using a ruler.

Additional information extracted from each paper included study type (field, greenhouse/glasshouse or survey), crop type, crop rotation sequence, soil characteristics (soil type, soil pH and available P), type of tillage, crop protection, weed control, fertilisation regime and type and unit of measured parameters. For each study type the sample size was considered to be (1) the number of field replications for experimental field studies, (2) the number of sites sampled or the number of farms under different management systems for farm surveys. Some

data reported at different growth stages (tillering and flowering) or different sampling times (e.g. week, months or sampling season). The flowering stage and last sampling (e.g. last week, month or season) were only included in the meta-analysis. Some observations of AM fungal parameters were excluded from analysis due to insufficient data to conduct a statistical analysis.

2.2.4. Characterisation of the data

Because level of P supply is hypothesized to influence AM fungal populations and colonisation, organic and conventional systems were classified to account for P supply. The organic system in each trial was classified into one of four types (Table 2.1). The organic farms that applied animal manure based on production by livestock, expressed as livestock units (LU) were classified depending on livestock units (LU). Where no information was provided regarding the livestock unit, the applied P level was used for the classification of organic systems.

Table 2.1. Organic system classes according to livestock unit (LU ha^{-1})and where no information was provided regarding the livestock unit, the applied P level (kg P ha^{-1}) was used for the classification of both organic systems (biodynamic and organic). These organic systems were classified as follows: organic high (ORG_H), biodynamic high (BDYN_H) (livestock unit equal to 1.4 or applied P level > 30 kg P ha^{-1}), organic low (ORG_L) and biodynamic low (BDYN_L) (livestock unit equal to 0.7 or applied P level < 30 kg P ha^{-1}). In cases where no information was provided regarding the livestock unit and applied P level, organic and biodynamic systems were classified as high systems (ORG_H and BDYN_H).

Organic System	Symbol	Livestock unit (LU ha^{-1})	P level (kg P ha^{-1})	#
Organic high	ORG_H	1.4	> 30	68
Organic low	ORG_L	0.7	< 30	37
Biodynamic high	BDYN_H	1.4	> 30	24
Biodynamic low	BDYN_L	0.7	< 30	13
Total				142

Number of observations.

These organic systems were as follows: organic high (ORG_H), biodynamic high (BDYN_H) (livestock unit equal to 1.4 or applied P level > 30 kg P ha^{-1}), organic low (ORG_L) and biodynamic low (BDYN_L) (livestock unit equal to 0.7 or P level < 30 kg P ha^{-1}). In cases where no information was provided regarding the livestock units and applied P level, organic and biodynamic systems were classified as high systems (ORG_H and BDYN_H).

Furthermore, the four classes of conventional farming system (Table 2.2) were as follows: conventional system exclusively applying mineral fertilisers was divided as conventional high (CONV_H) (applied P level > 30 kg P ha^{-1}) and conventional low (CONV_L) (applied P level < 30 kg P ha^{-1}), while integrated conventional systems applying both farmyard manures FYM and exclusively mineral fertilisers were divided into conventional-FYM -high (CONV_FYM_H) (applied P level > 30 kg P ha^{-1}) and conventional-FYM -low (CONV_FYM_L) (applied P level < 30 kg P ha^{-1}). Conventional systems with no information provided regarding the applied P level were classified as high systems (CONV_H).

Table 2.2. Conventional system classes according to applied P level (kg P ha^{-1}). Both conventional farming systems were characterised as follows: conventional system with exclusively applying mineral fertilisers was classified as conventional high (CONV_H) (applied P level > 30 kg P ha^{-1}) and conventional low (CONV_L) (applied P level < 30 kg P ha^{-1}). While the integrated conventional system with applying both farmyard manures (FYM) and exclusive mineral fertilisers was classified as conventional-FYM-high (CONV_FYM_H) (applied P level > 30 kg P ha^{-1}) and conventional-FYM-low (CONV_FYM_L) (applied P level < 30 kg P ha^{-1}). Conventional systems with no information provided regarding the applied P level were classified as conventional high systems (CONV_H).

Conventional system	Symbol	P level (kg P ha^{-1})	#
Conventional high	CONV_H	> 30	57
Conventional low	CONV_L	< 30	53
Conventional-FYM-high	CONV_FYM_H	< 30	20
Conventional-FYM-low	CONV_FYM_L	> 30	12
Total			142

Number of observations.

There are a range of different techniques used to assess AM fungal populations, diversity and colonisation. In this meta-analysis AM fungal parameters were grouped into three classes (Table 2.3). Spore density and mycorrhizal inoculum potential (MIP) in soil were classified as one class named soil spore density. This is because both measurements reflect the density of AM fungal propagules in the soil. As mycorrhizal inoculum potential (MIP) is defined as a bioassay experiment (trap culture) to measure total density of AM fungal propagules in the soil from field experiments including spores, soil hyphae and infected roots (Daniels and Skipper, 1982; Ohtomo *et al.*, 2018; Lehman *et al.*, 2019).

The root colonisation class included both arbuscule colonisation and total root colonisation. This is because both AM fungal colonisation measurements reflect the abundance of AM fungi inside crop roots.

Measures of diversity included the molecular measurements such as polymerase chain reaction (PCR) based methods to amplify DNA from soil and morphological description of species provided by the International Collection of (Vesicular) AM fungi (http://invam.caf.wvu.edu) converted to indices of diversity e.g. Shannon's diversity index (H') and species richness. This resulted in 142 observation pairs of AM fungal parameters as illustrated in (Table 2.3).

Table 2.3. Classes of response (parameters) of AM fungi.

Biological parameter reported	AM fungal Parameter class	*Class no.	#
Number of spores in soil	soil spore density	1	74
A bioassay: Mycorrhizal inoculum potential (MIP)	soil spore density	1	
AM fungal richness	diversity	2	15
Species diversity Index (H')	diversity	2	
Species richness	diversity	2	
Arbuscule colonisation (%)	root colonisation	3	53
Root colonisation (%)	root colonisation	3	
Total			142

\# Number of observations

*Class number of AM fungal parameter.

There were several observation pairs (different treatment comparisons) within each study for each AM fungal parameter including soil spore density, species diversity and root colonisation. The total number of observation pairs of AM fungal parameters within a study depended on different factors such as system, P supply level, P fertiliser type, crop type, experimental year, location, and AM fungal measurement used.

For each observation pair within a study, descriptive information about the management and environmental conditions were recorded. These factors were main crop, crop rotation class and fertilisation management classes. The main crops were classified into (11) classes, with wheat being the most common crop (48 observations) as illustrated in (Table 2.4).

Table 2.4. Classes of crop types cultivated in organic and conventional farming for studies included in the standard weighted meta-analysis.

Crop type	Crop type class	Class no.	#
Wheat, winter wheat	Wheat	1	48
Clover, red clover, and white clover	Clover	2	9
Grass	Grass	3	8
Red pepper, and Onion	Vegetables	4	6
Rice	Rice	5	1
Potato	Potato	6	1
Maize	Maize	7	12
Vetch-rye	Cover crop	8	4
grass-clover	perennial pastures	9	4
Winter-wheat/vetch-rye	[a] Mixed	10	12
Apple orchards	Apple	11	36
ns	ns	12	1
Total			142

\# Number of observations for all AM fungal parameters (Root colonisation, spore density and diversity). [a] Means of two observations in winter wheat and two observations in vetch-rye; ns, there was no information about crop type in paper.

Crop rotations were allocated to one of four types as used in Cooper *et al.* (2016) i.e. horticulture with ley periods, arable with ley periods, intensive arable (i.e. no ley crops) and ley crop. A ley period was defined as a full season of a soil building crop such as clover/grass. Whereas fertiliser management classes included organic fertiliser type classes and chemical fertiliser classes in conventional systems, including P fertiliser classes, N fertiliser classes and K fertiliser classes. Organic fertiliser types were assigned to one of seven classes: composted manure, composted manure and leaf mulch, composted manure and slurry, green manure, mixed manure, reactive rock phosphate (RP), and where no information was provided, organic fertiliser was classified as no organic fertiliser. Meanwhile, nitrogen, phosphorus and potassium fertilisers were classified into three levels (low, medium and high). Additional management factors were classified such as weed control classes and pest protection classes in conventional systems. Weed control and disease protection were characterised as yes/no depending on whether they were applied or not.

Environmental variables were assigned as factors, including soil pH and soil type. Soil types were classified into three groups according to USDA texture classes (Soil Survey Division Staff, 1993): light, which included all soils with greater than 50% sand and less than 40% clay (loamy sand, sandy clay loam, sand, and sandy loam); heavy, which included all soils with a

clay content higher than 40% (clay, sandy clay and silty clay); and loamy, which included other soils (clay loam, silt, silty clay loam, silty loam and medium loam). Meanwhile, soil pH was divided into three classes: acidic, neutral and alkaline. In addition, the data were characterised by aspects of the experimental design: field experiment or survey data (FE/SR); repeated measures (yes/no); and measures of variability available (yes/no). All outdoor studies were categorised as "field", including trap cultures when they were used to measure levels of AM fungal propagules in the soil.

2.2.5. Data synthesis

Effect sizes were generated to summarise the results of each study using mean effects, standard error, standard deviation, sample size and number of replications. For each study, the effect size (ES) of the standardised mean difference (SMD) was calculated together with its weight derived from the sample variance and the number of samples. These individual study effects were then pooled using random effects meta-analysis. A random effects model includes both the variance of estimates within studies (due only to sampling variance or sampling error) as well as the variance of effects between studies (due to random differences in their true effect sizes) (López-García *et al.*, 2014). The model was used for all analyses that accounts for both between study variability (I^2) and within study sampling variance. Heterogeneity tests were carried out using (Q statistics and I^2 statistics) on the overall effect size. The I^2 index referees to percentage of total heterogeneity, while Q referees to total heterogeneity (Rosenberg, 2013). The graphical format used to display these effects was Forest plots which display overall results of effect size from statistical results of a number of scientific studies addressing the same question and a visual representation of the amount of study heterogeneity (Palmer *et al.*, 2008).

Mixed effect models were used to explore the impact of including multiple effects within individual studies, such as different crop type, system classes (organic and conventional), experimental year, crop rotation, fertiliser type.

The risk of publication bias was assessed by visual inspection of the funnel plot and Egger's test of funnel plot asymmetry (Palmer *et al.*, 2008). Publication bias assessment is used to assess the quality and reliability of the magnitude of the effect size in meta-analysis because it is impossible to have located every study to answer the same question. The time of publication, language of publication, country and journal can all affect the chance that a study is located for

a meta-analysis. Also, the publication bias can estimate from published studies if there has been a failure to report some results which can generate a systematic bias in assessment of effect sizes (Cassey *et al.*, 2004; Chan *et al.*, 2004). Some published studies are based on statistical tests applied inappropriately, or only report a subset of the data. Even though the scientific literature is massive, the location of a study can affect its visibility and this can rely on the relative reputation of the research group that conducted it or the range to which their study is cited by others (Koricheva and Leimu, 2005). Some data has been analysed but never written up or may be research which is publicly available such as conference papers, theses, or governmental reports, all of which can affect publication bias of meta-analyses.

Meta-analysis calculations were conducted using the statistical software R (Wickham and 2009) and the package "metafor" (Viechtbauer, 2010).

2.3. Results

2.3.1. AM fungal root colonisation

The comparison of AM fungal colonisation between organic and conventional crop management practices was illustrated in a forest plot (Fig 2.2). The diamond at the bottom of the plot shows the standardised mean difference (SMD) calculated using a random effect model. It is displayed to the right side (positive side) of the dashed line of null effect illustrating that AM fungal colonisation was slightly favoured by organic farming management irrespective of crop type or other variables, *(p<0.0001)*. AM fungal colonisation was higher in organic than conventional crop roots and the SMD±SE was (1.71 ± 0.14) with a 95% confidence interval (CI) of 1.43 to 1.99 (Fig 2.2). Each observation pair (organic vs conventional) within each study is represented as a horizontal line with a black box in the middle with bigger boxes showing a larger sample size than smaller boxes, reflecting the weight of each observation pair. The position of the box shows the corresponding effect of the study and the 95% confidence interval (CI) boundaries of the estimate are represented by the horizontal line. For the observation pairs which have their horizontal line of 95% CI crossing the line of null effect there is no statistically significant difference between organic and conventional systems. Most studies had a mean SMD of greater than one, but in many cases the 95% confidence interval lines crossed the line of null effect (Table 2.5 and Fig 2.2). However, small studies showed larger effects than large

studies, leading to the suspicion of publication bias. Therefore, the overall effect may be smaller than suggested and probably over-estimated.

Many factors can affect the results of the studies such as researcher bias and for this reason, publication bias was tested in this meta-analysis. The I^2 (I-squared) statistic was used to assess the consistency of the papers analysed illustrating that the heterogeneity was low (Q=123.73, df=52, $p<0.0001$; I^2=53.21%) between observation pairs of studies comparing AM fungal colonisation over both systems.

The meta-analysis (Table 2.5 and Fig 2.2) revealed that there were three studies with non-significant results (Ryan, 1998; Nelson *et al.*, 2011a; Meyer *et al.*, 2015). The meta-analysis also detected that there were six studies with statistically significant results as their horizontal line of 95% CI did not cross the vertical line of null effect (Ryan *et al.*, 1994; Dann *et al.*, 1996; Eason *et al.*, 1999; Ryan *et al.*, 2000; Lumini *et al.*, 2011; Dai *et al.*, 2014) (Table 2.5 and Fig 2.2).

However, many studies have presented multiple observation pairs with a range of significant and non-significant effects (Table 2.5 and Fig 2.2), such as (Mader *et al.*, 2000; Hildermann *et al.*, 2010; Ryan and Kirkegaard, 2012; Bedini *et al.*, 2013). For example, the meta-analysis showed that the study of Galvan *et al.* (2009) presented two effects; the first effect indicated that the organic system tended to have lower, but non-significant, AM fungal colonisation compared to conventional farming, whereas, the second observation pair from the same study indicated that the organic system enhanced AM fungal colonisation and the effect was statistically significant (Table 2.5 and Fig 2.2).
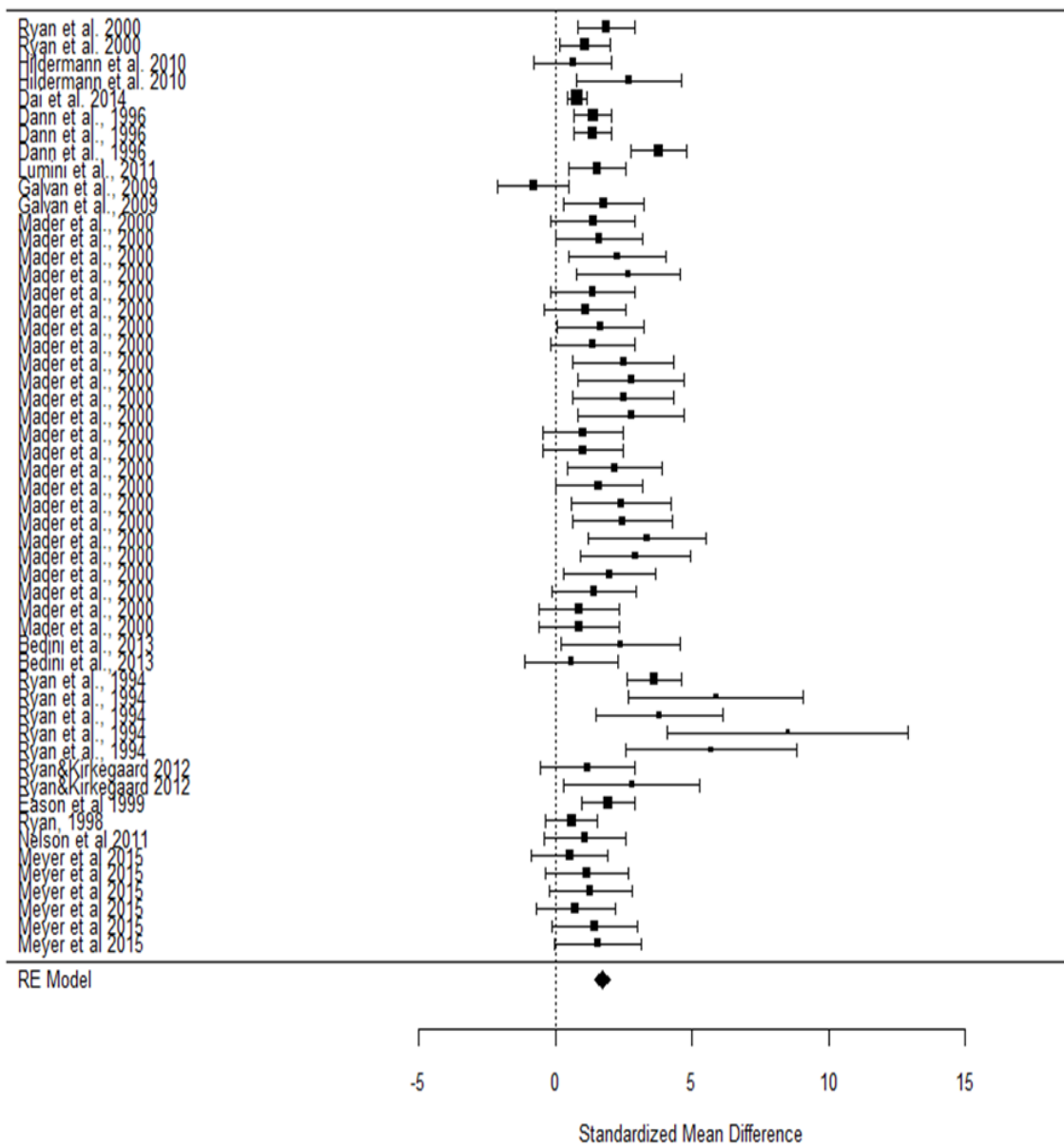
Fig 2.2. Forest plot showing the results of the comparison of AM fungal root colonisation in organic and conventional systems across a range of crop types showing standardised mean difference (SMD; black boxes), 95% confidence intervals (95% CI; horizontal lines), and line of null effect (dashed vertical line) with a value of zero showing no difference between organic and conventional systems. The overall estimated SMD from a random-effects (RE) model for all studies is indicated by the black diamond at the bottom of the figure. Full details of characteristics for each observation pair are shown in (Table 2.5). Heterogeneity was assessed across all the observation pairs of studies by I^2 test (I^2 >50%).

Analysis using mixed effects models indicated that crop type was not a significant factor affecting AM fungal root colonisation. However, a qualitative analysis of crop effects on patterns of AM fungal root colonisation (Table 2.5) showed that there was a trend towards larger effects for grain crops especially wheat compared to fruit and vegetables in organic compared to conventional farming (Table 2.5). The highest significant SMD (8.51, CI: 4.11, 12.90, p= 0.0001) was estimated for wheat in the Ryan *et al.* (1994) study (Table 2.5). Additionally, perennial pastures including clover and grass tended to present higher AM fungal root colonisation level than other crop types in organic than conventional farming (Table 2.5). The highest significant SMD with 95%CI was estimated for perennial pastures 2.78 (0.84, 4.73), p=0.005 in Mader *et al.* (2000) study and clover 2.80 (0.31, 5.29), p=0.0278 in Ryan and Kirkegaard (2012) study (Table 2.5).

A further mixed effects model analysis was conducted to identify multiple effects of different factors including, study type and different crop management practices such as fertiliser type, crop rotation, weed control, pest protection as well as different environmental conditions such as soil properties (e.g. soil pH, soil type) (Hedges *et al.*, 2010). However, none of these models provided clear results on effects of these factors on AM fungal root colonisation.

Strong funnel plot asymmetry consistent with publication bias (Fig 2.3) was detected (p<0.001) among observation pair results within studies for AM fungal root colonisation. Each point shows the mean effect size for a single observation pair result in the study and the funnel plot usually shows observation pair results of larger studies (the most precise estimates) clustered around the top of the figure plot whereas, results of smaller studies (less precise estimates) distribute across the base of the funnel plot. The funnel plot below shows that the most points were clustered around the top of the funnel plot indicating that these are results of larger studies while the points around the base of the plot represented results from smaller studies. The middle solid line shows the overall effect of standardised mean differences from the weighted meta-analysis. The two dotted lines either side represent the pseudo 95% confidence intervals, while the points outside the range of confidence intervals of the funnel plot indicate that the mean effect size of these observations of studies were overestimated.

Table 2.5. References for studies, characteristics of each observation pair and standardised mean difference (SMD) with 95% confidence intervals (95% CI) and *p*-value for comparison of AM fungal root colonisation in organic and conventional systems for studies included in the standard weighted meta-analysis.

Paper	Country	EXP. Year	S. Type	Crop type	ORG class	CONV class	SMD (95% CI)	*p*-value
Ryan et al., 2000	Australia	1993-1996	SR	Clover	BDYN_L	CONV_L	1.86 (0.81, 2.91)	0.0005
Ryan et al., 2000	Australia	1993-1996	SR	Grass	BDYN_L	CONV_L	1.08 (0.14, 2.02)	0.0241
Hildermann et al., 2010	Switzerland	2006-2007	FE	Wheat	BDYN_L	CONV_H	0.65 (-0.77, 2.00)	0.3677
Hildermann et al., 2010	Switzerland	2006-2007	FE	Wheat	BDYN_H	CONV_H	2.71 (0.79, 4.63)	0.0057
Dai et al., 2014	Canada	2009-2011	SR	Wheat	ORG_H	CONV_L	0.81 (0.46, 1.16)	<.0001
Dann et al., 1996	Australia	1992	FE	Wheat	ORG_L	CONV_L	1.38 (0.69, 2.06) [a]	<.0001
Dann et al., 1996	Australia	1992	FE	Wheat	ORG_L	CONV_L	1.36 (0.67, 2.05) [b]	0.0001
Dann et al., 1996	Australia	1992	FE	Wheat	ORG_L	CONV_L	3.78 (2.75, 4.82) [c]	<.0001
Lumini et al., 2011	Italy	2003	FE	Rice	ORG_L	CONV_H	1.54 (0.49, 2.59)	0.0042
Galvan et al., 2009	Netherlands	2004	SR	Vegetables	ORG_H	CONV_H	-0.81(-2.10, 0.48) [d]	0.2174
Galvan et al., 2009	Netherlands	2004	SR	Vegetables	ORG_H	CONV_H	1.76 (0.30, 3.22) [e]	0.0182
Mader et al., 2000	Switzerland	1989-1990	FE	Cover crop	BDYN_H	CONV_H	1.39 (-0.16, 2.93)	0.0783
Mader et al., 2000	Switzerland	1989-1990	FE	Cover crop	ORG_H	CONV_H	1.60 (0.01, 3.19)	0.0492
Mader et al., 2000	Switzerland	1989-1990	FE	Cover crop	BDYN_H	CONV_FYM_H	2.28 (0.50, 4.07)	0.012
Mader et al., 2000	Switzerland	1989-1990	FE	Cover crop	ORG_H	CONV_FYM_H	2.67 (0.77, 4.58)	0.006
Mader et al., 2000	Switzerland	1990-1991	FE	Wheat	BDYN_H	CONV_H	1.37 (-0.17, 2.91)	0.0806
Mader et al., 2000	Switzerland	1990-1991	FE	Wheat	ORG_H	CONV_H	1.10 (-0.39, 2.59)	0.1475
Mader et al., 2000	Switzerland	1990-1991	FE	Wheat	BDYN_H	CONV_FYM_H	1.65 (0.04, 3.25)	0.044
Mader et al., 2000	Switzerland	1990-1991	FE	Wheat	ORG_H	CONV_FYM_H	1.37 (-0.17, 2.91)	0.0806
Mader et al., 2000	Switzerland	1993	FE	perennial pastures	BDYN_H	CONV_H	2.49 (0.65, 4.34)	0.0082
Mader et al., 2000	Switzerland	1993	FE	perennial pastures	ORG_H	CONV_H	2.78 (0.84, 4.73)	0.005
Mader et al., 2000	Switzerland	1993	FE	perennial pastures	BDYN_H	CONV_FYM_H	2.49 (0.65, 4.34)	0.0082
Mader et al., 2000	Switzerland	1993	FE	perennial pastures	ORG_H	CONV_FYM_H	2.78 (0.84, 4.73)	0.005

Table 2.5. (continued)

Paper	Country	EXP. Year	S. Type	Crop type	ORG class	CONV class	SMD (95% CI)	*p*-value
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	BDYN_H	CONV_H	1.01 (-0.46, 2.48)	0.1796
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	ORG_H	CONV_H	1.02 (-0.45, 2.49)	0.1747
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	BDYN_L	CONV_H	2.17 (0.42, 3.91)	0.015
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	ORG_L	CONV_H	1.58 (-0.01, 3.17)	0.051
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	BDYN_H	CONV_FYM_H	2.40 (0.58, 4.21)	0.0097
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	ORG_H	CONV_FYM_H	2.46 (0.63, 4.30)	0.0086
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	BDYN_L	CONV_FYM_H	3.36 (1.21, 5.51)	0.0022
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	ORG_L	CONV_FYM_H	2.94 (0.94, 4.94)	0.004
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	BDYN_L	CONV_FYM_L	1.99 (0.29, 3.68)	0.0214
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	ORG_L	CONV_FYM_L	1.40 (-0.15, 2.95)	0.0761
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	BDYN_H	CONV_FYM_L	0.87 (-0.58, 2.32)	0.2409
Mader et al., 2000	Switzerland	1989-1990	FE	Mixed	ORG_H	CONV_FYM_L	0.87 (-0.58, 2.32)	0.2403
Bedini et al., 2013	Italy	2007	FE	Maize	ORG_L	CONV_H	2.40 (0.23, 4.58) [f]	0.0304
Bedini et al., 2013	Italy	2007	FE	Maize	ORG_L	CONV_H	0.58 (-1.14, 2.31) [g]	0.5077
Ryan et al., 1994	Australia	1992	FE	Wheat	ORG_L	CONV_L	3.62 (2.61, 4.62) [h]	<.0001
Ryan et al., 1994	Australia	1992	FE	Wheat	ORG_L	CONV_L	5.89 (2.69, 9.09) [i]	0.0003
Ryan et al., 1994	Australia	1992	FE	Wheat	ORG_L	CONV_L	3.79 (1.48, 6.11) [j]	0.0013
Ryan et al., 1994	Australia	1992	FE	Wheat	ORG_L	CONV_L	8.51 (4.11, 12.90) [k]	0.0001
Ryan et al., 1994	Australia	1992	FE	Wheat	ORG_L	CONV_L	5.70 (2.58, 8.82) [l]	0.0003
Ryan&Kirkegaard, 2012	Australia	1995	SR	Clover	ORG_L	CONV_L	1.18 (-0.56, 2.91)	0.1835
Ryan&Kirkegaard, 2012	Australia	1995	SR	Clover	BDYN_L	CONV_L	2.80 (0.31, 5.29)	0.0278
Eason et al., 1999	UK	1993	FE	Clover	ORG_H	CONV_H	1.92 (0.95, 2.89)	0.0001
Ryan, 1998	Australia	1994	SR	Clover	ORG_L	CONV_H	0.60 (-0.34, 1.55)	0.2122
Nelson et al., 2011	Canada	2005-2006	FE	Wheat	ORG_L	CONV_L	0.60 (-0.34, 1.55)	0.2122

Table 2.5. (continued)

Paper	Country	EXP. Year	S. Type	Crop type	ORG class	CONV class	SMD (95% CI)	*p*-value
Meyer et al., 2015	South Africa	2006-2011	FE	Apple	ORG_H	CONV_L	0.52 (-0.89, 1.93)	0.4672
Meyer et al., 2015	South Africa	2006-2011	FE	Apple	ORG_H	CONV_L	1.15 (-0.34, 2.65)	0.1315
Meyer et al., 2015	South Africa	2006-2011	FE	Apple	ORG_H	CONV_L	1.28 (-0.24, 2.80)	0.1
Meyer et al., 2015	South Africa	2006-2011	FE	Apple	ORG_H	CONV_L	0.74 (-0.69, 2.17)	0.3109
Meyer et al., 2015	South Africa	2006-2011	FE	Apple	ORG_H	CONV_L	1.44 (-0.12, 2.99)	0.0698
Meyer et al., 2015	South Africa	2006-2011	FE	Apple	ORG_H	CONV_L	1.57 (-0.01, 3.16)	0.0519

Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic farming. ORG class and CONV class refer to input intensity class as described in (Table 2.1 and 2.2). EXP. Year, experimental year; S. Type, study type; SR, survey; FE, experimental field.

Fertility treatments: [a, i] (T1) 0 kg P ha^{-1}; [b, j] (T2) 30 kg ha^{-1} as reactive rock phosphate (RP) applied in both organic and conventional systems; [c, k] (T3) 30 kg ha^{-1} as RP applied in organic vs 30 kg ha^{-1} as superphosphate applied in conventional system ; [h] (T4) 122 kg ha^{-1} RP applied in both organic and conventional systems; [l] (T5) 30 kg ha^{-1} as RP applied in organic vs 30 kg ha^{-1} as superphosphate plus nitrogen fertiliser applied in conventional system.

Location: [d] Fevoland; [e] Zeeland; [f] old organic (established since 1991) vs conventional farming; [g] young organic (integrated farming from 1991 to 2000 and converted into organic management since 2001) vs conventional farming.

The plot displays the precision of the studies as standard error of mean (Y-axis) while the effect size of the mean is plotted on the X-axis. The figure shows that the larger studies were more precise than smaller studies. Also, the multiple effects of observation pair results within the study of Mader *et al.* (2000) are shown in the dotted curved line of the funnel plot, which has a slight lack of symmetry.
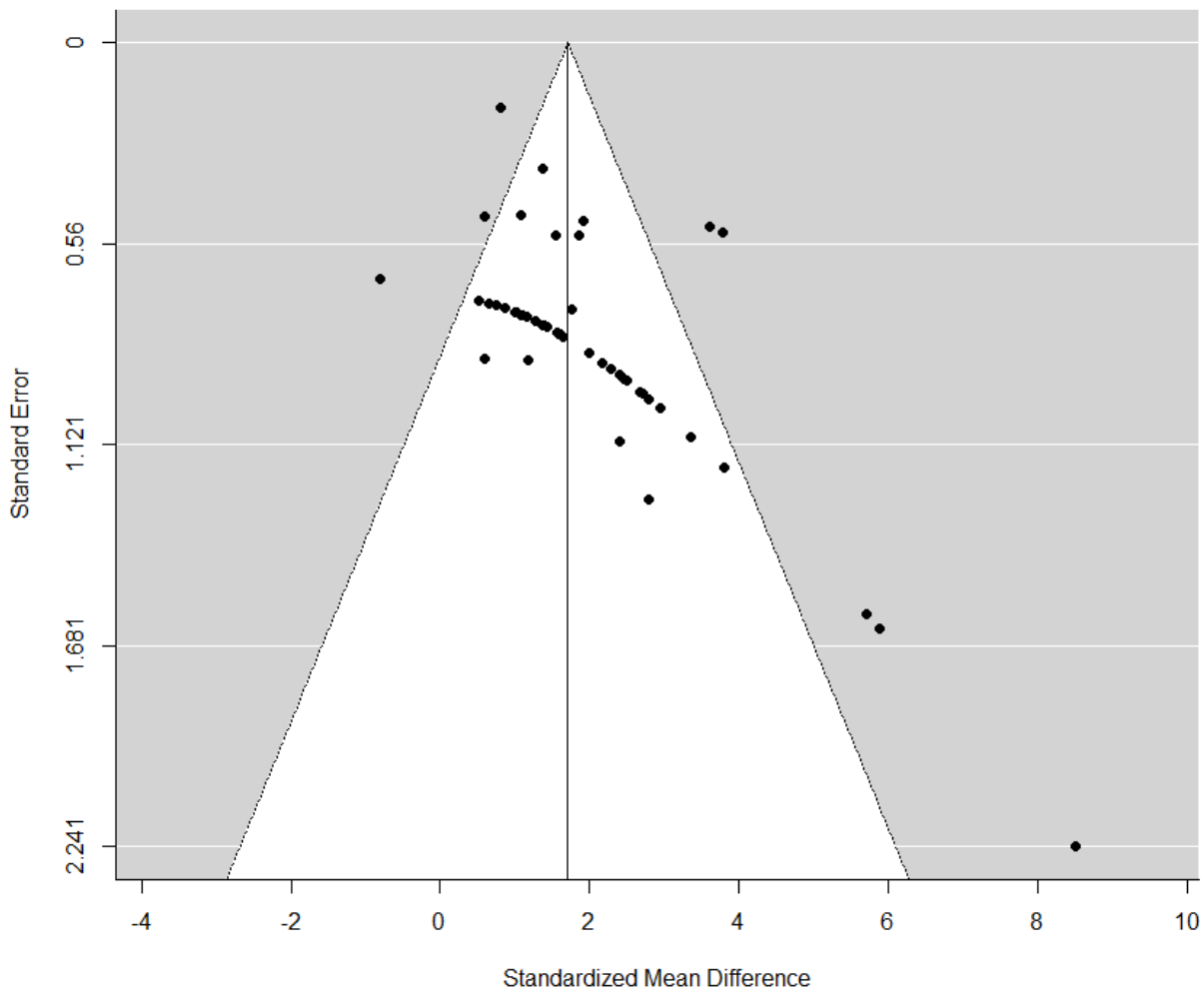
Fig 2.3. Funnel plot to visually detect the presence of publication bias among the observation pairs of studies in an organic and conventional system comparison on AM fungal colonisation (studies included in the standard weighted meta-analysis).A mixed effects meta-regression model was used to detect publication bias.

2.3.2. AM fungal soil spore density

According to the summary effects of SMD, it is clear that organic systems have significantly greater ($p<0.001$) AM fungal soil spore density than conventional systems, as most of the studies are located on the positive side of the line of null effect and the overall SMD$\pm$ SE was (1.63 ± 0.26) with a 95% CI of 1.12-2.14 (Fig 2.4). However, the analysis showed inconsistency between studies as the forest plot detected substantial heterogeneity (Q=450.79, df=73, $p<0.0001$; $I^2 = 88.95$ %) among the observation pair results comparing organic versus conventional within studies.

The studies presenting on the left of the vertical dashed line of null effect showed that in those cases organic crop management had lower soil spore density than the conventional system. Some non-significant results were observed in those studies, with their horizontal line (the 95% confidence interval) crossing the line of overall effect (Fig 2.4 and Table 2.6). However, the studies presenting on the right of the line of null effect showed that in those cases organic management resulted in greater soil spore density than conventional management. There were four studies with statistically significant observation pair results as their horizontal line of 95% CI did not cross the vertical line of null effect. The results of these studies indicated (Fig 2.4 and Table 2.6) that organic farming increased spore density (Ryan *et al.*, 1994; Eason *et al.*, 1999; Mader *et al.*, 2000; Oehl *et al.*, 2004). Whereas there were observation pair results of two studies that were non-significant (Scullion *et al.*, 1998; Lee *et al.*, 2008). However, the observation pair results of other studies have presented multiple effects with a range of significant (positive effect or negative effect) and non-significant effects, such as Ryan and Ash (1999), Purin *et al.* (2006), Bedini *et al.* (2013) and Meyer *et al.* (2015) (Fig 2.4 and Table 2.6). Moreover, Purin *et al.* (2006) was larger study (no. of replicates=24) than other studies (bigger black box and shorter 95% confident interval CI) (Fig 2.4). This study showed that there was one observation with lower numbers of spores and one observation with higher numbers of spores under organic management, but the other two observations showed no significant effects (Table 2.6). Whereas, all the Mader *et al.* (2000) results showed significantly higher spore densities for organic systems, even though they have wide CI, reflective of the size of this study (Fig 2.4 and Table 2.6).
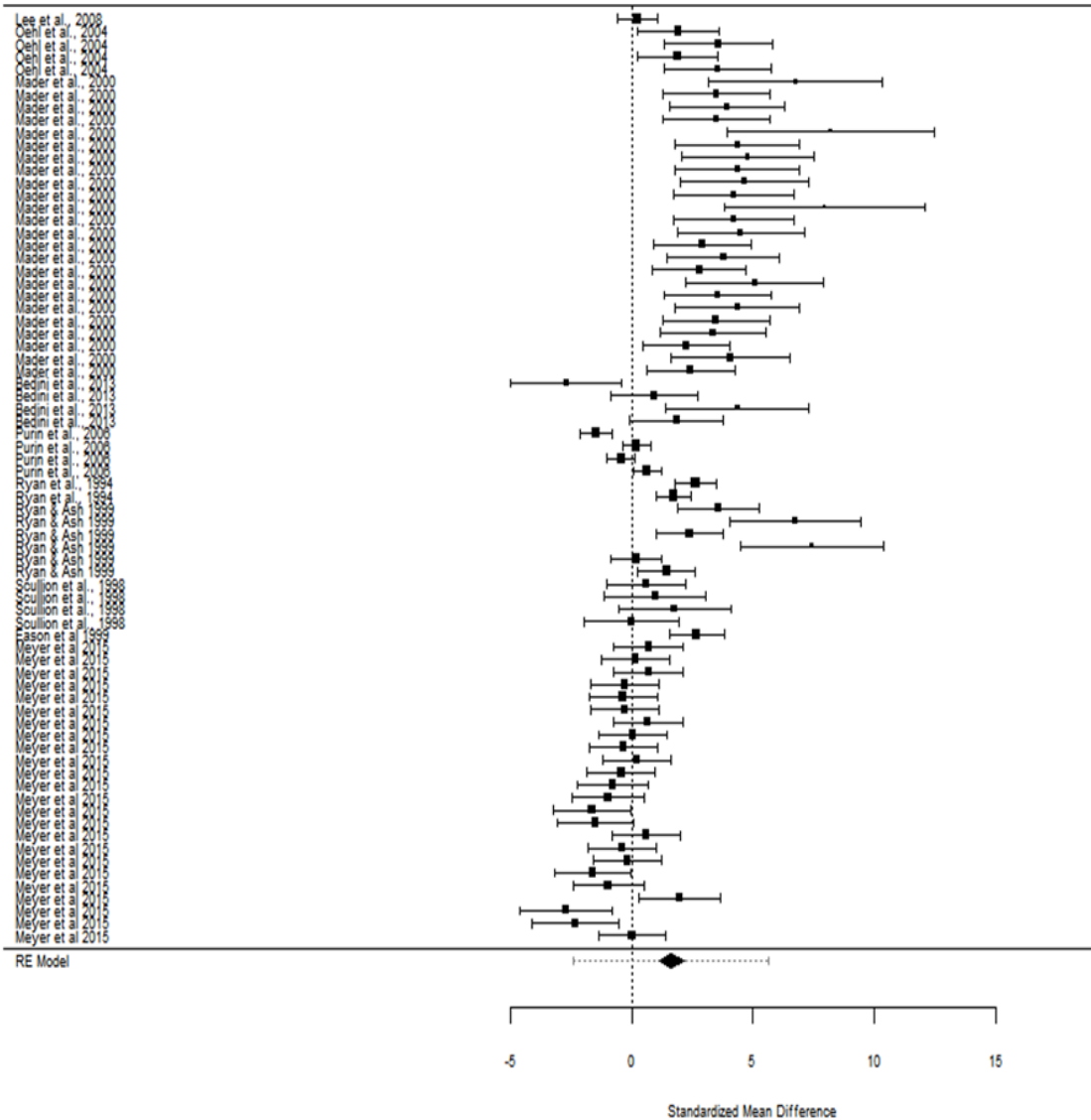
Fig 2.4. Forest plot showing the results of the comparison of AM fungal spore density in organic and conventional systems across a range of crop types showing standardised mean difference (SMD; black boxes), 95% confidence intervals (95% CI; horizontal lines), and line of null effect (dashed vertical line) with a value of zero showing no difference between organic and conventional systems. The overall estimated SMD from a random-effects (RE) model for all studies is indicated by the black diamond at the bottom of the figure. Full details of characteristics for each observation pair are shown in (Table 2.6). Heterogeneity was assessed across all the observation pairs of studies by I^2 test ($I^2 > 50\%$).

Mixed effect models indicated that crop type was not a significant factor affecting the impact of organic management on AM fungal spore density; however, there was a tendency for the impact of organic management on AM fungal spore density to be higher for grain crops especially wheat compared to fruit and vegetables (Table 2.6). The highest significant SMD (8.19, CI: 3.95, 12.44, $p=0.0002$) was estimated for wheat in the Mader *et al.* (2000) study. Additionally, clover and grass which were characterised as perennial pastures (Table 2.6) also tended to present higher of AM fungal spore density levels than other crop types in organic compared to conventional farming (Table 2.6). The highest significant SMD with 95% CI was estimated for clover 6.72 (4.02, 9.42), $p<.0001$ and grass 7.42 (4.48, 10.37), $p<.0001$ in Ryan and Ash (1999) study (Table 2.6). Furthermore, the SMD results indicated that AM fungal spore density tended to be higher in conventional than organic farming for apple in the Meyer *et al.* (2015) study.

Table 2.6. References for studies, characteristics of each observation pair and standardised mean difference (SMD) with 95% confidence intervals (95% CI) and *p*-value for comparison of AM fungal spore density in organic and conventional systems for studies included in the standard weighted meta-analysis.

Paper	Country	EXP. Year	S. Type	Crop type	ASDM	ORG class	CONV class	SMD (95% CI)	*p*-value
Lee et al., 2008	Korea	2008	SR	Vegetables	inoculum potential	ORG_H	CONV_H	0.23 (-0.60, 1.06)	0.5912
Oehl et al., 2004	Switzerland	2000	FE	Wheat	inoculum potential	ORG_H	CONV_H	1.94 (0.26, 3.62)	0.0237
Oehl et al., 2004	Switzerland	2000	FE	Wheat	inoculum potential	BDYN_H	CONV_H	3.57 (1.34, 5.80)	0.0017
Oehl et al., 2004	Switzerland	2000	FE	Wheat	inoculum potential	ORG_H	CONV_FYM_H	1.90 (0.23, 3.57)	0.0259
Oehl et al., 2004	Switzerland	2000	FE	Wheat	inoculum potential	BDYN_H	CONV_FYM_H	3.54 (1.32, 5.76)	0.0018
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_H	CONV_H	6.75 (3.16, 10.33) [a]	0.0002
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_H	CONV_H	3.50 (1.29, 5.70) [a]	0.0019
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_L	CONV_H	3.93 (1.56, 6.30) [a]	0.0012
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_L	CONV_H	3.50 (1.29, 5.70) [a]	0.0019
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_H	CONV_FYM_H	8.19 (3.95, 12.44) [a]	0.0002
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_H	CONV_FYM_H	4.36 (1.81, 6.90) [a]	0.0008
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_L	CONV_FYM_H	4.78 (2.06, 7.51) [a]	0.0006
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_L	CONV_FYM_H	4.36 (1.81, 6.90) [a]	0.0008
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_L	CONV_FYM_L	4.64 (1.98, 7.30) [a]	0.0006
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_L	CONV_FYM_L	4.21 (1.73, 6.70) [a]	0.0009
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_H	CONV_FYM_L	7.95 (3.82, 12.08) [a]	0.0002
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_H	CONV_FYM_L	4.21 (1.73, 6.70) [a]	0.0009
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_H	CONV_H	4.50 (1.89, 7.10) [b]	0.0007
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_H	CONV_H	2.91 (0.92, 4.90) [b]	0.0041
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_L	CONV_H	3.78 (1.47, 6.10) [b]	0.0014
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_L	CONV_H	2.78 (0.84, 4.72) [b]	0.0051
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_H	CONV_FYM_H	5.07 (2.23, 7.91) [b]	0.0005
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_H	CONV_FYM_H	3.54 (1.32, 5.76) [b]	0.0018

Table 2.6. (continued)

Paper	Country	EXP. Year	S. Type	Crop type	ASDM	ORG class	CONV class	SMD (95% CI)	*p*-value
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_L	CONV_FYM_H	4.36 (1.81, 6.90) [b]	0.0008
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_L	CONV_FYM_H	3.47 (1.28, 5.67) [b]	0.0019
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_L	CONV_FYM_L	3.36 (1.21, 5.51) [b]	0.0022
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_L	CONV_FYM_L	2.26 (0.48, 4.03) [b]	0.0126
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	BDYN_H	CONV_FYM_L	4.07 (1.64, 6.50) [b]	0.001
Mader et al., 2000	Switzerland	1989-1990	FE	Wheat	inoculum potential	ORG_H	CONV_FYM_L	2.44 (0.61, 4.27) [b]	0.009
Bedini et al., 2013	Italy	2007	FE	Maize	spore density	ORG_L	CONV_H	-2.68 (-4.96, -0.41) [c]	0.0209
Bedini et al., 2013	Italy	2007	FE	Maize	spore density	ORG_L	CONV_H	0.94 (-0.84, 2.72) [d]	0.3003
Bedini et al., 2013	Italy	2007	FE	Maize	inoculum potential	ORG_L	CONV_H	4.36 (1.42, 7.31) [c]	0.0037
Bedini et al., 2013	Italy	2007	FE	Maize	inoculum potential	ORG_L	CONV_H	1.85 (-0.06, 3.77) [d]	0.0577
Purin et al., 2006	Brazil	2003	FE	Apple	spore density	ORG_H	CONV_H	-1.46 (-2.10, -0.83)	<.0001
Purin et al., 2006	Brazil	2004	FE	Apple	spore density	ORG_H	CONV_H	0.20 (-0.37, 0.77)	0.4878
Purin et al., 2006	Brazil	2004	FE	Apple	inoculum potential	ORG_H	CONV_H	-0.43 (-1.00, 0.15)	0.1439
Purin et al., 2006	Brazil	2003	FE	Apple	inoculum potential	ORG_H	CONV_H	0.64 (0.06, 1.22)	0.0306
Ryan et al., 1994	Australia	1992	FE	Wheat	inoculum potential	ORG_L	CONV_L	2.64 (1.79, 3.48)	<.0001
Ryan et al., 1994	Australia	1993	FE	Wheat	inoculum potential	ORG_L	CONV_L	1.72 (1.00, 2.45)	<.0001
Ryan & Ash, 1999	Australia	1994	FE	Clover	inoculum potential	BDYN_H	CONV_L	3.55 (1.87, 5.24) [e]	<.0001
Ryan & Ash, 1999	Australia	1994	FE	Clover	inoculum potential	BDYN_H	CONV_L	6.72 (4.02, 9.42) [f]	<.0001
Ryan & Ash, 1999	Australia	1994	FE	Clover	inoculum potential	BDYN_H	CONV_L	2.39 (1.02, 3.76) [g]	0.0006
Ryan & Ash, 1999	Australia	1994	FE	Grass	inoculum potential	BDYN_H	CONV_L	7.42 (4.48, 10.37) [e]	<.0001
Ryan & Ash, 1999	Australia	1994	FE	Grass	inoculum potential	BDYN_H	CONV_L	0.19 (-0.86, 1.24) [f]	0.7269
Ryan & Ash, 1999	Australia	1994	FE	Grass	inoculum potential	BDYN_H	CONV_L	1.43 (0.26, 2.61) [g]	0.0167
Scullion et al., 1998	UK	1993	FE	Grass	inoculum potential	ORG_L	CONV_H	0.59 (-1.04, 2.23) [h]	0.478
Scullion et al., 1998	UK	1993	FE	Grass	inoculum potential	ORG_L	CONV_L	0.97 (-1.10, 3.04) [i]	0.3599
Scullion et al., 1998	UK	1993	FE	Grass	inoculum potential	ORG_L	CONV_H	1.76 (-0.55, 4.07) [j]	0.1345
Scullion et al., 1998	UK	1993	FE	Grass	inoculum potential	ORG_L	CONV_H	0.01 (-1.95, 1.97) [k]	0.9895

Table 2.6. (continued)

Paper	Country	EXP. Year	S. Type	Crop type	ASDM	ORG class	CONV class	SMD (95% CI)	*p*-value
Eason et al., 1999	UK	1993	FE	Clover	spore density	ORG_H	CONV_H	2.69 (1.58, 3.79)	<.0001
Meyer et al., 2015	South Africa	2006	FE	Apple	spore density	ORG_H	CONV_L	0.71 (-0.72, 2.13)	0.3329
Meyer et al., 2015	South Africa	2006	FE	Apple	spore density	ORG_H	CONV_L	0.17 (-1.22, 1.56)	0.811
Meyer et al., 2015	South Africa	2006	FE	Apple	spore density	ORG_H	CONV_L	0.71 (-0.72, 2.13)	0.3329
Meyer et al., 2015	South Africa	2006	FE	Apple	spore density	ORG_H	CONV_L	-0.28 (-1.67, 1.11)	0.6925
Meyer et al., 2015	South Africa	2006	FE	Apple	spore density	ORG_H	CONV_L	-0.35 (-1.75, 1.05)	0.6237
Meyer et al., 2015	South Africa	2006	FE	Apple	spore density	ORG_H	CONV_L	-0.28 (-1.67, 1.11)	0.6925
Meyer et al., 2015	South Africa	2007	FE	Apple	spore density	ORG_H	CONV_L	0.66 (-0.76, 2.09)	0.3613
Meyer et al., 2015	South Africa	2007	FE	Apple	spore density	ORG_H	CONV_L	0.06 (-1.33, 1.45)	0.9337
Meyer et al., 2015	South Africa	2007	FE	Apple	spore density	ORG_H	CONV_L	-0.32 (-1.72, 1.07)	0.6518
Meyer et al., 2015	South Africa	2007	FE	Apple	spore density	ORG_H	CONV_L	0.21 (-1.18, 1.60)	0.7625
Meyer et al., 2015	South Africa	2007	FE	Apple	spore density	ORG_H	CONV_L	-0.42 (-1.82, 0.98)	0.5553
Meyer et al., 2015	South Africa	2007	FE	Apple	spore density	ORG_H	CONV_L	-0.78 (-2.22, 0.66)	0.2873
Meyer et al., 2015	South Africa	2008	FE	Apple	spore density	ORG_H	CONV_L	-0.96 (-2.42, 0.50)	0.1982
Meyer et al., 2015	South Africa	2008	FE	Apple	spore density	ORG_H	CONV_L	-1.63 (-3.23, -0.03)	0.0456
Meyer et al., 2015	South Africa	2008	FE	Apple	spore density	ORG_H	CONV_L	-1.49 (-3.06, 0.08)	0.0624
Meyer et al., 2015	South Africa	2008	FE	Apple	spore density	ORG_H	CONV_L	0.59 (-0.83, 2.01)	0.4139
Meyer et al., 2015	South Africa	2008	FE	Apple	spore density	ORG_H	CONV_L	-0.40 (-1.80, 1.00)	0.5758
Meyer et al., 2015	South Africa	2008	FE	Apple	spore density	ORG_H	CONV_L	-0.16 (-1.55, 1.23)	0.8233
Meyer et al., 2015	South Africa	2010	FE	Apple	inoculum potential	ORG_H	CONV_L	-1.60 (-3.19, -0.01)	0.0492
Meyer et al., 2015	South Africa	2010	FE	Apple	inoculum potential	ORG_H	CONV_L	-0.96 (-2.42, 0.51)	0.1993

Table 2.6. (continued)

Paper	Country	EXP. Year	S. Type	Crop type	ASDM	ORG class	CONV class	SMD (95% CI)	*p*-value
Meyer et al., 2015	South Africa	2010	FE	Apple	inoculum potential	ORG_H	CONV_L	1.99 (0.29, 3.68)	0.0215
Meyer et al., 2015	South Africa	2010	FE	Apple	inoculum potential	ORG_H	CONV_L	-2.70 (-4.61, -0.78)	0.0058
Meyer et al., 2015	South Africa	2010	FE	Apple	inoculum potential	ORG_H	CONV_L	-2.30 (-4.08, -0.51)	0.0117
Meyer et al., 2015	South Africa	2010	FE	Apple	inoculum potential	ORG_H	CONV_L	0.03 (-1.36, 1.41)	0.9679

Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic farming. ORG class and CONV class refer to input intensity class as described in (Table 2.1 and 2.2). EXP. Year, Experimental year; S. Type, study type; SR, Survey; FE, experimental field; ASDM, AM fungal spore density measurement.

[a] Inoculum potential was measured for only indigenous AM fungi; [b] inoculum potential was measured for indigenous AM fungi plus inoculation with *Glomus mosseae*; [c] old organic (established since 1991) vs conventional farming; [d] young organic (integrated farming from 1991 to 2000 and converted into organic management since 2001) vs conventional farming; [e] pair A; [f] pair B; [g] pair C; [h,j] two locations of permanent grass (Denbigh, Wales) had different fertility management; [i] permanent grass (Bromyard, England); [k] permanent grass (Wick, England).

Strong funnel plot asymmetry consistent with publication bias (Fig 2.5) was detected ($p<0.001$) among studies for AM fungal spore density. The funnel plot showed that most points were clustered around the top of the funnel plot which reflected that these observation pairs are the results of large studies while fewer points were found at the bottom of the plot representing the results of smaller studies. Overall, this illustrates a bias towards publication of results from large studies. Also, the multiple effects found by Meyer *et al.* (2015) are shown in the dotted curved line of the funnel plot, which has a slight lack of symmetry.
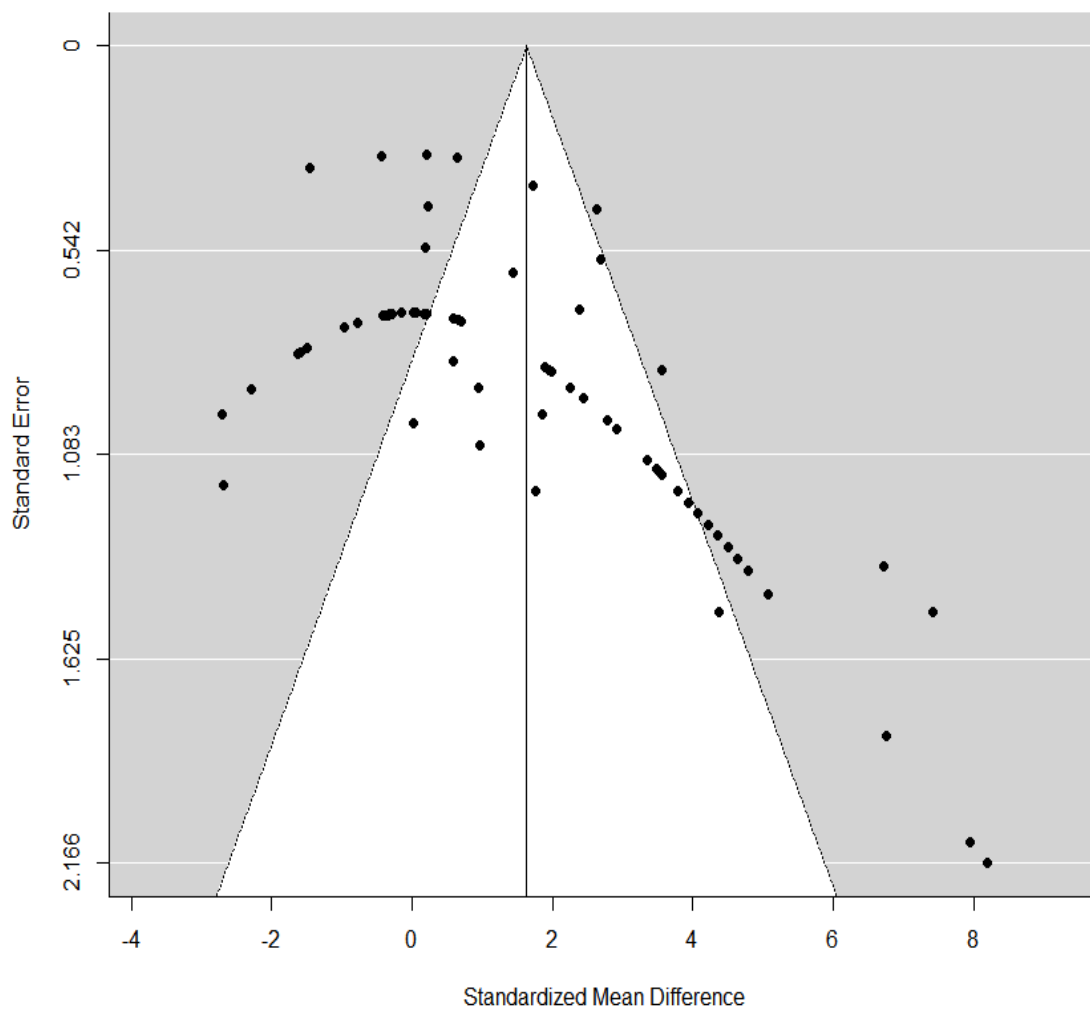
Fig 2.5. Funnel plot to visually detect the presence of publication bias among the observation pair results of studies in an organic and conventional system comparison on AM fungal spore density for studies included in the standard weighted meta-analysis. A mixed effects meta-regression model was used to detect publication bias.

2.3.3. AM fungal diversity

Meta-analysis indicated that the overall standard mean difference (SMD) for comparing organic versus conventional systems was a non-significant effect on AM fungal diversity (Fig 2.6). According to the summary effects of SMD, there were no significant differences between both systems (organic versus conventional) with regard to AM fungal diversity and the SMD±SE was (0.13 ± 0.35) with a 95% CI of -0.56-0.82. While there was no difference in fungal diversity overall, some individual studies showed (Fig 2.6 and Table 2.7) slightly higher levels of diversity under organic management (Lee *et al.*, 2008; Verbruggen *et al.*, 2010; Bedini *et al.*, 2013; Dai *et al.*, 2014), with other studies showing negative effects of organic farming on diversity of AM fungi compared to conventional farming (Purin *et al.*, 2006). There was a lot of heterogeneity (Q=121.7888, df=14, *p*=0.7184; I^2=90.67%) among studies comparing AM fungal diversity across both systems.

Mixed effect models indicated that crop type was not a significant factor affecting the impact of organic management on AM fungal diversity; however, there was a tendency for the impact of organic management on AM fungal diversity to be higher for grain crops, especially maize, when compared to conventional farming (Table 2.7). The highest significant SMD with 95% CI was estimated for maize 2.48 (0.28, 4.69), *p*=0.0272 in the (Bedini *et al.*, 2013) study (Table 2.7). Additionally, the SMD for AM fungal diversity also indicated that a significant negative effect of organic farming on AM fungal diversity in apples (SMD with 95% CI -2.59 (-3.36, -1.83) in 2003 and -2.64 (-3.41, -1.86) in 2004) (Purin *et al.*, 2006) (Table 2.7).

Effects of additional experimental factors such as different system classes (organic and conventional), experimental year, crop rotation, and fertiliser type were explored with mixed effects models but no clear consistent patterns emerged from these exploratory analyses and the complexity of the models resulted in considerable potential for model overfitting, making the results unlikely to be reliable. These results are therefore not presented.

The funnel plot indicated that there was no significant publication bias among studies for AM fungal diversity with large and small studies relatively evenly distributed within the pseudo 95% confidence intervals (white space within dotted lines; Fig 2.7).
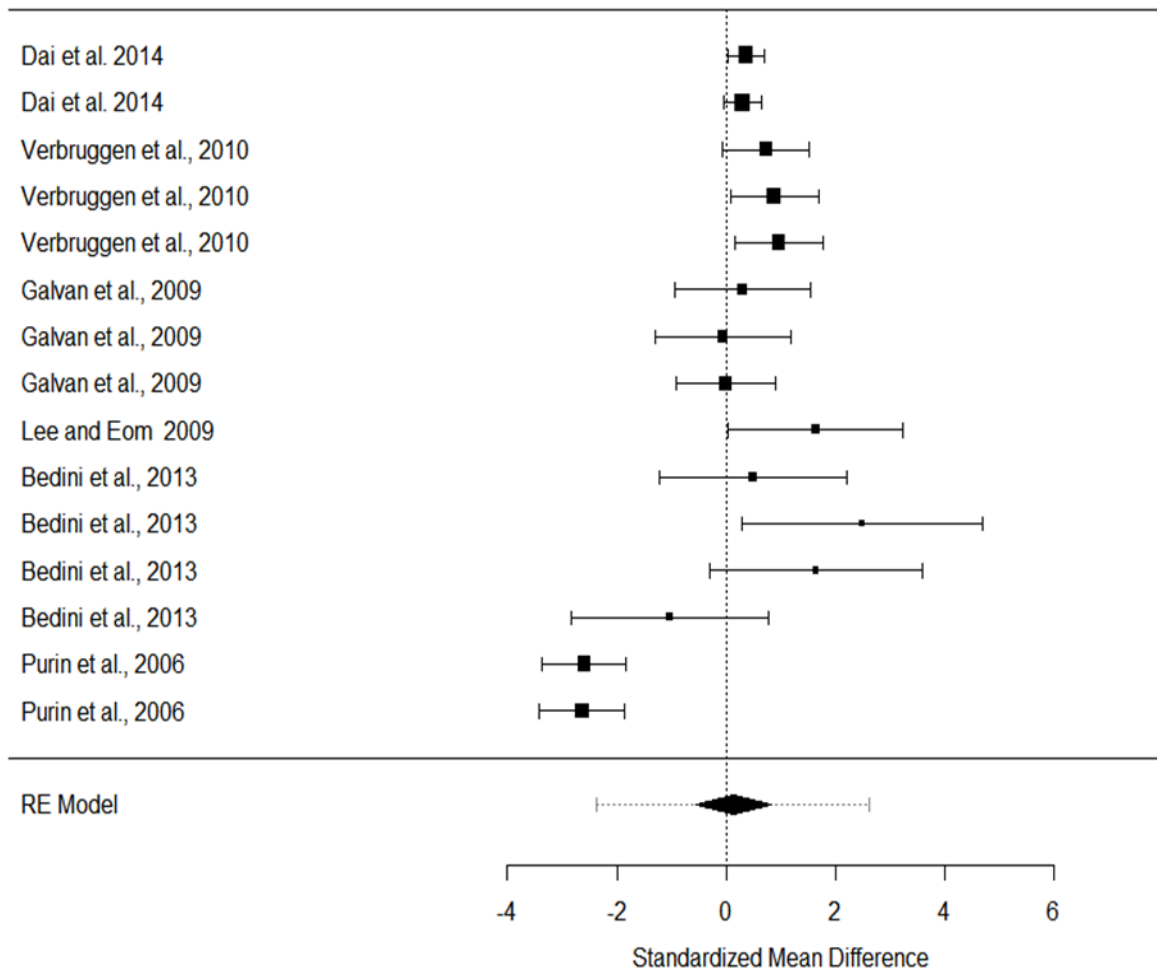
Fig 2.6. Forest plot showing the results of the comparison of AM fungal diversity in organic and conventional systems across a range of crop types showing standardised mean difference (SMD; black boxes), 95% confidence intervals (95% CI; horizontal lines), and line of null effect (dashed vertical line) with a value of zero showing no difference between organic and conventional systems. The overall estimated SMD from a random-effects (RE) model for all studies is indicated by the black diamond at the bottom of the figure. Full details of characteristics for each observation pair are shown in (Table 2.7). Heterogeneity was assessed across all the observation pairs of studies by I^2 test ($I^2 > 50\%$).

Table 2.7. References for studies, characteristics of each observation pair and standardised mean difference (SMD) with 95% confidence intervals (95% CI) and *p*-value for comparison of AM fungal diversity in organic and conventional systems for studies included in the standard weighted meta-analysis.

Citation	Country	EXP. Year	S. Type	Crop type	ADM	ORG class	CONV class	SMD (95% CI)	*p* value
Dai et al., 2014	Canada	2009-2011	SR	Wheat	Shannon-weaver	ORG_H	CONV_H	0.36 (0.02, 0.70)	0.0358
Dai et al., 2014	Canada	2009-2011	SR	Wheat	AM fungal Richness	ORG_H	CONV_H	0.30 (-0.04, 0.64)	0.0805
Verbruggen et al., 2010	Netherlands	2007	SR	Potato	AM fungal richness	ORG_H	CONV_H	0.73 (-0.07, 1.52)	0.0724
Verbruggen et al., 2010	Netherlands	2007	SR	Maize	AM fungal richness	ORG_H	CONV_H	0.88 (0.07, 1.68)	0.0326
Verbruggen et al., 2010	Netherlands	2008	SR	Maize	AM fungal richness	ORG_H	CONV_H	0.96 (0.15, 1.77)	0.0202
Galvan et al., 2009	Netherlands	2004	SR	Vegetables	Shannon Index (H')	ORG_H	CONV_H	0.30 (-0.94, 1.55) [a]	0.6343
Galvan et al., 2009	Netherlands	2004	SR	Vegetables	Shannon Index (H')	ORG_H	CONV_H	-0.06 (-1.30, 1.18) [b]	0.9243
Galvan et al., 2009	Netherlands	2005	SR	Vegetables	Shannon Index (H')	ORG_H	CONV_H	-0.01 (-0.91, 0.89) [a]	0.9785
Lee and Eom, 2009	Korea	2009	FE	ns	Shannon Index (H')	ORG_H	CONV_H	1.64 (0.03, 3.24)	0.0452
Bedini et al., 2013	Italy	2007	FE	Maize	Species richness	ORG_L	CONV_H	0.49 (-1.23, 2.21) [c]	0.5758
Bedini et al., 2013	Italy	2007	FE	Maize	Species richness	ORG_L	CONV_H	2.48 (0.28, 4.69) [d]	0.0272
Bedini et al., 2013	Italy	2007	FE	Maize	Shannon Index (H')	ORG_L	CONV_H	1.64 (-0.29, 3.58) [c]	0.0962
Bedini et al., 2013	Italy	2007	FE	Maize	Shannon Index (H')	ORG_L	CONV_H	-1.03 (-2.83, 0.76) [d]	0.2592
Purin et al., 2006	Brazil	2003	FE	Apple	Shannon Index (H')	ORG_H	CONV_H	-2.59 (-3.36, -1.83)	<.0001
Purin et al., 2006	Brazil	2004	FE	Apple	Shannon Index (H')	ORG_H	CONV_H	-2.64 (-3.41, -1.86)	<.0001

Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic farming. ORG class and CONV class refer to input intensity class as described in (Table 2.1 and 2.2). EXP. Year, Experimental year; S. Type, study type; SR, Survey; FE, experimental field; ADM, AM fungal diversity measurement.

Location: [a] Fevoland; [b] Zeeland; [c] old organic (established since 1991) vs conventional farming; [d] young organic (integrated farming from 1991 to 2000 and converted into organic management since 2001) vs c conventional farming.

The funnel plot also showed that all investigated studies are distributed around the overall effect line on both sides, while the points outside the range of the confidence intervals reflect that the mean effect sizes of these studies were overestimated.
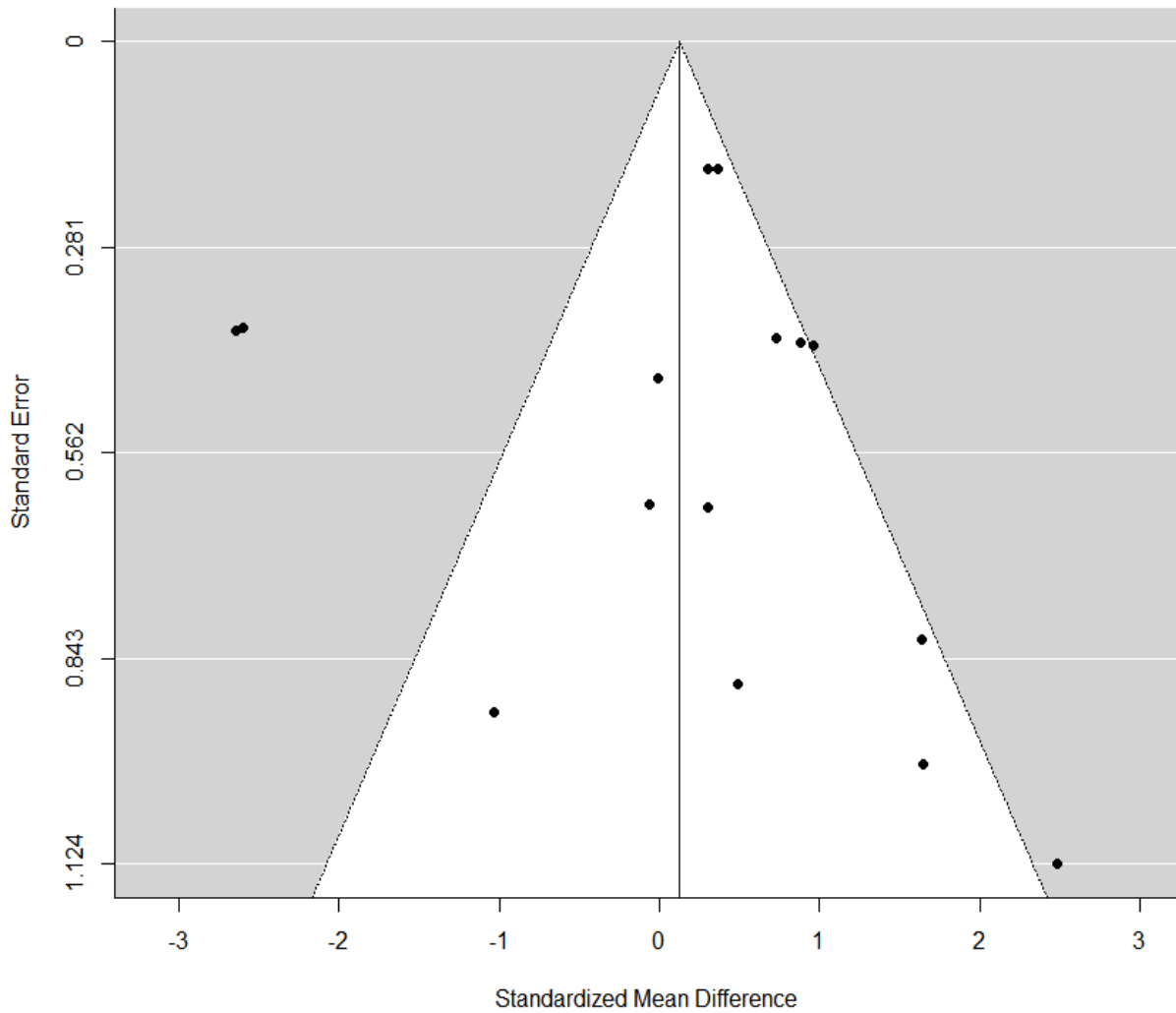

Fig 2.7. Funnel plot to visually detect the presence of publication bias among the observation pair results of studies in an organic and conventional system comparison on AM fungal diversity for studies included in the standard weighted meta-analysis.A mixed effects meta-regression model was used to detect publication bias.

2.4. Discussion

2.4.1. Do organic crop management practices increase AM fungal colonisation and soil spore density?

The results from these meta-analyses are useful for learning about the consequences of crop management practices (organic versus conventional) on AM fungal parameters. Moreover, the meta-analyses allowed quantification of the effects of both systems (conventional versus organic) on AM fungal root colonisation and soil spore density. In general, the weighted meta-analysis shows that the organic management approach supports the development of AM fungal colonisation and soil spore density (Fig 2.2 and 2.4).

The forest plots indicated that studies were consistent regarding the positive effect of organic farming on AM fungal colonisation of crop roots as most of the studies were on the same side of the null effect line.

The farming strategy applied in organic systems may support AM fungal development. For instance, the accumulation of organic carbon in organic farms due to applying organic fertiliser (Purin *et al.*, 2006; Gomiero *et al.*, 2011; Zhu *et al.*, 2016) such as compost (Purin *et al.*, 2006; Yang *et al.*, 2018a; Liu *et al.*, 2019), farm yard manure (Mader *et al.*, 2000) and green manure (Dai *et al.*, 2014) may promote colonisation by AM fungi and soil spore density (Allen *et al.*, 2001; Ryan and Tibbett, 2008). The high levels of soil organic matter can promote the survival of AM fungal propagules (Gollner *et al.*, 2011). Furthermore, organic matter can be an additional source of nutrients such as N (Sabine *et al.*, 1999) for AM fungi which can simultaneously get organic carbon from plants through photosynthate (Gavito and Olsson, 2003). The organic amendments can also indirectly promote AM fungal colonisation and spore density through enhancing soil properties such as soil aggregate stability, water retention, available nutrients and microbial activity (Gomiero *et al.*, 2011; Alam *et al.*, 2017).

The absence of readily soluble P fertiliser is the most common factor supporting AM fungal occurrence in organic farms. It is well known that organic farms apply phosphate rock, which is a relatively insoluble mineral, or organic materials such as compost or chicken manure as a source of P fertiliser (Douds *et al.*, 1997; Ryan and Tibbett, 2008) which can result in lower levels of soil available P compared to conventional farming (Derrick and Dumaresq, 1999; Oehl

et al., 2002; Knerr *et al.*, 2018). High levels of soil available P have been observed many times to decrease AM fungal colonisation (Hijri *et al.*, 2006; Smith and Read, 2008; Schalamuk *et al.*, 2011). In natural agro-ecosystems, a negative correlation is often observed between the P concentrations of plant tissues and AM fungal colonisation or soil spore density (Mårtensson and Carlgren, 1994; Ryan *et al.*, 2000). In this weighted meta-analysis, the Dann *et al.* (1996) observation pair results referred to three treatments: (T1) 0 kg P ha^{-1}, (T2) 30 kg ha^{-1} as reactive rock phosphate (RP) applied in both organic and conventional systems and (T3) 30 kg ha^{-1} as RP applied in organic versus 30 kg ha^{-1} as superphosphate applied in conventional. The largest SMD for AM fungal colonisation was for T3 (3.78, CI: 2.75, 4.82) suggesting that the soluble P in superphosphate in the conventional system inhibited AM fungi whereas the RP had no inhibitory effect relative to no P at all. Similarly, Ryan *et al.* (1994) presented results from the same treatments as Dann *et al.* (1996) (T1:SMD: 5.89, CI: 2.69, 9.09, T2: SMD: 3.79, CI:1.48, 6.11, T3: SMD: 8.51, CI: 4.11, 12.90), but with two additional fertiliser treatments (T4) 122 kg ha^{-1} RP applied in both systems (SMD: 3.62, CI: 2.61, 4.62) and (T5) 30 kg ha^{-1} as RP applied in organic versus 30 kg ha^{-1} as superphosphate plus nitrogen fertiliser applied in conventional (SMD: 5.70, CI: 2.58, 8.82) (see Table 2.5). These results also showed the largest SMD when organic systems were compared to systems using soluble P (superphosphate), confirming that increased available P when soluble P fertiliser is used can decrease AM fungal colonisation.

The fertiliser regime could be a determining factor in the differences in AM fungi in field trials (DOK) (Mader *et al.*, 2000; Oehl *et al.*, 2002; Oehl *et al.*, 2003; Oehl *et al.*, 2004; Hildermann *et al.*, 2010) initiated near Basel, Switzerland in 1978. The field trial treatments consisted of two organic farming treatments (bio-organic and biodynamic), two conventional systems (minerals only and minerals with farmyard manure) and a control (non-fertiliser added). The type and amount of fertiliser differed between organic and conventional farming as each treatment had two rates (low and high). However, in this meta-analysis study, the organic systems were categorised based on level of P input, but this categorisation did not help to explain the meta-analysis results. Therefore, whether inputs of P were high or low did not seem to affect the magnitude of the SMD when comparing organic and conventional systems for either AM fungal colonisation or spore density (Table 2.5 and 2.6). If organic matter inputs are the main factor driving enhanced root colonisation in organic systems, then we would expect no significant SMD when comparing organic systems with conventional manure-based systems. However, the results of the current weighted meta-analysis study detected lots of differences (a positive SMD) between observation pairs of conventional-FYM and organic

fertiliser type. This suggests that other aspects of organic management promote AM fungi colonisation apart from the use of organic matter inputs.

All of the studies included in this meta-analysis used pesticides of some type in the conventional systems, so it was not possible to determine whether differences in fertility source or pesticide inputs were driving the increased AM fungal colonisation and spore densities in organic management. The inhibitory effects of pesticides on AM fungal symbiosis may contribute to the reduced AM fungal colonisation and soil spore density in conventional compared to organic systems (Smith, 1980). Even though, the effect of pesticides is not clear in some cases, it is likely that the limited use of pesticides in organic systems compared to conventional systems could enhance AM fungal colonisation and spore density.

Organic farming tends to promote larger crop diversity such as crop rotation compared to conventional farming. This could be attributed to the greater importance of legumes in organic farms for providing N than in conventional farms (Gabriel *et al.*, 2006). Crop rotation is one of the fundamentals of organic farming practice which has an emphasis on diverse crop rotations with longer ley periods to control pests, diseases and weeds (Watson *et al.*, 2002). Several studies reported that diverse crop rotation can promote AM fungal root colonisation and spore density in organic farming (Oehl *et al.*, 2003; Gosling *et al.*, 2006; Maiti *et al.*, 2012). However, the mixed effects model in the current meta-analysis did not provide clear results on effects of crop rotation on AM fungal root colonisation and spore density.

Furthermore, increased weed populations which are likely favoured hosts for AM fungi may also support AM fungal development in organic farms (Nelson *et al.*, 2011b). Since organic farms show greater occurrence of weeds than conventional farms, this could contribute to an increase in AM fungal populations in organic farming. For example, apple trees managed organically in California, USA had greater AM fungal root colonisation than apples grown in conventional farms due to the occurrence of host weeds for mycorrhizae in the organic orchards (Werner, 1997).

Crop type may be one factor influencing the effect of organic management on AM fungal colonisation in roots and spore density in the soil. AM fungal colonisation and spore densities in the grain crops, especially wheat, seem to be more strongly affected by organic management than in fruit and vegetables with SMD for root colonisation in wheat as high as 8.51 (Ryan *et*

al., 1994) while the highest SMD for fruit was just 1.57 (Meyer *et al.*, 2015). The relatively small SMD for the fruit systems could be due to similarities in understorey management e.g. with perennial ground cover, and minimal tillage, compared to grain production where the crop rotation and inputs for conventional production are generally quite different from organic systems. The crop rotation in organic grain production is likely to be more diverse than conventional, resulting in more favourable conditions for AM fungi and a larger SMD than in the fruit systems. This can also explain the results for spore density from the Scullion *et al.* (1998) study (Table 2.6) which was conducted in four permanent grass locations and showed no significant difference in spore densities between organic and conventional systems. The lack of disturbance and similarity in crop rotation in the Scullion et al. (1998) study could have contributed to similarities in spore densities measured in organic and conventional systems.

There may also be geographic and environmental factors affecting the size and direction of the effect of organic farming on spore densities. For example, the comparison between organic versus conventional was conducted in the Galvan *et al.* (2009) study in two locations (Fevoland and Zeeland). Even though the two locations were cultivated with the same crop type (onion), AM fungal root colonisation were significantly higher under organic management in Zeeland (SMD 1.76, CI: 0.30, 3.22) while the opposite was true for the field in Fevoland (SMD: - 0.81 A, CI:-2.10,0.48) (Table 2.5).

The degree of variation between studies for the root colonisation data was low with most observation pairs showing a positive effect due to organic management, while more variability was detected in the results for spore density. Slight differences in effects within a given study may have been due to differences in crop type, system, experimental year and P supply as reported for the Mader *et al.* (2000) study (Table 2.5 and 2.6). Notably, the study by Mader *et al.* (2000) showed consistently positive effects of organic management on spore densities, while the study by Meyer et al. (2015) showed neutral or negative effects. One possible explanation for this variation is that the spore density may reflect the historical accumulation of AM fungal sporulation in the particular soil, and not necessarily the present symbiosis of the plant (Schalamuk *et al.*, 2013). Therefore, the source of variation between observation pair of spore density results may be attributed to the different historical crop management applied in these studies which included in this weighted meta-analysis study. The Mader *et al.* (2000) study was very long-term crop management trial established in 1978 and it was an arable and rotational study including crops which are good hosts of AM fungi. The many years of organic crop

management and host mycorrhizal plants could facilitate the accumulation of AM fungal spores in the Mader *et al.* (2000) study. Whereas the crop management of Meyer et al. (2015) was an orchard study (comparing three organic farms versus two conventional farms) which commenced in 2003, that took place over a shorter term and showed neutral effects of organic management on root colonisation. Similarly, the Bedini *et al.* (2013) study compared two organic farms (old and young organic) versus one conventional farm (Table 2.6 and 2.7). The SMD (4.36, CI:1.42, 7.31) of AM fungal inoculum potential for the observation pair comparing the old organic versus conventional farming was larger than the SMD (1.85, CI: -0.06, 3.77) comparing the young organic versus conventional farming. This result highlighted that differences between organic and conventional farming may be more apparent when comparing systems that have been established in the long-term compared to short-term studies.

We can conclude that organic farming significantly contributed to altered AM fungal parameters including colonisation and spore density when compared with conventionally managed soil whether under exclusively applied mineral fertilisation or an integrated fertilisation regime; this suggests that the crop rotation and pest management regimes used in organic farming are more important that the fertiliser type or rate in determining AM fungal activity.

2.4.2. Do organic crop management practices increase AM fungal soil diversity?

There was no conclusive evidence from this study that organic crop management practices enhanced AM fungal diversity. This was partly due to insufficient data for this parameter. The stringent criteria for conducting a weighted meta-analysis meant that even though some studies involved AM fungal diversity, they were excluded from the meta-analysis because of missing values for measures of variance or the number of replications. This may be because weighted meta-analysis is an important statistical approach for estimating heterogeneities of variance between experiments (Gurevitch and Hedges, 1999). However, in some studies the weighting effect size depended on the sample size due to this variable being inversely proportional to the variance within the experiment (Gurevitch and Hedges, 1999; Allison and Goldberg, 2002), but in this study, only measures of variance were employed for a weighted meta-analysis (Palupi *et al.*, 2012; Baranski *et al.*, 2014).

Moreover, the diversity and community structure of AM fungi tend to be supported by organic farms via the diverse crop rotations used in these farms. AM fungal diversity can be strongly affected by the host plant, and thus by crop rotation (Bever, 2002; Troeh and Loynachan, 2003). There is some evidence that an increase in crop diversity can lead to enhanced AM fungal diversity. For example, in western Kentucky, An *et al.* (1993) observed greater AM fungal diversity when plants were rotated than with continuous soybean growing. However, AM fungal diversity is not always affected by diverse rotations, as in a previous study reported by Franke-Snyder *et al.* (2001) involving a farming system experiment in Pennsylvania, USA. This study indicated that 15 years of large diverse crop rotations in low-input farming did not lead to a significant increase in AM fungal diversity.

Crop species can benefit from higher AM fungal diversity and the response of crop species to effective AM fungal species is a key step to integrate AM fungi in sustainable agriculture. The diversity of AM fungi is an important parameter as AM fungal community structures are different in their functions (Powell and Rillig, 2018; Frew, 2019). For example, some AM fungal species can offer more protection against pathogens and pests than other species (Thonar *et al.*, 2011; Hao *et al.*, 2019). Furthermore, some AM fungal species more effectively uptake P than other species and this may be because these species have extra-radical hyphae which are efficient in soil exploration and in P uptake and translocation to the plant roots (Jansa *et al.*, 2008; Frew, 2019). Therefore, more AM fungal diversity in soil can increase the chances for plants to be colonised by effective AM fungal species which may lead to enhancement of the AM fungal functions in plants (e.g. nutrient uptake and enhance plant resistance to abiotic and biotic stress) (Jansa *et al.*, 2008). For example, in a pot trial Frew (2019) found that the biomass was increased by10.2% when sorghum biocolor plant was inoculated with a single AM fungal species, while it increased 16.3% when inoculated with four species. The author found that a similar response was observed in P concentration in plant tissues. This could be attributed to higher colonisation in plants inoculated with four AM fungal species than those inoculated with single species and higher AM fungi species can present more AM fungal structures in host plant roots than single species. However, this study found that there were no further benefits from increasing AM fungal diversity (four AM fungal species treatment) to *Zea mays* plant, even though the plants responded positively to AM fungi. This may be because the *Zea mays* plant was colonised effectively by a single AM fungal species compared to plants inoculated with multiple AM fungal species as the colonisation was similar in both treatments. This could be also attributed to early colonising of AM fungal species which may suppress following

colonisers of AM fungal species (Werner and Kiers, 2015). However, this response depends on host plant species as it is an important factor (Powell and Rillig, 2018).

Furthermore, it seems that the pattern of crop type effect on AM fungal diversity data that included in this weighted meta-analysis study was very high uncertainty (large error bars on individual study effects), so understanding variation needs bigger sample sizes in future studies. Therefore, further studies may be needed in this area to clarify organic and conventional farming effect on AM fungal diversity.

2.5. Conclusion

The combined results of the weighted meta-analysis indicate that organic farming induces higher colonisation by AM fungi and soil spore density than conventional farming. AM fungal parameters that were used in this analysis are affected by crop management practices used in both systems. The pesticides used in conventional farming may decrease AM fungal root colonisation and soil spore density in some cases, although their impacts on AM fungal parameters may also be based on their type and rate of application, as well as on crop types. There are some indications that colonisation by AM fungi and soil spore density can be enhanced if there is high crop diversity within rotations. Thus, the repeated finding of greater AM fungal abundance on organic farms compared to conventional ones, and the tendency towards higher colonisation by AM fungi in organic farming, most likely results from the prohibition of soluble mineral fertilisers, and the implementation of more organic additions and diverse rotations. Forest plots revealed much heterogeneity among studies comparing organic and conventional systems on AM fungal spore density, while low heterogeneity among studies on AM fungal colonisation. Meanwhile, funnel plots detected high publication bias among studies comparing colonisation by AM fungi and soil spore density across both systems (organic and conventional). However, the weighted meta-analysis in this study did not detect any differences between organic and conventional farms in terms of AM fungal diversity. Similarly, a funnel plot did not reveal publication bias among studies on AM fungal diversity. This could be due to the low number of studies comparing organic and conventional systems with regard to the diversity of AM fungi. AM fungal colonisation and spore densities in the grain crops, especially wheat, seem to be more strongly affected by organic management than in fruit and vegetables. The meta-analysis study confirmed that organic farming enhances AM fungal development and that may contribute towards crop stability and resource efficiency in

the future. Moreover, this reflects that the AM fungi symbiosis is one of the ecological systems targeted by organic agriculture which may rely more on ecosystem services than on chemical-inputs. This can contribute to reduced environmental damage and increased crop production. The increased AM fungal development in organic farming is a feature which can be exploited in future to compensate for the yield gap between organic and conventional farming.

Chapter 3. Effects of Fertility Management Practices on Arbuscular Mycorrhizal Fungal Colonisation of Roots, Spore Density, Crop Yield and P Nutrition in Different Cultivars of Spelt (*Triticum spelta*)

3.1. Introduction

To meet the need for sustainable intensification of our food supply diversification of crop types and enhancement of biological processes to deliver resources is essential. Spelt (*Triticum spelta*) is a very old member of the wheat family, with a high demand particularly among organic food consumers, that offers potential for diversification of cropping systems (Arzani and Ashraf, 2017; Dumalasová *et al.*, 2017; Andruszczak, 2018). It is very nutritious, and some spelt varieties have also been shown to have higher protein and mineral content compared to modern wheats (Pospisil *et al.*, 2011; Arzani and Ashraf, 2017; Andruszczak, 2018). In addition, spelt has lower fertiliser requirements and its characteristic hull protects the grain from insects, disease and pollutants, retains nutrients in the kernel and enhance seed germination (Bonafaccia *et al.*, 2000; Lacko-Bartošová *et al.*, 2010). Due to these characteristics, spelt is well suited to growing with relatively low inputs and in organic production systems. Even though yields are lower and it requires additional post-harvest processing compared to modern wheat, demand for spelt from farmers and consumers has grown considerably due partially to the crop's ability to grow in varied/harsh climatic conditions (Bonafaccia *et al.*, 2000; Grausgruber, 2018).

Utilisation of the AM fungal symbiosis is an attractive strategy that can be used within sustainable agricultural systems. There are two options for the exploitation of AM fungi by farmers: (1) exploit management techniques to improve the functioning of AM fungi indigenous to the soil of the field, or (2) use an AM fungal inoculum in farms (Douds *et al.*, 2012). For both of these strategies, optimum functioning of AM fungi relies on a better comprehension of the effect of crop management practices on AM fungal functions and population dynamics (Harrier and Watson, 2003). Adjusting fertilisation strategies (Galvez *et al.*, 2001; Ryan and Kirkegaard, 2012) and the use of varieties more adapted to form associations with AM fungi than others (Yao *et al.*, 2001) are two aspects of crop management that could be optimised to enhance AM fungal function.

Genetic variance within a crop species (i.e., cultivar differences) can impact the functioning of the microbial community in the rhizophere, including AM fungi. It has been suggested that cultivars selected under high P fertilisation (e.g. modern varieties) are less adapted to form associations with AM fungi (Hetrick *et al.*, 1996). However, a study of modern durum wheat cultivars developed after the advent of fertilisers in agriculture compared to traditional landraces found that the modern cultivars had higher AM fungal colonisation than the landraces (Ellouze *et al.*, 2016). A study by Kirk *et al.* (2011) also showed that modern wheat cultivars had greater AM fungal colonisation and grain yield than older cultivars. This suggests that there is genetic variation in the potential to form associations with AM fungi that could be exploited, but that it may not be due to selection pressure from P fertilisation (Bazghaleh *et al.*, 2018).

3.1.1. Aim and objectives

Enhancement of AM fungi associations within diversified cropping systems (e.g. those including spelt) provides one strategy to address the challenge of sustainable intensification of the food system. Both fertilisation strategy (organic versus mineral, appropriate rates) and cultivar choice are important factors affecting levels of colonisation. Therefore, the main aim of this work was to test the effects of (and interactions between) contrasting spelt variety, fertiliser type (compost FYM and mineral N fertilisers) and fertiliser rate (equivalent to 50 and 100 kg N ha^{-1}) on AM fungal colonisation in spelt roots and spore density in the soil, crop yields and P nutrition. The following objectives have been established for this study:

1. Evaluate the effects of fertiliser type (mineral N vs compost) on AM fungal colonisation of spelt roots, AM fungal spore density in the soil, crop yield and P nutrition.

2. Evaluate the effects of fertiliser rates (Low fertiliser rate (50 kg N ha^{-1}) vs high rate (100 kg N ha^{-1})) on AM fungal colonisation of spelt roots, AM fungal spore density in the soil, crop yield and P nutrition.

3. Evaluate the effects of spelt cultivar on AM fungal colonisation of spelt roots, AM fungal spore density in the soil, crop yield and P nutrition.

4. Assess the effect AM fungal colonisation on grain yield, P uptake and P concentration in spelt at anthesis and harvest.

5. Assess the relationship between AM fungal colonisation of spelt roots and AM fungal spore density in the soil.

3.2. Methodology

3.2.1. Study site and experimental design

The field trial was established at Newcastle University's Nafferton Farm in northeast England (54:59:26.3 N; 1:54:37.4 W). The soil in the plots was a sandy clay loam with an average pH of 6.75, a P-index of 0, K-index of 1 and Mg-index of 3 at the start of the experiment in autumn 2014. The field experiments were part of the European Union's 7th Framework Programme Healthy Minor Cereals project (Grant agreement no. 613609) in the 2015 and 2016 harvest years. The trial plots were in a 4.8 ha field divided into four replicate blocks. The replicate blocks each contained ten 24 m x 24 m plots. This study utilised a subset of these plots representing mineral, compost and control fertiliser types applied at three rates (0, 50, and 100 kg N ha^{-1}). The factorial experiments included four varieties of spelt: Oberkulmer Rotkorn, Zuercher Oberlaender Rotkorn (ZOR), Rubiota and Filderstolz. Rubiota is a modern Czech variety. The Oberkulmer Rotkorn and ZOR varieties were sourced from Sativa Rheinau (Rheinau, Switzerland). Oberkulmer Rotkorn is an old Swiss landrace and ZOR is a modern variety first registered in 2012 and bred by the Peter Kunz breeding group (GZPK) for organic production systems. Filderstolz is a modern semi-dwarf German variety developed by the University of Hohenheim (Stuttgart, Germany) to have *Rht* dwarfing genes through a cross with the wheat variety Maris Huntsman. These varieties were selected to represent a range of genotypes expected to respond differently to fertility interventions and to differ in their potential to form associations with native populations of AM fungi. The trial had a split-split plot design. Fertiliser rate was the main plot factor and within each main plot were four (24 m x 6 m) sub-plots for each type of fertilisation and a further 4 sub-sub-plots for each spelt variety (Filderstolz, Oberkulmer Rotkorn, ZOR and Rubiota) sown across the whole plot (3 m x 24 m) (Fig 3.1).

Soils were sampled for P, K and Mg prior to the beginning of each experiment in the control (zero) and high rate main plot in each replicate (Table 3.1). Soil was sampled for mineral N in

March 2015 and March 2016 after compost application but prior to mineral N application. Samples were taken from plots designated as a zero control and high rate (100 kg N ha^{-1}) compost and mineral N plots to account for any variability within the experimental area prior to any fertiliser application (Table 3.2).
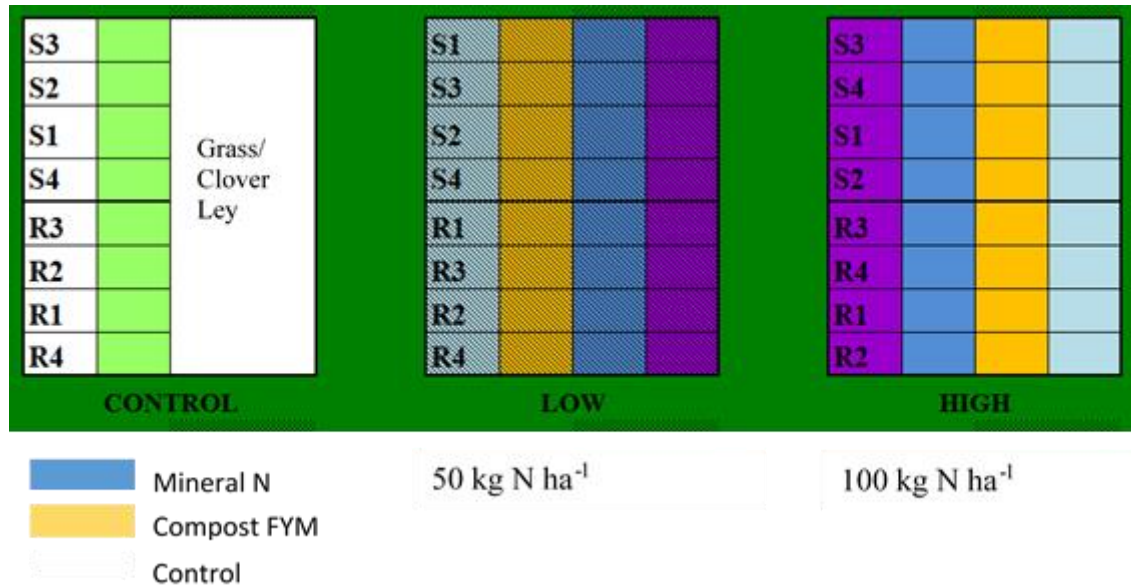
Fig 3.1. Field trial experimental design from a single block of the spelt experiment. Colours designate different fertiliser types (no colour is no input); The layout represents one replicate. The order of fertility types and varieties was randomized within the layout in each replicate (zero-input treatments were always alongside grass/clover); and letter/number combinations identify specific rye and spelt varieties (R = rye, S = spelt). Only spelt plots with mineral, compost or control treatments were used in this study.

Table 3.1. Soil P, K and Mg index in October 2014 and October 2015 (before planting and compost fertiliser application).

Year	Fertility Rate	Soil pH	P Index	P mg l^{-1}	K Index	K mg l^{-1}	Mg Index	Mg mg l^{-1}
2014/15	High	6.8 ± 0.11	0	8.6 ± 0.74	1	85.0 ± 5.12	3	155 ± 7.4
	Zero	6.7 ± 0.12	0	8.1 ± 0.33	1	82.5 ± 3.59	3	151 ± 6.1
2015/16	High	6.6 ± 0.03	0	7.3 ± 0.76	1	77.0 ± 7.18	3	162 ± 5.64
	Zero	6.5 ± 0.10	0	6.8 ± 0.78	1	74.5 ± 2.18	3	166 ± 2.92

Soil was analysed in October from plots designated for either high rate (100 kg N ha^{-1}) or zero rate fertiliser applications to account for any difference within the experimental area with soil samples collected prior to any fertiliser application.

Table 3.2. Soil N content at two depth (0-30 cm and 30-60 cm) in March 2015 and March 2016 (before mineral N application).

Year	Fertiliser type	Fertiliser rate	NO_3^- mg kg^{-1}	NH_4^+ mg kg^{-1}	Total available N Kg N ha^{-1}
0-30 cm					
2014/15	Compost	High	2.3 ± 0.24	1.2 ± 0.11	13.0 ± 1.20
	Zero	Zero	1.7 ± 0.38	1.1 ± 0.21	10.8 ± 2.13
2015/16	Compost	High	4.1 ± 0.64	1.5 ± 0.29	21.0 ± 2.06
	Zero	Zero	6.8 ± 1.08	1.6 ± 0.29	31.6 ± 4.47
30-60 cm					
2014/15	Compost	High	1.0 ± 0.19	0.8 ± 0.03	6.8 ± 0.74
	Zero	Zero	0.9 ± 0.23	1.0 ± 0.13	7.3 ± 0.62
2015/16	Compost	High	1.5 ± 0.43	0.7 ± 0.08	8.5 ± 1.84
	Zero	Zero	1.8 ± 0.18	0.8 ± 0.13	9.7 ± 0.24

Soil was analysed from plots designated as a zero control and high rate (100 kg N ha^{-1}) compost or mineral N applications to account for any difference within the experimental area, with samples collected in March after compost application but prior to mineral N application.

3.2.2. Agronomic management

Plots were established in a different area of the field in each year, but the previous crop in both seasons was the same (grass/clover, see Table 3.3). Plots were ploughed with a mouldboard plough in September 2014 and September 2015 and all varieties were sown on a single day during the first week of October 2014 and October 2015. In all plots weeds were controlled with standard herbicides as described in (Table 3.3), while no fungicides were applied in this trial. One application of mineral fertiliser (ammonium nitrate; 34.5% total N) was applied in each season (Table 3.3). Compost was added at the same total N-input levels as mineral fertilisers. The compost used in the experiment was produced on site at Nafferton Farm from dairy cattle manure and straw. The compost was tested for total N content (% dry matter) prior to sowing: analysis for the 2014/15 season showed a total N content of 3.05%, while analysis for the 2015/16 season showed total N as 3.63%. Fertilisation with low and high rates of compost provided 21.74 and 43.47 kg total P_2O_5 ha^{-1} and 34.03 and 68.06 kg total K_2O ha^{-1} in September 2014 and 16.47 and 32.93 kg total P_2O_5 ha^{-1} and 15.95 and 31.9 kg total K_2O ha^{-1} in September 2015 respectively. Differences in P and K application rates for the compost from year to year reflect differences in the nutrient composition of the compost relative to total N. No additional P and K was added to the mineral fertiliser treatments.

Table 3.3. Crop management details for spelt trials in 2014/15 and 2015/16 seasons.

	2014/15	2015/16
Previous crop	2 years grass/clover	3 years grass/clover
Sowing date	1 October 2014	5 October 2015
Biomass harvest date	1-3 September 2015	2-5 September 2016
Combine harvest date	8-9 September 2015	15-18 September 2016
Seeding rates (kg ha^{-1}) [a]		
Oberkulmer Rotkorn	368	315
ZOR[b]	403	300
Rubiota	277	320
Filderstolz	410	293
Herbicide application date and rate		
CleanCrop Gallifrey (fluroxypyr)	17 April 2015 (0.6 L ha^{-1})	11 April 2016 (0.35 L ha^{-1})
Isomec Ultra (dichloroprop-p)		11 April 2016 (1.5 L ha^{-1})
Fertiliser application date		
Compost FYM	29 September 2014	22 September 2015
Mineral N	17 April 2015	10 May 2016

[a] All varieties were drilled at 350 hulled seeds m^{-2} for season 2014/15 and 250 hulled seeds m^{-2} for season 2015/16.

[b] ZOR for season 2014/15 was sown at 300 seeds m^{-2} due to inadequate seed supply

3.2.3. Sampling strategy

Sampling for this project focussed on a subset of treatments that reflect the nutrient sources that were included in the experiments described in Chapter 4. Sampling was conducted to coincide with flowering (GS61; 1 July) in 2015 and 6-7 July in 2016. The following sampling procedure was used (as used by the Research Institute of Organic Agriculture, (FiBL) in Switzerland) with plots that represent the following treatments: 2 fertiliser types (compost FYM and mineral fertilisers) x 2 fertiliser rates (50 and 100 kg N ha^{-1}) x 4 spelt varieties x 4 replications plus 4 spelt varieties in the control plots per replicate to make the total number of plots sampled 80.

Two plants were sampled from each plot which reflects numbers sampled in studies on AM fungi in cereals e.g. Mader et al. (2000) used 3 root cores per plot in a field trial with 4 replicate blocks. It could be argued that only two plants per plot is an insufficient number to account for within plot variability or biases, but the factorial nature of the experimental design ensured a higher degree of replication for each fertiliser rate and type main plot and sub-plot and for each variety sub-sub-plot than just the two plants sampled in each plot. There were 8 fertiliser type x variety sub-sub-plots within each fertiliser rate main plot, meaning that a total of 16 plants

were sampled at each level of fertiliser rate within a block. Likewise, for each level of fertiliser type, a total of 16 plants were sampled at each level of fertiliser type within the block. For the variety sub-sub-plots, a total of 8 plants were collected within each block for each variety. As field blocks were replicated 4 times, means at the main effect level have a relatively high value for n which is reflected in the relatively low standard errors shown in the results tables (Table 3.5, 3.7, 3.11 and 3.16). The degree of replication for two-way interactions was lower: interaction means for fertiliser type x fertiliser rate represented the means of 8 plants/block or 32 plants in total; both fertiliser type x variety and fertiliser rate x variety represented the means of 4 plants/block or 16 plants in total. It was only at the level of fertiliser type x fertiliser rate x variety that plot means represented only 2 plants, or 8 plants in total for the mean. Ideally, larger numbers of plants would have been taken from each plot to ensure better representation of each plot, however, this had to be balanced against the time and resources required for root washing and analysis.

Roots were collected by digging a 7 cm diameter circle around the stem of the plant and extracting with a shovel to a depth of about 20 cm. The whole plant was extracted, including the roots, and transferred to a plastic bag. Two samples were randomly selected from each plot and pooled to form one sample. The plants were selected from outside the central harvest area of the plot, but not from the edge rows. At the same time, 100 g of soil from each sample was separated from the roots and stored at 4 °C for later assessments of AM fungal spore density.

To harvest the fine roots, the soil was carefully removed from the roots in a plastic container. The roots were placed on a sieving frame (2 mm sieve above a 1 mm sieve). The root systems were washed on the sieving frame with a running stream of tap water until no soil stuck to the roots anymore; tweezers were used to pick up the roots from the 1 mm sieve. The sieves were cleaned carefully between samples to prevent mixing of roots.

The roots were then briefly dried between paper tissues to remove adhering water. The roots were cut with scissors into 1 cm pieces (preferentially choosing fine roots) and around 500 mg of roots was randomly transferred into a falcon tube containing around 15 ml 80 % ethanol, and then stored at 4 °C until staining.

3.2.4. Plant Shoot and grain measurements

Crop mid-season biomass

The above-ground portion of the plants sampled for roots (as described above) was retained for assessment of dry weight and P analysis. The biomass was dried at 70 °C for 3 days and weighed. All samples were milled using a RETSCH SK300 machine to pass them through a 1 mm sieve mesh and kept at room temperature for P measurement.

Crop biomass

Prior to harvest, biomass samples were removed from each plot to assess the total biomass, harvest index (HI), moisture content and additional yield components. Plants from 4 x 0.5 m rows were counted and removed from each plot. In 2015 and 2016, spelt biomass was harvested in the first week of September. Biomass ear samples were dried, cleaned and threshed at Nafferton farm using a seed cleaner and thresher. Spelt was harvested with the hulls and was cleaned by threshing each sample 5 times to remove the husks. In 2016, dried spelt samples were de-hulled using a de-huller at Gilchesters Organic farms (Stamfordham, NE18 0QL).

Biomass harvest samples were individually processed for each plot. Sub-samples of straw (max. 50 g) and ears (max. 150 g) were weighed and dried (at 80 °C for 2 days or 70 °C for 3 days), then used to calculate moisture content and retained for further analysis. The flowering biomass, dry shoots (straw) and grain of spelt were ground before being passed through a 1 mm screen in a Wiley mill and stored at room temperature before laboratory analysis.

Phosphorus measurement

Plant phosphorus concentrations were assessed at the flowering stage for above-ground biomass and at harvest for straw and grain in both seasons. All samples were sent away to a commercial laboratory (SAC Commercial Ltd.) for analysis.

A nitric acid microwave digestion method (MARS™ 6, CEM Corporation) was used for digestion of the samples according to (Hansen *et al.*, 2009). Samples were digested with concentrated nitric acid in a microwave digestion apparatus. Blank and internal standards were prepared and used in each digestion run. The sample digest was then diluted with ultra-pure

water up to the 50 ml. The diluted digests were analysed for P concentration using inductively coupled plasma optical emission spectrometry ICP-OES (Perkin Elmer OES Optima 4300DV) and results were reported as the % P in the biomass, straw or grain of spelt.

Calculations

Where P was expressed as the concentration (mg g^{-1}) of dry matter, calculations were carried out as follows:

$$P \ (mg \ g^{-1}) = P \ (\%) \ x \ 10$$

Where P was expressed as the P uptake (kg ha^{-1}) of dry matter, calculations were carried out as follows:

$$P \ uptake \ (kg \ ha^{-1}) = \% \ P \ x \ 10 \ x \ DW \ (t \ ha^{-1})$$

Total P uptake (kg ha^{-1}) was calculated as the combined P uptake in straw and grain.

3.2.5. Mycorrhizal assessments

AM fungal colonisation

An ink-vinegar solution was used for root staining as described by Vierheilig *et al.* (1998). The percentage mycorrhizal colonisation was measured according to the magnified intersection method of McGonigle *et al.* (1990) and total fractional colonisation was separated into specific types of colonisation, including vesicles, hyphae and arbuscules. A water bath was used to maintain the temperature (80 °C). Part of the roots was transferred from the falcon tubes into scintillation vials. The roots were washed with deionized water to remove ethanol. Water and all chemicals used were added through a net and the root samples were at the bottom of the scintillation vials. Water and all chemicals were sucked from the scintillation vials using a vacuum pump with a suction flask. Root samples were soaked in 10 ml of boiling 10 % KOH solution for 30 minutes in order to digest the nuclei and cytoplasm of host plant cells. Then, the roots were soaked in 1 % HCl at room temperature for 45 minutes. HCl acid was added for acidification because the KOH treatment is very alkaline and must be acidified for the ink-vinegar dye to hold well to AM fungal structures. Finally, the roots were covered with 5 % ink-

vinegar solution for 30 minutes in a boiling water bath for staining. Deionized water was used to wash the roots from chemical materials after each step. In order to get rid of excessive stain, the roots were covered with 50 % glycerine and they were left for one week before assessment of root colonisation. This will allow blue-stained fungal tissue to be clearer and less dark under microscopic examination.

The stained roots were poured from a falcon tube into a petri dish. For each sample, 25 finer roots were selected and placed in parallel on a glass slide using forceps. A few droplets of glycerine were added, and a cover slide was pressed on the roots. The glass slide was placed under the compound Leica DMLB microscope and examined for colonisation by AM fungal structures, including vesicles, hyphae and arbuscules, with 100x and 400x magnification. The slide was moved horizontally and colonisation was assessed at each point along 4 parallel transects; parallel transects were 2 mm apart based on the scale integrated into the microscope eyepiece. At each of the 100 crossings between a root and a line, AM fungal root colonisation was assessed with regard to which vesicles, hyphae or arbuscules occurred at each point where the roots intersected a line. The plane of focus was moved completely through the root. The mean percentage of root colonisation by AM fungi was calculated after microscopic assessment. The scoring system used when examining each root segment is shown in (Table 3.4).

Table 3.4. Coding system for assessing arbuscular mycorrhizal fungal colonisation of stained roots.

Mycorrhizal observations	Code
Number of empty roots (not colonised)	a
Number of roots with hyphae only present	s
Number of roots with hyphae + arbuscules present	d
Number of roots with hyphae + vesicles present	f
Number of roots with hyphae + arbuscules + vesicles present	g

The following formula was used to calculate the percentage of root colonisation of 25 root samples on each slide read at four intersections/root:

Root colonisation (%) = (s + d + f + g) / 100

Furthermore, the percentages of the three structures of mycorrhizae – hyphae (H), arbuscules (A) and vesicles (V) respectively – were calculated as follows:

Total H = s + d + f + g

Total A = d + g

Total V =f + g

Total count = 1 * H + 2 * HA + 2 * HV + 3 * HAV

The proportion of the total number of these structures and the total count were calculated to produce actual counts of these structures as follows:

H count = total H / Total count

A count = total A / Total count

V count = total V / Total count

The final formula to calculate the percentage of the three structures (hyphae, arbuscules and vesicles) was as follows:

H% = (H count) * root colonisation (%)

A% = (A count) * root colonisation (%)

V% = (V count) * root colonisation (%)

In addition to total root colonisation, vesicle, hyphae and arbuscule formation in this study were reported (Fig 3.2) as important indicators of the effects of agricultural practices on AM fungal

colonisation; these AM fungal structures may respond differently to crop management practices (Sheng *et al.*, 2012; Schalamuk *et al.*, 2014; Lehman *et al.*, 2019; Liu *et al.*, 2019). Furthermore, data for the different AM fungal structures should be included as presenting only total root colonisation may hide a lot of useful and interesting information (Jin *et al.*, 2017).
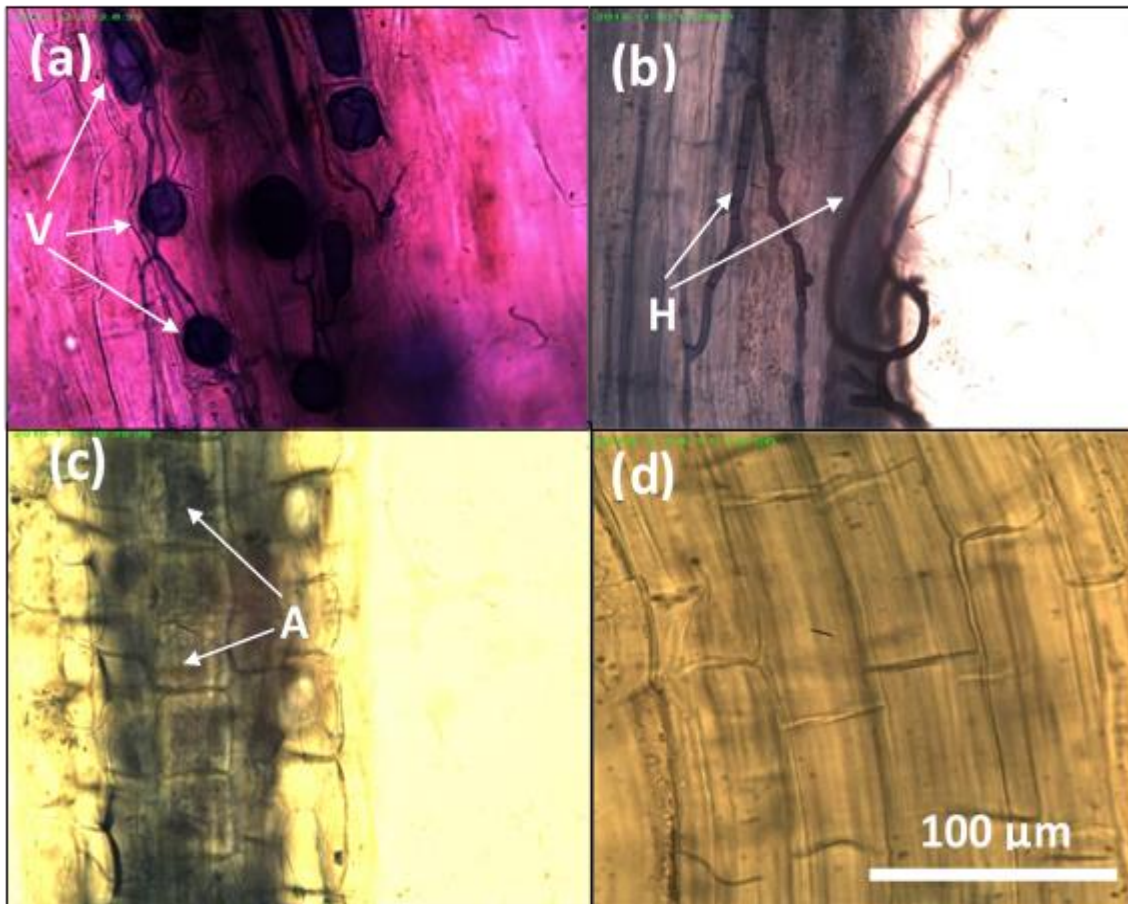
Fig 3.2. AM fungal colonisation and typical AM fungal structures formed with spelt. (a) vesicles for nutrient storage, (b) intra-radical hyphae for transfer nutrients, (c) arbuscules for nutrient exchange, and (d) Non-colonised root. V, vesicle; A, arbuscule; H, intra-radical hyphae. Root were stained by 5 % ink-vinegar solution; the scale bar is the same in panel a, b, c and d.

AM fungal spore density

Soil water content measurement

Soil water was removed by oven-drying (Craze, 1990). Fresh soil was passed through a 2 mm sieve and then a 30 g sample of sieved soil was weighed for each plot in a pre-weighed aluminium dish and dried at 105 ℃ for 24 hours. The weight of the dried soil plus dish was recorded, and then the gravimetric water content (GWC) was calculated.

Spore extraction and counting

Spore density was calculated using the wet sieving and sucrose density gradient centrifugation method (Daniels and Skipper, 1982) with slight modification. This method has routinely been used for assessing spore numbers in the soil since its introduction, and continues to be a standard method for counting AM fungal spores as evidenced by the number of recent publications (Liu *et al.*, 2019; Rasmussen *et al.*, 2019). The details of the method are described below.

A sample of soil equivalent to ~10 g dry soil was passed first through a 2 mm sieve then weighed (~10 fresh soil g) into a 50 ml falcon centrifuge tube and 15 ml of water was added until it was filled up to the 25 ml mark (half way up the tube). Then the soil-water mixture was stirred using a vortex shaker for a few minutes. Spores were separated from soil particles and organic debris by using sucrose density gradient centrifugation techniques. The pellet at the bottom of each tube was gently injected with equal amounts of a 60% (w/v) commercial sugar solution and homogenised to create a uniform suspension using a syringe with a plastic tube extension diluted the sugar solution concentration to 48%. Sucrose was used because it has a high density that allows the spores to float on the surface of the solution.

The suspension was spun at a speed of 3000 rpm (2000 x g) for 5 minutes. The supernatant sugar liquid was decanted onto a sieve with mesh size 38 μm and the soil pellet was discarded. Spores that were trapped by the sieves were carefully rinsed with tap water until the excess sucrose was completely removed and those caught on the sieve were washed into a petri dish with deionised water and made up to a small volume, before the contents of the petri dish were transferred into a falcon tube. The weight of solution in each tube was measured so that the total number of spores for each sample could be calculated based on counting an aliquot of the solution. A small aliquot (5 ml) of the solution was poured into a small petri dish underneath a

grid divided into 100 squares. This method allowed recovery of spores >38 μm in diameter; examination under a microscope (MEIJI 13066) with 40x magnification (Fig 3.3). All squares in the grid were counted and then spores per gram of dry soil were calculated depending on the total volume of solution in the falcon tube. Total spores in each sample were expressed as numbers per g dry soil.
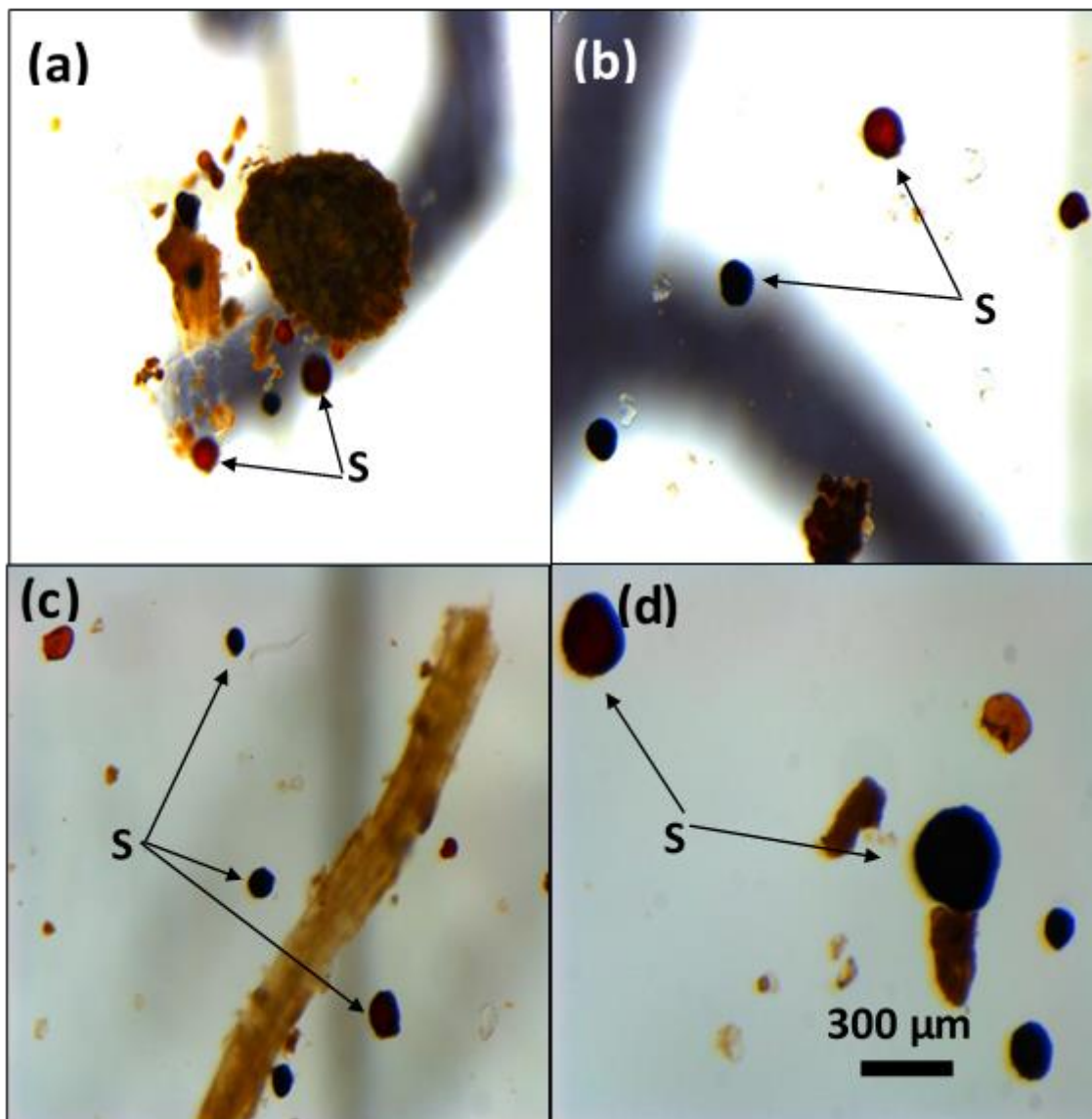
Fig 3.3. Spore (S) formed by AM fungi in the soil of the spelt plots. The scale bar is the same in panel a, b, c and d.

This method requires a high degree of skill to differentiate AM fungal spores from other spores in the soil; measures taken to ensure that spore counts were representative of AM fungal spores only are outlined here. The fresh, cleaned, healthy and bright AM fungal spores were identified according to several typical AM fungal spore characteristics as ball shaped, with attached hyphae, cluster forming and with typical colours (e.g. brown, black, light yellow, pinkish-red, white to dark yellow, pale greenish-yellow) (Gerdemann and Nicolson, 1963). Skills in identifying AM fungal spores were acquired through an intensive examination of a subset of samples. The AM fungal spores from 20 samples were examined under a high-quality stereo microscope (Leica M205) with different high magnification (160x, 120x, 80x and 40x) at the Ageing Research Laboratories, Medical School, Newcastle University. The AM fungal spores were photographed and identified morphologically by comparing spore shape, size, type of hyphae attachment and colour using the identification keys from the International Culture Collection of Vesicular-Arbuscular Mycorrhizal fungi website (INVAM http://invam.caf.wvu.edu). These pictures were used for reference when counting AM fungal spores in the larger sample set. This training allowed accurate identification and separation of AM fungal spores from the wider spore population in the samples.

3.2.6. Statistical analysis

Statistical analysis was conducted using the statistical software R (R Core Team, 2016). A database was imported from an excel sheet to the programming language R to set up a series of analyses. Treatment effects on AM fungal parameters, P concentration, P uptake and total P uptake were analysed to produce ANOVA *P*-values by using mixed-effect models, using the nlme function in R (nonlinear mixed effects package) (Pinheiro and Bates, 2000).

Experiments were designed in a split-split plot design with four replications, with fertiliser rate (high and low) as the main plot, two fertiliser type (compost and mineral) as the sub-plot and variety (Filderstolz, Oberkulmer Rotkorn, ZOR and Rubiota) as sub-sub-plots. Firstly, combined data for both seasons was analysed with year included as a factor in the model, followed by analysis of available data for each year separately where year interacted with one or more of the terms in the model. The combined year analysis used a mixed effects model with four fixed factors (year, fertiliser rate, fertiliser type and variety) with the random error structures of the model as: block/year/fertiliser rate/fertiliser type to reflect the nested structure of the experiment (Crawley, 2007). At each level of the interacting factors, further analyses

were performed where the interaction terms were significant. If interaction terms in the model were significant (*p*-value <0.05), general linear hypothesis tests (Tukey contrasts) were performed using the 'glht' function in the 'multcomp' package to compare differences among interaction means. The control plot (0 kg N ha^{-1}) measurements were not included in ANOVAs but means and standard errors are presented in the results tables.

The mean and standard error (SE) were calculated for each factor and interaction term. QQnorm was used to test the normality of the residuals of all models. Analysis of variance (ANOVA test) was used to determine significant differences (*p*-value < 0.05) between treatments for factors with two levels (e.g. fertiliser type, fertiliser rate and year). If significant differences (*p*-value < 0.05) occurred between varieties and/or interactions between factors with more than two levels, then Tukey's Honest Significant Difference (HSD) Test was performed in the general linear hypothesis testing (glht) function of the multcomp package procedure as described in Crawley (2007).

The Pearson correlation coefficient was assessed between different measured parameters. These measurements included AM fungal colonisation, AM fungal spore density, grain yield, P concentration at anthesis in crop biomass, harvest straw P concentration, grain P concentration, straw P uptake, grain P uptake and total P uptake at harvest. Data from both seasons was used in Pearson's product-moment correlation (Richard, 1990).

3.3. Results

3.3.1. AM fungal colonisation

The combined analysis over two seasons (2014/15 and 2015/16) (Table 3.5) showed that there was no significant effect of year on any of the measures of AM fungal colonisation. The combined analysis indicated that fertiliser rate (Table 3.5) only affected vesicle colonisation but did not have a significant effect on overall root colonisation or hyphae and arbuscules colonisation. The percentage of vesicles was highest where low rates (50 kg N ha^{-1}) of compost or mineral fertiliser were used and was numerically highest (not statistically tested) for the zero input (control) treatment. The lowest percentages of vesicles were observed where a high rate (100 kg N ha^{-1}) of mineral or compost fertiliser was used. In general, the type of fertiliser type and variety did not significantly affect any of the measures of AM fungal colonisation.

Table 3.5. Main effect means, ±SE and *p*-values for the effects and interactions of year, fertiliser type, fertiliser rate and spelt variety on root, hyphae, arbuscule, vesicle colonisation and AM fungal spore density (g^{-1} dry soil) in the spelt variety x fertility management field trial averaged over two seasons (2014/15 and 2015/16).

	Root colonisation (%)	Hyphae colonisation (%)	Arbuscule colonisation (%)	Vesicle colonisation (%)	Spore density (g^{-1} dry soil)
Main effect means					
Year (YR)					
2014/15	74.31 ± 2.79	34.01 ± 1.06	20.34 ± 0.98	19.96 ± 1.05	91 ± 3
2015/16	82.80 ± 1.41	38.54 ± 0.55	23.46 ± 0.59	20.79 ± 0.84	87 ± 3
Fertiliser type (FT)					
Compost	76.79 ± 2.33	35.34 ± 0.98	20.75 ± 0.80	20.70 ± 0.90	91 ± 3
Mineral N	80.32 ± 2.19	37.22 ± 0.78	23.05 ± 0.84	20.05 ± 1.00	87 ± 3
Fertiliser rate (FR)					
0 kg N ha^{-1}	86.00 ± 2.07	38.03 ± 0.74	23.25 ± 0.76	24.75 ± 1.05	82 ± 5
50 kg N ha^{-1}	81.78 ± 2.15	37.24 ± 0.81	22.09 ± 0.80	22.46 ± 0.98	88 ± 3
100 kg N ha^{-1}	75.33 ± 2.32	35.32 ± 0.95	21.71 ± 0.86	18.30 ± 0.84	90 ± 3
Variety (VR)					
Oberkulmer Rotkorn	77.84 ± 2.94	35.38 ± 1.16	21.75 ± 0.97	20.71 ± 1.41	91 ± 6
ZOR	75.25 ± 3.94	35.97 ± 1.63	20.58 ± 1.48	18.71 ± 1.40	94 ± 4
Rubiota	79.53 ± 3.01	35.67 ± 1.19	22.75 ± 1.14	21.11 ± 1.11	85 ± 5
Filderstolz	81.59 ± 2.84	38.10 ± 0.94	22.52 ± 1.05	20.97 ± 1.44	86 ± 3
ANOVA *p*-values					
YR	0.0977	0.0581	0.0803	0.6206	0.387
FT	0.3416	0.1292	0.0797	0.6763	0.2411
FR	0.1203	0.1478	0.7679	**0.0335**	0.7388
VR	0.4329	0.2868	0.4766	0.3616	0.3233
FT * VR	0.1245	0.1653	0.1174	0.4005	**0.0182**
FT * FR * VR	0.0658	**0.0404**	0.1533	0.1538	0.454
FT * FR * YR	0.9863	0.5271	0.6187	0.4066	**0.0497**
FT * VR * YR	0.5879	0.7024	0.1805	0.915	**<0.001**
FR * VR * YR	0.0585	0.0901	0.0779	0.0645	**0.0109**

Zero treatments were not included in the ANOVA.

Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

3.3.2. AM fungal spore density

The combined analysis over two seasons (2014/15 and 2015/16) (Table 3.5) showed that there was no effect of year on the fungal spore density ($p>0.05$). Although the main effect for fertiliser type was not significant, there was a significant interaction ($p=0.0182$) between fertiliser type and variety for spore density (Table 3.5). When mineral N was the fertiliser type, Oberkulmer Rotkorn had the highest spore densities, while Rubiota had the lowest spore densities; however, spore densities were significantly higher in compost compared to mineral N plots when the spelt variety was Rubiota (Table 3.6).

Table 3.6. Interaction means ± SE for the effects of fertiliser type and variety for AM fungal spore density (g^{-1} dry soil) in the spelt variety x fertility management field trial. Mean represent average over two seasons (2014/15 and 2015/16).

Fertiliser type	Variety			
	Oberkulmer Rotkorn	ZOR	Rubiota	Filderstolz
Compost	87 ± 6 Aa	100 ± 7 Aa	95 ± 8 Aa	82 ± 4 Aa
Mineral N	94 ± 9 Aa	88 ± 5 ABa	74 ± 6 Bb	90 ± 6 ABa

For each parameter assessed means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

In the combined analysis year interacted with several of the other experimental factors (FT x FR x YR, FT x VR x YR, FR x VR x YR) and so each year of data for fungal spore density was analysed separately. In each season (2014/15 and 2015/16) fertiliser type, fertiliser rate and variety did not affect spore density (Table 3.7). However, the fertiliser type by variety interaction had a significant effect on spore density in both seasons 2014/15 and 2015/16 (Table 3.7). There was no clear pattern to the results with variety significantly affecting spore density in different ways depending on the year and fertiliser type.

Table 3.7. Main effect means, ±SE and *p*-values for the effects and interactions of fertiliser type, fertiliser rate and spelt variety on AM fungal spore density (g^{-1} dry soil) in the spelt variety x fertility management field trial for each of two seasons: 2014/15 and 2015/16.

	AM Fungal spore density (g^{-1} dry soil)	
	2014/15	**2015/16**
Main effect means		
Fertiliser type (FT)		
Compost	93 ± 5	89 ± 4
Mineral N	88 ± 5	85 ± 5
Fertiliser rate (FR)		
0 kg N ha^{-1}	90 ± 7	73 ± 6
50 kg N ha^{-1}	92 ± 6	84 ± 4
100 kg N ha^{-1}	90 ± 4	90 ± 5
Variety (VR)		
Oberkulmer Rotkorn	95 ± 10	87 ± 5
ZOR	100 ± 6	88 ± 6
Rubiota	88 ± 6	81 ± 8
Filderstolz	81 ± 4	91 ± 5
ANOVA *p*-values		
FT	0.4132	0.3523
FR	0.6494	0.2449
VR	0.1662	0.5394
FT * FR	0.8384	**0.0022**
FT * VR	**0.003**	**<0.001**
FR * VR	**0.0251**	0.4104

Zero treatments were not included in the ANOVA.

Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

For example, in the 2014/15 season the highest spore density (*p*=0.003) was measured for the spelt variety ZOR when compost was used, ; however, spore density was significantly higher in mineral N compared to compost plots when the spelt variety was Oberkulmer Rotkorn (Table 3.8).

In contrast, in 2015/16, the highest spore density (*p*=<0.001) was measured for the spelt variety Rubiota with Oberkulmer Rotkorn slightly lower where compost rather than mineral fertiliser was used; however, the highest spore density was measured for the spelt variety Filderstolz with ZOR slightly lower where mineral rather than compost fertiliser was used (Table 3.8).

Table 3.8. Interaction means ± SE for the effects of fertiliser type and variety for AM fungal spore density (g^{-1} dry soil) in the spelt variety x fertility management field trial (2014/15) and (2015/16).

Fertiliser type	2014/15			
	Variety			
	Oberkulmer Rotkorn	ZOR	Rubiota	Filderstolz
Compost	80 ± 9 Bb	118 ± 6 Aa	89 ± 12 Ba	87 ± 4 Ba
Mineral N	110 ± 17 Aa	81 ± 4 Ab	88 ± 5 Aa	75 ± 7 Aa
	2015/16			
	Oberkulmer Rotkorn	ZOR	Rubiota	Filderstolz
Compost	95 ± 8 ABa	82 ± 7 Ba	102 ± 10 Aa	77 ± 6 Bb
Mineral N	79 ± 6 BCa	94 ± 9 ABa	61 ± 7 Cb	105 ± 6 Aa

For each parameter assessed means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

In 2015/16 there was a significant ($p=0.0022$) fertiliser type x fertiliser rate interaction (Table 3.7). The highest spore density occurred at a high rate compared to a low rate of compost; however, under mineral fertilisation, the rate of N application had no effect on spore density (Table 3.9).

Table 3.9. Interaction means ± SE for the effects of fertiliser type and fertiliser rate for AM fungal spore density (g^{-1} dry soil) in the spelt variety x fertility management field trial (2015/16).

Fertiliser type	Fertiliser rate	
	High	Low
Compost	99 ± 6 Aa	79 ± 4 Ba
Mineral N	80 ± 7 Ab	89 ± 6 Aa

For each parameter assessed means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

In 2014/15 the variety x fertiliser rate interaction was significant ($p=0.0251$) for spore density (Table 3.7). The results showed that the effects of varieties on spore densities was not the same under each fertiliser rate (Table 3.10). At low rates of N application spore density was

significantly higher in Oberkulmer Rotkorn with ZOR and Rubiota slightly lower compared to Filderstolz (Table 3.10).

Table 3.10. Interaction means ± SE for the effects of fertiliser rate and variety for AM fungal spore density (g^{-1} dry soil) in the spelt variety x fertility management field trial (2014/15).

Fertiliser rate	Variety			
	Oberkulmer Rotkorn	ZOR	Rubiota	Filderstolz
High	80 ± 12 Ab	102 ± 10 Aa	83 ± 6 Aa	93 ± 3 Aa
Low	109 ± 15 Aa	97 ± 7 Aa	94 ± 11 ABa	70 ± 5 Ba

For each parameter assessed means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

3.3.3. Phosphorus concentrations in plant tissue

The combined analysis for both seasons (2014/15 and 2015/16) indicated that year had a significant ($p<0.001$) effect on P concentration in straw and grain at harvest. The P concentration for the straw at harvest was higher in 2014/15 than 2015/16 while P concentration for grain at harvest was higher in 2015/16 than 2014/15 (Table 3.11). Over both seasons (2014/15 and 2015/16) compost treatments had significantly higher P concentrations in straw at harvest compared to mineral treatments (Table 3.11).

Additionally, the average of both seasons (2014/15 and 2015/16) results showed that the variety of spelt had a highly significant effect on P concentrations. At anthesis the P concentration in the crop biomass was highest for the spelt variety Filderstolz (Table 3.11). This was reflected in the straw at harvest when Filderstolz also had a high concentration of P equivalent to levels in the ZOR variety. However, grain P concentrations were lowest for these two varieties and highest for Oberklumer Rotkorn and Rubiota (Table 3.11). The highest grain P concentrations were for the Oberkulmer Rotkorn variety. In contrast, the average of both seasons (2014/15-2015/16) results showed that fertiliser rate did not affect P concentration for the all growth stages of spelt (Table 3.11).

Table 3.11. Main effect means, ±SE and *p*-values for the effects and interactions of year, fertiliser type, fertiliser rate and spelt variety on P concentration (mg P g^{-1}) at different spelt growth stages (crop biomass at anthesis, straw and grain at harvest) in the spelt variety x fertility management field trial, averaged over two seasons (2014/15 and 2015/16).

Spelt growth stage	P concentration (mg P g^{-1})		
	Anthesis	Harvest Straw	Harvest Grain
Main effect means			
Year (YR)			
2014/15	1.98 ± 0.04	0.88 ± 0.03	4.02 ± 0.04
2015/16	1.94 ± 0.03	0.67 ± 0.03	4.46 ± 0.05
Fertiliser type (FT)			
Compost	2.01 ± 0.04	0.87 ± 0.03	4.24 ± 0.05
Mineral N	1.92 ± 0.04	0.68 ± 0.03	4.24 ± 0.06
Fertiliser rate (FR)			
0 kg N ha^{-1}	2.22 ± 0.06	0.76 ± 0.03	4.29 ± 0.08
50 kg N ha^{-1}	1.99 ± 0.04	0.79 ± 0.03	4.25 ± 0.05
100 kg N ha^{-1}	1.94 ± 0.04	0.76 ± 0.03	4.23 ± 0.05
Variety (VR)			
Oberkulmer Rotkorn	1.83 ± 0.05 c	0.72 ± 0.04 bc	4.54 ± 0.07 a
ZOR	1.89 ± 0.04 bc	0.87 ± 0.03 a	3.71 ± 0.03 c
Rubiota	1.97 ± 0.05 b	0.69 ± 0.04 c	4.43 ± 0.05 ab
Filderstolz	2.16 ± 0.05 a	0.81 ± 0.05 ab	4.29 ± 0.05 b
ANOVA *p*-values			
YR	0.7809	**0.0105**	**0.0013**
FT	0.0881	**<0.001**	0.9123
FR	0.3499	0.3496	0.6376
VR	**<0.001**	**0.001**	**<0.001**
FR * VR	0.2775	0.6609	**0.0345**
VR * YR	0.2775	**0.0003**	**0.0006**
FT * VR * YR	**0.0334**	0.635	0.3906
FT * FR * VR * YR	0.4929	0.4057	**0.0481**

Main effect means for variety within the same column followed by the same letter are not significantly different (Tukey's HSD *p*<0.05). Zero treatments were not included in the ANOVA.

Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

The combined two season results (2014/15 and 2015/16) also detected a significant fertiliser rate x variety interaction (p=0.0345) for P concentration in grain at harvest (Table 3.11). The highest P concentration occurred where the spelt variety was Oberklumer Rotkorn at both fertiliser rates, but at the low rate Rubiota had a concentration of P equivalent to levels in Oberklumer Rotkorn; however, spelt variety ZOR had the lowest concentration of P concentration for grain at both fertiliser rates (Table 3.12).

Table 3.12. Interaction means ± SE for the effects of fertiliser rate and variety for the P concentration (mg P g^{-1}) of grain at harvest in the spelt variety x fertility management, average for two seasons (2014/15 and 2015/16).

Fertiliser rate	Variety			
	Oberkulmer Rotkorn	ZOR	Rubiota	Filderstolz
High	4.52 ± 0.10 Aa	3.70 ± 0.04 Ca	4.37 ± 0.06 Ba	4.34 ± 0.07 Ba
Low	4.56 ± 0.09 Aa	3.72 ± 0.05 Ca	4.48 ± 0.08 Aa	4.24 ± 0.06 Ba

For each parameter assessed means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test p<0.05).

All the main effects on P concentration were involved in the interactions with year (Table 3.11) and so the analysis was simplified by analysing results for each year separately. The results showed that fertiliser type had a significant effect on P concentrations for the straw at harvest in each season (p<0.05) 2014/15 (Table 3.13) and (p<0.01) 2015/16 (Table 3.14). The compost resulted in the highest concentration of P for the straw at harvest in each season. The results also showed that variety of spelt had a highly significant effect on P concentration in each season. In 2014/15 the P concentration in the crop biomass at anthesis was highest for the spelt variety Filderstolz (Table 3.13). This was reflected in the straw at harvest when Filderstolz also had a high concentration of P equivalent to levels in the ZOR variety. However, grain P concentrations were lowest for these two varieties and highest for Oberklumer Rotkorn and Rubiota (Table 3.13). In season 2015/16 the P concentration in the crop biomass at anthesis was highest for the spelt variety Filderstolz (Table 3.14), while spelt variety ZOR had the highest concentration of P for straw at harvest. However, grain P concentrations were lowest for the spelt variety ZOR and highest for Oberklumer Rotkorn (Table 3.14). In contrast, the results of

each season 2014/15 and 2015/16 showed that fertiliser rate did not affect P concentration at any growth stage of spelt (Table 3.13 and 3.14).

Table 3.13. Main effect means, ±SE and *p*-values for the effects and interactions of fertiliser type, fertiliser rate and spelt variety on P concentration (mg P g^{-1}) at different spelt growth stages (crop biomass at anthesis, straw and grain at harvest) in the spelt variety x fertility management field trial in one season (2014/15).

Spelt growth stage	P concentration (mg P g^{-1})		
	Anthesis	**Harvest Straw**	**Harvest Grain**
Main effect means			
Fertiliser type (FT)			
Compost	2.00 ± 0.07	0.95 ± 0.04	4.02 ± 0.05
Mineral N	1.96 ± 0.05	0.81 ± 0.03	4.02 ± 0.05
Fertiliser rate (FR)			
0 kg N ha^{-1}	2.23 ± 0.08	0.81 ± 0.05	4.03 ± 0.06
50 kg N ha^{-1}	2.00 ± 0.06	0.92 ± 0.04	4.02 ± 0.05
100 kg N ha^{-1}	1.96 ± 0.06	0.84 ± 0.03	4.02 ± 0.05
Variety (VR)			
Oberkulmer Rotkorn	1.83 ± 0.07 c	0.83 ± 0.05 bc	4.23 ± 0.03 a
ZOR	1.87 ± 0.07 c	0.91 ± 0.03 ab	3.55 ± 0.03 c
Rubiota	2.04 ± 0.09 b	0.76 ± 0.06 c	4.22 ± 0.03 a
Filderstolz	2.20 ± 0.09 a	1.03 ± 0.05 a	4.09 ± 0.02 b
ANOVA *p*-values			
FT	0.5564	**0.0252**	0.9912
FR	0.5667	0.275	0.9086
VR	**<0.001**	**0.0013**	**<0.001**

Main effect means for variety within the same column followed by the same letter are not significantly different (Tukey's HSD *p*<0.05). Zero treatments were not included in the ANOVA.

Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

Table 3.14. Main effect means, ±SE and *p*-values for the effects and interactions of fertiliser type, fertiliser rate and spelt variety on P concentration (mg P g^{-1}) at different spelt growth stages (crop biomass at anthesis, straw and grain at harvest) in the spelt variety x fertility management field trial in one season (2015/16).

Spelt growth stage	P concentration (mg P g^{-1})		
	Anthesis	**Harvest Straw**	**Harvest Grain**
Main effect means			
Fertiliser type (FT)			
Compost	2.02 ± 0.03	0.79 ± 0.03	4.47 ± 0.07
Mineral N	1.87 ± 0.05	0.55 ± 0.03	4.46 ± 0.08
Fertiliser rate (FR)			
0 kg N ha^{-1}	2.20 ± 0.10	0.71 ± 0.04	4.56 ± 0.11
50 kg N ha^{-1}	1.98 ± 0.04	0.66 ± 0.04	4.48 ± 0.08
100 kg N ha^{-1}	1.91 ± 0.04	0.67 ± 0.03	4.44 ± 0.07
Variety (VR)			
Oberkulmer Rotkorn	1.84 ± 0.07 b	0.62 ± 0.05 b	4.85 ± 0.06 a
ZOR	1.91 ± 0.05 b	0.84 ± 0.04 a	3.86 ± 0.03 c
Rubiota	1.91 ± 0.05 b	0.62 ± 0.05 b	4.63 ± 0.06 b
Filderstolz	2.12 ± 0.07 a	0.60 ± 0.04 b	4.50 ± 0.05 b
ANOVA *p*-values			
FT	0.0736	**0.001**	0.9046
FR	0.4369	0.7854	0.6013
VR	**0.0021**	**<0.001**	**<0.001**
FR * VR	0.5417	0.9339	**0.0278**

Main effect means for variety within the same column followed by the same letter are not significantly different (Tukey's HSD *p*<0.05). Zero treatments were not included in the ANOVA.

Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

The results of one season (2015/16) also indicated that there was a significant variety x fertiliser rate interaction effect (*p*=0.0278) on P concentration for grain at harvest (Table 3.14). The highest P concentration occurred where the spelt variety was Oberkulmer Rotkorn at both fertiliser rates, but there was an exception at the low rate where Rubiota also had the highest concentration of P equivalent to levels in the Oberkulmer Rotkorn; however, spelt variety ZOR had the lowest concentration of P for grain at both fertiliser rates (Table 3.15).

Table 3.15. Interaction means ± SE for the effects of fertiliser rate and spelt variety for the P concentration (mg P g^{-1}) of grain at harvest in the spelt variety x fertility management field trial (2015/16).

Fertiliser rate	Variety			
	Oberkulmer Rotkorn	ZOR	Rubiota	Filderstolz
High	4.82 ± 0.11 Aa	3.85 ± 0.04 Ca	4.52 ± 0.09 Bb	4.57 ± 0.06 Ba
Low	4.89 ± 0.07 Aa	3.87 ± 0.05 Ca	4.74 ± 0.06 Aa	4.42 ± 0.06 Ba

For each parameter assessed means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

3.3.4. Grain yield and P uptake

The main effects of fertiliser type, fertiliser rate and spelt variety on grain yield and P uptake at different spelt growth stages were investigated. In general, the grain yield and P uptake for the straw at harvest, as well as the total P uptake at harvest were higher in 2014/15 than 2015/16 season (Table 3.16). Over both seasons (2014/15 and 2015/16) fertiliser type had a significant effect on P uptake for the straw at harvest (Table 3.16). The compost resulted in the highest P uptake for the straw at harvest. Additionally, the combined analysis of both seasons (2014/15 and 2015/16) showed that variety of spelt had a highly significant effect on grain yield and P uptake. Furthermore, the results illustrated that Filderstolz had the lowest grain yield and P uptake for grain and total P uptake at harvest (Table 3.16). The highest P uptake for grain, total P uptake and grain yield were for the Oberkulmer Rotkorn variety of spelt. In contrast, the average of both seasons (2014/15 and 2015/16) results showed that fertiliser rate did not affect grain yield and P uptake for any growth stage of spelt (Table 3.16).

Table 3.16. Main effect means, ±SE and *p*-values for the effects and interactions of year, fertiliser type, fertiliser rate and spelt variety on grain yield (t ha^{-1}), P uptake (kg P ha^{-1}) for straw and grain at harvest and total P uptake (kg P ha^{-1}) (P uptake for straw plus grain at harvest), in the spelt variety x fertility management field trial, averaged over two seasons (2014/15 and 2015/16).

	P uptake (kg P ha^{-1}) at Harvest			Grain yield (t ha^{-1})
Spelt growth stage	**Straw**	**Grain**	**Total**	
Main effect means				
Year (YR)				
2014/15	4.18 ± 0.17	13.09 ± 0.36	17.27 ± 0.43	3.25±0.08
2015/16	2.45 ± 0.13	12.03 ± 0.46	14.48 ± 0.47	2.68±0.09
Fertiliser type (FT)				
Compost	3.65 ± 0.19	12.55 ± 0.38	16.20 ± 0.44	2.96±0.08
Mineral N	2.99 ± 0.18	12.56 ± 0.46	15.55 ± 0.53	2.97±0.10
Fertiliser rate (FR)				
0 kg N ha^{-1}	2.84 ± 0.27	11.93 ± 0.63	14.78 ± 0.68	2.79 ± 0.14
50 kg N ha^{-1}	3.38 ± 0.21	12.47 ± 0.40	15.86 ± 0.45	2.94±0.09
100 kg N ha^{-1}	3.25 ± 0.16	12.64 ± 0.44	15.89 ± 0.52	2.99±0.10
Variety (VR)				
Oberkulmer Rotkorn	3.44 ± 0.29	15.76 ± 0.44 a	19.19 ± 0.52 a	3.49±0.10 a
ZOR	3.61 ± 0.23	10.99 ± 0.36 c	14.60 ± 0.49 c	2.98±0.11 b
Rubiota	3.24 ± 0.28	13.76 ± 0.52 b	17.00 ± 0.66 b	3.12±0.12 b
Filderstolz	2.99 ± 0.25	9.72 ± 0.33 d	12.71 ± 0.45 d	2.27±0.08 c
ANOVA *p*-values				
YR	**0.0044**	0.2137	**0.0492**	**0.0282**
FT	**0.0073**	0.9728	0.1785	0.986
FR	0.5492	0.6908	0.9363	0.599
VR	0.1707	**<0.001**	**<0.001**	**<0.001**
FT * FR * VR	**0.0371**	0.5801	0.4458	0.577

Main effect means for variety within the same column followed by the same letter are not significantly different (Tukey's HSD *p*<0.05). Zero treatments were not included in the ANOVA.

Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

3.3.5. Relationships among AM fungi, grain yield and P nutrition

All the data from both years of the experiment were pooled and correlations among key variables were investigated and only figures with significant correlations have been presented. A Pearson correlation analysis revealed that root colonisation was negatively correlated with grain yield (Fig 3.4a). Furthermore, these results revealed that soil spore density was not correlated with AM fungal colonisation (Fig 3.4b). In addition, there was no relationship between root colonisation and P uptake in grain (data not shown), but there was a negative relationship between AM fungal root colonisation and P uptake in straw at the harvest (Fig 3.4d). P concentration in the grain was positively correlated with AM fungal hyphae colonisation (Fig 3.5a), as well as P concentration in crop biomass at anthesis having a strong positive correlation with AM fungal hyphae colonisation (data not shown). P concentration in crop biomass at anthesis also showed a positive correlation with AM root colonisation and vesicle colonisation (Fig 3.5b and d). In contrast, P concentration in crop biomass at anthesis showed a negative correlation with grain yield (Fig 3.4e). Furthermore, P concentration in straw at harvest showed a negative correlation with arbuscule colonisation (Fig 3.4c).
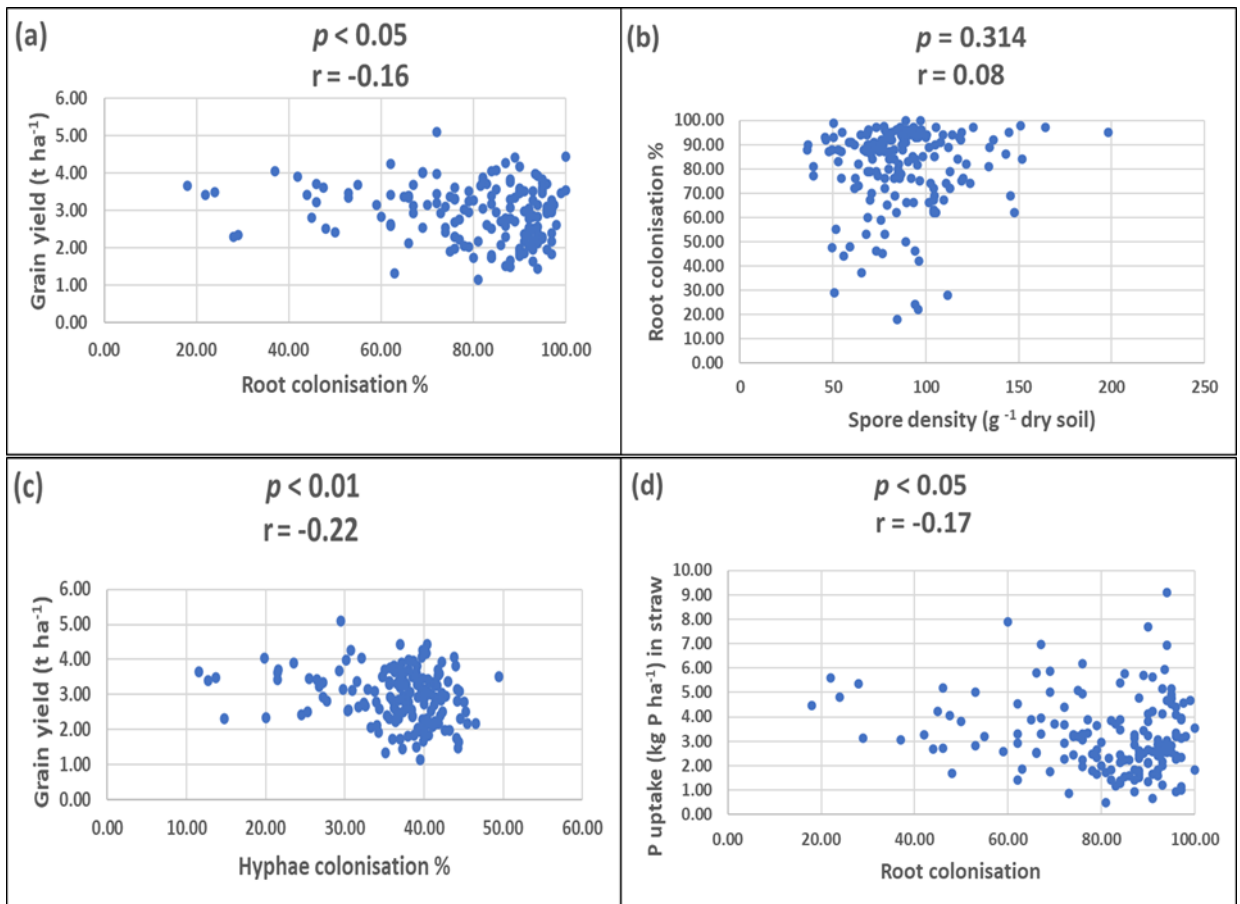
Fig 3.4. Pearson correlation coefficients (r) between all the individual sample values of (a) AM fungal total root colonisation and grain yield (b) AM fungal total root colonisation and spore density (c) hyphae colonisation and grain yield (d) AM fungal total root colonisation and P uptake (kg ha^{-1}) in straw at harvest, in the spelt variety x fertility management field trial (data pooled for 2014/15 and 2015/16 seasons).
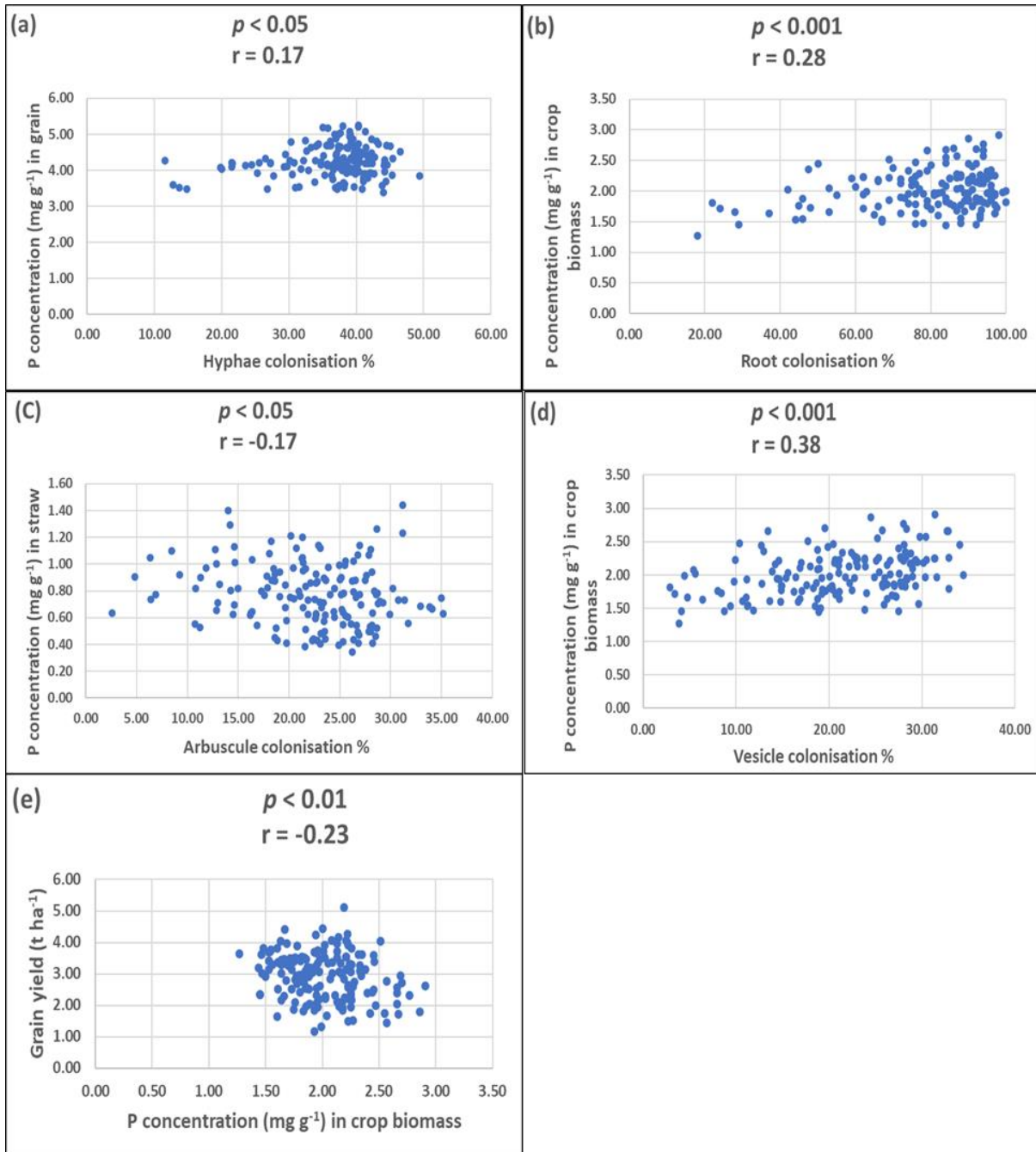
Fig 3.5. Pearson correlation coefficients (r) between all the individual sample values of (a) hyphae colonisation % and P concentration (mg g^{-1}) in grain (b) AM fungal root colonisation % and P concentration (mg g^{-1}) in crop biomass at anthesis (c) arbuscule colonisation % and P concentration (mg g^{-1}) in straw at harvest (d) vesicle colonisation % and P concentration (mg g^{-1}) in crop biomass at anthesis (e) P concentration (mg g^{-1}) in crop biomass at anthesis and grain yield (t ha^{-1}), in the spelt variety x fertiliser management field trial (data pooled for 2014/15 and 2015/16 seasons).

3.4. Discussion

3.4.1. How does fertility management (fertiliser type and rate) affect AM fungal colonisation and spore densities?

In this study, the main effect of fertiliser type on AM fungal colonisation and spore density was not significant (Table 3.5). However, there was a fertiliser type by fertiliser rate interaction in 2015/16 (Table 3.7 and 3.9) where the highest AM fungal spore density was measured at the high rate of compost addition.

The AM fungal colonisation characteristics are strongly controlled with host nutrient status and soil nutrient availability (Smith and Read, 2008; Prasad *et al.*, 2012). The compost treatments in this study provided phosphorous to the crop (21.74 and 43.47 kg total P_2O_5 ha^{-1} in 2014 for the crop 2014/15 season and 16.47 and 32.93 kg total P_2O_5 ha^{-1} for the crop 2015/16 season for low and high rates respectively) while no additional P was added with the mineral N fertiliser. The P additions applied in the compost plots may increase availability of soil P, as the initial availability of soil P was low in this study. This was reflected in higher P concentrations and P uptake in the spelt in the compost treatments compared to the mineral treatments at harvest, and slightly higher (non-significant results) values of P concentrations in the biomass from compost treatments at anthesis (Table 3.11, 3.13, 3.14 and 3.16). In general, the AM fungal root colonisation is inversely related to soil available P and crop P nutrition (Kahiluoto *et al.*, 2001; Wang *et al.*, 2017) and this was reflected in this study where there was a negative relationship between arbuscule colonisation and concentration of P at harvest (Fig 3.4c). These results are consistent with other studies which found that compost may not always have a positive effect on AM fungal colonisation and spore density. For example, Liu *et al.* (2019) observed that long-term (low and high) organic compost application led to low AM fungal colonisation levels due to the accumulation of P at the surface of soil.

The composition of P in organic fertilisers may affect AM fungal colonisation and spore density. Douds *et al.* (1997) reported that compost fertiliser improved the spore density of two groups of AM fungi (*G.etunicatum and Glomus spp.*) compared to raw dairy cow manure and conventional fertiliser. This may be due to lower concentrations of available P in compost and manure fertilisers. Tanu *et al.* (2004) indicated that poultry manure reduced AM fungal propagules in soil and attributed this to the high available P in these soils as the poultry manure

is known to increase the availability of P in soils. Therefore, availability of P in organic amendments may be as important as the total P contents in determining impacts on AM fungal colonisation.

The lack of a positive effect of compost on AM fungal colonisation and spore density may also be related to the AM fungal species present. Unfortunately, molecular techniques were not used to quantify AM fungal activity or species present in this study, so it is not possible to state the specific AM fungal species present in compost and mineral plots.

AM fungal species differ in their response to organic compost composition (Gryndler *et al.*, 2006; Liu *et al.*, 2019). The changes in environmental conditions such as organic matter (Yang *et al.*, 2018a), nutrient level (e.g. N, P) and soil pH (Wang *et al.*, 2011) due to organic compost addition may stimulate particular AM fungal species (Entry *et al.*, 2002; Alguacil *et al.*, 2011). One possible explanation is that the AM fungal species present in compost plots would not effectively colonise and provide benefits to host plants (Jansa *et al.*, 2008; Frew, 2019). Some AM fungal species may be sensitive to N accumulation due to organic compost addition including *Glomeraceae* (Bhadalung *et al.*, 2005). The variable response of different AM fungal species to compost addition may also refer to different life strategies of AM fungi (Hart and Reader, 2002; Liu *et al.*, 2019). For instance, (Liu *et al.*, 2019) observed that the AM fungal species *Acaulospora* were only present in organic compost plots and the authors pointed out that these species may be sensitive to the amount and presence of compost addition. Whereas other AM fungi may be sensitive to the organic compost rate, such as *Sclerocystis sinuosa* which was observed only in the low compost rate (Liu *et al.*, 2019). For instance, Yu *et al.* (2013) used 454 pyrosequencing in *Pisum sativum* roots and found that *Paraglomus sp.* were more abundant in treatments with low level rather than high levels of organic matter addition.

The AM fungal root colonisation may be more sensitive to long-term than short-term compost additions. The single application of compost amendment in this study may not have been enough to identify compost effects on AM fungal colonisation. For example, it has been found that 2-years of organic manure fertiliser did not affect AM fungal community composition and richness in maize compared to mineral fertiliser (Toljander *et al.*, 2008; Beauregard *et al.*, 2013) and *Avena sativa* (Zheng *et al.*, 2016).

This study showed significant interactions between fertiliser type and rate where the high rates of compost fertiliser enhanced spore density compared to low rates, but there were no differences in spore density between the two rates of mineral fertiliser (Table 3.9). Additionally, this effect was not evident for AM fungal colonisation. This result is consistent with previous studies for example, (Yang *et al.*, 2018a) reported that the highest rate of compost (45 Mg ha^{-1}) did not inhibit AM fungal growth and was efficient for increasing AM fungal spore density and Tanu *et al.* (2004) also found that AM fungal propagules were increased by higher application rates of composted manures. Compost application can result in patches with high levels of organic N that can enhance AM fungal spore density due to extra-radical hyphal proliferation (Bukovská *et al.*, 2016; Chen *et al.*, 2018a). Whereas AM fungi can get organic carbon from plant roots (Gavito and Olsson, 2003), they may also get additional nutrients such as N from organic sources, as reported by Sabine *et al.* (1999) and Hodge *et al.* (2001). The concentration of N in extra-radical hyphae of AM fungi is greater than in plant shoots and roots (Hodge and Fitter, 2010). Therefore, under high N conditions, AM fungi can get sufficient amounts of N for their extra-radical hyphae growth (Chen *et al.*, 2018a). This is one possible reason for increasing spore density of AM fungi due to enhanced extra-radical hyphae growth. In contrast, Copetta *et al.* (2011) conducted a pot trial and found a reducing trend of colonisation by AM fungi in *S. lycopersicum* along compost addition gradient (compost addition: 0, 25, 50, 75, 100%). However, several studies found that the compost addition stimulated colonisation by AM fungi (Tanwar *et al.*, 2013; Yang *et al.*, 2018a). This suggests that AM fungal colonisation and spore density may show varying responses to compost addition, This varying response may depend on the plant species, compost type (Muthukumar and Udaiyan, 2002; Copetta *et al.*, 2011; Cavagnaro, 2014), dosage of compost (Copetta *et al.*, 2011; Liu *et al.*, 2019) and AM fungal communities (Yang *et al.*, 2018a; Liu *et al.*, 2019).

In this study, over both seasons (2014/15 and 2015/16) lower rates of fertiliser input, whether from compost or mineral sources, promoted vesicle formation but not total colonisation, hyphae or arbuscules (Table 3.5). Vesicles are storage organs used to store carbohydrates and lipids (Jin *et al.*, 2017; Luginbuehl *et al.*, 2017). Under low levels of N supply, carbohydrates can accumulate in the plant as rates of production can exceed utilisation by growing organs of the plant (Cruz *et al.*, 2003; Jin *et al.*, 2015). Many studies have confirmed that carbohydrates can accumulate in plants under low N fertiliser conditions. For example, Braun *et al.* (2016) found that a low N level (0 and 50 kg N ha^{-1}) stimulated potato plants to accumulate more carbohydrate in the plant than at high N levels (100-300 kg N ha^{-1}). This has been reflected in the negative

relationship between the soluble carbohydrate concentrations within plant roots and concentration of N, P and K in shoots and roots (Muthukumar and Udaiyan, 2000). It may be that the high carbohydrate concentration that is allocated to roots when the N levels are lower stimulates fungi to form storage structures, including vesicles. It is thought that when mycorrhizal plants are subjected to stress conditions such as salt stress, drought stress and high temperatures, vesicles may survive (Jin *et al.*, 2017). In fact the existence of vesicles may be an indication that AM fungi are causing a decreased growth rate of crops through storing the carbohydrate resources in these structures (Jin *et al.*, 2017). One possible explanation is that when vesicles grow, AM fungi may need to obtain more C assimilate from the host plant, resulting in plant growth depression (Jin *et al.*, 2017).

While low fertility status affected vesicle colonisation of AM fungi, it does not always affect levels of AM fungal spore density in the soil. Schalamuk *et al.* (2011) found that AM fungal colonisation and arbuscule formation were greater with a low level of N (80 kg N ha^{-1}) compared to high N (160 kg N ha^{-1}) in wheat roots but that N fertiliser rate did not affect AM fungal spore density in the soil. Likewise, in this experiment, fertility management only affected spores in the 2015/16 season when the numbers were significantly higher in plots that received a high rate of compost application.

3.4.2. Do spelt cultivars differ in their level of AM fungal symbiosis and impacts on spore densities in the soil?

One strategy to improve AM fungal efficacy in cropping systems could be to select genotypes that more effectively form associations with these beneficial fungi. In this study the main effects of variety for both root colonisation and spore densities for the average for two seasons were not significant. However, the results of this experiment did not clearly identify which variety was better at forming associations with AM fungi as both the landrace Oberkulmer Rotkorn and the modern variety ZOR, which was bred for organic production systems, responded differently to AM fungal colonisation under different fertility management practices.

The fertiliser type x variety interaction for spore density on average for both seasons and within each season, indicates that some varieties may have no clear effect on spore density under specific fertility management systems. This may be related to different life-cycle strategies of AM fungal species colonised spelt roots (Bücking *et al.*, 2012). Spelt varieties may be colonised

by different AM fungal species, some of them may slowly colonise host roots and rapidly produce spores and conversely other species may rapidly colonise roots and slowly produce spores (Powell and Rillig, 2018).

It is well known, to assess AM fungal colonisation and spore density the seasonal variations and environmental conditions should be considered (Londoño *et al.*, 2019). Spore production starts when the plants are mature and before harvest season (Giovannetti, 1985), and when they are colonised by AM fungi. This could be related to the life-cycle of AM fungi as colonisation occurs before spore production. AM fungi may get the maximum colonisation in host roots at the flowering period, while producing lower spore density in this period compared to other phenological stages (e.g., harvest) of host plant (Prates Júnior *et al.*, 2019; Zhang *et al.*, 2019).

The lack of a clear pattern in the genotype effect on AM fungi in this study contrasts with other studies that have found genotypic differences in AM fungal colonisation within crop species. For example, different wheat genotypes inoculated with the AM fungus *Glomus mosseae* presented various degrees of AM fungal infection and the results were inconsistent with regard to variety effect on colonisation (Azcon and Ocampo, 1981). The mechanisms provided by those authors to explain the genotypic differences among wheat cultivars was that AM fungal colonisation was controlled by the amount of sugar existing in the exudates and extracts of plant roots. Some cultivars may exude more sugar from roots than other varieties which can help to develop AM fungal associations in these varieties. However, the different levels of AM fungal colonisation among wheat cultivars may not be directly attributed to the amount of sugar in both root extracts and exudates. The initial establishment of AM fungal colonisation requires a minimum amount of sugar and lipids in root exudates to encourage initial AM fungal growth (Luginbuehl *et al.*, 2017; Chen *et al.*, 2018a; Lanfranco *et al.*, 2018), and after that the level of AM fungal colonisation reached in wheat varieties could be regulated by a complexity of factors.

In addition to sugar, lipids are an important source of organic C delivered to the AM fungus from plant roots and this is necessary for AM fungal development (Luginbuehl *et al.*, 2017). The cultivars may have genetic variation for delivering lipids to the AM fungus which affect the level of colonisation (Luginbuehl *et al.*, 2017). In the current study, different P concentration in spelt tissues (Table 3.11) as a consequence of different fertility management may affect these mechanisms. This is because the P concentration in the shoots and roots of plants influences

the permeability of the cell phospholipid membranes of host plant roots, and in turn this could affect the amount of sugar and lipids delivered to AM fungi by different cultivars.

Varieties may have different influences on AM fungal colonisation (Kirk *et al.*, 2011; Singh *et al.*, 2012; Londoño *et al.*, 2019). Some varieties developed higher levels of AM fungal colonisation due to their compatibility with AM fungi being greater than other varieties. The ability to establish AM fungal colonisation vary according to landrace and/or modern varieties. For example, Kirk *et al.* (2011) reported that older wheat cultivars had lower total AM fungal colonisation and arbuscule formation than modern cultivars. However, these results were not in agreement with results of current study. There was no clear pattern of differences among the spelt varieties for AM fungal colonisation and spore density in this study as the main effect for variety was not significant and the three modern varieties (Filderstolz, ZOR and Rubiota) and one landrace (Oberkulmer Rotkorn) exhibited similar AM fungal colonisation and spore density. This is consistent with (Essiane-Ondo *et al.*, 2019) who found that there were no differences among the wheat landraces and all cultivars exhibited similar AM fungal colonisation. There is no evidence to suggest that landraces or organically bred varieties are better at forming AM fungal associations, therefore, no firm conclusion drawn.

3.4.3. Does AM fungal colonisation increase the grain yield and P nutrition of spelt?

Variety was the primary factor affecting P nutrition parameters and grain yield in spelt, in contrast to AM fungal parameters which were more influenced by fertility management. In the present study, it seems the old Swiss landrace, Oberkulmer Rotkorn was more suited to low input/organic conditions based on yield performance and P nutrition compared to the variety ZOR, even though it was bred for organic production systems. This result is consistent with Cosser *et al.* (1997) who found that old genotypes may be better suited to organic crop management, since they grow taller, accumulate more early dry matter and compete better for light than short-stemmed modern genotypes. In general, the landrace Oberkulmer Rotkorn seems to have the highest grain yield and P uptake. This does not appear to be related to AM fungal colonisation, since this landrace did not exhibit AM fungal colonisation that was significantly different from the other modern genotypes. Enhanced P levels in grain in this landrace may have been related to other mechanisms (e.g. root system size, number of fine root hairs) which allowed it to take up more P and successfully translocate it to the grain (Haling *et al.*, 2018).

The current study did not show a strong relationship between AM fungal colonisation and grain yield or P nutrition. There was a slight, weak negative relationship between grain yield and total root colonisation by AM fungi (Fig 3.4a) that could be related to changes in soil nutrient content due to fertiliser application (Knerr *et al.*, 2018). In this study the soil P level was low (P index=0) in both years and the compost plots received compost which provided N, P and K, but the mineral plots only received N fertiliser. Compost may supply a sustained release of P rather than a large single pulse of P and preserve moderate levels of soil available P which may explain the higher P concentrations and uptake for compost plots in straw at harvest compared to mineral N fertiliser (Yang *et al.*, 2017). Nonetheless P supply was not limiting yield since both mineral and compost plots had similar yields (see Table 3.16). This may have resulted in a slight negative or neutral effect of AM fungi due to demands for organic carbon from the host plant overtaking any benefits which might be produced from P or N transfer through the hyphal network of AM fungi (Johnson *et al.*, 1997; Jin *et al.*, 2017). This could not be confirmed in this study because nutrient supply to the spelt in all treatments was sufficient. Therefore, future studies of the effect of AM fungi on crop yields should take place along gradients of soil nutrient composition as this will improve understanding about what soil N and/or P conditions impact the AM fungal benefit to the host plant with regard to P nutrition and yield.

The poor relationship between AM fungal colonisation and crop yield in this study may also be due to competition for food resources with other soil microorganisms (Thakur *et al.*, 2019). The interactions between AM fungi and soil microorganism are complex and it is known that the rhizosphere of mycorrhizal plants can inhibit the growth of other beneficial microorganisms. On the other hand, some soil microorganisms may suppress AM fungal growth, thus constraining their benefits for crop yield and P nutrition (Thakur *et al.*, 2019), for example fungivorous organisms may consume mycorrhizal fungi (Hoeksema *et al.*, 2010). Some deleterious rhizosphere bacteria may produce unfavourable compounds (e.g. phytotoxins) (Klironomos and Hart, 2002) which contribute to reducing the ability of AM fungi to increase P nutrition and grain yield. It was beyond the scope of this study to explore rhizosphere communities, but a detailed bacterial and fungal community analysis using techniques such as next generation sequencing could be conducted in future research to elaborate on the community dynamics impacting on AM fungal function in cereal crops.

Significant AM fungal formation does not necessarily translate into higher crop yield. It is worthwhile to understand that while AM fungal colonisation can be beneficial, it does not

always improve P uptake and crop yield (Ryan *et al.*, 2016; Ipsilantis *et al.*, 2018). These results are consistent with those of Ryan and Angus (2003) who reported that autumn-sown wheat and field pea did not benefit in terms of P uptake and yield from enhanced AM fungal colonisation, even under P deficient conditions. The authors concluded that this may be attributed to the growing season moisture regime and temperature which impact P availability and crop yield. Those authors indicated that the low temperature may contribute to suppression of nutrient uptake (e.g. P, N and Zn) by AM fungi as P-translocation rates and P-flux in extra-radical hyphae of AM fungi may be reduced with declining temperature. In addition, the photosynthetic rate may also reduce under low temperature which could lead to transfer of less carbohydrate to AM fungi, thus reducing their development. In the present study, the low temperature (8.87 °C- 9.25 °C) may contribute to suppression of P uptake by AM fungi, thus reducing their ability to enhance crop yield. Environmental stress may be an important determinant of AM fungal colonisation and their population in soil and therefore should be considered when assessing factors affecting AM fungi under field conditions.

While P uptake differed in 2014/15 compared to 2015/16, there were no differences in AM fungal colonisation between the years. Other factors may affect P uptake such as environmental conditions (Roberts and Johnston, 2015). Since soil type and previous management as well as chemical properties were similar in both seasons, variations in weather between 2014/15 and 2015/16 are the most likely cause of the annual differences in grain yield and P nutrition. Rainfall in both seasons was within the normal range of rainfall in the UK (600 mm–1000mm) (MetOffice, 2018a) with 642 mm from September 2014 until September 2015 and 878 mm from September 2015 until September 2016 (Fig 3.6b). Average air temperatures during the growing period for the crop were 8.87 °C from September 2014 until September 2015 and 9.25 °C from September 2015 until September 2016 (Fig 3.6a); as these were within 1 °C temperature is not likely the important factor affecting the crop yield. Radiation levels may be the main factor contributing to higher yields in the 2014/15 season (Bilsborrow *et al.*, 2013), as 2015 had 6% more sunshine hours annually and 34% more sunshine during June in Northeast England compared to 2016 (MetOffice, 2018b). Based on the field station weather data collected at Nafferton farm, total radiation was higher over the full year and specifically over April, May, June and July in 2015 (1937 MJ m^{-2}) for the 2014/15 crop season compared to 2016 (1342 MJ m^{-2}) for the 2015/16 crop season. The higher yields in 2014/15 reflected a pattern across the UK that may have been due to higher sunshine hours (DEFRA, 2016). Higher yields resulted in higher P uptakes in 2014/15, even though P concentrations were actually lower in

the grain in 2014/15 (but higher in the straw). Sometimes higher yielding crops have lower concentrations of nutrients due to a dilution effect.

AM fungal arbuscule formation has been positively correlated with P concentration in the plant tissue of corn (Sheng *et al.*, 2012), but we did not observe a positive relationship between arbuscule formation and P concentration in spelt straw in the current study (Fig 3.5c). AM fungal total root colonisation reflects different proportions of arbuscule, vesicle and hyphae abundance in the roots. Increased AM fungal total root colonisation may not always reflect arbuscule formation. Sometimes arbuscule formation can decline with increasing nutrients (e.g. N and P) in the host as arbuscule formation tends to be greatest at a time of nutrient demand by the host (Muthukumar and Udaiyan, 2000). Whereas, the increasing concentration of nutrients in the host plant may not reduce and may even increase vesicle formation and/or hyphae (Muthukumar and Udaiyan, 2000). Therefore, a reduction in arbuscule colonisation by high concentrations of nutrients in the host plant may not always lead to reduced total root colonisation. Sheng *et al.* (2012) found that tillage reduced AM fungal total root colonisation and arbuscule colonisation, while it increased vesicle formation and did not significantly affect hyphae formation. This investment in vesicle formation is not beneficial to the host plant nutrition, while arbuscular growth is important for AM fungi to supply the host plant with resources (Sheng *et al.*, 2012; Voříšková *et al.*, 2017). Variations in patterns of AM fungal structure formation may be reflected in this study. There was a strong positive relationship between P concentration and AM fungal total root colonisation and vesicle colonisation (Fig 3.5b and d), excluding arbuscule colonisation in crop biomass at anthesis (data not shown). In addition, the significant negative correlation between P concentration in straw at harvest and arbuscules at anthesis may confirm that AM fungi did not provide nutritional benefits to the spelt which was also reflected in the negative relationship between grain yield and P concentration (Fig 3.4e).

It is possible that the method used to quantify AM fungi in the roots in this study did not reflect their level of function. Assessing the colonisation level using root staining and microscopy is not necessarily the best measure of assessing AM fungal activity in the host plant roots (Mensah *et al.*, 2015). Specifically, the vitality of colonising structures and the intensity of colonisation (i.e. number of hyphae crossing a root intersection) are not assessed during traditional microscopic techniques of measuring AM fungal colonisation. Recently, alternative approaches to microscopy have been developed to quantify and differentiate AM fungal taxa (Merryweather and Fitter, 1998). Quantitative PCR (qPCR) can be used to quantify

concentrations of DNA or RNA sequences from AM fungal taxa in mixtures (Thonar *et al.*, 2012; Voříšková *et al.*, 2017). It provides precise and rapid quantification of the abundance of specific AM fungal species in environmental samples such as field roots (Wagg *et al.*, 2011; Thonar *et al.*, 2012). It is quick (excluding DNA extraction) and reflects the absolute levels of root colonisation because it depends on DNA concentration per unit weight of roots between the different AM fungal species (Thonar *et al.*, 2012; Voříšková *et al.*, 2017) compared to the traditional staining/microscopic method which depends on percentage of root length colonised. Future studies could include alternative methods of AM fungal quantification, such as qPCR to quantify levels of active (RNA) AM fungal genes, thus confirming levels of functioning AM fungi in the roots.
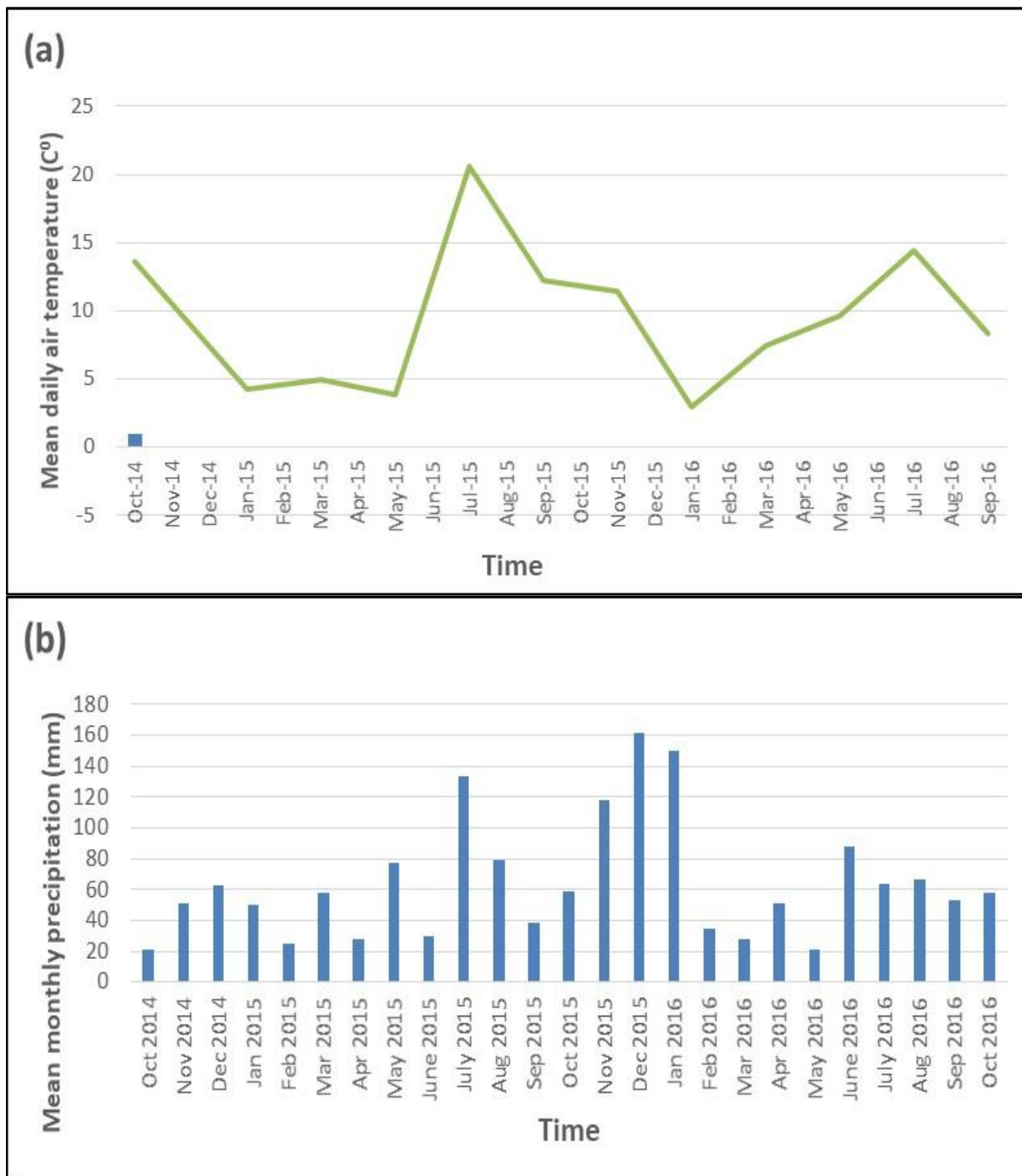
Fig 3.6. (a) Mean daily air temperature (°C) and (b) Mean monthly precipitation (mm) at Nafferton Farm throughout the growing season of spelt for the two growing seasons 2014/15-2015/16.

3.4.4. What is the relationship between AM fungal colonisation and spore density?

In theory there should be a high correlation between AM fungal colonisation in plant roots and spore formation in the soil (Jensen and Jakobsen, 1980), but this was not the case in this study (Fig 3.4b). There are different explanations for this situation. Firstly, there are additional AM fungal propagules in the soil besides spores, notably colonised root pieces and soil hyphae (Klironomos and Hart, 2002; Varela-Cervero *et al.*, 2016). Some AM fungal species produce spores within roots (Smith and Read, 2008). These propagules could have been promoting colonisation in the spelt roots in this study rather than the spores extracted from the soil. This agrees with previous studies for example, Rodríguez-Echeverría *et al.* (2008) who found that there was no relationship between AM fungal colonisation and spore density. Brundrett (1991) reported a poor relationship between AM spore densities and AM fungal formation. For those AM fungal species that do not form spores most often soil hyphae are the main source of AM fungal propagules and they can function as AM fungal propagules in many crops (Jasper *et al.*, 1989). Furthermore, dead root pieces which have been previously infected by AM fungal structures such as intra-radical hyphae and intra-radical vesicles are considered further active propagules for AM fungi in soil and can also initiate AM fungal association (McGee, 1987).

AM fungal species can differ in their ability to initiate new colonisation from different propagule types. AM fungal families have different colonisation strategies in terms of their ability to initiate new colonisation from each propagule type (Schalamuk and Cabello, 2010; López-García *et al.*, 2014; Varela-Cervero *et al.*, 2016). For example, *Pacisporaceae* and *Diversisporaceae* mainly initiate colonisation from spores and extra-radical hyphae, whereas *Glomeraceae* and *Claroideoglomeraceae* from colonised root fragments (Varela-Cervero *et al.*, 2016). Therefore, in this experiment, root colonisation may have been promoted by propagules other than spores, thus explaining the negative correlation between spores and root colonisation parameters.

The non-relationship between colonisation by AM fungi and spore density may related to the AM fungal life-cycle. Spore formation can occur from 3 – 4 weeks to up to 6 months after AM fungi colonise the roots of plants (Abbott and Gazey, 1994; Bücking *et al.*, 2012) and this depends on the AM fungal species (Bücking *et al.*, 2012). Spores are similar to vesicles as they are rich in lipids and require further supplies of carbon for their formation (Muthukumar and Udaiyan, 2000; Luginbuehl *et al.*, 2017). Therefore, spore production occurs after AM fungal

colonisation develops in the host plant roots which would be expected to provide more C assimilate to AM fungi, thus helping spore formation (Muthukumar and Udaiyan, 2000). Timing of sampling for the spores should also be considered. AM fungi produce the maximum spore density at the end of the growing season, after crop flowering has finished (Rodríguez-Echeverría *et al.*, 2008; Varela-Cervero *et al.*, 2016), yet soil sampling in this experiment occurred at the same time as flowering, possibly too early to detect impacts of higher levels of root colonisation on spore densities.

Another explanation is that the spore density may reflect the historical accumulation of AM fungal sporulation in the particular soil, and not necessarily the present symbiosis of the plant (Schalamuk *et al.*, 2013). Prior to spelt planting in each season the experimental area had been planted to an unfertilised grass/clover crop (see Table 3.3) The grass/clover might affect AM fungal spores as grass and clover are mycorrhizal plants (Mäder *et al.*, 1999) and the lack of tillage for this period would also have promoted a healthy population of mycorrhizae in the soil. Therefore, the spores which were counted in the present study could have resulted from the previous grass/clover crop and not directly resulted from colonisation of the present spelt crop.

Errors in spore identification also need to be considered. Spore counting methods used in this study were designed to minimise the risk of counting non-AM fungal spores (Abbott and Robson, 1991); however, every method has both limitations and advantages. The spore extraction method used in this study mainly extracts AM fungal spores, as other fungal spores such as Asco- and Basidiomycetes are much smaller and would just pass through the sieve and not be collected in the petri dish (Fischer *et al.*, 2010; Emygdio *et al.*, 2018). In some cases, spores from mosses may be collected, but they look so different in shape and colour, that they are easily distinguished (Henriques *et al.*, 2017; Alegro *et al.*, 2019). In spite of these limitations, many recent studies used the same method, (Bharadwaj *et al.*, 2007; Schalamuk *et al.*, 2013; Rasmussen *et al.*, 2019), demonstrating that it is a widely recognised and valid way for estimating densities of AM fungal spores in soils.

It is, however, possible that a significant proportion of spores counted were not viable. Some spores may be dead or dormant and so cannot colonise the plant roots. This could have resulted in negative correlations between spore densities and AM fungal colonisation. An alternative method that provides an indication of viable spores in soils is the inoculum potential measurement (infective bioassay) (Mader *et al.*, 2000; Lehman *et al.*, 2012; Coutinho *et al.*,

2019). It can also be used to multiply spores and enhance the accuracy of the species identification (Marinho *et al.*, 2019) and to increase the chance of discovering species that may not have sporulated at the time of soil sampling (Coutinho *et al.*, 2019; Marinho *et al.*, 2019). While not used in this study, parallel analyses of inoculum potential along with spore densities would provide a more robust suite of measurements to assess AM fungi populations in soils in future experiments.

3.5. Conclusions

Higher AM fungal colonisation did not lead to better crop yields, although it did improve plant P concentrations. Other factors may affect crop parameters (grain yield and P uptake) including genetic differences among spelt varieties, fertiliser type and environmental conditions. However, there were significant interactions which showed that variety effect did not form a clear pattern for AM fungal colonisation and spore density.

This study confirmed that AM fungal colonisation of roots did not always reflect AM fungal spore density in the soil. This phenomenon involved many potential factors such as 1) participation of AM fungal propagules other than spores, 2) historical accumulation of AM fungal spores, 3) spore dormancy for some AM fungal species, and 4) seasonal variation in AM fungal spores. Thus, further field tests are required to determine spelt variety and fertility management effects on AM fungal functions in sustainable agriculture. These further examinations of agronomic management effects will be useful to show how these management practices cause changes in AM fungal functions and in turn improve P nutrition and crop yield.

Chapter 4. Effects of Tillage Treatment, Fertiliser Type and Crop Protection Practices on Arbuscular Mycorrhizal Fungal Colonisation of Roots, Spore Density, Crop Yield and P Nutrition in Two Cultivars of Spelt (*Triticum spelta*)

4.1. Introduction

AM fungi are affected by a number of crop management practices and it is important to understand how these practices affect AM fungi colonisation and function if optimal management systems are to be designed. There is growing interest in reduced intensity tillage systems recently and these are the focus of this chapter. Furthermore, crop protection and fertiliser type (organic versus conventional practices) are also experimental factors in this chapter because of the interest in organic farming as an alternative, more environmentally friendly system of crop production. Tillage includes cultivation practices that disturb the soil to incorporate crop residues and prepare the land for planting. Tillage can range from the most intense deep inversion tillage methods using a mouldboard plough through to systems with minimal soil disturbance such as shallow non-inversion tillage and systems where no tillage is used at all (Cooper *et al.*, 2016). Even though using soil tillage can lead to improved soil conditions, thus enhance the productivity of the crop, it may suppress AM fungal symbiosis through disrupting AM fungal networks within the soil.

Tillage practices can affect AM fungal parameters, for example AM fungal colonisation and spore density were reduced by conventional tillage (deep inversion) compared to conservation tillage (reduce or shallow non-inversion and no-tillage) (Brito *et al.*, 2012; Säle *et al.*, 2015; Verzeaux *et al.*, 2016).

Conventional tillage can lead to reductions in the abundance of viable AM fungal propagules, including infective hyphae and spores. The influence of different long-term tillage treatments i.e. no-tillage for 6 years and 10 years, compared with conventional tillage using mouldboard plow on AM fungal propagules (total and active hyphae and spores) was investigated in a Mollisol in a field in central Chile (Curaqueo *et al.*, 2011). This field operated a spring wheat (*Triticum turgidum L.*) - maize (*Zea mays L.*) rotation. AM fungal propagules were greater with no tillage (6 years) than conventional tillage. Zhang *et al.* (2013) also reported that the

effectiveness and abundance of microbes, including AM fungi, were commonly greater under no tillage and ridge tillage than conventional tillage.

Variety choice can affect AM fungi function as some varieties may enhance their ability to uptake nutrients by associating with AM fungi to aid adaption to nutrient deficient conditions (Sawers *et al.*, 2010; Martín-Robles *et al.*, 2018; Ryan and Graham, 2018). The effects of variety on AM fungal colonisation, their functions and population in soil might interact with other agricultural practices such as fertiliser management , tillage and crop protection, thus spelt variety is an additional experimental factor in this study (Mao *et al.*, 2014; Ercoli *et al.*, 2017).

With increased interest and uptake of reduced intensity tillage practices (e.g. no-till, minimum tillage) as well as a move towards lower input/organic systems of production, there is a need to better understand how these practices affect AM fungal associations in crop plants, grain yield and P nutrition.

4.1.1. *Aim and objectives*

The aim of this work was to test the effects of (and interactions between) tillage, spelt variety, fertiliser type (compost and mineral fertilisers equivalent to 100 kg total N ha^{-1}) and 2 crop protection regimes (with and without fungicides and herbicides) on AM fungal colonisation in spelt roots and spore density in the soil, crop yields and P nutrition. This research was therefore designed to meet the following objectives:

1. To evaluate how tillage system (minimum vs conventional) affects AM fungal colonisation of spelt roots, AM fungal spore density in the soil, crop yield and P nutrition.

2. To evaluate how fertiliser type (mineral N vs compost) affects AM fungal colonisation of spelt roots, AM fungal spore density in the soil, crop yield and P nutrition.

3. To evaluate how spelt cultivars, affect AM fungal colonisation of spelt roots, AM fungal spore density in the soil, crop yield and P nutrition.

4. To evaluate how crop protection management (organic vs conventional) affects AM fungal colonisation of spelt roots, AM fungal spore density in the soil, crop yield and P nutrition.

5. To assess the relationships between AM fungal colonisation, grain yield, P uptake and P concentration of spelt.

4.2. Methodology

4.2.1. Study site and experimental design

The field trials were located at Nafferton Farm in northeast England (54:59:26.3 N; 1:54:37.4 W) and were a part of a long-term experiment established in 2001 that compares organic and conventional crop management practices using a factorial design. This trial (Nafferton Factorial Systems Comparison or NFSC trial) is described in detail in (Cooper *et al.*, 2011a; Bilsborrow *et al.*, 2013; Palmer *et al.*, 2013). In 2012 an additional tillage factor (deep inversion conventional tillage versus shallow non-inversion minimum tillage) was introduced into the conventional rotation plots of two of the experiments within the trial. In both years of the experiments (2015/16 & 2016/17), the trial design was a split-split-split plot design with tillage as the main plot (6 x 96 m). Each tillage main plot was split into two crop protection sub-plots (6 x 48 m) which were further split into two fertiliser type sub-sub-plots (6 x 24 m). The variety treatments were overlaid longitudinally across the full length of each main plot, creating 16 1.5 x 24 m sub-sub-sub plots in each replicate; the experiment was replicated four times in the field resulting in a total of 64 plots. The soil in the plots was a sandy clay loam with initial soil chemical properties for each crop season 2015/16 and 2016/17 shown in (Table 4.1, 4.2 and 4.3). For basic soil analysis (available P, K, Mg), samples were collected from the top 30 cm of each tillage sub-sub-sub plot (32 plots) to assess any historic effects of these treatments before drilling in early October, while for total and available N soil samples were collected in March before the main vegetative period.

Table 4.1. Soil available P, K and Mg analysis for the 2015/16 season and for the 2016/17 season. Means ±SD of four replicates.

plot	Soil pH	P Index	P mg l^{-1}	K Index	K mg l^{-1}	Mg Index	Mg mg l^{-1}
2015/16							
Main effect means							
Tillage system							
Minimum	6.7 ± 0.08	1	17.1 ± 1.1	1	86.6 ± 9.3	3	173.0 ± 5.4
Conventional	6.8 ± 0.08	2	17.6 ± 0.9	1	77.3 ± 6.1	3	168.3 ± 4.9
Crop protection							
Conventional	6.8 ± 0.08	2	17.5 ± 0.9	1	83.8 ± 8.5	4	178.0 ± 4.9
Organic	6.7 ± 0.08	1	17.2 ± 1.1	1	80.1 ± 7.4	3	163.3 ± 4.7
Fertiliser type							
Compost	6.9 ± 0.06	1	17.3 ± 1.0	1	102.0 ± 7.5	4	176.9 ± 4.4
Mineral N	6.6 ± 0.07	2	17.4 ± 1.0	1	61.9 ± 4.2	3	164.3 ± 5.3
2016/17							
Tillage system							
Minimum	6.5 ± 0.07	2	16.5 ± 1.1	1	72.6 ± 5.9	3	161.2 ± 5.6
Conventional	6.6 ± 0.09	2	17.6 ± 1.5	1	75.7 ± 5.9	3	153.4 ± 4.3
Crop protection							
Conventional	6.6 ± 0.08	2	18.2 ± 1.5	1	77.0 ± 6.4	3	158.6 ± 5.4
Organic	6.4 ± 0.09	1	15.8 ± 1.1	1	71.3 ± 5.3	3	156.0 ± 4.7
Fertiliser type							
Compost	6.6 ± 0.08	2	18.1 ± 1.4	1	87.7 ± 3.7	3	162.3 ± 3.8
Mineral N	6.4 ± 0.08	1	15.9 ± 1.2	0	60.6 ± 5.7	3	152.4 ± 5.8

Table 4.2. Soil mineral N content at two depths (0-30 cm and 30-60 cm) in March 2016 (before mineral N application).

plot	NO_3^-	NH_4^+	Total available N
	mg kg^{-1}	mg kg^{-1}	kg ha^{-1}
0-30 cm			
2015/16			
Main effect means			
Tillage system			
Minimum	2.9 ± 0.3	1.8 ± 0.2	17.4 ± 1.5
Conventional	3.3 ± 0.3	2.1 ± 0.3	20.3 ± 1.4
Crop protection			
Conventional	3.1 ± 0.3	1.7 ± 0.1	18.0 ± 1.3
Organic	3.1 ± 0.3	2.2 ± 0.3	19.8 ± 1.6
Fertiliser type			
Compost	3.3 ± 0.3	2.2 ± 0.3	20.4 ± 1.4
Mineral N	2.9 ± 0.3	1.8 ± 0.2	17.4 ± 1.4
30-60 cm			
Tillage system			
Minimum	0.7 ± 0.1	1.1 ± 0.1	6.9 ± 0.7
Conventional	1.0 ± 0.2	1.0 ± 0.1	7.4 ± 0.8
Crop protection			
Conventional	0.6 ± 0.1	1.0 ± 0.1	5.8 ± 0.6
Organic	1.1 ± 0.2	1.2 ± 0.1	8.5 ± 0.7
Fertiliser type			
Compost	0.6 ± 0.1	1.0 ± 0.1	6.2 ± 0.5
Mineral N	1.1 ± 0.2	1.1 ± 0.1	8.1 ± 0.9

Table 4.3. Soil mineral N content at two depth (0-30 cm and 30-60 cm) in March 2017 (before mineral N application).

plot	NO$_3^-$	NH$_4^+$	Total available N
	mg kg^{-1}	mg kg^{-1}	kg ha^{-1}
0-30 cm			
2016/17			
Main effect means			
Tillage system			
Minimum	3.6 ± 0.5	8.2 ± 1.2	44.4 ± 4.7
Conventional	3.4 ± 0.4	8.0 ± 1.4	42.4 ± 5.2
Crop protection			
Conventional	3.4 ± 0.4	7.5 ± 0.9	40.8 ± 3.6
Organic	3.6 ± 0.5	8.7 ± 1.6	46.0 ± 5.9
Fertiliser type			
Compost	3.8 ± 0.5	10.2 ± 1.6	52.3 ± 5.8
Mineral N	3.2 ± 0.3	6.0 ± 0.7	34.5 ± 2.2
30-60 cm			
Tillage system			
Minimum	2.0 ± 0.4	1.3 ± 0.3	12.4 ± 2.0
Conventional	1.9 ± 0.3	1.6 ± 0.3	13.2 ± 1.7
Crop protection			
Conventional	1.8 ± 0.3	1.7 ± 0.3	12.9 ± 2.0
Organic	2.1 ± 0.4	1.2 ± 0.2	12.6 ± 1.7
Fertiliser type			
Compost	1.6 ± 0.3	1.5 ± 0.3	11.5 ± 2.0
Mineral N	2.3 ± 0.4	1.5 ± 0.2	14.1 ± 1.7

4.2.2. Agronomic management

Spelt was established within the NFSC as part of the Healthy Minor Cereals project in autumn 2015 and 2016. In the factorial experiment an additional factor was included with two varieties/genotypes of spelt, Filderstolz and Oberkulmer Rotkorn. The following agronomic factors were included in the field experiments: two fertiliser input types (a compost and mineral N fertiliser) applied at the same total N-input rate equivalent to (100 kg N ha^{-1}) (Table 4.4). The compost had been applied routinely to the compost plots since 2004. The compost was tested for total N content (% dry matter) prior to sowing: analysis for the 2015/16 season showed a total N content of 3.63% while analysis for the 2016/17 season showed total N content as 2.74 %. One application of mineral N fertiliser (ammonium nitrate; 34.5% total N) was applied in each season. The compost was produced on site at Nafferton Farm from cow manure and straw

and applied prior to sowing. Also, two crop protection treatments were used (organic and conventional) i.e. with and without standard fungicide and herbicide spraying protocols (see Table 4.4 for timing and rates of application). Plots were tilled prior to spelt planting (Table 4.4). Minimum tillage treatment was shallow (<20 cm depth) non-inversion tillage using a Dyna-Drive cultivator (Bomford Turner Ltd.) while conventional tillage treatments were mouldboard ploughed to a depth of 25-30 cm. All plots were sown with a 1.5 m wide Sow-Lite seed drill (Jordan Engineering Ltd.).

The minimum tillage treatments were only applied during the cereal years of the rotation, excluding the first year following the ley phase when conventional tillage was used to destroy the ley.

Table 4.4. Crop management details for tillage trials in 2015/16 and 2016/17 seasons.

	2015/16	2016/17
Previous crop	potatoes 2014/wheat 2015	Grass/clover 2013/14/wheat 2015/16
Sowing date	15 October 2015	20 October 2016
Biomass harvest date	15 August 2016	16 August 2017
Combine harvest date	2 September 2016	31 August 2017
Seeding rates (kg ha^{-1}) [a]		
Oberkulmer Rotkorn	315	291
Filderstolz	293	298
Tillage date		
Minimum	8 October 2015	18 October 2016
conventional	8 October 2015	18 October 2016
Herbicide application date and rate		
CleanCrop Gallifrey (fluroxypyr)	11 April 2016 (0.35 L ha^{-1})	16 April 2017 (0.35 L ha^{-1})
Isomec Ultra (dichloroprop-p)	11 April 2016 (1.5 L ha^{-1})	16 April 2017 (1.5 L ha^{-1})
Fungicide application date and rate		
Bravo (chlorothalonil)	10 May and 3 June 2016 (2 L ha^{-1})	10 and 22 May 2017 (2 L ha^{-1})
Cortez (epoxiconazole)	10 May and 3 June 2016 (1 L ha^{-1})	10 and 22 May 2017 (1 L ha^{-1})
Fertiliser application date		
Compost FYM	6 October 2015	18 October 2016
Mineral N	21 April 2016	3 May 2017

[a]All varieties were drilled at 250 hulled seeds m^{-2} for 2015/16 and 2016/17 seasons.

4.2.3. Sampling strategy

Root sampling in both years was conducted to coincide with flowering (GS61; in July). The sampling procedure used is described in detail in Chapter 3. Plots from all treatments were sampled on 4-5 July 2016 and 7-10 July 2017.

4.2.4. Plant shoot and grain measurements

Crop yields, P concentrations as well as P uptake were measured using the same methods already described in Chapter 3. Briefly, the spelt crop biomass at flowering and the crop biomass at time of harvest were recorded each year (2016 and 2017). Prior to harvest, biomass samples were removed from each plot to assess total biomass, harvest index (HI), moisture content and additional yield components. Plants from 4 x 0.5 m rows were counted and removed from each plot.

Phosphorus concentrations were assessed in above-ground biomass at anthesis and in the straw and grain of spelt at harvest for both years. All samples were sent to the Analytical Services Department, Central Analytical Laboratory, SAC Commercial Ltd for analysis. Nitric acid microwave digestion of plant samples was used to assess P content as described in Chapter 3.

4.2.5. Mycorrhizal assessments

AM fungal colonisation

AM fungal colonisation of roots and spore density were measured as described in Chapter 3.

4.2.6. Statistical analysis

Experimental factor effects on AM fungal colonisation, AM fungal spore density, P concentration, P uptake and total P uptake were analysed using linear mixed effects models in the nlme package of R (Pinheiro and Bates, 2000). An ANOVA was used to generate *p*-values for the main effects and interaction effects. Means and the standard error (SE) for all treatment effects and interactions were calculated.

The cropping years 2016 for the 2015/16 crop and 2017 for the 2016/17 crop were included as a fixed effect in an analysis that combined data for both years. Fixed effects were year, crop protection, fertiliser type, tillage system and variety. The random effect term of block/tillage/crop protection/fertiliser type was specified to reflect the nested structure of the design (Crawley, 2007). If significant differences (p-value <0.05) occurred between interactions between factors, general linear hypothesis tests (Tukey contrasts) were performed using the 'glht' function in the 'multcomp' package.

Year was included as a random term in the combined year analysis. Where there were significant interactions with the year factor data for each individual year was analysed separately. At each level of the interacting factor, further analyses were performed where the interaction terms were significant and that factor was removed from the random error term (Cooper *et al.*, 2011a). Where interactions with tillage were found, a tillage system subset was conducted for each individual year. Fixed and random effects were the same as those used for analysis in each year but excluding tillage.

QQnorm was used to test the normality of the residuals of all models. The differences between treatments were detected by ANOVA test (p-value). Pearson's product-moment correlations (Richard, 1990) were calculated to assess relationships between different crop growth parameters and AM fungal parameters.

4.3. Results

4.3.1. AM fungal colonisation

When data was combined for both years (Table 4.5), fertiliser type had a significant effect on AM fungal root colonisation ($p<0.05$), with higher levels of colonisation where compost rather than mineral fertiliser was used. Root, hyphae and arbuscule colonisation were also higher for the spelt variety Filderstolz on average over both years (Table 4.5). Tillage had no significant effect on any AM fungal colonisation parameters. There were significantly more AM fungal arbuscules in 2016/17 compared to 2015/16. Root colonisation was slightly higher in 2016/17 than 2015/16, but it was not significantly different. Crop protection practices had no significant effect on AM fungal colonisation in the combined year analysis.

Table 4.5. Main effect means, ±SE and *p*-values for the effects and interactions of year, crop protection, fertiliser type, tillage system and spelt variety on root, hyphae, arbuscule and vesicle colonisation and spore density of AM fungi in the Nafferton Factorial Systems Comparison (NFSC) field trial, averaged over two seasons (2015/16 and 2016/17).

	Root colonisation (%)	Hyphae colonisation (%)	Arbuscule colonisation (%)	Vesicle colonisation %	Spore density (g⁻¹ dry soil)
Main effect means					
Year (YR)					
2015/16	74.63 ± 1.88	37.47 ± 1.06	18.40 ± 0.73	18.75 ± 0.80	87±3
2016/17	85.44 ± 1.23	39.04 ± 0.49	23.00 ± 0.57	23.40 ± 0.79	58±2
Tillage system (TI)					
Minimum	78.47 ± 1.83	37.34 ± 0.81	19.60 ± 0.72	21.53 ± 0.89	77 ± 3
Conventional	81.59 ± 1.60	39.17 ± 0.84	21.80 ± 0.69	20.62 ± 0.80	69 ± 3
Crop protection (CP)					
Conventional	80.45 ± 1.80	38.39 ± 0.86	20.49 ± 0.72	21.57 ± 0.93	67 ± 3
Organic	79.61 ± 1.65	38.12 ± 0.80	20.91 ± 0.71	20.58 ± 0.76	78 ± 3
Fertiliser type (FT)					
Compost	82.34 ± 1.71	38.92 ± 0.77	21.37 ± 0.75	22.05 ± 0.92	77 ± 3
Mineral N	77.72 ± 1.70	37.59 ± 0.88	20.03 ± 0.66	20.09 ± 0.75	69 ± 3
Variety (VR)					
Filderstolz	84.42 ± 1.45	40.65 ± 0.81	21.66 ± 0.66	22.11 ± 0.80	69 ± 2
Oberkulmer Rotkorn	75.64 ± 1.81	35.86 ± 0.73	19.74 ± 0.75	20.04 ± 0.88	77 ± 4
ANOVA *p*-values					
YR	0.0629	0.3923	**0.0292**	0.07	**0.0013**
TI	0.1236	0.1168	0.0704	0.423	**0.0128**
CP	0.6375	0.7937	0.6857	0.3654	**0.0007**
FT	**0.014**	0.1953	0.1076	0.0759	**0.0036**
VR	**<0.001**	**<0.001**	**0.0055**	0.0556	**0.0018**
CP * FT	**0.0172**	0.2146	0.076	0.1176	0.0744
CP * VR	**0.0399**	0.3209	0.1532	0.1083	0.47
FT * VR	0.6823	0.658	0.1149	0.2127	**0.0392**
CP * YR	**0.037**	0.1753	0.1395	0.3315	0.099
FT * YR	0.065	0.549	0.2349	0.1019	**0.0013**
TI * YR	0.324	0.2385	0.9838	0.5971	**0.0088**
VR * YR	**0.004**	**0.002**	**0.0128**	0.7663	**0.0006**
CP * FT * TI	**0.0276**	**0.0408**	0.1973	0.4154	**0.0144**
FT * TI * VR	0.1346	0.1331	0.9411	0.3109	**0.0042**
CP * FT * YR	0.4283	0.2477	0.273	0.5302	**0.0125**
FT * TI * YR	0.4808	0.2975	0.962	0.8895	**0.0263**
TI * VR * YR	0.3847	0.3103	0.4144	0.9733	**0.0016**
CP * FT * TI * VR	0.2294	0.0556	0.6375	0.8891	**0.0155**
FT * TI * VR * YR	0.4244	0.2148	0.6199	0.6506	**0.0003**
CP * FT * TI * VR * YR	**0.0287**	0.1516	**0.0296**	0.3472	**0.0171**

[b] Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

There was a significant crop protection x fertiliser type interaction effect on AM fungal root colonisation (Table 4.5), as well as for variety of spelt and crop protection.

Year interacted with several experimental factors (CP, VR, and CP x FT x TI x VR), for root colonisation as well as hyphae and arbuscule colonisation, so each year of data was analysed separately. The results (Table 4.6) showed that fertiliser type had a significant effect on AM fungal root colonisation, as well as on arbuscule colonisation in 2016/17, and these were both higher where compost was applied; but these effects were absent in 2015/16. AM fungal root, hyphae and arbuscule colonisation were significantly higher for the Filderstolz variety in 2015/16, but there were no variety effects on these parameters in 2016/17. As reported for the combined year analysis, crop protection practices and tillage system did not affect AM fungal colonisation in either 2015/16 or 2016/17. The significant interaction between fertiliser type and variety for arbuscule colonisation (p=0.0318) in 2016/17 (Table 4.6). There were no differences between the two varieties at a given level of fertility type; however, for the Oberkulmer Rotkorn variety, arbuscule colonisation was significantly higher when compost was the fertiliser type (Table 4.7).

In 2015/16 the interaction between tillage and other experimental factors affected root colonisation (CPxFTxTI, CPxFTxTIxVR) and arbuscule colonisation (CPxTI) so an analysis at each level of tillage for 2015/16 was conducted (Table 4.8). In 2015/16 for both tillage systems root colonisation was higher for the Filderstolz variety of spelt; arbuscule colonisation was also higher for Filderstolz under minimum tillage, but there was no variety effect under conventional tillage in 2015/16 (Table 4.8). There was also a significant crop protection and fertiliser type interaction for AM fungal root colonisation (p=0.0206) under minimum tillage in 2015/16 (Table 4.8). Root colonisation was significantly higher in organic compared to conventional crop protection plants when mineral was used as fertiliser; however, the use of compost significantly increased AM fungal root colonisation under conventional crop protection relative to mineral fertilisation (Table 4.9).

Table 4.6. Main effect means, ±SE and *p*-values for the effects and interactions of crop protection practices, fertiliser type, tillage system and spelt variety on root, hyphae and arbuscule colonisation of AM fungi in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16 and 2016/17).

	2015/16			2016/17		
	Root colonisation (%)	**Hyphae colonisation (%)**	**Arbuscule colonisation (%)**	**Root colonisation (%)**	**Hyphae colonisation (%)**	**Arbuscule colonisation (%)**
Main effect means						
Crop protection (CP)						
Conventional	73.00 ± 2.65	36.89 ± 1.43	17.50 ± 0.99	87.91 ± 1.55	39.89 ± 0.59	23.59 ± 0.70
Organic	76.25 ± 2.69	38.06 ± 1.58	19.44 ± 1.06	82.97 ± 1.82	38.19 ± 0.76	22.41 ± 0.90
Fertiliser type (FT)						
Compost	75.25 ± 2.50	37.84 ± 1.39	18.66 ± 1.00	89.44 ± 1.55	40.01 ± 0.61	24.15 ± 0.90
Mineral N	74.00 ± 2.85	37.11 ± 1.62	18.28 ± 1.08	81.44 ± 1.64	38.07 ± 0.74	21.84 ± 0.64
Tillage system (TI)						
Minimum	72.13 ± 2.73	35.91 ± 1.46	17.38 ± 1.08	84.81 ± 1.90	38.78 ± 0.64	21.89 ± 0.75
Conventional	77.13 ± 2.56	39.04 ± 1.51	19.56 ± 0.96	86.06 ± 1.58	39.30 ± 0.75	24.11 ± 0.82
Variety (VR)						
Filderstolz	81.66 ± 2.39	41.50 ± 1.47 a	20.22 ± 1.05	87.19 ± 1.52	39.80 ± 0.70	23.11 ± 0.72
Oberkulmer Rotkorn	67.59 ± 2.35	33.45 ± 1.17 b	16.72 ± 0.92	83.69 ± 1.90	38.28 ± 0.67	22.89 ± 0.89
ANOVA *p*-values						
CP	0.2796	0.5343	0.1554	0.0779	0.1424	0.4782
FT	0.6558	0.664	0.7586	**0.0027**	0.0793	**0.0419**
TI	0.1649	0.1698	0.164	0.6281	0.6412	0.2466
VR	**<0.001**	**00.001**	**0.0013**	0.1133	0.0912	0.8116
CP * TI	0.1712	0.6717	**0.0425**	0.8975	0.6994	0.8396
FT * VR	0.314	0.5161	0.9488	0.5246	0.8941	**0.0318**
CP * FT * TI	**0.0448**	0.1388	0.3153	0.3518	0.1486	0.38
CP * FT * TI * VR	**0.0364**	0.064	0.0719	0.403	0.5669	0.2005

[b] Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

Table 4.7. Interaction means ± SE for the effects of fertiliser type and spelt variety on AM fungal arbuscule colonisation (%) in the Nafferton Factorial Systems Comparison (NFSC) field trial (2016/17).

	Variety	
Fertiliser type	Filderstolz	Oberkulmer Rotkorn
Compost	23.25 ± 1.10 Aa	25.06 ± 1.44 Aa
Mineral N	22.96 ± 0.98 Aa	20.73 ± 0.77 Ab

Means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

Table 4.8. Main effect means, ±SE and p-values for the effects and interactions of crop protection practices, fertiliser type and spelt variety under minimum and conventional tillage on the root and arbuscule colonisation of AM fungi in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16).

	Minimum tillage		Conventional tillage	
	Root colonisation (%)	**Arbuscule colonisation (%)**	**Root colonisation (%)**	**Arbuscule colonisation (%)**
Main effect means				
Crop protection (CP)				
Conventional	68.38 ± 4.12	14.88 ± 1.33	77.63 ± 3.17	20.13 ± 1.17
Organic	75.88 ± 3.45	19.88 ± 1.50	76.63 ± 4.13	19.00 ± 1.54
Fertiliser type (FT)				
Compost	73.63 ± 4.04	16.69 ± 1.40	76.88 ± 3.02	20.63 ± 1.29
Mineral N	70.63 ± 3.76	18.06 ± 1.68	77.38 ± 4.24	18.50 ± 1.40
Variety (VR)				
Filderstolz	79.44 ± 3.55	19.75 ± 1.74	83.88 ± 3.21	20.69 ± 1.23
Oberkulmer Rotkorn	64.81 ± 3.32	15.00 ± 1.02	70.38 ± 3.27	18.44 ± 1.44
ANOVA p-values				
CP	0.1462	0.0559	0.8433	0.6735
FT	0.4649	0.4354	0.9178	0.2288
VR	**0.0025**	**0.0048**	**0.0024**	0.1205
CP * FT	**0.0206**	0.0698	0.9588	0.5048
CP * FT * VR	0.3334	0.7898	**0.0372**	**0.0327**

[b] Boldface is used for a significance of $p<0.05$.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

Table 4.9. Interaction means ± SE for the effects of fertiliser type and crop protection on root colonisation (%) under minimum tillage in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16).

	Crop protection	
Fertiliser type	Conventional	Organic
Compost	75.88 ± 6.21 Aa	71.38 ± 5.46 Aa
Mineral N	60.88 ± 4.25 Bb	80.38 ± 3.92 Aa

Means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

4.3.2. AM fungal spore density

The combined analysis over both years showed that a range of experimental factors as well as sample year all affected spore densities (Table 4.5). Spore densities were higher in 2015/16 compared to 2016/17. Minimum tillage, organic crop protection practices and use of compost as a fertiliser input all resulted in higher average spore densities. Densities were also higher on average where Oberkulmer Rotkorn was the spelt variety grown. All the main effects interacted with year and so the analysis was simplified by analysing results for each year separately (Table 4.10). Spore densities were higher under conventional crop protection in 2015/16 but did not significantly differ due to crop protection practices in 2016/17. In contrast, in 2016/17 spore densities were significantly higher when compost was the fertiliser type, but these effects were absent in 2015/16. Minimum tillage resulted in higher AM fungal spore densities than conventional tillage in 2015/16, while these effects were not detected in 2016/17. Also, in 2015/16 spore densities were significantly higher where the spelt variety was Oberkulmer Rotkorn rather than Filderstolz, while these effects were absent in 2016/17 (Table 4.10). Two- and three-way interactions among experimental factors varied depending on the year. In 2015/16 there were significant FTxVR, TIxVR and CPxFTxTIxVR interactions, while in 2016/17 CPxFT, FTxTI, CPxFTxTI and FTxTIxVR were all significant. Since several of these interactions were with the tillage factor, the effects of crop management practices on spore densities were investigated at each tillage level within each year (Table 4.11).

Variety was the dominant factor affecting spore densities in minimum tillage systems in 2015/16 with significantly higher spore densities when the Oberkulmer Rotkorn variety was grown. Furthermore, there was a significant CPxFTxVR interaction (Table 4.11) for AM fungal spore densities under minimum tillage in 2015/16.

Table 4.10. Main effect means, ±SE and *p*-values for the effects and interactions of crop protection practices, fertiliser type, tillage system and spelt variety on AM fungal spore density (g^{-1} dry soil) in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16 and 2016/17).

	AM fungal spore density (g^{-1} dry soil)	
	2015/16	**2016/17**
Main effect means		
Crop protection (CP)		
Conventional	80 ± 3	55 ± 4
Organic	95 ± 4	62 ± 2
Fertiliser type (FT)		
Compost	87 ± 4	67 ± 3
Mineral N	88 ± 4	50 ± 3
Tillage (TI)		
Minimum	96 ± 4	58 ± 3
Conventional	78 ± 3	59 ± 4
Variety (VR)		
Filderstolz	79 ± 2	59 ± 3
Oberkulmer Rotkorn	96 ± 5	58 ± 4
ANOVA *p*-values		
CP	**0.0073**	0.1019
FT	0.7947	**0.001**
TI	**0.0195**	0.8369
VR	**0.0002**	0.7673
CP * FT	0.6098	**0.002**
FT * TI	0.4678	**0.0101**
FT * VR	**0.0294**	0.5921
TI * VR	**0.007**	0.087
CP * FT * TI	0.2352	**0.0153**
FT * TI * VR	0.5473	**<0.001**
CP * FT * TI * VR	**0.0038**	0.9737

[b] Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

Table 4.11. Main effect means, ±SE and *p*-values for the effects and interactions of crop protection practices, fertiliser type and spelt variety under minimum and conventional tillage on AM fungal spore density (g^{-1} dry soil) in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16 and 2016/17).

	AM fungal spore density (g $^{-1}$ dry soil)			
	2015/16		2016/17	
	Minimum tillage	Conventional tillage	Minimum tillage	Conventional tillage
Main effect means				
Crop protection (CP)				
Conventional	88 ± 6	72 ± 3	53 ± 4	57 ± 7
Organic	105 ± 6	85 ± 5	63 ± 3	60 ± 3
Fertiliser type (FT)				
Compost	97 ± 6	76 ± 3	62 ± 4	71 ± 5
Mineral N	95 ± 6	80 ± 5	54 ± 3	46 ± 4
Variety (VR)				
Filderstolz	82 ± 4	76 ± 2	61 ± 4	57 ± 4
Oberkulmer Rotkorn	110 ± 6	81 ± 6	55 ± 4	61 ± 7
ANOVA *p*-values				
CP	0.0553	0.0969	0.0653	0.7031
FT	0.7512	0.4831	0.0749	**0.0006**
VR	**0.0002**	0.3016	0.1457	0.305
CP * FT	0.2692	0.6127	0.4395	**0.0028**
FT * VR	0.2376	0.0601	**0.0004**	**0.0022**
CP * FT * VR	**0.0024**	0.429	0.8968	0.9376

[b] Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

In 2016/17 the use of compost increased spore densities relative to mineral fertiliser under conventional tillage; this effect was absent under both tillage systems in 2015/16 and under minimum tillage in 2016/17. There was also a significant crop protection x fertiliser type interaction for AM fungal spore densities (*p*=0.0028) under conventional tillage in 2016/17 (Table 4.11). AM fungal spore densities were significantly higher in conventional compared to organic crop protection plots when compost was used as a fertiliser; however, AM fungal spore densities were significantly higher in organic compared to conventional crop protection plots when mineral was used as a fertiliser (Table 4.12).

Table 4.12. Interaction means ± SE for the effects of fertiliser type and crop protection on AM fungal spore density (g^{-1} dry soil) under conventional tillage in the Nafferton Factorial Systems Comparison (NFSC) field trial (2016/17).

Fertiliser type	Crop protection	
	Conventional	Organic
Compost	80 ± 7 Aa	63 ± 5 Ba
Mineral N	35 ± 4 Bb	57 ± 5 Aa

Means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

The fertiliser type x variety interaction had a significant effect on spore densities (Table 4.9) under minimum tillage ($p=0.0004$) and conventional tillage ($p=0.0022$) in 2016/17. When minimum tillage was used, the highest spore densities occurred where the spelt variety was Filderstolz with the compost fertiliser while with mineral fertiliser the highest spore densities were obtained with spelt variety Oberkulmer Rotkorn (Table 4.13). In contrast, when conventional tillage was used, the spelt variety Oberkulmer Rotkorn outperformed Filderstolz to give greater AM fungal spore densities under compost (Table 4.13).

Table 4.13. Interaction means ± SE for the effects of fertiliser type and spelt variety on AM fungal spore density (g^{-1} dry soil) under different tillage systems in the Nafferton Factorial Systems Comparison (NFSC) field trial (2016/17).

Minimum tillage		
	Variety	
Fertiliser type	Filderstolz	Oberkulmer Rotkorn
Compost	74 ± 4 Aa	50 ± 5 Ba
Mineral N	48 ± 3 Bb	60 ± 5 Aa
Conventional tillage		
	Variety	
Fertiliser type	Filderstolz	Oberkulmer Rotkorn
Compost	62 ± 5 Ba	81 ± 6 Aa
Mineral N	52 ± 6 Aa	41 ± 5 Ab

Means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

4.3.3. *Phosphorus concentrations in plant tissue*

The results of the combined analysis for two years (2015/16 and 2016/17) are shown in (Table 4.14). Overall, year had significant effects on P concentration, and this was higher for biomass at anthesis and straw at harvest in 2016/17 than 2015/16. In contrast, the P concentration for grain was higher in 2015/16 than 2016/17. The use of compost significantly increased biomass P concentration at anthesis and in straw at harvest compared to mineral fertiliser. Biomass P concentration at anthesis was also significantly higher where the spelt variety was Filderstolz, while grain P concentration was enhanced where the variety was Oberkulmer Rotkorn. Crop protection practices and tillage system did not affect P concentration when years were combined. The fertiliser type x variety interaction had a significant effect on P concentration of straw (p=0.0233) at harvest (Table 4.14). This was enhanced in compost compared to mineral fertiliser where the spelt variety was Filderstolz, while there was no significant difference in P concentration between compost and mineral fertiliser where the spelt variety was Oberkulmer Rotkorn (Table 4.15).

Table 4.14. Main effect means, ±SE and *p*-values for the effects and interactions of year, crop protection practices, fertiliser type, tillage system and spelt variety on P concentration (mg g^{-1}) in plant tissue at different spelt growth stages (anthesis crop biomass, straw and grain at harvest) in the Nafferton Factorial Systems Comparison (NFSC) field trial, averaged over two seasons (2015/16 and 2016/17).

Spelt growth stage	P concentration (mg g^{-1})		
	Anthesis	**Harvest Straw**	**Harvest Grain**
Main effect means			
Year (YR)			
2015/16	1.69 ± 0.05	0.74 ± 0.03	3.52 ± 0.08
2016/17	2.31 ± 0.05	1.27 ± 0.06	3.26 ± 0.03
Crop protection (CP)			
Conventional	1.95 ± 0.07	1.01 ± 0.07	3.32 ± 0.06
Organic	2.05 ± 0.05	1.00 ± 0.04	3.47 ± 0.06
Fertiliser type (FT)			
Compost	2.13 ± 0.06	1.13 ± 0.06	3.47 ± 0.06
Mineral N	1.87 ± 0.06	0.87 ± 0.05	3.31 ± 0.06
Tillage system (TI)			
Minimum	1.95 ± 0.06	0.96 ± 0.05	3.44 ± 0.07
Conventional	2.05 ± 0.06	1.04 ± 0.06	3.34 ± 0.05
Variety (VR)			
Filderstolz	2.12 ± 0.06	0.96 ± 0.05	3.28 ± 0.06
Oberkulmer Rotkorn	1.88 ± 0.06	1.04 ± 0.06	3.50 ± 0.06
ANOVA *p*-values			
YR	**<0.001**	**<0.001**	**0.0013**
CP	0.2205	0.891	0.1798
FT	**0.0088**	**0.0026**	0.1275
TI	0.0505	0.1732	0.2211
VR	**<0.001**	0.1414	**0.0058**
FT * VR	0.2755	**0.0233**	0.7151
CP * YR	**<0.001**	**<0.001**	**0.0497**
FT * YR	0.543	**0.0061**	**0.0214**
CP * FT * YR	**0.0263**	0.6122	0.6391
CP * FT * TI * YR	**0.0217**	0.3941	0.258

[b] Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

Table 4.15. Interaction means ± SE for the effects of fertiliser type and spelt variety on P concentration (mg g^{-1}) in straw at harvest in the Nafferton Factorial Systems Comparison (NFSC) field trial, averaged over two seasons (2015/16 and 2016/17).

	Variety	
Fertiliser type	Filderstolz	Oberkulmer Rotkorn
Compost	1.16 ± 0.08 Aa	1.11 ± 0.09 Aa
Mineral N	0.77 ± 0.05 Ab	0.97 ± 0.08 Aa

Means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

However, year interacted with several of the other experimental factors (CP, FT, CP x FT, CP x FT x TI) and so each year of data for P concentration was analysed separately. In 2015/16, the results showed that crop protection practices had a significant effect on P concentrations for biomass at anthesis and straw at harvest and that they were higher with organic than conventional crop protection (Table 4.16). A significant effect was also observed on P concentration at anthesis due to fertiliser type, with slightly higher concentrations with compost compared to mineral fertiliser additions. The Filderstolz variety had a higher P concentration in the biomass at anthesis compared to Oberkulmer Rotkorn, while the P concentration for grain was slightly higher for Oberkulmer Rotkorn. The tillage system used did not affect P concentration in 2015/16, although there was a significant CPxFTxTI interaction for anthesis P concentrations.

There were many interactions among experimental factors for P concentration in 2015/16. There was a crop protection x variety interaction effect on P concentration for biomass (p=0.0368) at anthesis (Table 4.16). The higher P concentration occurred where the spelt variety was Filderstolz rather than Oberkulmer Rotkorn under organic crop protection; however, overall P concentration was higher for both varieties under organic compared to conventional crop protection (Table 4.17).

Table 4.16. Main effect means, ±SE and *p*-values for the effects and interactions of crop protection practices, fertiliser type, tillage system and spelt variety on P concentration (mg g^{-1}) at different spelt growth stages (crop biomass at anthesis, straw and grain at harvest) in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16).

Spelt growth stage	P concentration (mg g^{-1})		
	Anthesis	**Harvest Straw**	**Harvest Grain**
Main effect means			
Crop protection (CP)			
Conventional	1.51 ± 0.05	0.59 ± 0.02	3.37 ± 0.11
Organic	1.87 ± 0.07	0.89 ± 0.04	3.68 ± 0.10
Fertiliser type (FT)			
Compost	1.83 ± 0.06	0.80 ± 0.04	3.69 ± 0.10
Mineral N	1.55 ± 0.06	0.68 ± 0.05	3.35 ± 0.10
Tillage system (TI)			
Minimum	1.64 ± 0.07	0.73 ± 0.04	3.61 ± 0.12
Conventional	1.75 ± 0.06	0.75 ± 0.05	3.44 ± 0.09
Variety (VR)			
Filderstolz	1.79 ± 0.08	0.75 ± 0.05	3.37 ± 0.11
Oberkulmer Rotkorn	1.60 ± 0.05	0.73 ± 0.04	3.68 ± 0.10
ANOVA *p*-values			
CP	**0.0337**	**0.0138**	0.1179
FT	**0.0252**	0.0992	0.0542
TI	0.1054	0.6089	0.2571
VR	**0.0007**	0.6357	**0.0427**
CP * VR	**0.0368**	0.2537	0.3786
FT * VR	0.2139	**0.0404**	0.6816
CP * FT * TI	**0.0481**	0.861	0.7228

[b] Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

There was also an interaction between fertiliser type and variety for P concentration in straw (*p*=0.0404) at harvest in 2015/16 (Table 4.16). P concentration of straw at harvest was significant higher in compost compared to mineral fertiliser where the spelt variety was Filderstolz (Table 4.18).

Table 4.17. Interaction means ± SE for the effects of crop protection and spelt variety on P concentration (mg g^{-1}) in crop biomass at anthesis in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16).

	Variety	
Crop protection	Filderstolz	Oberkulmer Rotkorn
Conventional	1.56 ± 0.07 Ab	1.47 ± 0.07 Ab
Organic	2.03 ± 0.11 Aa	1.72 ± 0.07 Ba

Means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

Table 4.18. Interaction means ± SE for the effects of fertiliser type and spelt variety on P concentration (mg g^{-1}) in straw at harvest in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16).

	Variety	
Fertiliser type	Filderstolz	Oberkulmer Rotkorn
Compost	0.85 ± 0.06 Aa	0.74 ± 0.05 Aa
Mineral N	0.65 ± 0.07 Ab	0.72 ± 0.06 Aa

Means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

In 2016/17, fertiliser type had a significant effect on P concentration, with compost giving a higher P concentration for straw at harvest (Table 4.19). The P concentration for biomass at anthesis was enhanced where the spelt variety was Filderstolz. In contrast, the P concentration for grain was higher where the spelt variety was Oberkulmer Rotkorn. However, crop protection practices and tillage system did not affect P concentration in 2016/17.

Table 4.19. Main effect means, ±SE and *p*-values for the effects and interactions of crop protection practices, fertiliser type, tillage system and spelt variety on P concentration (mg g^{-1}) at different spelt growth stages (crop biomass at anthesis, straw and grain at harvest) in the Nafferton Factorial Systems Comparison (NFSC) field trial (2016/17).

Spelt growth stage	P concentration (mg g^{-1})		
	Anthesis	**Harvest Straw**	**Harvest Grain**
Main effect means			
Crop protection (CP)			
Conventional	2.39 ± 0.08	1.43 ± 0.09	3.26 ± 0.04
Organic	2.23 ± 0.06	1.10 ± 0.07	3.26 ± 0.06
Fertiliser type (FT)			
Compost	2.42 ± 0.06	1.47 ± 0.08	3.24 ± 0.05
Mineral N	2.20 ± 0.07	1.06 ± 0.07	3.27 ± 0.04
Tillage system (TI)			
Minimum	2.26 ± 0.07	1.20 ± 0.08	3.28 ± 0.05
Conventional	2.36 ± 0.06	1.33 ± 0.09	3.24 ± 0.05
Variety (VR)			
Filderstolz	2.45 ± 0.06	1.18 ± 0.08	3.19 ± 0.05
Oberkulmer Rotkorn	2.17 ± 0.07	1.35 ± 0.08	3.33 ± 0.05
ANOVA *p*-values			
CP	0.2322	0.0864	0.9761
FT	0.0738	**0.004**	0.6175
TI	0.2066	0.1675	0.5626
VR	**0.0003**	0.0646	**0.0184**
CP * FT * TI	0.2788	0.3032	**0.0422**

[b] Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

4.3.4. Grain yield and P uptake

There were significant differences in final straw P uptake and total P uptake as well as grain yield between 2015/16 and 2016/17; straw P uptake, total P uptake and total grain yield were all significantly higher in 2016/17 compared to 2015/16 (Table 4.20). Crop management practices affected P uptake and grain yield over both years (average for two years 2015/16 and 2016/17). The P uptake for grain, total P uptake and grain yield were all higher when mineral fertiliser was used. Also, conventional tillage significantly increased straw, grain and total P uptake and grain yield. Additionally, the Oberkulmer Rotkorn variety had significantly greater uptake of P in straw and in total compared to Filderstolz. However, year interacted with several

of the other experimental factors (CP, FT, CP x FT, CP x FT x TI), and so each year of data for P uptake was analysed separately.

Table 4.20. Main effect means, ±SE and *p*-values for the effects and interactions of year, crop protection practices, fertiliser type, tillage system and spelt variety on P uptake (kg ha^{-1}) for straw and grain at harvest and total P uptake (kg ha^{-1}) (P uptake for straw plus grain at harvest) in the Nafferton Factorial Systems Comparison (NFSC) field trial, averaged over two seasons (2015/16 and 2016/17).

Spelt growth stage	P uptake (kg ha^{-1}) at Harvest			Grain yield (t ha^{-1})
	Straw	**Grain**	**Total**	
Main effect means				
Year (YR)				
2015/16	1.36 ± 0.15	5.84 ± 0.54	7.20 ± 0.65	1.72 ± 0.16
2016/17	4.68 ± 0.38	6.66 ± 0.39	11.33 ± 0.68	2.04 ± 0.12
Crop protection (CP)				
Conventional	3.27 ± 0.40	6.86 ± 0.49	10.13 ± 0.75	2.09 ± 0.15
Organic	2.77 ± 0.30	5.63 ± 0.45	8.40 ± 0.66	1.67 ± 0.13
Fertiliser type (FT)				
Compost	2.73 ± 0.36	4.13 ± 0.29	6.86 ± 0.60	1.22 ± 0.09
Mineral N	3.30 ± 0.35	8.37 ± 0.48	11.67 ± 0.69	2.54 ± 0.14
Tillage (TI)				
Minimum	2.40 ± 0.29	4.94 ± 0.41	7.35 ± 0.62	1.48 ± 0.12
Conventional	3.64 ± 0.40	7.55 ± 0.48	11.18 ± 0.72	2.28 ± 0.14
Variety (VR)				
Filderstolz	2.35 ± 0.26	5.99 ± 0.48	8.33 ± 0.60	1.86 ± 0.15
Oberkulmer Rotkorn	3.69 ± 0.42	6.51 ± 0.48	10.20 ± 0.80	1.89 ± 0.14
ANOVA *p*-values				
YR	**<0.001**	0.0858	**<0.001**	**0.0105**
CP	0.4431	0.1899	0.2772	0.21
FT	0.1458	**0.001**	**0.0004**	**<0.001**
TI	**0.0035**	**0.001**	**0.001**	**<0.001**
VR	**0.0002**	0.2693	**0.0086**	0.7895
TI * VR	**0.0196**	0.3106	0.0655	0.5216
CP * YR	0.1426	0.0517	0.5423	**0.0067**
FT * YR	**0.0444**	0.0838	**0.0311**	**0.0439**
TI * YR	0.4007	**0.0022**	0.0869	**0.0003**
VR * YR	**0.0072**	0.1077	**0.0159**	**0.0291**

[b] Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

In 2015/16, P uptake for straw and grain, total P uptake and grain yield were significantly higher with the mineral fertiliser than with compost (Table 4.21). In addition, tillage had an effect on P uptake, with P uptake for straw and grain at harvest, total P uptake and grain yield greater

under conventional than minimum tillage. Crop protection practices also had a significant effect on grain P uptake and grain yield where they were higher under conventional compared to organic crop protection. In contrast, spelt variety did not affect P uptake in 2015/16. The results in 2015/16 revealed several significant interaction effects on P uptake. The interaction between tillage and variety had a significant effect on P uptake for straw (p=0.009) at harvest. The higher P uptake occurred where the spelt variety was Oberkulmer Rotkorn compared to Filderstolz under conventional tillage (Table 4.22).

Table 4.21. Main effect means, ±SE and p-values for the effects and interactions of crop protection practices, fertiliser type, tillage system and spelt variety on P uptake (kg ha^{-1}) for straw and grain at harvest and total P uptake (kg ha^{-1}) (P uptake for straw plus grain at harvest) in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16).

	P uptake (kg ha^{-1}) at Harvest			Grain yield (t ha^{-1})
Spelt growth stage	**Straw**	**Grain**	**Total**	
Main effect means				
Crop protection (CP)				
Conventional	1.35 ± 0.18	6.92 ± 0.82	8.27 ± 0.93	2.10 ± 0.25
Organic	1.37 ± 0.25	4.76 ± 0.67	6.13 ± 0.88	1.34 ± 0.18
Fertiliser type (FT)				
Compost	0.73 ± 0.10	3.30 ± 0.35	4.03 ± 0.41	0.93 ± 0.11
Mineral N	2.00 ± 0.25	8.37 ± 0.82	10.37 ± 0.94	2.51 ± 0.22
Tillage system (TI)				
Minimum	0.89 ± 0.14	3.79 ± 0.56	4.68 ± 0.66	1.09 ± 0.15
Conventional	1.83 ± 0.25	7.88 ± 0.79	9.72 ± 0.93	2.35 ± 0.23
Variety (VR)				
Filderstolz	1.16 ± 0.16	5.96 ± 0.78	7.12 ± 0.88	1.84 ± 0.24
Oberkulmer Rotkorn	1.56 ± 0.26	5.72 ± 0.76	7.28 ± 0.97	1.60 ± 0.21
ANOVA p-values				
CP	0.9688	**0.0475**	0.082	**0.0129**
FT	**0.0037**	**0.0003**	**0.0003**	**<0.001**
TI	**0.0012**	**<0.001**	**<0.001**	**<0.001**
VR	0.0678	0.7023	0.8097	0.1076
CP * FT	0.4908	0.1713	0.3579	**0.0396**
FT * TI	0.3489	0.0723	0.0594	**0.0242**
FT * VR	0.434	0.2356	0.3726	**0.0423**
TI * VR	**0.009**	0.1801	**0.0351**	0.5634

[b] Boldface is used for a significance of p<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

Table 4.22. Interaction means ± SE for the effects of tillage management and spelt variety on P uptake (kg ha^{-1}) in straw at harvest in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16).

Tillage	Variety	
	Filderstolz	Oberkulmer Rotkorn
Minimum	0.99 ± 0.23 Aa	0.79 ± 0.18 Ab
Conventional	1.33 ± 0.22 Ba	2.33 ± 0.41 Aa

Means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

Also, there was a significant interaction between tillage and variety for total P uptake ($p=0.0351$) at harvest in 2015/16 (Table 4.21), although none of the interaction means were significantly different (Table 4.23). There was, however, a different pattern to the effects at each level of tillage with slightly higher P uptake for Oberkulmer Rotkorn compared to Filderstolz under minimum tillage while the opposite was true under conventional tillage ($P>0.05$).

Table 4.23. Interaction means ± SE for the effects of tillage management and spelt variety on total P uptake (kg ha^{-1}) in straw plus grain at harvest in the Nafferton Factorial Systems Comparison (NFSC) field trial (2015/16).

Tillage	Variety	
	Filderstolz	Oberkulmer Rotkorn
Minimum	5.33 ± 1.11 Ab	4.03 ± 0.70 Ab
Conventional	8.91 ± 1.23 Aa	10.53 ± 1.40 Aa

Means labelled with the same capital letter within the same row or the same lowercase letter within the same column are not significantly different (Tukey's honestly significant difference test $p<0.05$).

In 2016/17, the use of mineral fertiliser significantly increased grain P uptake, total P uptake and grain yield compared to compost fertiliser (Table 4.24). Also, tillage had a significant effect on P uptake and grain yield, with conventional tillage resulting in dramatically higher P uptake for straw and grain, total P uptake and grain yield than minimum tillage. Furthermore, spelt

variety Oberkulmer Rotkorn had a significantly higher total P uptake and for straw and grain as well as grain yield compared to Filderstolz. Also, the results revealed that crop protection had no significant effect on P uptake and grain yield (Table 4.24).

Table 4.24. Main effect means, ±SE and *p*-values for the effects and interactions of crop protection practices, fertiliser type, tillage system and spelt variety on P uptake (kg ha^{-1}) for straw and grain at harvest and total P uptake (kg ha^{-1}) (P uptake for straw plus grain at harvest) in the Nafferton Factorial Systems Comparison (NFSC) field trial (2016/17).

Spelt growth stage	P uptake (kg ha^{-1}) at Harvest			Grain yield (t ha^{-1})
	Straw	Grain	Total	
Main effect means				
Crop protection (CP)				
Conventional	5.18 ± 0.63	6.80 ± 0.57	11.98 ± 1.09	2.08 ± 0.17
Organic	4.18 ± 0.42	6.51 ± 0.55	10.68 ± 0.81	2.00 ± 0.17
Fertiliser type (FT)				
Compost	4.74 ± 0.52	4.95 ± 0.42	9.69 ± 0.87 b	1.50 ± 0.12
Mineral N	4.61 ± 0.57	8.37 ± 0.51	12.98 ± 0.97	2.58 ± 0.17
Tillage system (TI)				
Minimum	3.91 ± 0.43	6.10 ± 0.54	10.01 ± 0.82	1.87 ± 0.17
Conventional	5.44 ± 0.61	7.21 ± 0.56	12.65 ± 1.05	2.21 ± 0.17
Variety (VR)				
Filderstolz	3.53 ± 0.39	6.01 ± 0.55	9.54 ± 0.77 b	1.89 ± 0.17
Oberkulmer Rotkorn	5.82 ± 0.59	7.30 ± 0.54	13.12 ± 1.04	2.19 ± 0.17
ANOVA *p*-values				
CP	0.4681	0.8647	0.6768	0.8796
FT	0.8175	**0.0004**	**0.0081**	**0.0003**
TI	**0.0142**	**0.0364**	**0.009**	**0.0378**
VR	**0.0002**	**0.0053**	**0.0003**	**0.0325**

^{b} Boldface is used for a significance of *p*<0.05.

Where there were no significant effects for an interaction term for any of the response variables, this row was left out of the table.

4.3.5. Relationships between AM fungi, grain yield and P nutrition

A Pearson correlation analysis revealed that arbuscule colonisation and vesicle colonisation were not correlated (data not shown). P uptake in the straw was positively correlated with root, arbuscule and vesicle colonisation (Fig 4.1d, e and f). P concentration in grain at harvest was weakly negatively correlated with arbuscule colonisation (Fig 4.1c). Spore density was

negatively correlated with root colonisation (Fig 4.2a), hyphae colonisation and arbuscule colonisation (data not shown). Spore density negatively correlated with P uptake at harvest (in grain, straw and total uptake) (Fig 4.2b and c) (data not shown for total P uptake), as well as with P concentration in crop biomass at anthesis and in straw at harvest (Fig 4.2e and f), but it was positively correlated with P concentration in grain at harvest (Fig 4.2d). Grain yield negatively correlated with spore density (data not shown) and P concentration in grain at harvest (Fig 4.1b), but there was no significant relationship between grain yield and AM fungal colonisation (Fig 4.1a). P concentration in crop biomass positively correlated with AM fungal root, arbuscule and vesicle colonisation at anthesis (Fig 4.3a, b and c) and in straw at harvest (Fig 4.3d, e and f).
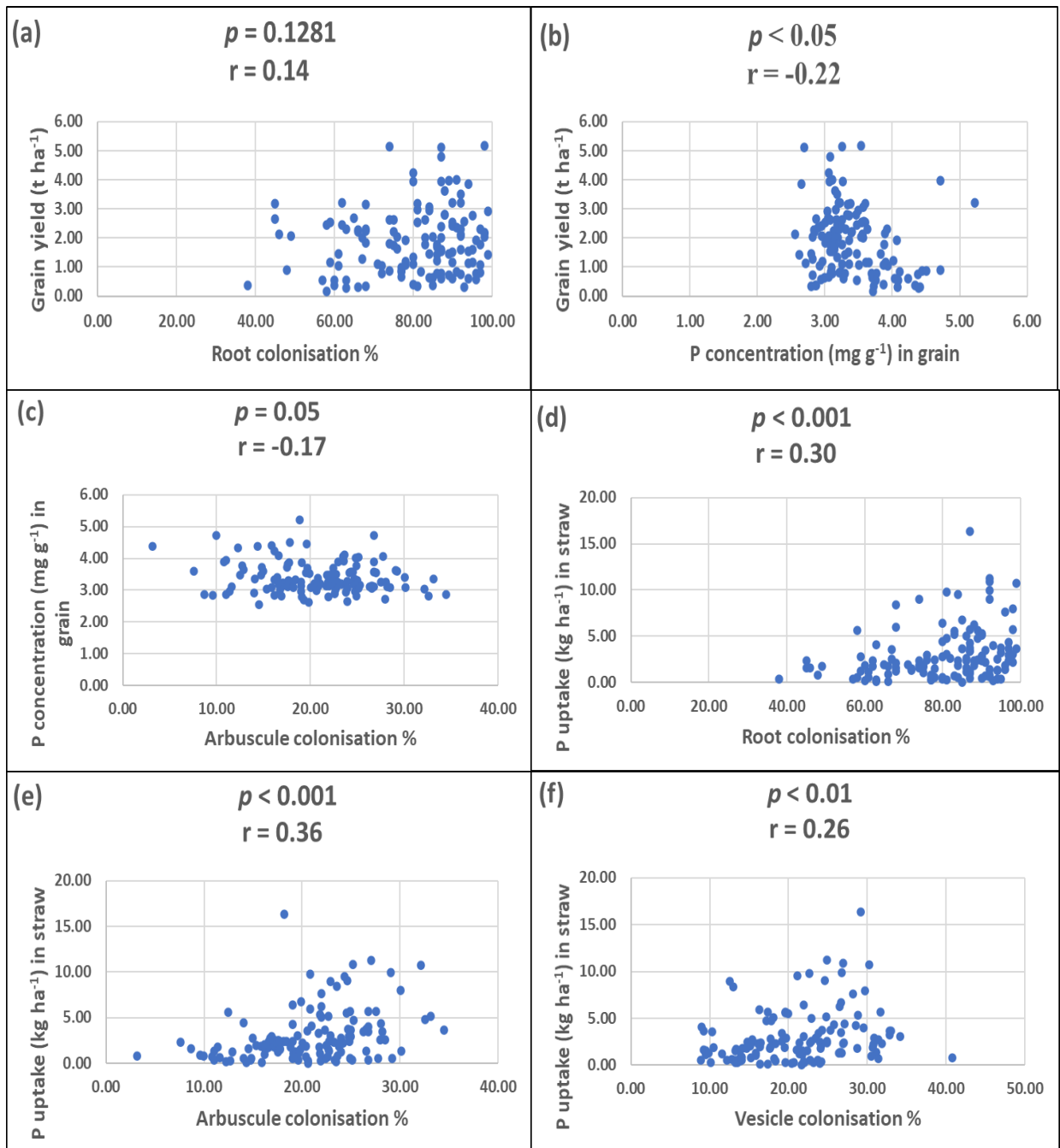
Fig 4.1. Pearson correlation coefficients (r) between all the individual sample values of (a) AM fungal root colonisation and grain yield (t ha^{-1}) (b) grain yield (t ha^{-1}) and P concentration (mg g^{-1}) in grain (c) arbuscule colonisation and P concentration (mg g^{-1}) in grain (d) root colonisation and P uptake (kg ha^{-1}) in straw at harvest (e) arbuscule colonisation and P uptake (kg ha^{-1}) in straw at harvest (f) vesicle colonisation and P uptake (kg ha^{-1}) in straw at harvest in the Nafferton Factorial Systems Comparison (NFSC) field trial (data pooled for 2015/16 and 2016/17 seasons).
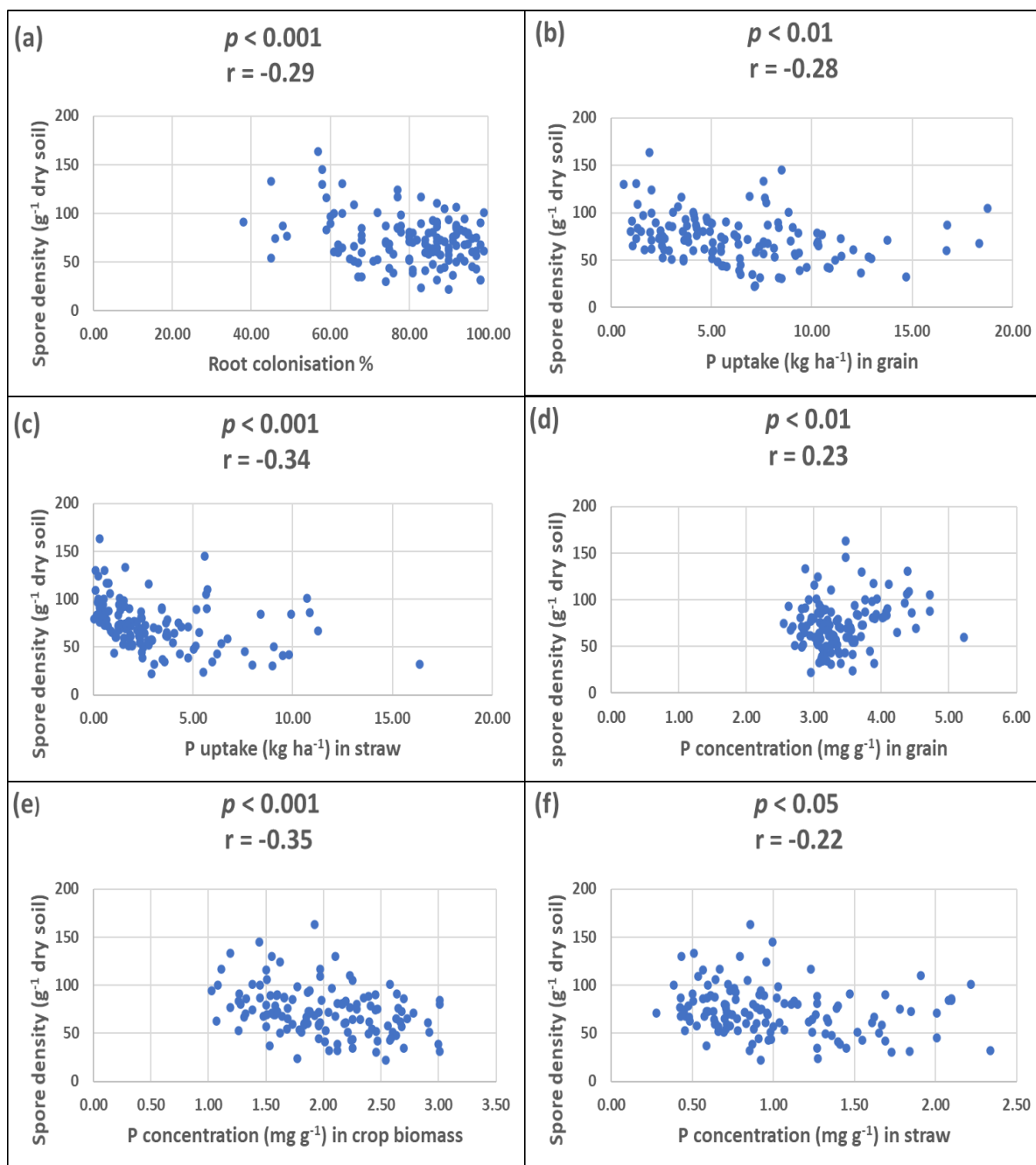
Fig 4.2. Pearson correlation coefficients (r) between all the individual sample values of (a) AM fungal spore density (g^{-1} dry soil) and root colonisation (b) AM fungal spore density (g^{-1} dry soil) and P uptake (kg ha^{-1}) in grain (c) AM fungal spore density (g^{-1} dry soil) and P uptake (kg ha^{-1}) in straw at harvest (d) AM fungal spore density (g^{-1} dry soil) and P concentration (mg g^{-1}) in grain (e) AM fungal spore density (g^{-1} dry soil) and P concentration (mg g^{-1}) in crop biomass at anthesis (f) AM fungal spore density (g^{-1} dry soil) and P concentration (mg g^{-1}) in straw at harvest in the Nafferton Factorial Systems Comparison (NFSC) field trial (data pooled for 2015/16 and 2016/17 seasons).
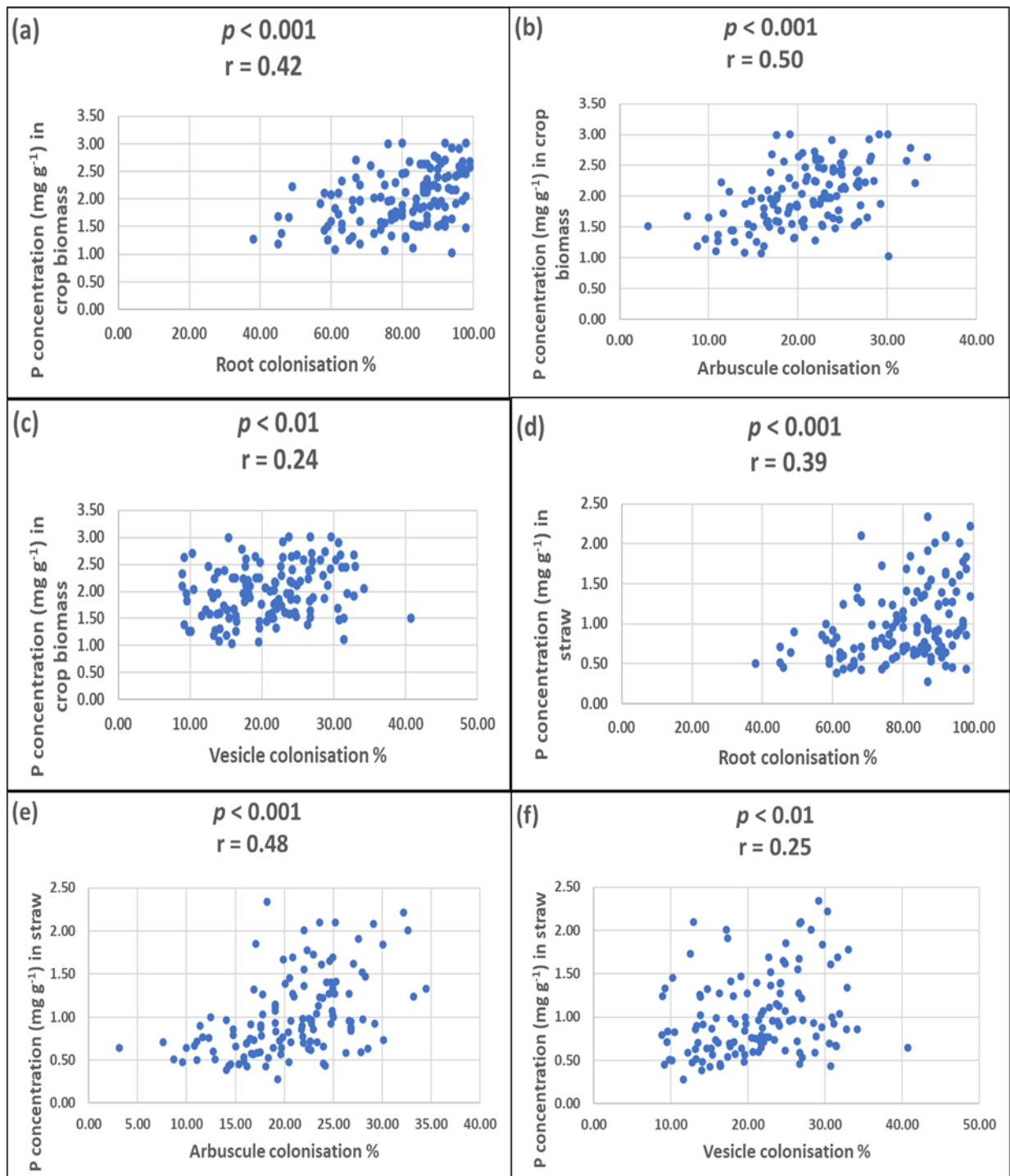
Fig 4.3. Pearson correlation coefficients (r) between all the individual sample values of (a) AM fungal root colonisation and P concentration (mg g^{-1}) in crop biomass at anthesis (b) arbuscule colonisation and P concentration (mg g^{-1}) in crop biomass at anthesis (c) vesicle colonisation and P concentration (mg g^{-1}) in crop biomass at anthesis (d) root colonisation and P concentration (mg g^{-1}) in straw at harvest (e) arbuscule colonisation and P concentration (mg g^{-1}) in straw at harvest (f) vesicle colonisation and P concentration (mg g^{-1}) in straw at harvest in the Nafferton Factorial Systems Comparison (NFSC) field trial (data pooled for 2015/16 and 2016/17 seasons).

4.4. Discussion

4.4.1. Do different tillage treatments affect AM fungal colonisation and spore densities?

In this study minimum tillage increased the soil spore density of AM fungi. This could be due to the different layers of soil being mixed together when the tillage treatment was conventional, which resulted in increased soil available P. This increased soil available P may affect AM fungal development, including spore density (Lekberg *et al.*, 2008; Ryan and Tibbett, 2008). For example, Sheng *et al.* (2013) reported that conventional tillage led to mixing of different layers of soil at different depths (0-15 and 15-30 cm), which resulted in soil layers with similar soil properties and high soil available P. They indicated that larger available P levels is a barrier to AM fungal colonisation.

Minimum tillage only increased spore densities in one year, but not in the other. This may be related to different historical crop management practices applied in the field. Prior to this experiment, the 2015/16 field trial was planted to potatoes (2014) followed by wheat (2015). The 2016/17 field trial followed two years of wheat after a 2-year grass/clover ley phase. This different historical rotation applied in different years may affect AM fungal propagules which may interact with tillage management effect on spore density. The different historical tillage management applied in this study may also cause the differences of tillage effect on one year than another.

Tillage effects on spore density in one season, but not in the other may be related to differences in weather conditions as 2015/16 was wetter than 2016/17 (892 mm versus 706 mm of rainfall) (Fig 4.4b), whereas the temperature in both years was very similar (9.47 $^{\circ}$C versus 9.67 $^{\circ}$C) (Fig 4.4a). This wetter weather would have favoured the activity of soil microorganisms (Monreal *et al.*, 2011), influencing the abundance of AM fungal propagules during the 2015/16 season. It seems that the environmental effects on spore density may interact with tillage. Therefore, an effect of tillage on spore density was observed in 2015/16 rather than the 2016/17 season.

Dilution of the AM fungal propagules including spores in greater soil volumes by ploughing is another mechanism that causes reduced AM fungal spore density (Galvez *et al.*, 2001; Kabir, 2005; Schalamuk *et al.*, 2011; Schalamuk *et al.*, 2013). The lower AM fungal spore density under conventional tillage management could also be interpreted in terms of the strong effect

of the soil disturbance created by ploughing (Galvez *et al.*, 2001; Curaqueo *et al.*, 2011). Conventional tillage, especially with deep inversion, is also likely to bury AM fungal propagules further down than the depth of early seedling crop root development (Kabir, 2005; Verzeaux *et al.*, 2016). In this study, the depth of sampling was 20 cm, and AM fungal spores could have been buried by conventional tillage deeper than our sampling depth. Sheng *et al.* (2013) found that tillage decreased AM fungal spore density at a depth range of 0-15 cm. Some studies have indicated that conventional tillage may alter AM fungal spore density indirectly through changing soil properties such as organic matter content and soil moisture (Beena *et al.*, 2000; Burrows and Pfleger, 2002; Castillo *et al.*, 2006). In contrast, the hyphae network under minimum tillage remains undamaged and thus the active hyphae density is generally larger than in soil under conventional tillage (Cornejo *et al.*, 2009). Also, minimum tillage may lead to increased soil organic matter, which could play a role in increasing AM spore density in soil. Bilalis *et al.* (2010) reported that conservation tillage – including minimum and no tillage – led to greater soil organic matter and total N than conventional tillage. However, the main effect of tillage practice did not affect AM fungal colonisation in this study, even though there were significant interactions between tillage management and variety on AM fungal root colonisation and arbuscule formation. Although, conventional tillage may disturb the network of AM fungal hyphae, the native AM fungal hyphae may survive winter temperatures and recover their ability to colonise spelt roots (Kabir *et al.*, 1997). Unfortunately, the contribution of each type of propagule (hyphae and spores) to AM fungal percent colonisation was not measured in this study, but this could be one possible reason for the absence of differences in colonisation by AM fungi between tillage treatments. These results are consistent with results reported by Monreal *et al.* (2011) in a study conducted at two sites with cultivated flax plants in Manitoba, Canada, to find out the effect of different agriculture practices – including two tillage systems (reduced and conventional) – on AM fungal colonisation. They indicated that the type of tillage management did not impact the AM fungal colonisation of flax in either study year. In contrast, several studies have observed that AM fungal colonisation was lower under conventional tillage as compared to under reduced tillage (Galvez *et al.*, 2001; Schalamuk *et al.*, 2011; Verzeaux *et al.*, 2017b). One explanation provided was regarding the negative effect of conventional tillage in terms of the decreased density of the AM fungal propagules such as active soil hyphae of indigenous AM fungi that survive the winter season in a field study (Kabir *et al.*, 1997; Avio *et al.*, 2013; Verzeaux *et al.*, 2017b), which could affect early AM fungal colonisation of crops. In this case, soil ploughing may lead to the breakup of AM fungal hyphae which may result in a reduced chance of AM fungi colonising plant roots (Alguacil *et al.*, 2008; Verbruggen *et al.*, 2013). Therefore, AM fungal colonisation could be reduced by conventional tillage practices

through destruction of the hyphae network (Álvaro-Fuentes *et al.*, 2008). Gao *et al.* (2010) reported that conventional tillage can damage the soil hyphae network established by AM fungi in current crop roots, resulting in a reduction in colonisation by AM fungi of the following crop roots. However, this is not the case in this study as the tillage management effect (conventional versus reduced) was similar on AM fungal colonisation and only spore density was affected by tillage treatement.

4.4.2. *Does organic fertiliser increase AM fungal colonisation and spore densities?*

Fertiliser management practices can induce changes in AM fungal development. The results of this study suggested that compost fertiliser promoted AM fungal root colonisation and spore density, but this effect depended on the whole management system. Compost additions may stimulate AM fungi to colonise spelt roots through several processes. Firstly, this could be related to increased soil organic matter (Wang *et al.*, 2018b) which promotes AM fungal development (Ryan and Tibbett, 2008). Whereas AM fungi can get organic carbon from plant roots through photosynthate (Gavito and Olsson, 2003), they may also get additional nutrients such as nitrogen from organic matter, as reported by Sabine *et al.* (1999) and Hodge *et al.* (2001). The concentration of N in extra-radical hyphae of AM fungi is greater than in plant shoots and roots (Hodge and Fitter, 2010). Therefore, under low N conditions, AM fungi do not supply the plant with N as they need this N for their growth (Chen *et al.*, 2018a). Compost addition is rich in humic acid and organic N which stimulate AM fungal growth (Gryndler *et al.*, 2009). Therefore compost may compensate the N which is required in high amounts for AM fungal growth (Yang *et al.*, 2018a). AM fungi can utilize soil organic N sources, despite the frequent assumptions that due to their obligate biotrophic life-cycle AM fungi only obtain C from their host plant to complete their life-cycle. Some AM fungi can exploit up to 30% of the organic N present in organic soil patches (Leigh *et al.*, 2009). Soil containing a greater organic N content can stimulate both AM fungal root colonisation and N transfer to the plant, therefore the type of N fertiliser is an important factor for controling both biological activity and AM fungal colonisation in terms of N uptake efficiency (Leigh *et al.*, 2009; Thirkell *et al.*, 2016). Furthermore, the greater soil organic matter likely encourages survival of AM fungal propagules before spelt sowing (Gollner *et al.*, 2011).

Secondly, compost fertiliser may enhance plant growth (Lee *et al.*, 2004) and increase carbon allocation to plant roots (Donn *et al.*, 2014), thus releasing more carbohydrate in the root exudates to attract AM fungal colonisation. In the present study, the root colonisation by AM

fungi was measured at anthesis where the compost amendment positively affected AM fungal colonisation. Unfortunately, the AM fungal colonisation was not measured at harvest stage where mineral fertilisation increased spelt growth and also spelt growth at anthesis was not measured to find out the effect of compost on this crop parameter at this growing stage. However, in this study compost increased P concentration in crop biomass at anthesis and harvest straw compared to mineral fertiliser. It is possible that the compost could also positively affect spelt growth and/or P uptake at this growth stage which can contribute to the allocation of more C to spelt roots, thus enhancing AM fungal colonisation.

Thirdly, compost can enhance the soil's physical characteristics (McCoy, 1998). For example, Caravaca *et al.* (2002) found that compost additions enhanced physical properties of the soil rhizosphere such as aggregate stability and water soluble carbon. This could support soil microorganism activities, as rhizosphere aggregates affect nutrient cycles, permeability, aeration and serve as a refuge for soil microorganisms and fauna in microsites. These conditions may promote the release of growth-stimulating substances from soil microorganisms, where AM fungi can get benefits from these substances (Fauci and Dick, 1994).

Fourthly, compost may have lower adverse effects on AM fungal development than equivalent amounts of mineral fertiliser. This could be due to temporal variations in soil P availability between organic and mineral fertiliser as compost supplies a sustained release of P rather than a large amount in a single pulse (Yang *et al.*, 2017). As is well known, soil available P can negatively affect AM fungal development in roots or soil (Lanfranco *et al.*, 2018; Wang *et al.*, 2018b) through increasing P concentration levels in plant tissues (Thomson *et al.*, 1991; Grant *et al.*, 2005). The advantage of using organic additions with lower or moderate level of soil available P consists in the slow release of P that can prevent levels of P rising in plant tissues, which gives a chance for crop symbiosis with AM fungi. These results are consistent with previous studies which observed that compost increased AM fungal colonisation and spore density at moderate levels of soil available P (Treseder and Allen, 2002; Yang *et al.*, 2017; Yang *et al.*, 2018a). Therefore, AM fungi can develop when the soil available P level is sufficient for AM fungal growth, but not for the crop.

In addition, this study presented several significant interactions among crop management factors regarding fertiliser effects which have not been reported before. These interactions pointed out that the compost effect depended on the whole management system. Some interactions confirmed that compost can support AM fungal colonisation. For example, the

combination of compost with conventional crop protection under minimum tillage promoted root colonisation for the 2015/2016 season (Table 4.9). In addition, in the current study the main effect of compost for the 2016/17 season increased AM fungal root and arbuscule colonisation compared to mineral fertiliser, while this effect was absent for the 2015/16 season. This effect may relate to the initial available N levels in soil as these N levels may have promoted AM fungal root colonisation. The differences of initial total available N level between compost (20.4 kg ha^{-1}) and mineral plots (17.4 kg ha^{-1}) in 2015/16 was smaller than differences between compost (52.3 kg ha^{-1}) and mineral (34.5 kg ha^{-1}) in 2016/17 at the 0-30 cm depth (Table 4.2 and 4.3). Overall, the initial total soil available N level for all plots in 2016/17 was higher than for the 2015/16 season in the current study. This suggested that AM fungi also require other nutrients such as N for their own growth and these soil N levels may promote AM fungal growth, but not crop growth (Hodge and Fitter, 2010; Hodge and Storer, 2015). Therefore, compost amendment promoted AM fungal root colonisation, but this effect depended on interaction with other crop management practices and initial soil nutrients.

In general, in this study spore density the effects of fertiliser type on spore density depended on other interacting crop management practices such as crop protection, tillage and/or variety. For example, the main effect of compost for the 2016/17 season and the combination of compost with conventional crop protection under minimum tillage promoted AM fungal root colonisation (see Table 4.9) However, these interactions reflected that there was no consistent pattern for fertiliser type on AM fungal colonisation and spore density. This could be attributed to the fact that the response of AM fungal spores to fertiliser types may differ to that of AM fungal colonisation. As spore density of AM fungi reflect the long-term crop management and not the current colonisation. For example, Gryndler et al. (2006) found that AM fungal spore density did not follow the same pattern as AM fungal colonisation and hyphae length regarding fertiliser effects. They indicated that AM fungal colonisation and hyphae length were increased by manuring and decreased by mineral fertiliser, whereas AM fungal spore density was not affected by both fertiliser types. This suggested that AM fungal root colonisation does not always reflect the spore density of AM fungi in soil.

In general, mineral fertiliser can negatively affect AM fungal colonisation. Mineral fertiliser with P or N reduced AM fungal colonisation (Schalamuk et al., 2011; Sheng et al., 2012). This may be because mineral additions can modify soil available P and N which can strongly affect microorganism functions (Cruz et al., 2009). Mineral fertiliser can reduce AM fungal colonisation (Ercoli et al., 2017) through affecting soil nutrient availability and nutrient

concentrations in plant tissues (Corkidi *et al.*, 2002; Wang *et al.*, 2017), but that is not always the case (Wang *et al.*, 2018a). This could be because the high availability of nutrients such as N or P in the soil due to mineral additions can qualitatively change the composition of root exudates and thus reduce AM fungal colonisation (Verzeaux *et al.*, 2016). Recently, it has been indicated that N fertilisation negatively impacts the production and exudation of strigolactones that are the main host recognition signalling molecules involved in AM fungal colonisation (Venice *et al.*, 2017; Lanfranco *et al.*, 2018; Tavarini *et al.*, 2018). In the present study, the mineral plots had lower AM fungal root colonisation compared to compost plots, even though the initial total available N level in soil was lower for mineral than compost plots. The initial soil available P was low for the all compost and mineral plots. However, in this study the soil mineral N analysis was conducted before mineral fertiliser applied, while the root sampling was conducted after mineral fertiliser applied. Therefore, the negative effect of mineral fertiliser may be related to increase level of soil available N and/or P in mineral plots which led to reduce AM fungal root colonisation (Chen *et al.*, 2018a; Lanfranco *et al.*, 2018). These results are consistent with Verzeaux *et al.* (2016) who found that the absence of mineral N fertiliser strongly enhanced AM fungal colonisation of wheat roots in an experiment conducted in a field trial over 5 years. This is related to the fact that AM fungal colonisation is suppressed when plants are cultivated under high levels of available P and N (Lanfranco *et al.*, 2018) due to reduced C allocation to AM fungi.

4.4.3. Do spelt cultivars differ in their response to AM fungal symbiosis and spore densities in the soil?

This study suggested that there were considerable differences between spelt varieties for AM fungal colonisation, which was enhanced when the spelt variety was the modern variety Filderstolz. Meanwhile, spore densities were increased where the spelt variety was the old Swiss landrace, Oberkulmer Rotkorn (Table 4.5, 4.6 and 4.10). Several studies have found that the modern wheat varieties are highly compatible with AM fungi (Kirk *et al.*, 2011; Ellouze *et al.*, 2016; Ercoli *et al.*, 2017), although Hetrick *et al.* (1993) found that wheat landraces were more responsive to AM fungal colonisation than modern cultivars, reflecting results of other studies (Sangabriel-Conde *et al.*, 2014; Cobb *et al.*, 2016). The variance in the response of both spelt cultivars to AM fungal colonisation may be related to variation in the genetic basis of receptivity to AM fungal symbiosis (Sawers *et al.*, 2017; Lanfranco *et al.*, 2018). AM fungal colonisation may be related to amount of sugar and lipid existing in the root exudates and the

genetic differences in patterns of release of monosacccharides among crop genotypes that affect carbohydrate uptake by AM fungi (Lanfranco *et al.*, 2018).

Differences in crop varieties' formation of symbioses with AM fungi may also be attributed to differing nutritional requirements between cultivars. Some crop cultivars adapt to deficient nutrient conditions, such as P deficient conditions, better than other cultivars (Liu *et al.*, 1995; Ryan *et al.*, 2016). Since AM fungi play an important role in P uptake, it would be credible to expect that cultivar differences in terms of compatibility with AM fungi might also occur.

Furthermore, the variation in AM fungal colonisation between crop varieties could arise from alterations in the biochemical and physiological properties of root systems that are controlled by genetic variation among crop cultivars (Thomas and Ghai, 1987). Plants produce inhibitory substances as a consequence of AM fungal colonisation, such as phytoalexins and phenolics (Krishna *et al.*, 1985). The varietal differences in the growth rates of AM fungi within the root cortex may be due to variation in the production of these substances among crop cultivars (Ryan *et al.*, 2016).

In this study, higher colonisation by AM fungi was found for the modern variety Filderstolz, and greater soil spore density occurred with the Swiss landrace, Oberkulmer Rotkorn. The spelt variety was only in the field for less than one year (from Oct 2015 to harvest 2016), and the soil sampling in this experiment occurred at the time of flowering. Therefore it was possibly too early in the season to detect the impacts of higher levels of root colonisation on spore densities. This phenomenon could be attributed to a reduction in the spore density of AM fungi due to spore germination prior to root colonisation, which led to depletion of spores in the plots where Filderstolz was cultivated relative to Oberkulmer (Varela-Cervero *et al.*, 2016).

Variation among native AM fungal populations is another explanation for this phenomenon. There may be genetic differences among the AM fungal species in the natural soil populations which result in enhanced colonisation of one variety of spelt compared to the other (Van Der Heijden *et al.*, 2008; Yang *et al.*, 2018b). Differences have been found in microbial communities associated with different wheat cultivars (Siciliano *et al.*, 1998). For example, in a greenhouse experiment, Seguel *et al.* (2016) observed different wheat cultivars showing variation in their response to AM fungi. They indicated that the spore density and glomalin production of AM fungi were different among four wheat crop cultivars, although different wheat genotypes showed high AM fungal colonisation at the first phenological stage (11 days

after sowing). Sangabriel-Conde *et al.* (2014) suggested that the differences in the responses of different maize genotypes to AM fungal colonisation may be due to these genotypes not associating with the same group of AM fungal species.

In this study, there were several significant interactions which have not been reported before for spelt. However, the performane of both modern Filderstolz and the Swiss landrace, Oberkulmer Rotkorn on AM fungal colonisation and spore density depended on interaction with other crop management practices. These interactions reflected the fact that the spelt cultivars differed in their response to AM fungal symbiosis under different crop management practices. Genetic variability among different crop cultivars for compatibility with AM fungi may depend on genotype-environmental condition interactions (Sawers *et al.*, 2017). For example, in a greenhouse experiment, Ellouze *et al.* (2016) found that AM fungal colonisation was lower (21%) in landraces than modern wheat cultivars (27%), and that the differences were clearer at low levels of soil fertility. However, the response of modern and old spelt cultivars to AM fungal symbiosis under different crop management practices is complex, therefore, further research is required.

4.4.4. Does organic crop protection increase AM fungal colonisation and spore densities?

The results of this study suggest that AM fungal spore densies were increased more where organic crop protection was applied than under conventional management (Table 4.5 and 4.10). Organic management can have a positive effect on AM fungal development since it does not allow the use of herbicides or fungicides, both of which can inhibit fungal development (Mader *et al.*, 2000; Ryan and Tibbett, 2008).

Herbicides can directly reduce AM fungal symbiosis by lowering AM fungal spore viability and hyphal length due to inhibitory effects on spores (Verzeaux *et al.*, 2017b). Herbicides can also work indirectly by removing the weeds that can work as host plants for AM fungi (Moorman, 1989; Mader *et al.*, 2000; Santos *et al.*, 2006). However, in this experiment, the combination of fungicides and herbicides was one factor referred to as crop protection management and it is therefore difficult to tell which pesticides were causing the reduction in spore density under conventional management.

It seems that herbicides do not have as strong an effect on AM fungal develoment as fungicides. It was hypothesized that weeds may play an important role in enhancing AM fungal

colonisation (Nelson *et al.*, 2011a). Weeds were present in all plots in autumn and would have had an equal effect on colonisation and they were only controlled by herbicides in the spring. Weeds are important hosts for AM fungal establishment and they are faster colonising from soil inoculum of AM fungi than cereal crops (Nelson *et al.*, 2011a). These conditions can build a soil network of AM fungi hyphae through weeds (Bücking *et al.*, 2016) with spelt at the beginning of the season in autumn. If weeds are controlled by herbicides and the extra-radical hyphae is still kept in contact with spelt roots, the AM fungal colonisation of roots of young spelt would be enhanced by autumn germinating weeds.

However, the effect of herbicide on AM fungal development may depend on its type and rate. (Smith *et al.*, 1981). For example, it has been reported that if di-allate (trade name: Avadex), diuron (trade name: Karmex) and trifluralin (trade name: Treflan) herbicides are applied at a normal rate, they will not have a negative effect on the functioning and establishment of AM fungi (Smith *et al.*, 1981). There are two types of herbicides: contact herbicides that kill the part of the plant in contact with the herbicide but the roots may survive whereas, systemic herbicides are absorbed and translocated through the plant's system, killing the entire plant (Kogan and Bayer, 1996). Brito *et al.* (2013) reported that herbicide type is an important factor to control the level of benefit from AM fungi. The authors found that the type of herbicides such as paraquat (trade name; Gramoxone) and glyphosate (trade name; Roundup) had no impact on AM fungal colonisation of wheat. These herbicides have contrasting modes of action as paraquat is a contact herbicide which kills the part of the plant in contact with the chemical, whereas, glyphosate is systemic meaning it is absorbed and translocated through the plant's vascular system, killing the entire plant (Brito *et al.*, 2013). In this study, plots were sprayed with standard herbicides Isomec Ultra (dichloroprop-p) and Cleancrop Galifrey (fluroxypyr) and they were applied at normal rates. Although both dichloroprop-p and fluroxypyr herbicide are systemic herbicides (Durkin, 2009; IUPAC, 2019), these herbicides did not lead to reduced AM fungal colonisation. The dichloroprop-p herbicide is environmentally friendly and is also used as a plant growth regulator (IUPAC, 2019), therefore it may not have adverse effects on AM fungal colonisation. However, there were no consistent results regarding herbicide effects on AM fungal development across many studies; these depended on experimental conditions, crop species and herbicide type (Lenoir *et al.*, 2016). For example, the herbicide isoxaflutole did not inhibit colonisation by AM fungi *Glomus intraradices* of corn roots when it was applied equivalent to recommended field rates, in a greenhouse experiment (Stokłosa *et al.*, 2011). Whereas, the herbicide dicamba (active ingredinet) significantly reduced the extra-radical hyphae and spore density of AM fungal *Funneliformis mosseae* in chicory plants in an *in vitro*

experiment (de Novais *et al.*, 2019). Both herbicides gramoxone (Paraquat) and Brominal (bromoxynil or Labuctrill-25) significantly reduced AM fungal spore density and colonisation of legume roots in a pot experiment (Abd-Alla *et al.*, 2000). However, all these studies were conducted under controlled conditions (e.g. *in vitro* and greenhouse experiments) which may offer the advantages that the environmental conditions can be restricted to a limited number of variables, allowing more accurate investigation of the herbicide effect on the plant-AM fungus symbiosis. Moreover, the herbicide types and crop species used in these studies were different compared to this experiment. Therefore, it is important to examine herbicide effects on AM fungal development under field conditions as this effect can be variable under such conditions due to interactions with other field factors.

It is more likely that fungicides may inhibit AM fungal development than herbicides. In the current study, the use of conventional crop protection which included fungicides decreased spore densities but did not affect root colonisation. The fungicide epoxiconazole used in this study is a systemic fungicide from the class of triazoles developed to provide broad spectrum control of major fungal pathogens as well as actively stopping spore production and fungal growth (Xu *et al.*, 2007). Whereas, the fungicide chlorothalonil that was also used in this study is a contact (non-systemic) and broad-spectrum fungicide (Agchem, 2019). It can lead to the inhibition of enzymes responsible for cellular respiration (Baćmaga *et al.*, 2018). It seems these fungicides affected more extra-radical structures of AM fungi such as spores more than intra-radical structures (hyphae, arbuscules and vesicles). In the present study, it seems that the chlorothalonil fungicide may not affect AM fungal colonisation as it is a non-systemic fungicide. This is consistent with Bary *et al.* (2005) who found that a non-systemic fungicides – including chlorothalonil, fenarimol and iprodione – did not affect AM fungal colonisation of annual grass in a temperate region. This could be due to degradation of fungicides on the soil surface where an undecomposed layer of plant material occurs. In this sense, an inadequate amount of fungicide reached the plant roots, and consequently it had no impact on AM fungal colonisation. Even though epoxiconazole is a systemic fungicide, it is one fungicide from a class of triazoles which don't have the level of systemic movement of many fungicides; they are locally systemic (the active ingredient is absorbed by leaves and moves within the leaf) (Kjøller and Rosendahl, 2000). Therefore, it could be that the epoxiconazole did not also have adverse effects on AM fungal colonisation in this study. A previous greenhouse study showed that systemic fungicides belonging to the triazoles such as epoxiconazole applied at the recommended rate do not affect AM fungal colonisation of *Cucumis sativus* L. plants (Kjøller and Rosendahl, 2000). Those authors also found that propiconazole, another broad-spectrum

(triazole) fungicide, decreased the alkaline phosphatase activity of the extra-radical hyphae. This study suggested that extra-radical hyphae have a higher sensitivity to this fungicide as compared to the intra-radical hyphae colonisation which was not affected. Furthermore, in the present study, the fungicides epoxiconazole and chlorothalonil were applied at recommended rates and this is a possible reason why we did not observe negative effects of these fungicides on AM fungal colonisation, as high rates of fungicides can affect enzymatic pathways of AM fungi (Buysens *et al.*, 2015). This study is consistent with previous studies, for example an *in vitro* study where AM fungal structures were in direct contact with the compounds; Buysens *et al.* (2015) found that azoxystrobin (trade name Amistar®) fungicide caused a reduction in the spore density and extra-radical hyphae of AM fungal *Rhizophagus irregularisin* in potato plants, while the root colonisation was not affected by this fungicide.

The different effects of fungicides on AM fungal growth may be related to fungicide types and mode of action (Buysens *et al.*, 2015; de Novais *et al.*, 2019). As azoxystrobin is systemic and a broad-spectrum fungicide (Buysens *et al.*, 2015). In the present study, epoxiconazole and azoxystrobin are broad-spectrum fungicides and this may explain why these fungicides could affect only extra-radical structures such as spores. The sterol biosynthesis inhibitors fungicides fenpropimorph and fenhexamid which also have broad spectrum antifungal activity reduced spore density, but had no impact on colonisation by AM fungi *Glomus intraradices* in carrot roots grown in monoxenic cultivation system (Zocco *et al.*, 2008). It seems that these fungicide types had direct contact with extra-radical structures of AM fungi including spores, but they did not affect colonisation by AM fungi.

The effects of different crop protection treatments may depend upon crop management practices. The results of this study indicate that applying intensive agricultural practices together could lead to reductions in AM fungal colonisation and spore density. In the present study the crop protection effect on AM fungal colonisation and spore density depended on the fertiliser and tillage system. It seems that the negative effect of pesticides including herbicides and fungicides on AM fungal development can be minimised by use of a suitable fertiliser such as compost which could positively affect AM fungal development (Pimmata *et al.*, 2013).

4.4.5. Does AM fungal colonisation increase the grain yield and P nutrition of spelt?

This study suggested that fertiliser type and tillage treatment were strong factors affecting grain yield and P uptake for grain and straw. In general, conventional tillage and mineral N fertiliser

increased grain yield and P uptake for grain and straw at harvest. This could be related to how conventional tillage may enhance the soil properties. Conventional tillage can enhance organic matter turnover and reduce bulk density (Sheng *et al.*, 2012). It serves to prepare the seedbed and incorporates and distributes organic matter through the topsoil providing conditions suitable for mineralising nutrients, particularly N (Peigné *et al.*, 2007). Furthermore it can control the pressure from grass weeds which can reduce the competition for nutrient sources, thus increasing crop yield (Peigné *et al.*, 2007).

Furthermore, the high grain yield and P nutrition achieved by mineral fertiliser may be attributed to the prompt release of nutrients from mineral fertilisers, which can make these nutrients immediately available to the crop, thus enhancing P uptake and crop yield (Cruz *et al.*, 2009; Hu *et al.*, 2010).

The concentration of P was enhanced in spelt biomass at anthesis by compost additions and with the spelt variety Filderstolz, which may be reflected later in increased P concentration in spelt straw at harvest. This could be related to AM fungal colonisation, which is increased by compost additions, and to the spelt variety Filderstolz at this phenological stage (anthesis). The positive relationship between AM fungal colonisation and P concentration for crop biomass at anthesis and harvest (Fig 4.3a and d), and with P uptake for straw at harvest (Fig 4.1d), reflected these effects. The enhancement in P concentration in crop biomass at anthesis and straw at harvest with compost addition rather than mineral fertiliser may be related to change in the terms of P-C exchange under mineral fertilisation irrespective soil P status. Under mineral fertilisation conditions, plants allocate an increasing amount of C to AM fungi while receiving relatively less P (Williams *et al.*, 2017). Organic additions can support AM fungal colonisation of crops – for example, the effects of an organic addition (green manure as living mulch) on AM fungal colonisation has been examined in a greenhouse experiment by Deguchi *et al.* (2012). This organic addition led to increased P concentration and P uptake in corn plant shoots compared to where no living mulch was applied due to the promotion of AM fungal colonisation by living mulch treatments (Deguchi *et al.*, 2012). Current results supported the results of this study, as organic compost addition increased P concentration through enhancing AM fungal colonisation in spelt crop biomass at anthesis.

However, the compost and Filderstolz effects on AM fungal colonisation did not result in higher grain yield in this study. There is not always a positive relationship between colonisation by AM fungi and grain yield and P uptake (Ryan *et al.*, 2016; Koch *et al.*, 2017; Sawers *et al.*,

2017; Thirkell *et al.*, 2017) and AM fungi may not compensate for reductions in fertiliser inputs (Berdeni *et al.*, 2018). This study's results are consistent with previous studies which observed no response or a negative effect of AM fungal colonisation on P uptake and plant growth (Ryan and Angus, 2003; Hildermann *et al.*, 2010; Sawers *et al.*, 2010; Smith and Smith, 2012). Although, the colonisation by AM fungi increased P concentration in this study, AM fungal colonisation did not translate into other benefits such as increased crop yield or growth. Under some conditions, crop yield may not benefit from high colonisation and AM fungi may become parasitic which could reduce plant growth (Ryan and Angus, 2003). This is because high AM fungal C use with a lack of P benefit can cause plant growth depression. It therefore seems that exploration into the contribution of AM fungi to crop P uptake and grain yield under field conditions is required before a conclusion can be drawn about the impacts of colonisation by AM fungi on crop yield.

Furthermore, variety was an additional factor affecting grain yield and P uptake in the 2016/17 field trial (Table 4.24), whereas the P concentration for grain was affected by variety in the 2015/16 field trial (Table 4.16) and 2016/17 field trial (Table 4.19). In general, grain yield, grain P concentration and P uptake in grain and straw were enhanced for the spelt variety Oberkulmer Rotkorn. Although higher AM fungal colonisation occurred with Filderstolz than Oberkulmer Rotkorn, this effect was not reflected later on in grain yield. Therefore, the effects of Oberkulmer Rotkorn on grain yield and P nutrition could be attributed to a variety of causes. Some varieties have more efficient root systems with longer root hairs than other varieties for exploring and absorbing nutrients from the soil (Haling *et al.*, 2016; Haling *et al.*, 2018).

In general, modern varieties are higher yielding than landraces (Kirk *et al.*, 2011), but in the current study Oberkulmer Rotkorn produced higher grain yields than Filderstolz. This is because the rates of fertiliser applied (100 kg N ha^{-1}) were quite low in this trial. It is possible that the Filderstolz was bred for higher rates of mineral application. These results are in agreement with those of Cobb *et al.* (2016) who reported that landraces generated grain yields higher by 285%, and 206% greater straw biomass than hybrids in non-fertilised soil.

Moreover, the higher P concentration for the crop biomass of spelt at anthesis and harvest in 2016/17 compared to 2015/16 may be due to greater AM fungal colonisation and arbuscule formation, although root colonisation was only slightly higher in 2016/17. Since grain yield did not correlate with colonisation by AM fungi, the higher grain yield and P uptake for straw in 2016/17 may be related to differences in weather conditions. However, the rainfall does not

seem to be the main factor affecting grain yield since the rainfall was within the normal range of rainfall in the UK (600 mm – 1000mm) (MetOffice, 2018a) for both seasons (892 mm from September 2015 until September 2016 and 706 mm from September 2016 until September 2017, based on the field station weather data collected at Nafferton farm) (Fig 4.4b). Therefore, both trial years were not unusual in terms of precipitation. Furthermore, the average temperature in both years was very similar (9.67 ℃ versus 9.47 ℃) (Fig 4.4a). Radiation levels may be the main factor contributing to higher yields in the 2016/17 season (Bilsborrow *et al.*, 2013). Based on the field station weather data collected at Nafferton farm, total radiation was higher over the full year and specifically over April, May, June and July in 2017 (1784.16 MJ m^{-2}) compared to 2016 (1342 MJ m^{-2}). The higher yields in 2016/17 reflected a pattern across the UK that may have been due to higher sunshine hours (DEFRA, 2017). Higher yields resulted in higher P uptakes in 2016/17, even though P concentrations were actually lower in the grain in 2016/17 (but higher in the straw). Sometimes higher yielding crops have lower concentrations of nutrients due to a dilution effect.
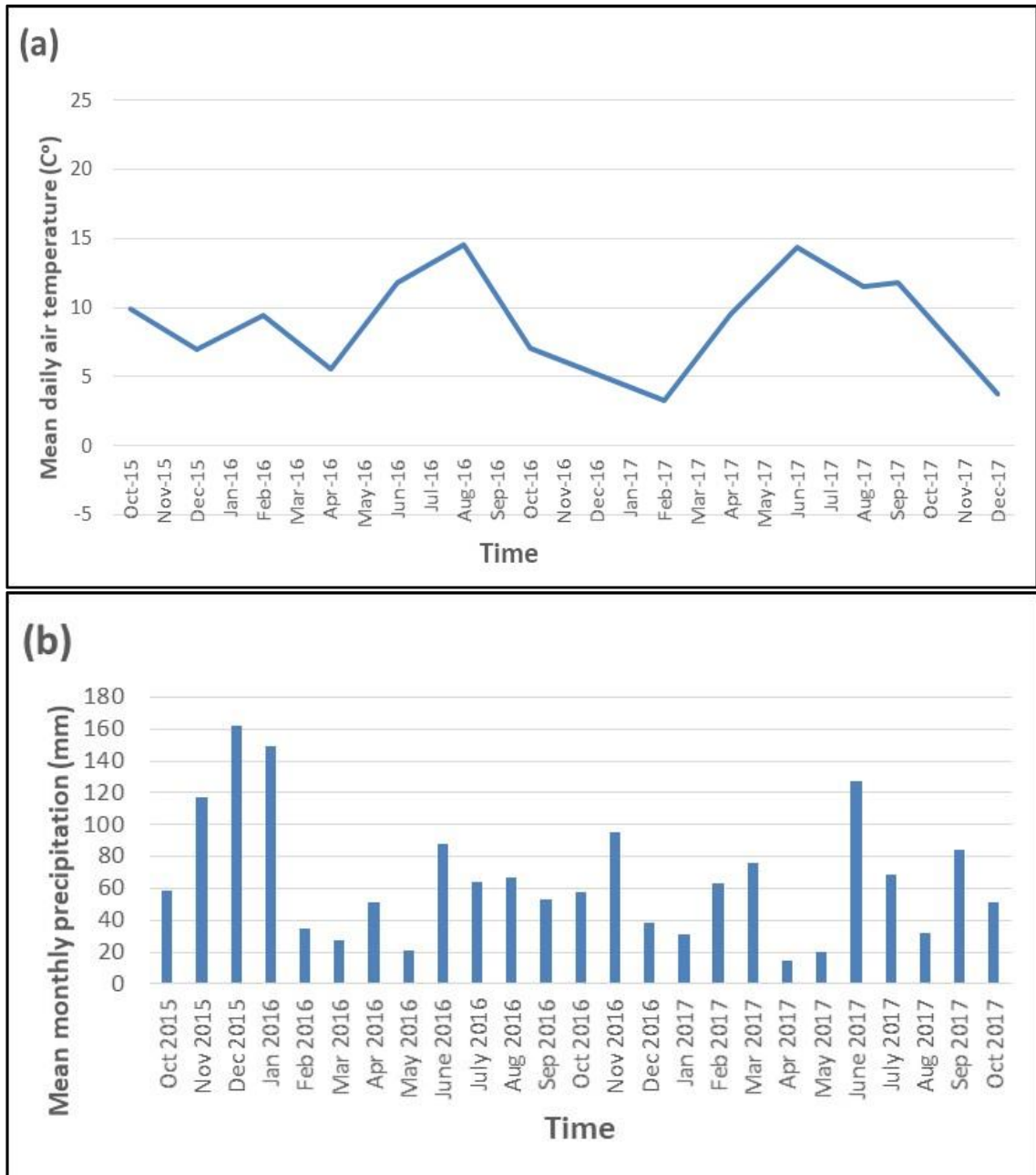
Fig 4.4. (a) Mean daily air temperature (°C) and (b) Mean monthly precipitation (mm) at Nafferton Farm (2015/16 and 2016/17).

4.4.6. Do AM fungal spore densities in soil reflect AM fungal colonisation in crop roots?

The AM fungal spore densities were higher in 2015/16 than 2016/17, but arbuscule colonisation was greater in 2016/17 than 2015/16 (Table 4.5). The spore density of AM fungi is not always positively correlated with AM fungal colonisation (Brundrett, 1991; Schalamuk *et al.*, 2013) for several reasons. The environmental conditions are an important factor and they can affect AM fungal development. Cool and warm seasons can affect mycorrhizal symbioses with plants and mycorrhizae can be highly active during warm times of the year (Hetrick *et al.*, 1989). However, AM fungal colonisation can be reduced by cool weather, especially when the temperature range is 5-10 ^{o}C (Andersen *et al.*, 1987). This could be attributed to low temperatures leading to reduced photosynthesis, which results in decreased AM fungal symbiosis due to the reduction of carbohydrate production (Son and Smith, 1988). The low winter temperatures of less than 10 ^{o}C could also decrease the spread and P flow through extra-radical hyphae of AM fungi (Gavito *et al.*, 2003). This may also lead to reduced AM fungal development as the plant will stop providing AM fungi with fixed C to avoid parasitism (Kobae *et al.*, 2016). The amount of precipitation is another factor which can affect AM fungal symbiosis. Mycorrhizal colonisation is more active during the wet season than the dry season (Osmond *et al.*, 1987). In this study, as described in Sec. 4.4.5 rainfall and temperature was similar and within normal ranges for the UK. Therefore, the higher AM fungal colonisation in 2016/17 was probably not related to environmental conditions but could be related to different historical rotations between the years. In 2015/16 the spelt followed five years of arable cropping, while in 2016/17 spelt followed two years of arable cropping after a 2-year grass/clover ley phase. Therefore, the presence of grass clover might affect AM fungal colonisation. Grass and clover are mycorrhizal plants (Mäder *et al.*, 1999) and the lack of tillage for this period would also have promoted a healthy population of mycorrhizae in the soil and could have affected colonisation in the present spelt crop.

Seasonal variation is another important factor affecting AM fungal spore density in the soil (Schalamuk *et al.*, 2013). Mostly, AM fungal spores are less abundant throughout the period of AM fungal inoculation, but they become more plentiful during the period of maturity of the roots (Varela-Cervero *et al.*, 2016). Spore germination may cause reductions in AM fungal spore density which leads to depletion of spores in the soil as an immediate effect of colonisation of the spelt roots with AM fungi (Varela-Cervero *et al.*, 2016). The peak period of AM fungal spore density is generally when the crops are harvested for agricture or when a long dry season interrupts root activities (Rodríguez-Echeverría *et al.*, 2008).

In addition, spore counting is difficult because some spores may represent the accumulation of old residual spores (Hijri *et al.*, 2006; Schalamuk *et al.*, 2013). Additionally, there are other AM fungal propagules in soil besides spores, notably colonised root fragments and soil hyphae (Schalamuk and Cabello, 2010; de Novais *et al.*, 2019). Spores counted may not accurately reflect the AM fungal propagule levels in the soil because colonisation can occur from different AM fungal propagules (Douds *et al.*, 1997; Schalamuk and Cabello, 2010; Verzeaux *et al.*, 2017b; de Novais *et al.*, 2019) whilst some spores show dormancy (Abbott and Robson, 1982). For example, extra-radical hyphae of AM fungi are a more effective source of AM fungal inoculum than spores under undisturbed soil conditions (Kabir, 2005; Brito *et al.*, 2013). These hyphae can also maintain the AM fungi's ability to establish AM fungal associations for at least 5 months after host root death (Pepe *et al.*, 2018).

Furthermore, the lack of correlation between spore density and AM fungal colonisation of spelt roots in this study could be attributed to different AM fungal species which would have taken different times to colonise the plants and also grow through the soil to reach roots (Smith *et al.*, 2015). Spores of different AM fungal species differ in their effectiveness at germinating and infecting crop roots (Koch *et al.*, 2017; Verzeaux *et al.*, 2017b; Bruns *et al.*, 2018; Yang *et al.*, 2018b). AM fungal species exhibit different colonisation development in the plant roots and many studies have reported variation in AM fungal colonisation among different AM fungal species (Van Der Heijden *et al.*, 2008; Mensah *et al.*, 2015; Yang *et al.*, 2018b). For example, some AM fungal species prefer to develop extra-radical hyphae in soil (Denison and Kiers, 2011), whereas, others prefer to form extensive inner hyphae within roots (Antunes *et al.*, 2006).

4.5. Conclusions

This study confirmed that crop management practices – including tillage, fertiliser type, crop protection and crop variety – influence AM fungal parameters, whether colonisation or soil spore density. AM fungal spore densities were enhanced by minimum tillage, whereas the tillage treatment did not affect AM fungal colonisation. Compost additions induced higher AM fungal colonisation and spore density. The modern variety Filderstolz was more responsive in terms of forming symbioses with AM fungi than Oberkulmer Rotkorn, but this was not reflected later in grain yield. However, the old Swiss landrace, Oberkulmer Rotkorn showed greater P uptake and grain yield, even though it had lower AM fungal colonisation. One possible explanation is that the enhanced P uptake in grain in the landrace may have been related to other

mechanisms (e.g. root size and the number of fine root hairs) which allowed it to take up more P and successfully translocate it to the grain. Organic crop protection seems to encourage AM fungal development more than conventional protection, as the soil spore density of AM fungi was enhanced by organic crop protection. However, colonisation by AM fungi was not affected by this management practice suggesting that the rates of the fungicides and herbicides used in this experiment were not high enough to inhibit colonisation by AM fungi. Selection of an organic addition as a source of N fertiliser could be a good strategy to exploit available services in the agriculture system, including those of AM fungi. Additionally, genotype selection based on a larger ability to form symbioses with AM fungi could be integrated into breeding programmes to generate lines or genotypes that are more efficient at forming symbioses with AM fungi.

Chapter 5. General Discussion

This chapter synthesises the major findings of the project by addressing each of the agronomic factors originally listed at the conclusion of each Chapter. Detailed discussion of specific results was contained in earlier chapters and is generally not repeated. Suggestions for further research are made throughout the chapter and summarised at the end. Therefore, the aim of this chapter was to synthesise the findings from Chapters 1-4 and draw some general conclusions about optimal management practices to enhance AM fungal colonisation and crop yields and nutrient uptake in spelt production systems. It also explores the underlying biophysical processes and mechanisms that explain AM fungal colonisation and its impacts in spelt production systems and the wider implications of the study's findings.

5.1. Fertility management

Fertility management is a major factor that can impact AM fungal colonisation and soil spore density. In general, it seems that organic compost promoted AM fungal colonisation in spelt roots and soil spore density of AM fungi compared to mineral fertiliser in this study, but only under long-term application (Chapter 4 results from the Nafferton Factorial Systems Comparison trial), while application in a single year did not positively affect AM fungal parameters (Chapter 3 results). In the long-term trial, AM fungal colonisation in the compost treatments over the two years of the study averaged 82.34% which was significantly higher than the long-term mineral N treatments (76.79%). In contrast, average root colonisation in mineral and compost treatments after a one-time application of fertiliser in the fertility input trial (Chapter 3) only differed by 3% and this difference was not significant.

While, overuse of organic manure may lead to an accumulation of nutrients in soil, especially P which may negatively affect AM fungal development (Gosling *et al.*, 2006; Dai *et al.*, 2014; Liu *et al.*, 2019), in this study long-term compost amendment may have had a positive effect on colonisation by AM fungi and spore densities. The results of our meta-analysis study confirmed these results and indicated that that AM fungal colonisation and spore density were higher in organic treatments that rely on organic matter inputs for fertility, compared to conventional systems. This is because all organic treatments in the meta-analysis were from long-term experiments (e.g. 8 years) with a long history of manure/compost application (Dann *et al.*, 1996).

Additions of organic matter added to soil have been reported to promote AM fungal development (Hodge and Fitter, 2010). This could be related to increased soil organic matter (Wang *et al.*, 2018b) which can enhance survival of mycorrhizal soil propagules in the absence of plant roots through colonisation of organic pieces by the AM fungus (Warner, 1984; Smith and Read, 2008). Adding compost to soil can increase supplementary nutrients such as total organic carbon, available P, and total N in the rhizosphere of plants (Alguacil *et al.*, 2011). Additions of compost may promote biological activity in terms of fungal populations, microbial biomass, and bacteria (Noyd *et al.*, 1995) through increasing soil organic matter. Enhanced soil organic matter ameliorates soil properties such as soil structure which can increase water-holding capacity (Cotching, 2018). This improvement enhances microbial activity for nutrient cycling, which can enhance the crop productivity, thus supplying energy to the soil through root exudation, and residue decomposition (Cotching, 2018). This may create an environment suitable for AM fungal colonisation by increasing root exudation.

The composition of fertiliser type may have determined the effects on AM fungal colonisation and their soil populations. In this study the compost plots of both experiments only received compost which has P and K as well as N and all micronutrients. Whereas mineral fertility plots only received mineral N fertiliser in both trials. In general, increased soil available P due to mineral fertilisation can reduce the AM fungal root colonisation due to increase P concentration in plant tissues (Kahiluoto *et al.*, 2001; Wang *et al.*, 2017). However, mineral N fertiliser alone has not been shown to adversely affect AM fungal colonisation. For example, Tian *et al.* (2013) reported that high rates of N application do not obstruct the AM fungal colonisation of maize roots, implying that N fertiliser is not the main factor impeding AM fungi. This could explain the lack of a difference in AM fungal parameters due to fertiliser type in the fertility trial (Chapter 3).

In general, there was an inconsistent pattern for the effect of fertiliser type on AM fungal colonisation and soil spore density which could be attributed to the fact that the response of AM fungal spores to fertiliser types may differ to that of AM fungal colonisation (Martinez and Johnson, 2010; Sharma and Buyer, 2015). Spore density measurements of AM fungi may reflect long-term crop management and not the current colonisation. Lack of correlation between colonisation and spore populations has been reported in other studies e.g. (Kurle and Pfleger, 1994; Schalamuk *et al.*, 2013). Therefore, AM fungal colonisation in host plant roots may not always reflect the AM fungal population in soil.

Furthermore, the effects of fertiliser on AM fungal colonisation, and soil spore densities may also relate to the fertiliser rate. Low and high levels of N fertiliser (50 and 100 kg N ha⁻¹) regardless of the input source were applied in the fertility experiment, while only the high N level (100 kg N ha⁻¹) was applied in the tillage experiment. There were larger AM fungal populations in the compost compared to mineral N treatment in the tillage experiment, while this difference was absent in the fertility experiment. Additionally, in this study the low levels of fertilisers applied in the fertility experiment increased vesicle colonisation but not total colonisation by hyphae or arbuscules. The existence of vesicles may be an indication that AM fungi are causing a decreased growth rate of crops through storing the carbohydrate resources in these structures (Jin *et al.*, 2017). One possible explanation is that when vesicles grow, AM fungi may need to obtain more C assimilate from the host plant, resulting in plant growth depression (Jin *et al.*, 2017).

5.2. Spelt variety

Filderstolz and Oberkulmer Rotkorn were common varieties in both field experiments in this thesis. It seems that the modern spelt variety Filderstolz showed highly favourable compatibility with AM fungi compared to the Swiss landrace, Oberkulmer Rotkorn in this study with higher total root and arbuscule colonisation. Several studies have found that the modern wheat varieties are highly compatible with AM fungi (Kirk *et al.*, 2011; Ellouze *et al.*, 2012; Brito *et al.*, 2013; Ellouze *et al.*, 2016; Ercoli *et al.*, 2017). These results are in agreement with those of Zhu *et al.* (2001) who found modern wheat genotypes had greater AM fungal colonisation than the older genotypes. However, these results are also in contrast to Hetrick *et al.* (1993) who found that the growth and AM fungal colonisation of modern wheat cultivars were lower than landraces (cultivars released before 1950). Additionally, other previous studies observed that landraces had more AM fungal colonisation than modern varieties in crops such as maize (Martinez and Johnson, 2010) and sorghum genotypes (Cobb *et al.*, 2016). In some cases, no difference in AM fungal colonisation levels have been observed between modern and older genotypes (Hildermann *et al.*, 2010). Filderstolz is a modern dwarf variety bred conventionally. It is thought that the genes that are responsible for mycorrhizal associations could be reduced when the plant is bred under high-input agriculture conditions (Hetrick *et al.*, 1996). However, the current study did not support this hypothesis as Filderstolz showed higher colonisation by AM fungi than the old Swiss landrace, Oberkulmer Rotkorn. However, the modern Filderstolz variety was not able to benefit from AM fungal colonisation. This could be attributed to AM fungal functions with regard to enhanced P uptake and yield in cultivars which may decrease

with the year of release of the cultivar (Zhu *et al.*, 2001). This suggests that modern breeding programs may have unwittingly selected genotypes that do not benefit from AM fungal colonisation (Zhu *et al.*, 2001). Therefore, it is necessary to include AM fungi in breeding programmes to maximise their beneficial effects on crop nutrition (e.g. P) and yield (Smith *et al.*, 1992). The current study also suggests that assessments of AM fungal colonisation may provide an indication of AM fungal association with plants, but do not effectively indicate whether the plant is benefiting from AM fungal symbiosis (Kirk *et al.*, 2011). Therefore, current results tend to support the theory that AM fungal colonisation is not a strong indicator of the benefit the crop is receiving from AM fungal symbiosis (Hetrick *et al.*, 1993).

The differences in the response of both spelt cultivars to AM fungal colonisation may be related to several reasons. The differences in response of spelt varieties to AM fungal colonisation may be related to variation among the crop varieties in terms of amounts of sugar and lipids in plant root exudates (Wang *et al.*, 2017; Lanfranco *et al.*, 2018). Differences in crop varieties' formation of symbioses with AM fungi may also be attributed to differing nutritional requirements between cultivars. The crops adapted to nutrient deficient conditions are more likely to form associations with AM fungi (Liu *et al.*, 1995; Ryan *et al.*, 2016). Since AM fungi play an important role in P nutrition (Elbon and Whalen, 2015), it would be credible to expect that cultivar differences in terms of compatibility with AM fungi might also occur.

The variation in AM fungal colonisation may be attributed to differences in root morphology between genotypes such as root length and number and length of root hairs (Eissenstat *et al.*, 2015; Kramer-Walter *et al.*, 2016). Variation among native AM fungal populations is another explanation for this phenomenon. There may be genetic differences mong the AM fungal species in the natural soil populations which result in enhanced colonisation of one variety of spelt compared to the other (Mao *et al.*, 2014; Sangabriel-Conde *et al.*, 2015; Ryan *et al.*, 2016). The crop that exhibits a higher AM fungal colonisation than other crops may have lower ability to mobilise nutrients from nutrient deficient soils (Gao *et al.*, 2012). Genetic variation in the association of spelt with AM fungi suggests the possibility of increasing the sustainability of cropping systems through the use of spelt genotypes that select highly effective AM fungal taxa residing in agricultural soils.

However, there was no consistent pattern of the effect of spelt variety on spore density across both trials. In the fertility experiment, both the modern variety Filderstolz and the Swiss landrace, Oberkulmer Rotkorn did not affect spore density of AM fungi. While in the tillage

experiment the spore, density was higher where the landrace, Oberkulmer Rotkorn was cultivated rather than the modern variety Filderstolz. The variety effect on spore density may not be clear in this study as the spelt variety has only been in the plot for a short time.

5.3. Tillage treatments

This study indicated that tillage treatment did not play a significant role in determining the levels of AM fungal colonisation. In this study, the fertility trial had only conventional tillage while in the tillage trial there were both conventional and minimum tillage treatments. The average AM fungal colonisation under conventional tillage in both experiments was very similar (79% $\pm$ 2.1 average for two years) under conventional tillage in the fertility experiment, and (82% $\pm$ 1.60 average for two years) under conventional tillage in the tillage experiment. AM fungal colonisation was only slightly lower (78% $\pm$ 1.83) under minimum tillage in the tillage experiment. Therefore, this study found that tillage system (conventional versus minimum) had no effect on AM fungal colonisation when compared across experiments.

The impact of tillage on AM fungal colonisation has been inconsistent over many of studies, and the reasons for these variation of tillage effects is not clear. As a previous study observed that extra-radical hyphae net of AM fungi was more influenced by conventional tillage than by reduce tillage and no-tillage, especially in the upper soil layers of field grown maize (Kabir *et al.*, 1997). The negative effects of conventional tillage on AM fungal colonisation were also reported (McGonigle and Miller, 1996; Galvez *et al.*, 2001). However, those authors were comparing conventional tillage with no-tillage. A previous study found that conventional tillage led to reduce colonisation by AM fungi compared to minimum tillage (Gao *et al.*, 2010), but this comparison was conducted under different experimental conditions with a different crop (flax). In this study, the minimum tillage treatment still involves considerable disturbance of the soil. Unfortunately, this study did not provide activity assessment of other types of AM fungal propagules (soil hyphae and infected root fragments) which could be a better indicator for AM fungi affected by tillage treatment especially soil hyphae (Álvaro-Fuentes *et al.*, 2008). Although, conventional tillage may disturb the network of AM fungal hyphae in this study, the native AM fungal hyphae may survive winter temperatures and recover their ability to colonise spelt roots (Kabir *et al.*, 1997; Krauss *et al.*, 2010). For example, the colonisation of new roots may occur by soil hyphae network more than spores. Martinez and Johnson (2010) suggested that soil hyphae network may be an important source of AM fungal inoculum particularly under a low input environment where spore density is found to be low. The absence of tillage

treatment effects on AM fungal colonisation may be because of coincidence that tillage disturbs only some AM fungal propagules most possible the hyphae network, but other AM fungal propagules such as infected root fragments, and spore remained active and are a considerable source for soil inoculum in this study. In some instances, AM fungal colonisation may not delay, and reduce due to tillage treatment (Gavito and Miller, 1998). Therefore, the tillage effects on these types of AM fungal propagules remained obscure, but it could be one possible reason for the absence of differences in colonisation by AM fungi between both tillage treatments.

The absence of tillage effect on AM fungal colonisation may also be attributed to AM fungal species. Unfortunately, this study did not provide information about AM fungal community structures. In agricultural soils there is a range of AM fungal species with the genus *Glomus spp.* being most predominant, most likely based on its ability to produce spores relatively faster than the other AM fungal species and perhaps survive conventional tillage conditions (Oehl *et al.*, 2009; Voříšková *et al.*, 2016). These AM fungal species may be prevalent in conventional tillage plots which could contribute to recovery of AM fungal populations after ploughing. This could be one possible reason for the absence of differences in AM fungal colonisation between conventional and minimum tillage treatments in this study.

However, the tillage effect on AM fungal development in spelt relied on interaction with other crop management practices. In the tillage trial, there were significant interactions between tillage management and variety on AM fungal root colonisation and arbuscule formation. Reduce tillage enhanced AM fungal total root colonisation and arbuscule formation where Filderstolz was cultivated. Therefore, AM fungi may enhance their host plant nutrition (e.g. P) from the soil due to produce higher arbuscule (Luginbuehl *et al.*, 2017) under reduce than conventional tillage.

Furthermore, the average spore density in the fertility experiment under conventional tillage (89 ± 3 spores g^{-1} dry soil) was higher than spore density under conventional tillage (69 ± 3 spores g^{-1} dry soil) and minimum tillage (77± 3 spores g^{-1} dry soil) in the tillage experiment. These differences in spore density of AM fungi between both experiments (fertility trial versus tillage trial) may be related to long-term in tillage experiment compared to short-term crop management practices in the fertility experiment. As the long-term of tillage system whether conventional or minimum in the tillage experiment may lead to reduce spore density of AM fungi compared to fertility experiment (Säle *et al.*, 2015). Statistical analysis of the tillage experiment of this study found that AM fungal spore density was lower under conventional

than minimum tillage. However, this effect is not clear as reduce tillage increased spore density in one year, but not in the other. This could be related to accumulation of AM fungal activity in the previous crop (Schalamuk *et al.*, 2013) or reflect long-term crop management (Hijri *et al.*, 2006; Schalamuk *et al.*, 2013). Therefore, this effect may interact with previous rotation management in each year which led to accumulation of spore in one year over another (e.g. grass/clover ley phase) (Mäder *et al.*, 1999). However, lower spore density of AM fungi under conventional tillage compared to minimum tillage in the tillage experiment may be attributed to several reasons. Minimum tillage can accumulate the organic matters in soil (Grandy *et al.*, 2006) which can positively affect AM fungal spore density (Allen *et al.*, 2001; Bilalis *et al.*, 2012). Dilution of the AM fungal propagules in greater soil volumes, including spores by ploughing, is another mechanism that causes reduced AM fungal spore density in the area of seedlings established by ploughing (Kabir, 2005; Schalamuk *et al.*, 2013). AM fungal spores may also have been buried by conventional tillage. Therefore, under long-term of crop management practices, minimum tillage is useful management compared to conventional tillage to support AM fungal populations in soil, thus sustainable agricultural management.

5.4. Crop protection practices

Organic crop protection promoted AM fungal spore densities but did not affect total root colonisation. In this study, conventional crop protection was applied where only herbicides were used in the fertility trial, while in the tillage trial both herbicides and fungicides were used in the conventional crop protection treatments, compared to organic crop protection with no pesticides used. To avoid seasonal fluctuations in AM fungal colonisation and spore density, AM fungal development was compared across both experiments in the common year (2015/16). The average AM fungal colonisation in 2015/16 was (82.8% ± 1.4) in the fertility trial under conventional crop protection (where only herbicides were applied). Whereas, the average AM fungal colonisation in 2015/16 under conventional crop protection (herbicides + fungicides) in the tillage trial was (73.0% ± 2.7), while under organic crop protection it was (76.3% ± 2.7) Since the average of AM fungal colonisation where only herbicides were applied in the common year of the fertility trial was slightly higher than both the conventional and organic crop protection treatments in the same year in the tillage trial, this suggests that herbicides do not negatively affect AM fungal colonisation. Weeds may have also played an important role in promoting AM fungal colonisation in the fertility trial (Nelson *et al.*, 2011a) as weeds are an important host for AM fungal establishment and they are faster colonising from soil inoculum of AM fungi than cereal crops (Nelson *et al.*, 2011a). The extra-radical hyphae of AM fungi

that colonised weed roots may have contacted the spelt roots in the early season in autumn and before herbicides were applied.

The average of AM fungal spore density in 2015/16 was (87 ± 3 spores g^{-1} dry soil) in the fertility trial under conventional crop protection (only herbicides applied; Chapter 3), whereas, the average of AM fungal spore density in the same year in a different field under conventional crop protection (herbicides + fungicides; Chapter 4, NFSC trial) was 80 ± 3 spores g^{-1} dry soil, while under organic crop protection in the same trial it was 95 ± 4 spores g^{-1} dry soil. This shows a general trend with highest numbers of spores in plots with no history of pesticide application (organic crop protection plots in the NFSC trial), compared to somewhat lower numbers in the herbicide-only plots, and lowest numbers where both fungicides and herbicides were applied, and suggests that repeated application of fungicides in the long-term NFSC trial is negatively affecting AM fungal spores. It also may reflect the long-term effects of plant species composition (Schalamuk *et al.*, 2013) as, over the 14 years of the NFSC trial, weeds have been controlled in the conventional crop protection plots while there has been abundant weed growth in organic crop protection plots, potentially acting as host plants, promoting AM fungi and leaving higher spore densities in these plots (Schreiner *et al.*, 2001).

In general, the experiments of this study did not show any effect of the two fungicides (chlorothalonil and epoxiconazole) applied in the tillage trial and the two herbicides (fluroxypyr and dichlorprop-P) applied in both fertility and tillage trials, on AM fungal colonisation levels when they are applied at recommended rates. In both field trials of this study, the herbicides were applied over a 5-month (from May to September) period, which should have been sufficient for any detrimental effect of the herbicides on root colonisation levels to become apparent (Bary *et al.*, 2005), especially in the tillage trial where these products had been applied to all arable crops grown in the rotation since 2004. However, only spore density of AM fungi was affected by crop protection treatment in the tillage trial. It could be these types of fungicides, and herbicides do not have adverse effects on AM fungal colonisation. Therefore, this study recommends these dose rates of both pesticides. Moreover, this study found that the crop protection effect on AM fungal colonisation and spore density was inconsistent under different crop management practices such as fertility management and tillage system. Therefore, the crop protection effect on AM fungal development under different crop management practices need further research.

5.5. Phosphorus nutrition, and grain yield

AM fungal colonisation enhanced tissue P concentrations in the tillage trial, but not in the fertility trial in this study. P concentration and uptake were measured in an effort to assess AM fungal functions in the spelt cultivars in this study. Soil P levels in the present study were particularly low. The higher level of P concentration was measured in the modern variety Filderstolz compared to the Swiss landrace, Oberkulmer Rotkorn as well as for compost compared to mineral N fertiliser at anthesis in the tillage trial. This may be related to higher AM fungal colonisation in Filderstolz compared to the landrace, Oberkulmer Rotkorn as well as for compost treatments compared to mineral N at anthesis in the tillage trial. Whereas, in the fertility trial, even though the P nutrition was enhanced by compost amendment and for the Swiss landrace, Oberkulmer Rotkorn, this enhancement in P nutrition did not relate to AM fungal colonisation. The inconsistent results across both trials of this study may be related to previous crop management as the compost was applied for many years in previous crops of tillage trial compared to only once in the fertility trial.

The relationship between arbuscule colonisation and P concentration was inconsistent in this study, as it was sometimes positive, negative and in some cases, there was no relationship. However, this study tends to support the theory that arbuscular colonisation may not be a good indicator of the benefit spelt is receiving from AM fungi (Hetrick *et al.*, 1993).

A positive relationship between AM fungi and crop yield was expected in low P conditions, however, current results did not show a clear link between AM fungal colonisation in spelt genotypes and P nutrition or crop yields. The difference between study results presented here and other studies e.g. (Hetrick *et al.*, 1996) may be attributed to different experimental conditions or to the small number of cultivars used in current study. Hetrick *et al.* (1996)'s study was conducted in a greenhouse with five mycorrhizal fungal inoculants and 10 wheat genotypes under three phosphorus regimes. This study found that the relationship between wheat biomass production and AM fungal root colonisation was positive for six genotypes, which responded favourably to the symbiosis, and negative for other genotypes, which responded negatively or were nonresponsive to AM fungal inoculation. However, AM fungal inoculation was applied in this study and in most cases the inoculation by AM fungi may effectively enhance crop growth and nutrition (Hijri, 2016). In this study the only focus was on indigenous AM fungi. The current study tends to support the hypothesis that studies of agricultural management treatments on indigenous AM fungi often find no positive relationship

between AM fungal root colonisation and crop performance parameters (yield and nutrient uptake) (Verbruggen *et al.*, 2012; Köhl *et al.*, 2014; Ryan and Graham, 2018). Maintaining AM fungal soil inoculum levels and sustaining more effective AM fungal communities may require particular agricultural practices which effectively enhance the functioning of indigenous AM fungi (Douds *et al.*, 2016). Achieving high yield may require selection of cultivars and AM fungi that form associations rapidly, thus improving early crop P requirements (Zhu and Smith, 2001; Singh *et al.*, 2012). An AM fungal inoculation study conducted over a 4-year period by Hijri (2016) in Europe and North America under field conditions used the same AM fungal species (*Rhizophagus irregularis*) applied to potato and in 231 field trials. This study found that the average potato yield for inoculated fields (42.2 tons ha^{-1}) was higher by 3.9 tons ha^{-1} compared with non-inoculated controls (38.3 tons ha^{-1}). The author explained that the beneficial effect on plant growth and nutrition in the early stages of growth may be attributed to introducing fresh and active AM fungal inoculants that establish mycorrhizal symbioses much earlier than would occur with native AM fungal populations. The indigenous AM fungal communities may not be effective enough to achieve significant crop yield benefits for particular reasons (Leiser *et al.*, 2016). It appears that it is more commonly reported to find a positive relationship between plant performance and AM fungal colonisation in inoculation studies (Dai *et al.*, 2014; Zhang *et al.*, 2018b). It should not be assumed that there is always a positive relationship between colonisation by AM fungi and grain yield and P uptake (Ryan *et al.*, 2016; Koch *et al.*, 2017; Sawers *et al.*, 2017; Thirkell *et al.*, 2017; Ilyas, 2019). For example, Kirk *et al.* (2011) did not observe significant correlations between AM fungal colonisation and yield. AM fungal symbiosis generally promotes plant growth, but growth reduction in plants due to AM fungal symbiosis may also occur (Ryan *et al.*, 2005; Li *et al.*, 2008; Ryan and Kirkegaard, 2012). In the fertility trial of this study, there was weak negative relationship between AM fungal colonisation and grain yield of spelt. The depression in growth associated with AM fungal symbiosis demonstrates that colonisation of roots does not guarantee benefit from the symbiosis (Hetrick *et al.*, 1992). The growth reduction caused by AM fungi may be attributed to a reduction in the amount of C available for growth in plants unable to satisfy their C assimilation level to match the increased demand generated by the AM fungal sink (Dai *et al.*, 2014). The results based on colonisation by AM fungi should be interpreted carefully. It therefore seems that exploration into the contribution of AM fungi to crop P uptake and grain yield under field conditions is required before a conclusion can be made about the impact of colonisation by AM fungi on crop yield.

In the present study, variety had a major effect on increased grain yield, P nutrition where Swiss landrace, Oberkulmer Rotkorn produced more grain yield, and P nutrition than modern variety Filderstolz in the fertility trial (Magistrali, 2019) and tillage trial of this study. Since the landrace, Oberkulmer Rotkorn did not exhibit high colonisation by AM fungi, enhanced P levels in grain in this variety may have been related to other mechanisms (e.g. root system size, numbers of fine root hairs) which allowed it to take up more P and successfully translocate it to the grain (Haling *et al.*, 2018). In general, the modern varieties are higher yielding than landraces (Kirk *et al.*, 2011) but in this study the landrace Oberkulmer Rotkorn had a higher yield compared to the modern variety Filderstolz. This could be attributed to the low rates of mineral fertiliser applied (100 kg N ha^{-1}). It is possible that the modern variety Filderstolz was selected under higher rates of N application. The landrace, Oberkulmer Rotkorn may be particularly recommended for organic systems due to its enhanced P uptake and high yields. While other spelt varieties included in the fertility trial may also be viable depending on management system (e.g. Rubiota and ZOR may yield more when fungicides are applied which was not applied in this trial).

In summary, modern cultivars were found to have higher arbuscular and total colonisation than older cultivars in the one year of tillage trial in this study. The current study did not support the hypothesis that the older cultivars have superior AM fungal colonisation over modern cultivars developed under low levels of mineral N fertiliser. Future research should focus on the yield and P uptake benefits the cultivars are receiving from AM fungi instead of only considering AM fungal colonisation. The current study also demonstrated that in spite of the higher AM fungal colonisation for the modern cultivars, P uptake and grain yield was greater for older than modern cultivars in two site years of the fertility trial and one year of the tillage trial in this study.

In the present study, grain yield, and P uptake were also promoted by conventional crop protection and mineral fertiliser (Ryan and Ash, 1999; Corkidi *et al.*, 2002). Furthermore, the environmental conditions, particularly higher levels of solar radiation, played a positive role in promoting crop yield.

5.6. Comparisons between farms under organic and conventional management

The meta-analysis study comparing effects of organic and conventional crop management on AM fungal development indicated that AM fungal colonisation in roots and spore density in

soil were higher in organic than conventional farming. According to the results of both experiments fertility management played an important role in AM fungal colonisation and spore density. The meta-analysis in this study supported these results as all organic systems have had a long history of manure/compost application. Since AM fungi is largely controlled by nutrient availability to plants, organic management conditions can promote AM fungal symbiosis (Hartmann *et al.*, 2014; Knerr *et al.*, 2018). Organic fertilisers used in organic farming including compost additions can positively affect AM fungal development. The results of these experiments provided evidence that compost amendment can promote AM fungal development. The high amount of organic C in organic amendments (e.g. compost) is a possible reason for higher AM fungal colonisation and spore densities in organic than conventional farming (Ryan and Kirkegaard, 2012). However, the P in organic compost fertilisers may affect AM fungal development (Liu *et al.*, 2019). A high level of AM fungal colonisation can occur after applying a high rate of compost if the level of soil P remains low (Ryan and Kirkegaard, 2012), however, in some cases organic compost can act as mineral P fertiliser if it results in rapid mineralisation of P in soil, and inhibit AM fungal colonisation (Douds *et al.*, 1997; Liu *et al.*, 2019).

The meta-analysis revealed that the AM fungal soil diversity did not differ significantly between contrasting management (organic vs conventional) practices, although the number of studies was insufficient to draw firm conclusions. Unfortunately, the current study did not use molecular techniques to find out the effect of different crop management practices on AM fungal community composition. Therefore, further studies are required to clarify the influence of different crop management systems (organic vs conventional) on AM fungal community structure.

The potentially enhanced levels of fungal colonisation in organic agriculture or in biodynamic systems may not compensate for decreased yields due to lack of fertilisation (Ryan and Ash, 1999). The tillage trial results indicated that there was no link between AM fungal colonisation and grain yield of spelt while in the fertility trial this relationship was weak negative. This suggests that AM fungal root colonisation does not always translate to high yield and may sometimes negatively impact production (Thirkell *et al.*, 2017; Ryan and Graham, 2018). Therefore, the high colonisation by AM fungi in organic farming may have no positive effect on crop yield and/or biomass (Dann *et al.*, 1996) and may be negative due to the large plant C investment in the AM fungal sink. For example, (Dai *et al.* (2014)) found that the higher colonisation by AM fungi in organic compared to conventional systems was associated with low productivity of organic wheat. The authors attributed this phenomenon to the high

concentration of nutrients observed in organic farming which may cause wheat growth limitation associated with insufficient C assimilation in wheat. Better understanding of the mechanisms responsible for generating beneficial, neutral or parasitic effects from AM fungi on host plants in organic systems is important before fungi can be efficiently managed to maximize yield.

5.7. Future research

The present study provided evidence to show that genotypes of spelt can vary in their degree of AM fungal colonisation; however, this effect was not consistent under different crop management practices (e.g. fertility and tillage) and field conditions. Therefore, further research is required to determine which spelt varieties are most effective at forming AM fungi associations that positively affect crop yields under specific management conditions.

AM fungal colonisation was promoted by organic compost amendment over several years in the tillage (NFSC) trial compared to the fertility trial which had only received a single application of compost. This suggests that single (one-time) applications of compost are not enough to promote AM fungi, whereas the repeated applications of compost in the tillage trial may have resulted in conditions that enhanced AM fungal populations. Further investigation of compost effects on AM fungal development under field conditions is required to determine the required frequency of compost applications for enhancement of AM fungi colonisation in crops.

Crop protection management effect on AM fungi is complex as effects are dependent on target pest (weeds or disease) and the active ingredient and mode of action of the pesticide. No clear pattern of mycorrhizal suppression by pesticides such as herbicides and fungicides has been demonstrated in the literature. The fertility trial had only herbicides, while in the tillage trial both herbicides and fungicides were used. The levels of AM fungal colonisation for the fertility trial (herbicides only) was higher than the tillage trial (herbicides+fungicides). This suggests that fungicides are the crop protection product having the largest impact on AM fungal colonisation. Even though this comparison could provide us with some clues, it is difficult to make firm conclusions based on this limited evidence. Therefore, further research may focus on fungicides and herbicides as individual factors as well as possibly on their types, mode of action or active ingredients. As this study design did not allow us to answer most questions about many of these factors.

The relationship between crop parameters (e.g. yield and P) and AM fungal colonisation was considered in this study across both trials. Although these AM fungi may not give plants a benefit of increased yield and P uptake under low soil P levels detected in these fields, the fungi may serve other beneficial functions. Perhaps, the greatest benefit of high AM fungal colonisation in plants is non-nutritional through resistance to water or heat stress, improvements in soil structure and increased resistance or tolerance to certain diseases and insect pests.

In addition to total root colonisation, AM fungal structures (e.g. arbuscule, vesicle, hyphae and spore) were investigated across both fertility and tillage trials in this study. These AM fungal structures responded differently to agricultural practices in this study. Data for the different AM fungal structures may be useful to include in future research as presenting only total root colonisation may hide a lot of useful and interesting data (e.g. existence of vesicles that indicate a growth depression of the crop).

More focussed research is necessary to quantify the carbon-nutrient exchange (e.g. N and P) between mycorrhizal fungus and crops and how this may be affected by interactions with environmental factors such as nutrient availability, temperature and drought conditions and/or interaction with other organisms. Few studies have successfully tested the contribution of AM fungi to crop yield under field conditions. Future research needs to focus on traits of plants and AM fungi for optimal symbiotic functioning under different environmental conditions and management practices in order to integrate AM fungi in sustainable agriculture.

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