Fostering Populations of Arbuscular Mycorrhizal Fungi Through Cover Crop Choices and Soil Management

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Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text. It is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text. It does not exceed the prescribed word limit for the Faculty of Biology Degree Committee.

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Abstract

Over 70% of land plants, including many key agricultural crops, form a beneficial, symbiotic relationship with arbuscular mycorrhizal (AM) fungi. This has triggered interest in the potential role of these fungi in sustainable food production for an increasing population. However, it is known that many common farming practices can negatively influence both the diversity, and abundance of the AM fungi. It is therefore desirable to identify farming practices or amendments that can foster these fungal populations to increase crop and soil benefits, including yield. Cover cropping, the growing of non-food crops outside of regular crop production for the role of protecting and improving soil, has also been suggested to influence both the diversity and abundance of AM fungi. A large-scale analysis of AM fungal diversity in UK agriculture provided a framework for further analysis of how cover crops, and soil amendments influence AM fungal communities. Replicated trials in both glasshouse and field conditions have shown evidence that multiple iterations of cover crops can increase the extent to which plants are colonised by AM fungi, although this had no measurable impact on yield. In the same trial, it was shown that long term application of nitrogen fertiliser influenced AM fungal community composition, but this observation was not made in a shorter validation experiment conducted at the field scale. In a separate trial, addition of a commercial AM fungal inoculum had little impact on the AM fungal community, crop growth, or yield in field conditions, further suggesting that multiple iterations of soil amendments are required to cause measurable, long-term shifts in AM fungal diversity and benefit.

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1 Chapter One – Farming for the 21st Century: Cover Cropping and Beneficial Fungi to Improve Sustainability and Crop Yields

Global population is predicted to reach 9.8 billion by 2050 (United Nations Department of Economic and Social Affairs 2017). One of the key challenges when supporting a larger and more effluent population is the production of more food on the same, or smaller area, of the world's surface; and studies have suggested that world food production will have to increase by at least 70% to satisfy extra demand (Godfray et al. 2010; Alexandratos and Bruinsma 2012; Keating et al. 2014; Grafton et al. 2015).

The agricultural achievements of the green revolution were considerable; with production of many grain-crops, including wheat, rice, and maize increasing at a rate faster than population growth (Tilman 1999). This near doubling of food production was attributed to combinations of plant breeding for higher yielding varieties; development of herbicides and pesticides; better agricultural practices; increase in cultivated and irrigated land; and application of major nutrient fertilisers, especially nitrogen (N) and phosphorus (P). Analysis by the FAO shows that an increase in N and P fertiliser of 7.5- and 3.3- fold respectively has been required to attain present levels of yield (Tilman 1999; FAO 2018). Almost 60 years later, yields have plateaued for many important crop species throughout the world (Knight et al. 2012; Fischer et al. 2014). Furthermore, it is becoming increasingly apparent

that the practices that enabled such impressive yield increases are unsustainable in the medium to long term due to many factors including soil erosion and quality, resistance of pests and diseases to chemical treatments, damage to ecosystem services, and pollution/toxicity issues (Smil 1999; Bennett et al. 2001; Tilman et al. 2002; FAO and ITPS 2015).

Central to the unsustainable nature of modern farming is the increasing pressure and resulting degradation and erosion of soils (FAO and ITPS 2015). Soils are fundamental to life on earth and support a range of ecosystem services, including food and biomass production; climate mitigation; storage and filtering of water and nutrients; production of raw materials; hosting and supporting biodiversity; and other cultural services, all of which are all intricately linked. The capacity of a soil to provide these services is inherently controlled by many factors: the soil texture, nutrient composition, depth, structure, pH, accumulation of salts, and toxicity, which in themselves control secondary characteristics such as water holding ability, cation exchange capacity, and associated supporting biodiversity.

Another component of modern food production under stress is macronutrient delivery. It has been suggested that reserves of phosphate rock, used as a crop fertiliser, will become exhausted within 300 to 400 years, if not sooner (Van Kauwenbergh 2010; Dawson and Hilton 2011). Furthermore, excessive application of N and P fertiliser can be detrimental to terrestrial and aquatic

ecosystems through diffuse pollution, and contribute to greenhouse gas emissions through volatilisation of certain nitrogen species such as ammonia (Smil 1999; Bennett et al. 2001; Tilman et al. 2002).

The UK Department for Environment, Food & Rural Affairs (DEFRA) acknowledges the unsustainable nature of modern agriculture and identifies key regions for improvement, including biodiversity, water and air quality, climate change mitigation, and soil health, in the policy statement 'Health and Harmony: The future for food, farming and the environment in a Green Brexit' (DEFRA 2018). These areas, and their interaction will be considered throughout this PhD thesis, not only in terms of agricultural sustainability, but also food production, economics, and ease of adoption.

1.1 The Importance of Soil Health to Sustainable, Economically Viable Farming

Soil health can be defined as 'the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, maintain or enhance the quality of air and water, and promote plant, animal and human health' (Doran et al. 1996; Doran 2002). There are complex interactions between the physical, chemical, and biological aspects of soil, which affect, and are affected by one another, alongside the plants that it supports.

Physical Aspects of Soil Health

Good soil structure is integral to the proper functioning of soil as a growth medium; it impacts crop establishment, root growth, water infiltration, microbial communities, and ultimately crop yields (Bronick and Lal 2005; Vrindts et al. 2005; Munkholm et al. 2013). Soil aggregation, the arrangement of sand, silt, and clay around Soil Organic Matter (SOM, see below), is controlled by several factors. These include the bedrock material, plant rooting, macro- and microbiota, and can be altered by compaction, caused by weather or farming activities such as driving heavy machinery over the land, from grazing by sheep or cattle, or flooding (Hamza and Anderson 2005). The resultant compaction is generally worse if the soil has a low SOM content and is wet.

Compaction can be alleviated by cultivating the soil, but these effects may be short lived; have undesirable impacts on soil biota, erosion, and greenhouse gas emissions; and are costly in terms farmer inputs; including fuel, equipment and time (House and Parmelee 1985; Kessavalou et al. 1998; Calonego and Rosolem 2010).

Chemical Aspects of Soil Health

SOM is the organic component of soil, made up of flora and fauna at differing stages of decomposition. Amongst other benefits to soil structure and water

retention, SOM is closely linked to nutrient cycling and availability, including both macronutrients N, P, and sulphur (S) and many micronutrients such as manganese (Mn), zinc (Zn), iron (Fe), and others (Verma et al. 2010). This is due to the ability of SOM to offer cation exchange sites for these nutrients originating from parent material or applied in the form of fertilisers.

SOM is also important for the sequestration of carbon and represents a significant global carbon sink. It is estimated that the total global soil carbon of 2500 gigatons (Gt) is comprised of 1550 Gt of soil organic carbon (SOC) and 950 Gt soil inorganic carbon (SIC), approximately 3.3 times the amount of carbon contained in the atmosphere (Lal 2004; Batjes 2014). Increasing the soil carbon pool of degraded soils therefore has two important quantifiable, and economically beneficial impacts relating to climate mitigation and increased crop yield.

Macro and Micronutrient Supply from Soil for Crop Growth

Access to macro and micronutrients stored in the soil is central to plant nutrition. The production of inorganic nitrogen by the Haber Bosch reaction has been pivotal to the yield increases since the industrial revolution. Application of N, together with P and K fertiliser, alongside other agronomic advancements, has resulted in a near doubling of food production since the 1960s (Tilman 1999; FAO 2018). FAO data shows that an increase in N and P fertiliser of 7.5- and 3.3- fold respectively has been required to attain these

increases in yield, yet it is estimated that a further 40 and 20 Tg of N and P fertiliser respectively, is required to meet food production needs in 2040, based on current farming practices (Balu L. Bumb & Carlos A.Baanante 1996; Frink et al. 1999; Vance 2001). This level of fertiliser application is both environmentally, and economically unsustainable in the medium to long term, and policies are being introduced nationally to address some of these issues (Smil 1999; Bennett et al. 2001; Tilman et al. 2002; DEFRA 2018).

Biological Aspects of Soil Health

Soil is a rich microcosm supporting a breadth of meso and micro fauna, which play important roles in functioning of a healthy ecosystem. Movement and cycling of carbon, nitrogen and other macro and micronutrients in the soil are influenced by movement of small insects, fungi, and bacteria. Soils are known to support an enormous diversity of bacteria and fungi, which are of central importance to nutrient cycling in all forms. Saprophytic fungi and bacteria, for example, contribute to the decomposition of non-living plant and animal debris, and in the process, liberate nutrients for uptake by plants. Using N as an example, ammonium in the form of ammonium hydroxide released by saprophytic organisms is nitrified into nitrites and subsequently nitrates in the presence of oxygen, which are readily assimilated by plants. Alternatively, ammonium may be lost by volatilisation as ammonia gas or reduced to nitrogen gas by denitrifying bacteria and lost to the atmosphere.

In a mature, undisturbed soil, this community of soil microbiota will be balanced to cycle and recycle carbon, and other macro and micronutrients. These soils are less prone to extreme change in composition of the microbial community, and may be better equipped to deter invasion by soil pathogens (Van Elsas et al. 2012; Coelho et al. 2020). This offers a more stable environment for plant growth, and may contribute to increases in biomass and productivity.

An understanding of the importance of bacterial and fungal diversity has stimulated increasing discussion about the importance of biodiversity and key groups within the soil microbial community, how these organisms contribute to crop yields, and how modern agronomic practices can positively or negatively impact their populations (Peterson et al. 1984; Lehman et al. 2012).

1.2 Biology of Arbuscular Mycorrhizal Fungi and the Impact of Agriculture on AM Fungal Populations

Over 70% of land plants form symbioses with arbuscular mycorrhizal (AM) fungi of the Glomeromycotina (Spatafora et al. 2016; Brundrett and Tedersoo 2018). These obligate, mutualistic fungi convey benefits including nutrient uptake, increased pest and pathogen resistance, and drought tolerance, in exchange for plant derived carbon (Jakobsen and Rosendahl 1990;

Marschner and Dell 1994; Augé 2001; Jung et al. 2012; Rapparini and Peñuelas 2014).

History and Evolution of AM fungi

This ubiquitous interaction between plant and fungus became established in the earliest land plants around 460 million years ago, and fossils which strongly resemble AM fungal structures in liverworts, hornworts, lycophytes, and ferns, suggest that the AM symbiosis predates development of true root systems (Remy et al. 1994; Redecker et al. 2000; Brundrett 2002). The widespread occurrence of AM symbiosis, across plant families, habitats, and life histories, is consistent with a single origin and multiple subsequent losses, with such losses more common in specialised taxa, such as parasitic, or insectivorous plants (Wang and Qiu 2006).

Several studies have postulated that the symbiosis helped plants to survive in their new, terrestrial environment, where the fungal symbiont provided the plant with increased tolerance to abiotic stresses, as well as insoluble, and potentially scarce nutrients (Gryndler 1992; Selosse and Le Tacon 1998; Wang et al. 2010). In exchange for these nutrients, the plant symbiont would provide the fungus with sugars and lipids fixed by photosynthesis, which may have been limiting to heterotrophic organisms unable to fix carbon, but in abundance for autotrophic plants living in a high CO_2 world (Brundrett 2002). It is likely that these early land plants would have been colonised by

a range of fungi, and other microorganisms, ranging from symbiotic, to parasitic (Saikkonen et al. 1998). There is evidence that the genes involved in the perception and establishment of AM symbiosis were present in the ancestors of land plants, predisposing plants to preferentially engage with beneficial symbionts (Wang et al. 2010). It is speculated that it is this interaction between plant and fungus was pivotal to the successful radiation of land plants in the early Devonian period.

The AM fungal symbiosis is likely to have had major impacts on ecosystem structure and function since its inception, and studies have shown that the presence, and diversity of AM fungi have an impact on above-ground plant community composition (Gange et al. 1993; Van Der Heijden et al. 1998a; Klironomos et al. 2000; Hart et al. 2003; Bennett et al. 2017). Depending on the mycorrhizal status of the plants in an ecosystem, AM fungi can promote coexistence of less abundant mycorrhizal plant species, or facilitate dominance by one or a few highly mycorrhizal plant species (Janos 1980; Grime et al. 1988; Hart et al. 2003). Regulation of plant diversity and coexistence is also likely to have wider reaching impacts on faunal diversity, nutrient cycling, carbon sequestration, and plant evolution.

Establishment of AM Symbiosis at the Pre-Contact Phase

The formation, and maintenance of mycorrhizal symbiosis is highly regulated, and involves the transfer of signals between plant and fungus through the duration of the symbiosis. Plants can become colonised by AM fungi of

multiple species concurrently, via interaction with spores, hyphae, or colonised root fragments (Smith and Read 2008). In the pre-contact phase of the interaction, plants perceive AM fungi by detecting a number of molecules, which in turn are upregulated upon fungal perception of a nearby plant host. These AM fungal signals consist of molecules based around an N-acetylglucosamine backbone, and include a mixture of chito-oligosaccharides (COs) and lipochito-oligosaccharides (LCOs) (Maclean et al. 2017). These molecules are known more commonly as Myc-factors, due to their similarity to rhizobacterial Nod-factors. These signals together constitute the common symbiosis signalling pathway, and research has shown that it is likely that the rhizobial symbiosis has 'hijacked' the pathways associated with the more ancient AM fungal symbiosis (Oldroyd 2013). It is unknown how plants can distinguish between AM fungi, Rhizboia, and other pathogenic organisms, which also produce these signalling molecules, however, the ratio of COs and LCOs may play an important role (Choi et al. 2018).

Plant recognition of these oligosaccharides involves LysM-containing receptor complexes, inducing calcium spiking which is perceived by a calcium- and calmodulin-dependent serine/threonine protein kinase (CCaMK), inducing transcriptional regulation of a number of genes involved with communication, accommodation, and nutrient exchange with the fungus (Hayashi et al. 2010; Miller et al. 2013). In a hormonal crosstalk with the fungus, plants release carotenoid-derived strigolactones into the rhizosphere, which, if perceived by a nearby AM fungal partner, induces hyphal branching. Simultaneously, detection of fungal signals induces morphological changes in plant

root structure, including the formation of new large lateral roots, both of which increase the likelihood of physical contact between plant and fungus (Akiyama et al. 2005). The AM symbiosis is highly regulated by plants, and the strigolactone signalling pathway is downregulated when nutrients, especially phosphate, is not limiting to plant growth (Yoneyama et al. 2007b, a). This prevents the unnecessary payment for nutrients which the plant could have sourced through the non-symbiotic route.

Maintenance and Control of AM Symbiosis at the Post-Contact Phase

At the point of physical contact, a fungal hyphopodia is formed on the root surface, and the plant cell in contact with the fungus produces a pre-penetration apparatus, guiding the fungus through the outer cells to the inner cortex (Rosewarne et al. 1997; Genre et al. 2005). Here the fungus spreads longitudinally along the root via intercellular spaces, before invaginating many cortical cells in the form of highly branched arbuscules (Figure 1.1), regarded as the primary site of nutrient exchange (Smith and Read 2008). Formation of new arbuscules is controlled by GRAS protein *Reduced Arbuscular Mycorrhiza 1 (RAM1)* which is regulated by CCaMK in concert with other regulatory proteins in the nucleus (Gobbato et al. 2013).

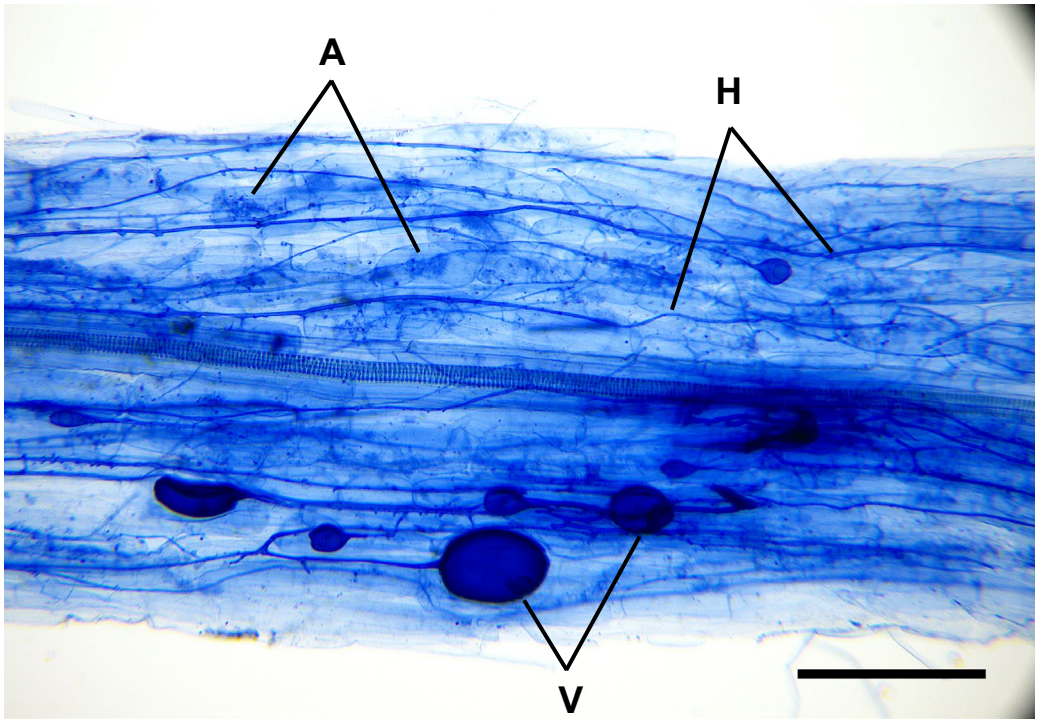

Figure 1.1 Mycorrhizal hyphae (H), arbuscules (A) and vesicles (V) stained with trypan blue, present within a large lateral root of leek (*Allium ampeloprasum*). Root material was sourced from Allpress Farm, UK during January 2019. Scale bar represents 200 µm.

Nutrient exchange at the arbuscule is highly regulated in space and time; and involves expression of nutrient transporter genes that are only present in arbusculated cortical cells during AM symbiosis. Mined phosphate is transported through AM fungal hyphae as polyphosphate, before arriving at the plant-fungus interface known as the periarbuscular membrane, where it is catabolised and released into the periarbuscular space as inorganic phosphate (Pi). Conserved plant phosphate transporters such as MtPT4/OsPT11 enable uptake of fungus-delivered phosphate, and its expression is integral to maintenance of the arbuscule, and the symbiosis (Paszkowski et al. 2002; Javot et al. 2007). As well as the more commonly researched occurrence of specialised phosphate transport in AM symbiosis,

AM specific ammonium (NH_4) and Zinc (Zn) transporters have been identified. Three AMT2 ammonium transporters are induced during mycorrhizal symbiosis in *M. truncatula*, and wild type arbuscules are maintained in MtPT4 mutants in low nitrogen conditions, suggesting that nitrogen is both transferred from fungus to plant, and that MtAMT2;3 plays a key role in maintenance of the symbiosis (Breuillin-Sessoms et al. 2015). Conversely, analysing gene expression of the AM fungi *Glomus intraradicies* (now *Rhizophagus irregularis*) revealed the cation diffusion facilitator (CDF) family transporter GintZnT1, with implications for membrane Zn transfer (González-Guerrero et al. 2005).

In exchange for nutrients, plants invest as much as 20% of the carbon fixed through photosynthesis to their fungal partner (Bago et al. 2000). Sugars and lipids are transferred to the fungus via plant transporters such as SlSUT2 and MtSTR/STR2, respectively (Zhang et al. 2010; Gutjahr et al. 2012; Bitterlich et al. 2014; Roth and Paszkowski 2017). Not only is this carbon exchange important for the maintenance of the symbiosis, it also represents a considerable carbon sink, and is likely to be important in local carbon dynamics, and global carbon sequestration and cycling (Johnson et al. 2002; Zhu and Miller 2003). A summary of AM fungal colonisation is shown in Figure 1.2.

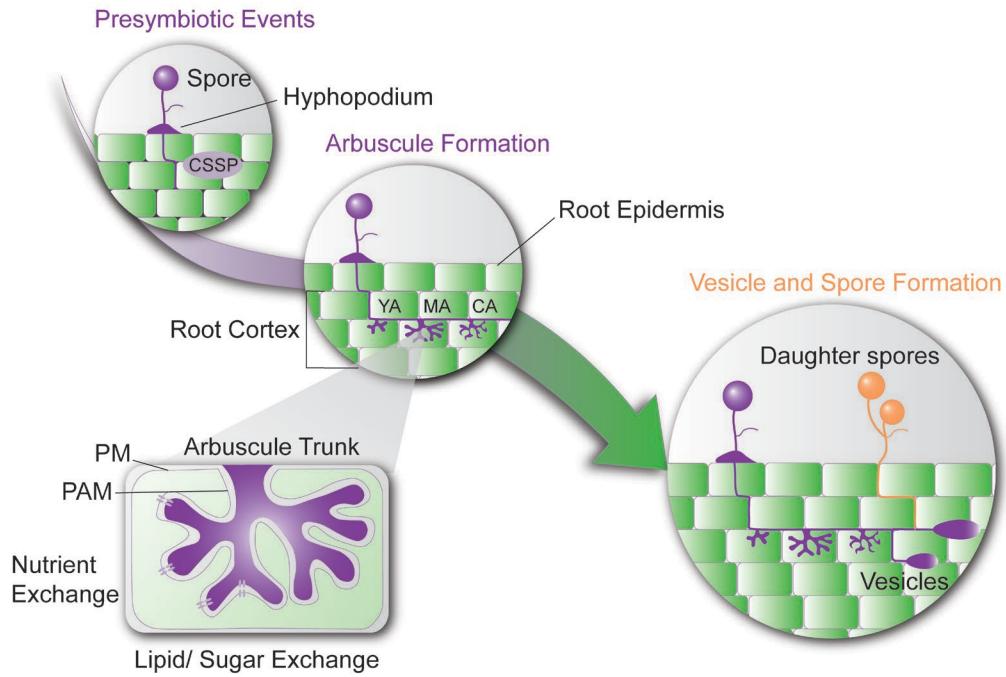

Figure 1.2 Diagram representing the stages of AM colonisation in plant roots. Figure represents the three stages of AM fungal symbioses, including early symbiosis, formation of Young (YA), Mature (MA), and Collapsing (CA) Arbuscules, and finally formation of vesicles and spores.

1.3 Impact of AM Fungi on Plant Growth in Agriculture

Studies have shown a positive relationship between AM fungal diversity and colonisation, and increases in plant productivity, or biomass in natural systems (Van Der Heijden et al. 1998b; Klironomos et al. 2000). Furthermore, Veresoglou et al. (2012) showed that AM colonisation significantly decreased root:shoot ratios in a meta-analysis of 516 trials, however, this outcome was not consistent amongst all plant families considered. The authors also reported differences following inoculation with different fungal isolates, and that AM fungal mixtures were generally more effective than single isolates.

Colonisation by AM fungi has been shown to induce a growth response in a meta-analysis of 435 controlled experiments, where total crop biomass was on average 34.9% higher in inoculated vs non-inoculated crops (Van Geel et al. 2016). Similarly, a meta-analysis by Lekberg and Koide (2005) indicated that increased AM colonisation resulted in a 23% increase in crop yield (Figure 1.3). AM fungi can also improve soil structure, carbon sequestration, and water retention through production of the stable glycoprotein glomalin (Wilson et al. 2009; Van Der Heijden 2010). Glomalin is produced by mycorrhizal hyphae, and has been linked to remediation of polluted soils, soil structural improvement, and climate mitigation (González-Chávez et al. 2004; Singh et al. 2013).

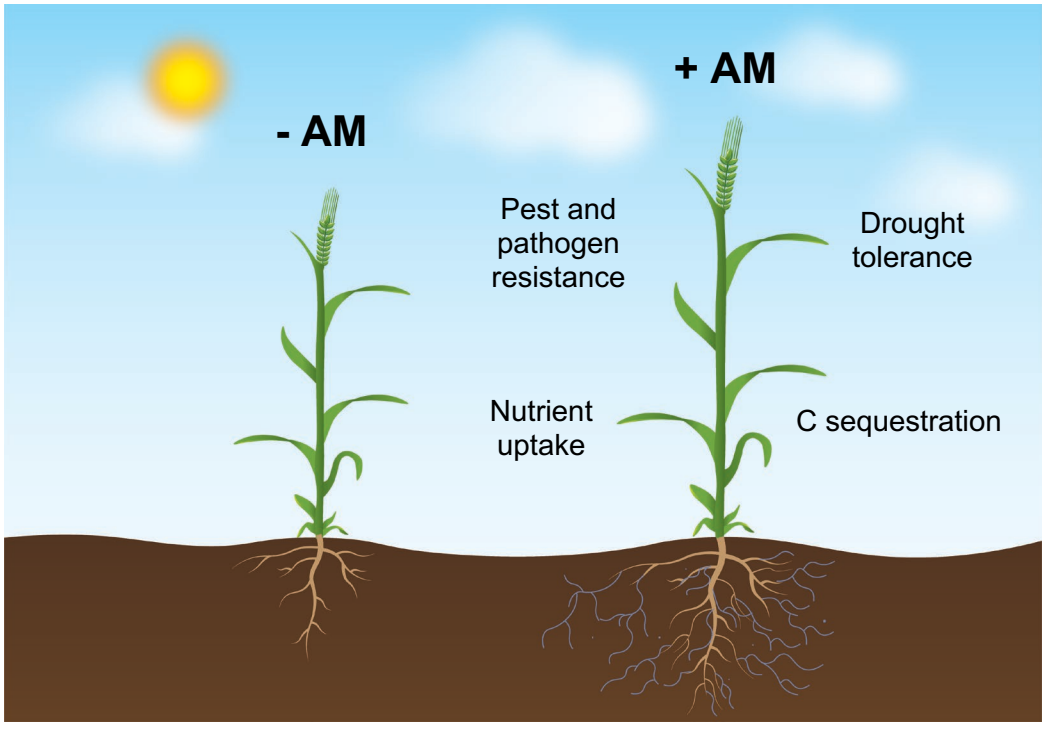

Figure 1.3 Diagram representing the plant benefit from increasing interaction with AM fungi.

Over 384 species of mycorrhizal fungi have been identified; initially by spore morphology, and more recently by molecular approaches, such as analysis of the commonly used 18S SSU rDNA region (Öpik et al. 2010). Through this method, Öpik et al. (2016) estimate that there may be as many as 2000 distinct taxa within the Glomeromycotina. AM fungi are found throughout the world, and colonise many plant families, with the notable exception of plants from the families Chenopodiaceae, Brassicaceae, Caryophyllaceae, Polygonaceae, Juncaceae, and Proteaceae. This means that several agricultural crops, including radish, mustard, beet, spinach, buckwheat, and oilseed rape have lost the ability to form a mycorrhizal symbiosis (Remy et al. 1994; Maherali et al. 2016).

AM Fungi Colonising Plants are not Random Assemblages, and have Variable Impacts on Plant Growth and Yield

Davison et al. (2011) found evidence for selectivity between AM fungi and plants in a temperate forest in Estonia, both at the level of individual plant species and plant ecological groups. This finding was in support of Öpik et al. (2009), who also found AM specificity within plant ecological groups. The authors found that generalist plants appeared to be colonised by generalist AM fungi, and that seasonal assemblages of AM fungi were stable. Similar findings have been shown by Gollotte et al. (2004) in upland grasslands, and Uibopuu et al. (2009) in a glasshouse experiment involving soil inoculum taken from a young forest, an old forest, and an arable field. It has also been shown that neighbouring plants may influence the AM fungal community

hosted in plant roots. Neighbours may override the effect of plant species selectivity, create a novel AM community not previously observed in either host, or have no effect (Hausmann and Hawkes 2009).

In a recent study, a range of maize cultivars were colonised at different levels by the model species *Rhizophagus irregularis,* which was correlated with growth variation under controlled conditions (Gutjahr *pers. comm.*). Individual crop cultivars colonised by different species of AM fungi have also shown variation in their levels of colonisation, and subsequent growth. Klironomos (2003) inoculated a range of plant species with 'local' and 'exotic' AM fungi. Klironomos found that interactions in an ecosystem could range from parasitic to mutualistic, and of the plant species tested, no single AM taxon provided the greatest benefit to all plant partners. Equally, the same AM fungal taxon could exhibit highly mutualistic or parasitic behaviour based on its plant host. One taxon, *Gigaspora rosea*, increased growth of *Plantago lanceolata* by 41%, but reduced the growth of *Rudbeckia hirta* by 40%, compared to the uninoculated control. When 64 plant species were inoculated with a single AM fungal isolate (*Glomus etunicatum*), plant host responses followed a normal distribution across the full spectrum of symbioses. Klironomos also found that exotic AM fungi had greater consistency in their impact on their plant partner, and this impact was more likely to be negative than positive. This suggests some evidence for local adaptation of the plant-AM symbiosis.

Li et al. (2019) showed that different AM fungal taxa had variable impacts on biomass of maize, and four weed species in field conditions. AM fungal taxa had positive, negative, and neutral impacts on maize biomass, and using a statistical approach, Li et al. identified AM fungal taxa which were positively correlated with maize growth, but had a negative impact on adjacent weeds. This further suggests a role for plant-fungus specificity, and shows potential for exploitation in field conditions.

In natural ecosystems, multiple species of AM fungi are normally present, and will concurrently colonise plants of the same, and different species. Bennett and Bever (2007) investigated the effect of three species of AM fungal inoculum on the growth of *Plantago lancelota*, and how herbivory by the specialist lepidopteran herbivore, *Junonia coenia*, influences this growth. *Glomus 'white'* (*Glomus sp. D1)* exhibited the greatest growth benefit but did not alter plant response to herbivory. Conversely, *Archaeospora trappei* provided less growth promotion, but maintained greater plant growth in the herbivory treatment. Inoculation with *Scutellospora calospora* did not influence plant biomass in the control or herbivory treatments. Plant responses in the three species mixed inoculum were most similar to *Glomus white*, which led authors to believe that this species is a dominant compatible symbiont for *P. lancelota,* although neither root length colonisation nor relative abundance of each species was determined.

AM fungal taxa have been shown to occupy distinct niches, whether spatially, temporally, or in transfer of specific nutrients or benefits to the plant

(Aerts 2003; Lekberg et al. 2007; Johnson et al. 2010; Bahram et al. 2015). The fact that the plant-AM symbiosis has persisted for over 540 million years suggests that the symbiosis is highly regulated at the whole plant, and/or cellular level, and there is molecular evidence in support of this claim (Paszkowski et al. 2002; Akiyama et al. 2005; Javot et al. 2007; Yoneyama et al. 2007b, a). Symbiotic selectivity is likely to be determined by the plant, which allocates photosynthate preferentially to more beneficial fungal partners (Bever et al. 2009; Kiers et al. 2011). However, the plant-AM symbiosis is not immune to 'cheaters', as there is evidence that some AM fungal taxa can be highly competitive, at the cost of providing benefit to the plant host (Pearson et al. 1993; Bennett and Bever 2009).

Impact of Agriculture on the Abundance and Diversity of AM Fungi

Reduction in the abundance and diversity of AM fungi in modern agriculture has been attributed to several factors. Increasing soil macro and micronutrients through application of artificial fertiliser, reduces the plant's benefit from participation in the symbiosis and creates competition between AM fungal species for photosynthate (Gosling et al. 2013; Liu et al. 2015). Plants in phosphate-rich soils exhibit a reduction in root exudates, which in turn reduces root colonisation (Akiyama et al. 2005). Application of fertiliser to increase crop yield elevates soil phosphate to many times the levels found at mature, undisturbed sites, and causes considerable reduction in the symbiosis at the pre-contact phase (Thomson et al. 1986; Bruce et al. 1994; Balzergue et al. 2011).

Intensive food production, coupled with larger and heavier machinery can result in compaction of soils, which may be overcome by cultivations. Disruption of the soil damages mycorrhizal networks, impacting their ability to scavenge for nutrients, colonise crops, and subsequently transfer nutrients to the plant partner (Galvez et al. 2001; Kabir 2005). Lastly, modern agricultural practice can include long periods where the land is left fallow (Figure 1.4). Absence of living plant hosts means that these obligate biotrophic symbionts cannot persist, except in the form of resting spores, and subsequently hyphal networks break down. In a farmer's rotation, the following crop is likely to be sown into a soil devoid of a mycorrhizal network, and therefore colonised solely by spores in the upper soil profile. Initially, the resulting hyphal networks will be small and unconnected, reducing mycorrhizal benefit.

Figure 1.4 A fallow field at North Moor Farm, UK in January 2018

In a study by Öpik et al. (2006), the authors found that agricultural sites hosted the lowest AM fungal diversity of all ecosystems studied. Verbruggen et al. (2010) found that modern agriculture only supported around 50% of the diversity of a nearby grassland, where 25% of total diversity was restored by low-input organic farming, possessing a community composition more similar to the grassland habitat. Growing crops in monoculture is also likely to influence the number of AM fungal taxa, when compared to more diverse host sites. This may be due to a reduction in ecological niches that can be inhabited by different AM fungal taxa, and has been demonstrated in experiments, showing dominance of one AM fungal species in an agricultural context (Menéndez et al. 2001; Daniell et al. 2001; Oehl et al. 2003; Öpik et al. 2006; Verbruggen and Kiers 2010). The relative contribution of mycorrhizal species richness and evenness, on crop growth is not known (Rodriguez and Sanders 2014).

Variation in mycobiont growth and development have been shown be largest at the genus level (Hart et al. 2001). AM fungi which are adapted to highly disrupted soils are likely to be characterised by fast germination, and a large investment in reproductive spores. It has been suggested that because of this, species commonly found in agricultural sites may be less beneficial to their plant hosts (Johnson 1993; Menéndez et al. 2001; Verbruggen and Kiers 2010; Van Geel et al. 2016). Ruderal *Glomus* species have different life history strategies, compared to other AM genera, and it has been postulated that occurrence of several AM genera with different characteristics may re-

sult in increased plant benefits (Van Der Heijden and Scheublin 2007). Furthermore, it has been shown that a diverse pool of AM fungal taxa occupy a range of spatial or functional niches, providing complementary plant benefits (Maherali and Klironomos 2007; Verbruggen et al. 2010).

1.4 Role of AM Fungi in Plant Nitrogen Uptake and Cycling

The AM fungi are often associated with their role in plant P_i uptake, with numerous labs exploring the genetic mechanisms of P_i transfer, as well as P_i-mediated suppression of symbiosis in controlled and natural systems. However, there is increasing evidence, albeit intractable and inconsistent, for a role of AM fungi in plant N uptake. This has largely been overlooked in the literature, and may play a key role in plant growth, and environmental N cycling (Tobar et al. 1994; Govindarajulu et al. 2005; Leigh et al. 2009; Hodge and Fitter 2010; Kobae et al. 2010; Hodge and Storer 2014; Breuillin-Sessoms et al. 2015).

Molecular Evidence for a Role of AM Fungi in Symbiotic Nitrogen Uptake

Despite inconsistencies in experimental evidence, there is more robust molecular evidence demonstrating the importance of AM fungi in plant N uptake. Stable isotope labelling experiments, such as by Govindarajulu et al.

(2005), have shown that inorganic nitrogen can be taken up by AM fungi, translocated within the fungus as arginine, before being transferred to the plant without carbon, and potentially bound to polyphosphate. Further molecular evidence includes the discovery of AM inducible ammonium transporters in soybean. *GmAMT4.1* showed specific expression in arbusculated cells, and was shown to localise to the branch domain of the periarbuscular membrane (Govindarajulu et al. 2005; Kobae et al. 2010). These findings suggest that in addition to contributing up to 100% of plant phosphate requirement, AM fungi have a role in nitrogen scavenging and uptake, via the symbiotic route (He et al. 2003; Smith et al. 2003). The inconsistencies around N uptake by AM fungi have been perplexing scientists for several years, but it has been speculated that due to the fungi's own requirement for N, there may be conflict for resources between partners, with the fungal partner only providing N for photosynthetically derived carbon when P is not limiting (Hodge and Fitter 2010; Hodge and Storer 2014). Considering that as much as 30% of AM fungal N is sourced from decomposition of organic matter, and up to 30% of root N is sourced via the symbiotic route, AM fungi may have a considerable, and previously overlooked role in global N cycling (Hodge et al. 2001; Govindarajulu et al. 2005; Hodge and Fitter 2010; Smith and Read 2010).

1.5 Taxonomy of AM Fungi

Taxonomy of AM fungi within the fungi, as well as AM fungal taxa within the clade has been subject to countless revisions since their discovery. The following section gives an overview of key advances and revisions to classification of AM fungi.

AM fungi were first described in the 19th century, as clusters of spores (sporocarps) found in the upper layers of soil (Tulasne and Tulasne 1844), which were later associated with formation of arbuscules and vesicles within plant roots (Janse 1897). Barbara Mosse first showed experimentally that non-sterile sporocarps of a fungus then known as *Endogone mosseae,* was able to colonise the roots of strawberry, recreating the vesicular-arbuscular colonisation illustrated by Janse in 1897 (Mosse 1953). Mosse also went on to show colonisation in apple, tomato, lettuce, wheat, and some other grasses, demonstrating the diverse host range of this newly described endosymbiont.

Historically, taxonomy of AM fungi was determined by spore morphological traits. Spores are small and exhibit modest variation, which made speciation difficult, even for the expert eye (Young 2012). In 1974, Reid et al. split the AM fungi within the genus *Endogone* into four genera in the order Endogonales: *Glomus, Sclerocytis, Gigaspora, and Acaulospora.* In 1990, AM fungi were moved to the order Glomerales, within the phylum Zygomycota*,* which contained two suborders and three families. Spores without obviously defining features were assigned to the genus *Glomus*, which represented a large

number of the known diversity of AM fungi. The *Glomus* were later suggested to be non-monophyletic, representing multiple genera or families (Morton and Benny 1990; Walker 1992; Schüßler et al. 2001).

Following the molecular revolution in biology, it was possible to classify taxonomy based on DNA sequence variation. Analysis of SSU rRNA gene sequences provided clear evidence that the AM fungi form a monophyletic phylum, the Glomeromycota, and should be removed from the polyphyletic Zygomycota (Schüßler et al. 2001; Schwarzott et al. 2001). However, the claim that the Glomeromycota were monophyletic was dependent on inclusion of *Geosiphon pyriforme*, a fungus forming endocytobiosis with a Cyanobacteria (Gehrig et al. 1996). Although this fungus was not typical of other Glomeromycota, it did represent a candidate for a fungal partner engaging in primitive symbioses with green alga, during the evolution of land plants in the early Devonian. Furthermore, it was suggested that the AM fungi diverged from the same common ancestor as the Ascomycota and Basidiomycota, with the authors describing three new orders, the Archaeosporales, Paraglomerales, and Diversisporales within the Glomeromycota (Schüßler et al. 2001).

In 2004, the Diversisporales were amended to contain the new families Pacisporaceae and Diversisporaceae, which contained some species formerly described as *Glomus* (Walker and Schler 2004; Walker et al. 2004). In 2006, a multigene phylogeny of fungi including basal lineages, placed Glomeromycota as a sister group to Ascomycota and Basidiomycota (James et al. 2006).

Next, Oehl et al. (2008) split the genus *Scutellospora* into three new families containing six genera: *Scutellospora in the* Scutellosporaceae*; Racocetra and Cetraspora in the* Racocetraceae*; and Dentiscutata, Fuscutata, and Quatunica in the* Dentiscutataceae*.* This was in response to previous evidence that *Scutellospora* was polyphyletic, although inconsistencies between morphological and molecular characteristics had prevented the authors from clearly separating these species (De Souza et al. 2005). Except for the genus *Racocetra*, Morton and Msiska (2010) rejected these amendments, criticising the methodology of Oehl et al. (2008). Morton & Msiska instead suggest reversion to the prior classification of two genera, *Gigaspora* and *Scutellospora*, with a third addition of the *Rococetra*, in the family Gigasporaceae (Schüßler et al. 2001; Redecker and Raab 2006).

To further understand the mechanisms of the AM symbiosis, the production of a complete and annotated genome of *Glomus intraradicies* was undertaken by the United States Department of Energy's Joint Genome Institute (JGI) in 2004. Difficulties in aligning regions with high levels of polymorphism meant that four years later, in 2008, the genome was not complete (Martin et al. 2008). In 2009, phylogenetic analyses comprising the partial SSU, entire ITS region, and the partial LSU rDNA aimed to characterise the model AM taxon *Glomus intraradices.* The authors showed that *Glomus intraradices* DAOM197198, the strain in the process of genome sequencing, was more closely related to *Glomus irregulare* than *Glomus intraradices,* despite lacking the irregular shaped spores characteristic of its name (Stockinger et al. 2009). Results revealed that AM isolates DAOM197198 and BEG195

believed to be *Glomus intraradices*, were in fact *Glomus irregulare,* and was later validated by Sokolski et al. (2010).

In 2009, the complete mitochondrial genome of *Glomus intraradices* isolate 494 was published, covering 70,606 bp comprising 26 introns (Lee and Young 2009). The authors produced a phylogeny based on 14 mitochondrially encoded proteins and denounce that although placement within the fungal tree is uncertain, it is clearly outside the Dikarya, which comprise the Ascomycetes and Basidiomycetes. This is in contrast to Schüßler et al. (2001), who proposed that the Glomeromycota are the sister group of Dikarya.

In 2010, Öpik et al. (2010) released the Glomeromycota specific database MaarjAM, which when released, described 282 SSU rRNA VT, based on phylogenetic analysis of 2238 records from 102 publications. VT were clustered at 97% similarity, due to sequence variation of up to 15% observed within single isolates, or even single spores (Koch et al. 2004; Pawlowska and Taylor 2004; Hijri and Sanders 2005; Stockinger et al. 2009; Lindahl et al. 2013; Bruns et al. 2018), and corresponded to approximately to the traditional binomial nomenclature of species level (Lee et al. 2008). The authors do however identify discrepancies between the classification of VT and the previously observed number of morphospecies. Firstly, in some cases, single morphospecies are represented by multiple VT. Secondly, the number of VT greatly exceeds the observed number of recorded morphospecies, however it is suggested that this is more likely to be because of geographic under sampling than erroneous inflation of diversity (Öpik et al. 2010, 2013). Finally,

SSU rRNA sequences are considered to have limited ability to resolve *Ambispora*, *Diversispora* and *Scutellospora* taxa to the species level (Gamper et al. 2009; Öpik et al. 2010). Not only does this reduce the estimated diversity of these groups, but the authors urged caution when assigning species names to environmental sequences. The database, though not without flaws, represented a centralised, publicly available resource aiming to standardise classification of AM taxa from environmental samples. It allowed for comparability between studies, and aided insight into the biogeography and ecology of AM fungi for the first time.

In 2011, species in the orders Glomerales and Diversisporales were reorganised based on rRNA sequence data and morphological analysis relating to spore formation. Two genera, *Septoglomus* and *Simiglomus* were added to the Glomeraceae, and the genus *Viscospora* was added to the Claroideoglomeraceae (Oehl et al. 2011). In 2012, Krüger et al. (2012) conducted a large scale phylogenetic analysis of AM fungi, amplifying two overlapping rDNA regions totalling ~3kb. The two regions included the SSU rRNA gene (~1800bp), and a 1.5kb region covering 250bp of the SSU, the complete ITS region (~ 475–520bp) and ~800bp of the LSU. The results further split the Glomerales into the Glomeraceae (previously *Glomus Group A*) and Claroideoglomeraceae (previously *Glomus Group B*). The new genera in the Glomeraceae included *Funneliformis* and *Septoglomus* (previously *Glomus Group Aa*), *Rhizophagus* and *Sclerocystis* (previously *Glomus Group Ab*), with only a few species remaining in the *Glomus* (previously *Glomus Group Ac*). The remaining sequences were tentatively assigned to the *Simoglomus,*

but the authors acknowledged the need for more data to validate this group. The former *Glomus Group C* was reclassified into the family Diversisporaceae. In total, the authors analysed sequences for 109 named species, and a further 27 cultures of species without name. This still represented only half of the 230 species of AM fungi estimated at the time.

Increasing numbers of studies looking at molecular diversity of the AM fungi resulted in conflicting methods of taxonomic classification, which were often based on little evidence or received inappropriate peer review. Redecker et al. (2013) proposed an evidence-based consensus for classification, based on molecular phylogenetic evidence, as well as morphological characters. Some new AM taxa were rejected, and others were synonymised. Redecker et al. did not support splitting the phylum Glomeromycota into three classes Glomeromycetes, Archaeosporomycetes, and Paraglomeromycetes (Oehl et al. 2011), instead placing all Glomeromycota into the class Glomeromycetes. The Glomeromycetes included the four orders, Glomerales, Diversisporales, Archaeosporales, and Paraglomerales, supported by sequence data as sister groups as originally proposed by Schüßler et al. (2001). Redecker rejected *Simiglomus, Viscospora, Albahypha, Kuklospora,* and *Orbispora* as genera, and Scutellosporaceae, Dentiscutataceae, Racocetraceae, and Intraornatosporaceae as families.

In 2013, nine years after the project's inception, the 153-Mb haploid genome of *Rhizophagus irregularis* was published. The genome contained 28,232 genes and provided clues to the cryptic life history of the fungus, and the

Glomeromycota more generally. Comparison of gene families placed *Rhizophagus irregularis* close to the Mucuromycotina, and mating-related genes suggested that this fungus was not limited to clonal reproduction only. However, the complete genome was unable to illuminate the obligate biotrophic nature of the fungus, lacking no evidence for genome erosion or any loss of complexity in metabolic processes. The authors did identify a lack of genes encoding enzymes for plant cell wall degradation, as well as genes involved in toxin and thiamine synthesis (Tisserant et al. 2013). The complete genome represented an invaluable resource for future studies on plant-AM signalling as well as ecological genomics.

The most recent major revision to the phylum level classification of AM fungi was published by Spatafora et al. (2016). The authors conducted phylogenetic analysis of genomic data for 46 taxa from the phylum Zygomycota, which had lost support for monophyly following molecular phylogenies based on one or more genes. Spatafora et al. split the Zygomycota into two phyla, Mucoromycota and Zoopagomycota, which were further arranged into six subphyla, four classes and 16 orders. Mucoromycota comprised Glomeromycotina, Mortierellomycotina, and Mucoromycotina and is sister to Dikarya. The Mucoromycota is the more derived clade of zygomycetes, and along with AM fungi in the Glomeromycotina, contains root endophytes and plant decomposers. There is still not complete agreement in phylogenetic classification of AM fungi, but it is the classification of Glomeromycotina, as well as the species level classification of Öpik et al. (2010) that will be used throughout this PhD thesis.

1.6 Cover Cropping as a Method for Soil Improvement

Cover crops are grown for the purpose of 'protecting or improving' soils between periods of regular crop production (Stobart and Gosling 2015, Figure 1.5). They can be effective at improving soil fertility and structure, reducing weeds and pests, and environmental management. The choice of cover crop species and subsequent management will depend on several factors, including the desired outcomes, climate, and soil type (Snapp et al. 2005; Abdollahi and Munkholm 2014; Stobart and Gosling 2015).

Figure 1.5 A cover crop of radish, oat, and vetch, at Euston Farm in January 2018

Cover Crop Species Selection to Address Soil Improvement Aims

Leguminous cover crops are often selected due to their ability to fix N via their interaction with rhizobia (Figure 1.6), but can also be used to improve soil structure, increase SOM, and attract beneficial insects, such as pest-predators and pollinators (Cossio et al. 2007). Common leguminous cover crops include vetch, clover, black medick, peas, and beans. Gramineous cover crops, such as rye and oats have relatively shallow roots which are effective at scavenging residual nutrients left from the previous crop. They establish ground cover quickly after autumn sowing and are therefore effective at suppressing weeds (Cossio et al. 2007; Stobart et al. 2015). Brassica species include mustards and radishes, which establish quickly and have deep, strong root systems, which can improve soil structure (Williams and Weil 2004; Chen and Weil 2010). It has been found that species in the Brassicaceae family (especially mustard species) exhibit biofumigation properties (the suppression of soil born pests and diseases and weed seed germination) which make them a popular choice for farmers with pest and disease issues (Haramoto and Gallandt 2004; Motisi et al. 2009). This biofumigation activity is related to Brassica's production of glucosinolates, which in the presence of water and myrosinase enzymes, contained in the plant cells, are transformed to isothiocyanates, which have also been shown to negatively impact the mycorrhizal symbiosis in some studies (Paul Schreiner and Koide 1993; Hill 2006; but see Pellerin et al. 2007; White and Weil 2010).

Figure 1.6 Root nodules on the roots of Vetch (*Vicia sativa)*, containing rhizobia bacteria.

Impact of Cover Crop Mixtures on the Realised Benefits to Soil Improvement

Mixing cover crop species in a polyculture has been shown to result in complementary and sometimes additive benefits. Total biomass of cover crop mixtures can be increased by occupying more above and belowground niches,

for nutrients, water, and light (Ofori and Stern 1987). Furthermore, N fixation by rhizobia associated with legumes can be utilised by non-legumes to increase growth. Soil depletion of N by non-legumes, may also provide conditions conducive to increasing bacterial N fixation (Ofori and Stern 1987; Giller et al. 1991). Cover crop mixtures may also be selected based on the requirements of the site. For example, a farmer may be interested in fixing additional N via the symbiotic route, and deterring weeds, so may opt to mix a legume species with a cover crop with allelopathic properties. However, other studies have shown that the benefit of increased cover crop diversity does not sufficiently improve soil conditions or crop yields to justify the additional price (Wortman et al. 2012; Finney et al. 2016).

1.7 Wider Implications of Cover Cropping in an Arable Rotation

Cover cropping may simultaneously create favourable soil conditions for crop growth, and improve soil health in the longer term (Figure 1.7). However, it is also important to have modest short-term expectations for cover cropping. It is possible that creating favourable soil conditions and improving populations of symbiotic mycorrhizal fungi will have significant, and notable increases in crop yields, however, it is more likely that modest improvements will be observed, at least during the first few crop cycles. It is important not to forget that although individual crops function on yearly cycles, improvements to soil health is a slow and iterative process. This does

not diminish the merit of cover cropping as a method to improve soil quality and function, but highlights the importance of long term trials such as the NIAB New Farming Systems (NFS), and Sustainability Trial in Arable Rotations (STAR). These trials will allow for measurement of incremental improvements to soil health over time, and are an invaluable resource for soils research.

Figure 1.7 Soil cracking in response to drought at Northampton UK, in July 2018.

1.8 Economics of Cover Cropping in an Arable Rotation

Unlike cash crops, cover crops are not normally expected to be harvested and sold for profit by farmers, but still incur the same costs, both in monetary and time investments, although some farmers with livestock or access to them are increasingly using cover crops as an early season source of forage. Cover crop seeds are sold as single species, simples mixes, and complex multi-species packages, ranging in cost from approximately £50 to £200 per hectare (Cotswold Seeds, Gloucestershire; Kings Crops, Norfolk; Hurrels Seeds, East Yorkshire). Further, the costs associated with preparing a seed bed, drilling the crop, and subsequently desiccating the crop amount to approximately £60/ha (Agro Business Consultants 2021). Drilling of cover crops coincides with the end of a busy harvest period for farmers, adding extra strain to staff already working long harvest hours. Considering financial costs alone, a modest £50/ha cover crop mix will cost approximately £110/ ha after diesel, herbicide, and machinery costs. This is equivalent to an increase in economic returns from malting barley yield of approximately 130%, an unrealistic expectation in the short term. In the longer term, it is expected that improvements to soil health will positively impact crops throughout a rotation, and will improve sustainability of yields in the medium to long term. Therefore, based on a six-crop rotation, or projected over the expected lifespan of a single field, a more modest yield improvement is required per cropping year.

This simplistic view omits the long-term incremental benefits that cover crops provide for soil health and other ecosystem services, which are less easily assigned a monetary value, and difficult to include in a farmer's balance sheet. Farming, like any other business, must maximise the ratio of incomings to outgoings. In recognition of the catchment scale benefits to soil and environment, farmers can receive financial support (up to £114 per hectare) for adoption of over winter cover cropping, as part of the Countryside Stewardship scheme (DEFRA 2020).

1.9 Cover Crops to Improve Populations of Mycorrhizal Fungi

It is widely accepted that cover crops contribute to soil health. Some cover crop species provide a 'bridge' for persistence of actively growing AM fungi between cash crops, as hyphal networks will die without interaction with a plant host (Kabir et al. 1999; Lehman et al. 2012). A meta-analysis by Bowles et al. (2017) found that cover cropping increased colonisation of the subsequent summer cash crop by 28.5%, compared to fallow. Many studies have found positive effects of cover copping on mycorrhizal abundance in the following crop, with mixed results in terms of the resulting benefit (Galvez et al. 1995; Boswell et al. 1998; Kabir and Koide 2002; Sorensen et al. 2005; White and Weil 2010; Higo et al. 2013, 2017; Bowles et al. 2017; Davidson-Lowe et al. 2021). Bowles et al. (2017) found that legumes had a

greater effect on cash crop root colonization than grasses or non-legume dicots, and surprisingly, even non-AM cover crop species, such as brassicas, have been shown to increase cash crop colonisation when compared to fallow. This may be due to presence of weeds germinating from the soil seed bank which can partake in the AM symbiosis, compared to bare soil sprayed with broad spectrum herbicides, or that non-AM cover crop species create soil conditions more favourable to the fungus (Drew et al. 2006; Njeru et al. 2014b). Conversely, other studies such as Karasawa and Takebe (2012) showed that mycorrhizal sunflower and wild oat cover crops increased AM colonisation of following maize, but this effect was not seen following non-mycorrhizal buckwheat.

Bowles et al. reported that colonisation of cash crops following cover crops was not influenced by cultivations. This is an encouraging result for farmers who depend on cultivations for control of agricultural weeds or fungal pathogens on crop debris. A study by Kabir and Koide (2002) found that mycorrhizal inoculum potential following a rye or oat cover crop was not significantly different, despite oat being killed by winter frost. However, a cover crop mixture of rye and oat significantly increased colonisation, P uptake, and yield in maize in the USA. This may be due to an increase in the number of ecological niches that can be inhabited by different AM fungal species, increasing the propagule diversity available to the following crop (Maherali and Klironomos 2007; Verbruggen et al. 2010).

Despite promising evidence linking the benefit of cover crops to AM fungi and crop yields in the literature, the effects of cover crops on mycorrhizal populations, and the benefits they convey to subsequent cash crops in UK agriculture is not fully understood (White et al. 2016). It has been documented that plant species show variation in levels of AM colonisation, and subsequent benefits from the symbiosis (Hoeksema et al. 2010). Although AM fungi are not host specific, there is evidence that both plant and fungi can associate preferentially with specific hosts (Van Der Heijden et al. 1998b; Johnson et al. 2004; Sýkorová et al. 2007b). Many studies have found positive effects of cover cropping on mycorrhizal abundance in the following crop, but literature concerned with the importance of AM diversity is less common, although advances in high-throughput DNA sequencing has led to increased accuracy, and reduced price of microbiome studies, which are subsequently becoming increasingly abundant.

1.10 Next Generation Sequencing for Microbiome Metabarcoding

Metabarcoding involves high throughput sequencing (HTS) of up to millions of variable DNA barcodes that can be amplified from environmental samples. Sequences are aligned, compared to a reference database, and can be identified down to species, or near species level. A DNA barcode is a short fragment of DNA that has been amplified from a sample of interest and must

have high levels of sequence conservation for the organism or group of organisms of interest in the flanking region, but have at least one variable region between these conserved areas.

Affordable metabarcoding has been made possible by the advent of next generation HTS on platforms such as the Roche 454, Pacific Biosciences Single Molecule Real Time Sequencing (SMRT), and more recently, Oxford Nanopore and Illumina systems. Newer platforms are more accurate; offer increased sequence depth allowing for greater numbers of samples per run; and are increasingly affordable (Reuter et al. 2015). This has meant that metabarcoding is becoming more common in labs concerned with the diversity and function of microorganisms from a range of sources, such as the human gut, soil, and water.

The HTS platform of choice will depend on a number of factors, relating to the length of barcodes, number of samples, desired read coverage, and cost. The Illumina platform makes up around 70% of the market share, and boasts high read numbers and relatively low costs. However, this platform can currently only handle DNA barcodes up to 600bp on the Miseq system, and 500bp on higher throughput machines. The Pacific Biosciences SMRT platform improves on this, with read lengths of 5-6kbp, with maximums of up to 30kbp, but has lower read coverage and higher costs than Illumina per sample. The Oxford Nanopore MinION platform can produce reads of up to one million bp and individual sequencers can be combined to increase read coverage. The MinION system also has the advantage of being small enough

to be transported into remote field locations, or countries where HTS is not readily available. The Oxford Nanopore technology is continually being improved in terms of base calling accuracy, and may become an economically viable option in the future.

rDNA Marker Selection for Bacteria/ Fungi/ AM Fungi

Due to the increasing interest in understanding the microbiome of a number of environments, such as biodiversity drivers; impact of climate change or soil ecology relevant to food production, there are standardised protocols in place for the assessment of fungi and bacteria, which are followed with minor adaptations by many scientists. Assessment of fungi makes use of the ITS region of the rDNA (Schoch et al. 2012), whereas assessment of bacteria makes use of variation in the 16S rDNA region (Schwieger and Tebbe 1998).

Recent studies investigating the diversity of AM fungi often use regions of the 18S rDNA as barcodes, including the small subunit (SSU), ITS region (ITS1-5.8S-ITS2) and the large subunit (LSU) which includes the variable D1 and D2 regions. There is less consensus on which AM fungal primers are optimal, with trade-offs and preferences visible in different labs around the world. The Kruger primer set (Krüger et al. 2009), which amplifies a ~1500bp region of part of the SSU, ITS region and part of the LSU (pSSU-ITS-pLSU), captures the highest diversity of AM fungi. However, these primers are not suitable for the short read-lengths of the Illumina system and

therefore the number of samples that can be run on a PacBio machine make this an expensive option when large numbers of samples are required.

Of the molecular markers for identification of AM fungi, the SSU rRNA gene has been most commonly used for ecological studies. This is due to the ability of this region to distinguish between morphological species within the Glomeromycotina, as well as having the most comprehensive reference taxonomy spanning multiple geographies and ecosystems, in the MaarjAM database. The SSU, however, has difficulty in distinguishing AM taxa from the Diversisporales, and the LSU rRNA gene is often subsequently used in taxonomic studies (Öpik et al. 2010, 2014; Davison et al. 2015). Considering the attributes of the available markers, the SSU rDNA gene was chosen for taxonomic identification of AM fungi throughout this PhD thesis.

Bioinformatics of HTS Microbiome Data

As in other microbial ecology analysis, much of the work on AM fungi occurs on reputable pipelines including QIIME2 (Bolyen et al. 2018) and mothur (Schloss et al. 2009), which have excellent documentation and are relatively straightforward with some basic knowledge of working on the command line. These pipelines act as a wrapper for algorithms such as DADA2 (Callahan et al. 2016) and Deblur (Amir et al. 2017), which are de-noising and clustering algorithms for HTS data. However, these programs are more suited to working with Absolute Sequence Variants (ASVs), that offer increased, but redundant precision when used with the curated MaarjAM database,

which clusters sequences at a 97% sequence similarity. Bioinformatics of metabarcoding data is an increasing active area of research, and many stand-alone programs have been, and are being developed to allow users to have maximum control over their analysis. Programs such as SpAdes (Bankevich et al. 2012) can perform error correction on sequence data, and paired with other programs, allow users to control threshold values, and perform *de novo*, or closed reference OTU picking, as appropriate, with tools such as VSEARCH (Rognes et al. 2016), also implemented on the command line.

1.11 Influence of Cover Crops on the Molecular Diversity of AM Fungi in Agriculture

Studies including Ramos-Zapata et al. (2012) and Higo et al. (2013, 2017) found a positive effect of cover cropping on the AM fungal diversity of the following crop, when compared to fallow or non-AM cover crops. Other studies such as Higo et al. (2018) and Cloutier et al. (2020) have found that certain cover crops caused a shift in AM fungal community composition, but did not increase total diversity *per se*. Other studies have not found a significant impact of cover cropping on AM fungal diversity, and many of these studies concluded that seasonal or abiotic factors may play a large contribution to AM diversity, or that host selection was a stronger driver of AM species colonisation than previously thought (Njeru et al. 2014b, a; Higo et al. 2014, 2015, 2020; Heberle et al. 2015; Turrini et al. 2016; Pakarinen et al. 2021).

Many of these studies originate from the USA or Japan, and focus on maize, or soybean respectively, as the cash crop. One UK study by Detheridge et al. (2016), documented the legacy effect of cover cropping on all soil fungi in a cereal rotation. Clover cover crops, when compared to ryegrass supported different populations of fungi, including Ascomycota, Basidiomycota, Zygomycota, and Glomeromycota (now Glomeromycotina). Clover cover crops were found to support the highest soil nitrate-N, which was negatively correlated with AM fungal populations. This may demonstrate a potential interaction between bacterially fixed N from legume roots, and AM populations. Differences in total soil fungi were conserved in the following wheat, and subsequent barley. Due to the breadth of this study, AM sequence diversity was only determined to the family or genus level.

1.12 AM Fungal Inoculants as a Method to Artificially Improve Mycorrhizal Populations

The widespread understanding that AM fungi contribute to plant growth and health has resulted in the development of commercial AM fungal inoculants, leading to variable outcomes in RLC, biomass, and yield (Hart et al. 2018). A range of companies produce mycorrhizal inoculants for the commercial and private sector, including PlantWorks (Sittingbourne, UK) in the UK. Due to its obligate nature, PlantWorks culture mycorrhiza on nurse plants, and produce five species including *Funneliformis mosseae, Funneliformis geosporum, Claroideoglomus claroideum, Rhizophagus irregularis,*

and *Glomus microaggregatum.* These five species are grown individually, then mixed in approximately equal proportion before being bagged and sold.

Commercial Production of AM Fungal Inoculums

The first challenge of producing AM fungal inoculum lies in producing sufficient quantities of infectious propagules. Under lab conditions, spores of AM model species are cultured using a tailored nutrient agar, repeatedly transferring sections of colonised plant roots to fresh agar to multiply spore numbers (see Ceballos et al. 2013). This process is limited by the obligate nature of AM fungi; whereby the fungal spores cannot germinate and grow significant hyphal biomass without an association with living plant roots. Spores are removed through centrifugation and sieving before being quantified microscopically. This is a time consuming, but inexpensive way to multiply the fungus of interest, with low risk of introducing pathogens or weeds to the experimental system. On the other hand, the volume of AM fungi required for large scale application usually involves the production of crude inoculum. Production of crude inoculum involves inoculation and cultivation of a host trap plant with a known species of AM fungal isolate, in an inert medium optimised for AM fungal growth. The propagation of spores in a host trap plant system is demanding in terms of space and time, and requires careful biocontrol measures to prevent cultivation of pathogens that may be co-cultivated and undetected before the crude inoculums are used (Berruti et al. 2016).

Ideally, crude inoculum is introduced to the field via the seed drill alongside a cover crop to allow AM networks to establish before drilling the cash crop, however, applying inoculum with the cash crop is also an option. A seed drill may be an ineffective method for introduction of inoculum if it is wet when clay is used as a carrier for the inoculum (which will stick to the drill), or if seeds are small. In this case inoculum can be broadcast and incorporated by a shallow cultivation, but this incurs costs in terms of time, and money for additional diesel. In theory, the introduced AM network should persist indefinitely, so long as there are no deep cultivations, long fallow periods, or cultivation of non-mycorrhizal crop monocultures, but this remains to be proven. There is some counter evidence to suggest that soil microbiomes are resistant to the introduction of various microbiota, as shown by Coelho et al. (2020).

AM Fungal Inoculums and Their Effectiveness

A meta-analysis of AM inoculation was conducted by Berruti et al. (2016) for 127 publications ranging from 2001 to 2015, encompassing 164 inoculation experiments. The majority of these experiments (65%) were conducted in glasshouse conditions, while 24% were in field conditions. Increase in colonisation between the inoculated and control plants was significantly higher in the glasshouse than field conditions, although the authors did not standardise the response to account for colonisation by native inoculum in field conditions. Inoculation with native AM fungi exhibited higher rates of colonisation, biomass, and yield than use of 'exotic' species, in line with findings

by Klironomos (2003). Berruti et al. (2016) also show that multispecies inoculums increased shoot biomass compared to single species, but there was no overall increase in colonisation, nutrition, or yield. The authors observe that many experiments have been limited to inoculation with *Rhizophagus intraradices/ irregularis, and Funneliformis mosseae* AM fungi, which represent three generalist symbionts which are easily cultured, able to colonise a wide array of host plants, and are geographically widespread. They therefore may lack niche differentation, resulting in less benefit when grown as a mixed incoulum. Furthermore, they may only exhibit a fraction of the benefit that would be attained by a specialist, but more difficult to culture AM species.

In another glasshouse experiment, Gosling et al. (2016) showed that increasing AM inoculum diversity beyond three species did not further increase shoot biomass or P uptake. However, the authors argued that experimental glasshouse conditions can facilitate the dominance of a single species, and that a more diverse AM community would be more beneficial when there are multiple stressors, such as is found in field conditions. A recent publication by Crossay et al. (2019) investigated the effect of different species and families of AM fungal inoculum, whilst subjecting the host *Metrosideros laurifolia* to a number of abiotic stressors. Inoculation with different families of AM fungi exhibited strong synergistic effects under nutrient deficiencies, and increased heavy metal concentrations, which acted as stressors. Inoculation with different families of AM fungi increased plant growth and mineral P nutrition, and limited heavy metal translocation to

shoots. The authors found evidence for functional reduncancy, as inoculation with two species from the genus *Acaulospora* reduced plant biomass when compared to the same inoculum mix with only one species of *Acaulospora.* This study was in agreement with a meta-analysis by Yang et al. (2017), who found that plant performance was positively correlated with AM family diversity, and not total diversity *per se.* The authors investigated variation in plant response to the Glomeraceae, Gigasporaceae, and Claroideoglomeraceae across 902 articles, and found that the family glomeraceae was associated with highest P uptake, and drought stress tolerance. The Gigasporaceae were associated with lowest P uptake, but exhibited improved tolerance to heavy metals, and fungal pathogens. The family Claroideoglomeraceae exhibited intermediate benefit to nutrient uptake, drought and salt stress, low improvements to heavy metal tolerance, but was most effective at improving tolerace to attack by nematodes.

There is surprisingly little direct evidence for successful establishment of AM fungal inoculum (Hart et al. 2018). Without molecular tracking for the establishment of introduced taxa, changes to plant RLC, biomass, or yield are purely correlative. Furthermore, it is important to test the long term persistence and impact of introduced species, with some studies only reporting persistence up to 13 weeks (Köhl et al. 2016). Clearly, farmers will hope that investment in AM fungal inoculums would last at least one growing season, if not, a full crop rotation or more. Hart et al. (2018) also draw attention to the potential of unintended consequences resulting from AM fungal inoculation in nearby natural systems. There is evidence that AM inoculation can

influence AM fungal communities, albeit indirectly (Pellegrino et al. 2012; Berruti et al. 2017). There is also evidence that AM fungal community composition can influence aboveground plant composition, and broadly reduce overall diversity (Hartnett and Wilson 1999). These factors when considered together suggest the potential for unintended shifts in aboveground community composition, which could in turn impact faunal biodiversity; nutrient, water, and carbon cycling; and other associated ecosystem services.

Despite the interest in AM fungal inoculants in agriculture, Berruti et al. (2016) concluded from their review, that native populations of AM fungi were as good as, or more beneficial than introduced inoculum. Others have shown that native populations, as opposed to 'exotic' AM taxa are more effective at increasing plant biomass, and can improve resistance to arthropod herbivory (Middleton et al. 2015). It seems likely that increasing AM fungal abundance *per se* will provide less benefit than promoting species which are suited to a specific soil type, and/or mycobiont-plant host combination. The use of cover crops could help promote natural diversity of field soils, whilst providing other benefits to soil health (Faye et al. 2013, Figure 1.8).

Figure 1.8 Cover crop mixture of fodder radish, black oat, vetch, and buckwheat at Holkham Farm, UK in February 2018.

1.13 Project Overview

This PhD programme will explore how cover crops, and other amendments can influence the diversity and abundance of AM fungi, whether these populations are maintained in subsequent cash crops, and critically, the resulting effect on crop yield. Experiments will include crops important to UK agriculture, including barley (*Hordeum vulgare*), maize (*Zea mays*), and oat (*Avena sativa)* (DEFRA 2017). The research presented has been conducted at a number of spatial scales, from a national assessment of AM fungal diversity, to field scale experiments and glasshouse trials to address project aims, and finally, verification on farm at the field scale (Figure 1.9).

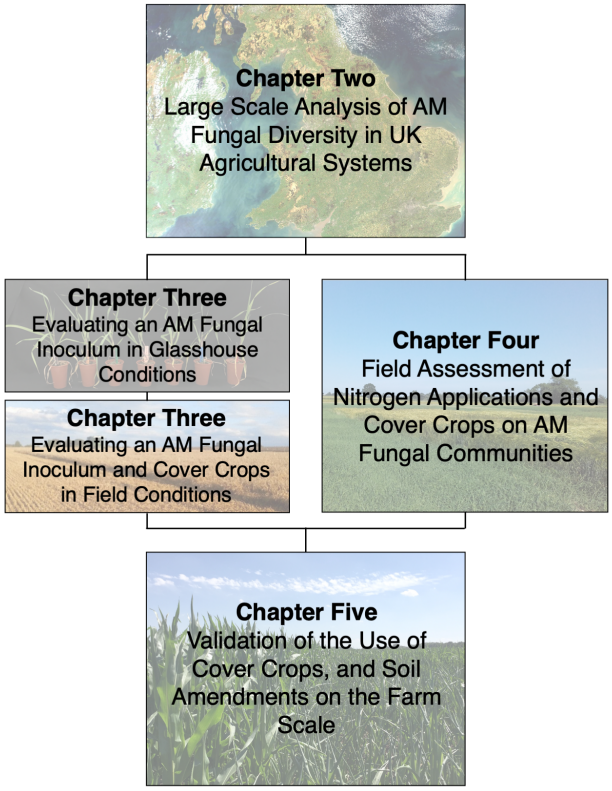

Figure 1.9 Summary of experimental chapters, conducted across a range of spatial scales.

1.14 Hypotheses

The key hypotheses the project will address include:

1. Certain AM fungal taxa are more beneficial to certain cash crops than others, and are often absent or lowly represented in high disturbance, conventional agriculture
2. The use of cover crops promotes the establishment of a diverse range of AM fungal species, which facilitates increased interaction with following cash crop
3. Increasing the diversity and abundance of AM fungi increases the likelihood of more beneficial plant-fungal interactions occurring. This will in turn increase crop growth and yield.

These hypotheses will be explored by considering the following aims:

1. Assess the diversity of AM fungi in UK agricultural systems, identifying farming practices which are detrimental to diversity *per se* or individual taxa
2. Assess the impact of cover crop species on soil health, including the diversity and abundance of AM fungi
3. Quantify the effect of increased diversity and abundance of AM fungal species on crop yield, under a range of soil, inoculation, and weather conditions.

To date, I am not aware of any comprehensive UK studies which have assessed the diversity of AM fungi in agricultural systems, or explored how

cover crops influence populations of AM fungi, beyond the family/ genus level. Further to this, studies outside of the UK considering how cover crops increase mycorrhizal diversity rarely report on the benefits to the following crop, such as biomass or yield. Considering previous literature, it seems likely that AM populations are governed by an interaction between abiotic and biotic factors. It will be important to determine whether specific AM fungal species can be promoted by cover crops, and whether these species are adopted by the following crop. Impacts of artificially adjusting the biotic or abiotic elements, by amendments, non-AM crops, or inoculation will be considered. Most importantly, the effect of cover crops, and AM fungal diversity and abundance will be quantified in terms of cash crop benefit, including yield. The project aims to benefit farmers and agronomists through improved understanding of how specific cover crop species influence AM populations, and the benefits that these cover crops, and fungi convey to the following cash crop (such as increased biomass/ yield). Findings will inform future cover crop species selection and other farm practice in relation to cover cropping and AM fungi.

2 Chapter Two – Large Scale Analysis of Arbuscular Mycorrhizal Fungi in UK Agricultural Systems, and Implications for Sustainable Agriculture

2.1 Introduction

Despite the importance of AM fungi to crop nutrition, there are no published comprehensive assessments of AM fungal diversity in UK agriculture. In order to assess the effectiveness of various amendments or inoculants, it is essential to establish a baseline understanding of the AM fungal diversity and abundance already present in agricultural sites across the UK. This baseline will provide a framework for the comparison of trials, and treatments within trials, to better understand the impact of experimental treatments on AM fungal diversity in UK agriculture.

Global Diversity of AM Fungi

In comparison to the worldwide diversity of other fungi, the arbuscular mycorrhiza of the phylum Glomeromycotina are very low in diversity compared to other soil microbial phyla. There are currently ~250 morphologically defined or 350 to 1000 molecularly defined AM fungi (Kivlin et al. 2011; Öpik et al. 2014).

AM fungi display very low endemism; this was reported in a study by Davison et al. (2015). The authors identified 246 AM VT from 836 samples from across the world, where 93% of these VT were found in more than one continent, and 34% were found on all six sampled continents. Similarly, 90% of VT were found in more than one climatic zone, and 79% of VT were found in both forests and grasslands. Davison et al. showed that distance had as strong an influence on community composition as environmental variables, such as temperature; and paleogeographic history and host-plant identity explained sample level composition, at least at a low level.

The top 10 most abundant taxa in this study (and the proportion of samples they occupied) were: VTX00113 *Glomus MO-G3* (67%), VTX00166 *Glomus MO-G4* (51%), VTX00115 *Glomus MO-G13* (51%), VTX00191 *Glomus MO-G31* (38%), VTX00193 *Claroideoglomus lamellosum* (34%), VTX00092 *Glomus Yamato09 A1* (34%), VTX00247 *Glomus Glo39* (32%), VTX00149 *Glomus MO-G1* (30%), VTX00199 *Glomus MO-G7* (30%), and VTX00222 *Glomus MO-G23* (29%).

Diversity of AM Fungi in Natural and Agricultural Systems

A metanalysis by Öpik et al. (2006) investigated the diversity of AM fungi colonising the roots of 36 host plant species from 25 locations from around the globe. The authors found that AM fungal diversity varied between habitats. Tropical forests hosted the largest diversity of AM taxa per plant species (18.2) followed by grasslands (8.3), temperate forests (5.6) and arable

and polluted sites (5.2). Verbruggen et al. (2010) also found that agricultural sites being used for maize (*Zea mays* L.) or potato (*Solanum tuberosum* L.) cultivation only supported around 50% of the diversity (3.9 taxa on average) of a nearby grassland (8.8). The authors show that at these sites, 25% of total diversity was restored by low-input organic farming approaches (6.4), additionally possessing a community composition more similar to that found in a grassland habitat.

In addition to a paucity of AM fungal species on arable sites, Daniell et al. (2001) also reported a lower Shannon Diversity (*H*), an index which takes into account both the number, and relative proportions of taxa in a community. In their UK study of AM diversity in an arable field, Daniell et al. (2001) found eight AM taxa by RFLP with *HinfI* and *RsaI* restriction enzymes. *Glomus sp. Glo1a*, which included known sequences of *Glomus mosseae* and *Glomus geosporum,* comprised 62% of the cloned reads. This is in contrast to AM fungal communities in an afromontane forest site in Ethiopia, where of 20 AM taxa colonising roots of *Prunus africana,* the two most abundant taxa comprised only 21% and 18% (Wubet et al. 2004). This may be due to a reduction in ecological niches that can be inhabited by different AM fungal species, or to sensitivity of some AM species for survival in high disturbance conditions. This phenomenon has been demonstrated in other experiments, which show dominance of one AM fungal species living in an agricultural context (Menéndez et al. 2001; Oehl et al. 2003; Öpik et al. 2006; Verbruggen and Kiers 2010). Menéndez et al. (2001) investigated the influence of tillage and crop monoculture on AM community composition

in Argentina. The authors used spore morphological traits to identify species and determine AM diversity, and identified 17 taxa. Soil used for continuous cultivation of wheat or barley contained considerably fewer spores, representing lower diversity, and lower Shannon Index, than grassland or a three-year red clover cover crop. The dominant species in the monoculture plots were VTX00067 (*Glomus mosseae)*, VTX00251 (*Scutellospora pellucida)*, and VTX00214 (*Glomus sp. 7)*. However, Menéndez et al. acknowledge the study is limited to sporulating AM species, and care should be taken when comparing these results to molecularly characterised communities.

Generalist and Specialist AM Fungi in Agricultural Systems

The AM fungi are often documented to be beneficial root endosymbionts, which provide soilborne nutrients for photosynthetically derived carbon. This is a somewhat naïve and simplistic view, and the Glomeromycotina can span the continuum of symbiosis, from parasitism, to commensalism, and the commonly described mutualism (DeBary 1879; Johnson et al. 1997). Attempts have been made to classify AM into functional groups, however most efforts are confounded by the fact that individual AM fungal taxa vary in the host benefits derived according to several biotic and abiotic factors, such as host plant identity, nutrient availability, disturbance, and pest pressure. Agricultural sites represent one of most disturbed habitats of AM fungi. Regular applications of nutrients, cultivations, and fallow periods, inflict intense selection pressures on AM in time frames rarely experienced in natural systems. Verbruggen and Toby Kiers (2010) applied the concept of r-

selection to the AM fungi, as a framework to understanding life-history evolution (Pianka 1970; Grime and Pierce 2012).

R-selection is known to benefit organisms that invest heavily in rapid reproduction and dispersal, and are therefore able to complete their life cycles within cycles of agricultural disturbance. The frequent 'resetting' of AM diversity based on propagation of spores overrides any potential adaptation to particular niches within an arable field, and selects against species that invest predominantly in hyphal growth for the benefit of the plant (Jakobsen and Rosendahl 1990; Sýkorová et al. 2007a; Verbruggen and Kiers 2010).

Cultivations represent one of the most extreme forms of disturbance for AM fungi and have been investigated at length in the literature. Cultivations broadly decrease diversity of AM fungi, and this effect may differ depending on the fungi's tolerance to disturbance (De La Providencia et al. 2007). Although some species within the family Glomeraceae are associated with being tolerant of soil disturbance, they may be less beneficial in terms of host nutrient acquisition (Jansa et al. 2002; Rosendahl and Matzen 2008).

Cultivations have been shown to negatively impact *Acaulospora, Scutellospora, Ambispora, and Gigaspora* species of AM fungi. Species within the *Gigasporaceae* are unusual in that they are unable to colonise host plants from hyphal fragments, unlike other AM fungal species, which makes them particularly vulnerable in cultivated systems (Biermann and Linderman 1983; Menéndez et al. 2001; De La Providencia et al. 2007). AM in both the *Glomeraceae* and *Gigasporaceae* families can repair their hyphal networks

following disturbance, though anastomosis formation was more common in *Glomus* species than *Gigaspora* and *Scutellospora*. This provides an alternative strategy for species that are not able to sporulate within the time constraints of an agricultural season, but may come at a fitness cost to both fungus and plant (De La Providencia et al. 2005).

Plant-AM Fungal Specificity as a Driver of AM Fungal Diversity

One particular area of interest is to determine whether there is consistency between AM fungal symbionts colonising the same crop species under different conditions. Although AM fungi are not host specific, there is evidence for host preference between partners, with plants receiving a variation in benefit from the symbiosis (McGonigle and Fitter 1990; Van Der Heijden et al. 1998b; Johnson et al. 2004; Sýkorová et al. 2007b; Öpik et al. 2009; Hoeksema et al. 2010; Martínez-García and Pugnaire 2011). This suggests that there are likely to be optimal combinations of plant and AM fungi, which will enable maximal nutrient uptake and therefore growth and yield.

AM Fungal Diversity in UK Agricultural Soils

In June 2020, there were 384 VT described in the MaarjAM database, and the database is constantly being updated to reflect newly discovered taxa, or taxa that should be split or merged according to newly discovered sequence variants. Of these VT, 84 have been found in studies in the UK, sourced from grassland, forest, gardens, sand dunes, and arable sites.

Unfortunately, although the MaarjAM database is expertly curated it does not contain all UK studies of AM diversity, so it is reasonable to believe that this figure, and the estimate of AM diversity in arable sites alone will be higher.

Background to the FERA Big Soil Community

As a platform to assess UK AM fungal diversity, this experiment makes use of 150 samples from FERA's Big Soil Community; where farmers have submitted soil samples and associated metadata on crop identity, yield, pesticide use, soil conditions, and other abiotic and biotic measurements. The Big Soil Community was focussed on broad range fungal and bacterial diversity, sequencing the ITS and 16S rDNA regions respectively, and made some interesting observations, including a near complete absence of AM fungi from agricultural soils. This was a highly unexpected and unlikely discovery, and highlighted some potential limitations of the protocol used, which at some stage (likely at the PCR or early in the bioinformatics pipeline) were excluding lowly abundant AM fungal rDNA sequences.

Using the same DNA samples, and published AM specific primers, it has been possible to amplify and sequence a hypervariable region of the 18S rDNA, which provides a measure of total AM diversity in UK agriculture, as well as highlighting potential farming practices, amendments, and soil conditions, which promote or impact AM diversity *per se,* or specific AM

families, genera, or species. An important aim therefore is to identify sufficient numbers, and diversity of samples that enable questions to be answered at the farm scale, which has been identified as an important shortcoming of studies exploring the interactions between soil microbes and plant responses, and indeed agricultural trials more generally.

Classical ecological analysis tools such as db-RDA will be used to identify factors underpinning AM fungal community composition. Analysis of differential abundance will identify those taxa which are significantly more or less abundant in a given treatment, which may then be used as markers indicative of certain biotic or abiotic conditions. These taxa may be useful indicators of soil health, which is a priority target of the UK Government's 2020 Agricultural Bill (Coe and Finlay 2020).

This study will provide an overview of AM fungal diversity in UK agricultural soils, and aims to quantify the effect of factors such as soil type, crop identity, and amendments such as fungicides, underpinning the AM fungal community. The results from this chapter will provide a framework for analysis of AM fungal community compositional data in further chapters, as well as other studies of AM fungal diversity in UK agriculture.

2.2 Methods

Study Sites and Sampling

Soil samples were collected and sent from farm sites as part of the FERA Big Soils project. This study utilised 150 of the original 258 samples, representing 67 individual field locations (Figure 2.1). The further 108 samples were omitted from this analysis as they did not have appropriate metadata. Soil was sampled by growers following a set protocol, which involved sampling points in a lazy W within the field of interest. Approximately 1kg of soil was sent via chilled courier to FERA Science (Sand Hutton, UK) for analysis.

Figure 2.1 Geographic distribution of the 67 independent sites included for analysis

UK Climatological Data for Study Sites

Weather data was extracted from the Met Office HadUK gridded climate observations dataset, at 1km resolution. Mean, minimum, and maximum temperatures; rainfall; wind speed; sunshine hours; relative humidity; pressure at sea level; partial pressure of water vapour; number of days with snow lying; and number of days with ground frost, were collected as an annual average for the experimental year. Data was collected from netCDF weather files using the raster (Hijmans 2020) and ncdf4 (Pierce 2019) packages in R (R Core Team 2019)

DNA Extraction from Big Soil Community Soil Samples

10g of soil was taken from soil samples at random for DNA extraction, using the PowerSoil DNA kit following the manufacturers protocol (QAIGEN, UK). Purified DNA was eluted in 2ml of solution C6, and stored at -20°C. Prior to PCR, DNA samples were diluted 100-fold to improve amplification of AM fungal DNA.

DNA Amplification of Soil Samples using AM fungal Specific Primers

DNA was amplified using the primers NS31 (Simon et al. 1992) and AML2 (Lee et al. 2008) which included the Illumina barcodes p5 and p7 at the 5' and 3' ends respectively. These primers amplify a ~550bp region of the 18S rDNA, and are specific to AM fungi. Reactions were performed in triplicates,

and included 0.1ul Phusion DNA Polymerase (NEB, UK), 2ul Phusion HF buffer, 0.2ul dNTP, 0.5ul of each primer, 5.7ul nuclease free water, and 1ul of the diluted DNA sample. Samples were amplified on an MJ Research PTC-225 thermocycler with the following conditions: 98°C for 3 minutes, then 30 cycles of 98°C for 10 seconds, 57°C for 30 seconds, and 72°C for 30 seconds, before a final extension of 72°C for 10 minutes. 5ul of the pooled triplicate samples were visualised on a 1% agarose gel, stained with ethidium bromide. The resulting PCR products were purified with AMpure beads (Beckman Coulter, US), followed by two washes of 80% EtOH.

Indexing and Library Preparation of Study Samples

Illumina Nextera XT indexes were attached to PCR amplicons following the manufacturers recommendations. The resulting PCR product was purified with AMpure beads, followed by two washes of 80% EtOH. DNA concentration was measured using a BMG CLARIOstar plate reader and equimolar concentrations of each indexed sample were pooled to a final concentration of 30nM. 2ul of the final pooled library was visualised on an Agilent Technology Bioanalyzer, to check for off-target amplification. Subsequently, the pooled library was run on a BluePippin using a 1.5% agarose cassette, to remove a small ~180bp amplicon. 5ul of the final pooled and indexed library was then denatured with an equal volume of fresh 0.2N NaOH at room temperature for 5 minutes. The denatured DNA was mixed with 990ul pre-chilled HT1 reagent, resulting in a 20 pM denatured library in 1 mM NaOH.

40% PhiX was added to the denatured library to increase heterogeneity of base calling, to improve read quality (Dumbrell, A. *pers. com.*). PhiX was denatured as template DNA, described above, then, 30 µl of the denatured and diluted PhiX control was added to 570 µl of the denatured and diluted amplicon library. The product was placed in a heat block at 96°C for 2 minutes, inverted twice, then stored on ice for 5 minutes before sequencing.

Illumina Sequencing of Indexed Library

DNA was sequenced on the Illumina MiSeq using the 2x300bp v3 sequencing kit. Raw reads were demultiplexed using bcl2fastq, and exported in zipped FASTQ format.

Bioinformatical Analysis of Study Sequence Data

Raw FASTQ files were quality trimmed using SICKLE version 1.33 (Joshi and Fass 2011) in paired-end mode using a minimum phred score of 25, trimming reads at the first ambiguous base (N) and only from the 3' end. Error correction of reads passing quality filtering was conducted using BayesHammer (Nikolenko et al. 2013) using the default parameters in SPAdes version 3.13.0 (Nurk et al. 2013). Forward and reverse reads were then aligned using the PEAR algorithm (Zhang et al. 2014) in PANDAseq version 2.11 (Masella et al. 2012), removing forward and reverse primers, and reads shorter than 95% of the target amplicon length. Chimeric reads were removed using the uchime function in VSEARCH version 2.14.2

(Rognes et al. 2016) in reference database mode against the MaarjAM database (Öpik et al. 2010).

All non-chimeric reads were assigned to VT using BLAST+ (Altschul et al. 1990) following the open reference OTU picking approach of Davison et al. (2015) which itself is based on the original method used by Öpik et al. (2010) to assign taxonomy to AM fungal reads in the MaarjAM database. Reads were assigned to VT based on the following criteria: sequence similarity ≥97%; query sequence coverage ≥97%; and a BLAST e-value < 1e-50, utilising soft masking with the DUST filter. Where a read received multiple hits, the best hit on the basis of the BLAST score was selected.

To negate the effect of sequence error on inflated measures of diversity, observations of AM VT which did not exceed 0.1% of the total reads for that sample were removed from analysis. Similarly, samples which failed to amplify (<100 reads) were also removed from further analysis. For sites with replicated samples, the sample with the greatest read depth was taken forward for further analysis. This was done to simplify statistical analyses, and to prevent bias for greater numbers of AM VT being observed in sites with greater numbers of samples.

Statistical Analysis of Factors Underpinning AM Fungal Diversity in UK Agriculture

Measures of total AM fungal diversity richness was recorded before rarefying samples to the median read depth. Although there has been much discussion around alternatives to rarefaction, it remains as a robust and widely used method of read standardisation, and has been shown to outperform other methods of read standardisation (deCárcer et al. 2011; Hiiesalu et al. 2014; McMurdie and Holmes 2014; Moora et al. 2014; Xiang et al. 2014; Varela-Cervero et al. 2015; Morgan and Egerton-Warburton 2017; Rodríguez-Echeverría et al. 2017; Faggioli et al. 2019). After rarefaction, the gamma diversity was not impacted, so measures of alpha and beta diversity were calculated on rarefied reads, in the Phyloseq (McMurdie and Holmes 2013) and vegan (Oksanen et al. 2013) packages in R (R Core Team 2019). Distance based analysis of community composition was calculated on Bray-Curtis distances in the R package vegan (Oksanen et al. 2013), and visualised by db-RDA and NMDS using ggplot2 (Wickham 2016). Differential abundance of AM taxa was calculated using the package DESeq2 (Love et al. 2014) in R, to identify individual virtual taxa which were more or less abundant when comparing biotic or abiotic conditions.

2.3 Results

AM Fungal Diversity in UK Agriculture

After stringent quality thresholding, 280,748 Glomeromycotinian DNA sequences remained for further analysis. In total, 84 AM fungal Virtual Taxa were identified from across the 67 independent sites in the trial, and this number remained the same when considering the additional 81 replicate samples. This set of 84 AM VT was comprised of: six *Acaulospora*; one *Ambispora*; eight *Archaeospora*; seven *Claroideoglomus*; seven *Diversispora*; 44 *Glomus*; seven *Paraglomus*, and three *Scutellospora* taxa. The distribution of AM fungal genera across the UK is shown in Figure 2.2.

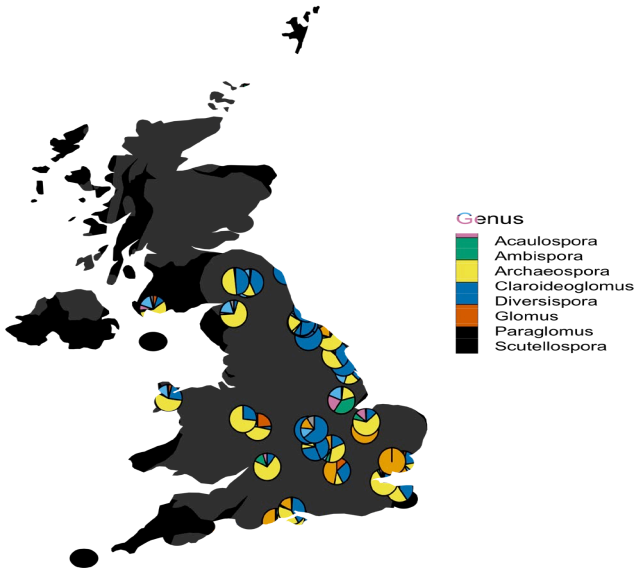

Figure 2.2 Proportion of AM Fungal Genera at the 66 farm sites
Pie charts represent proportion of AM fungal composition, at the genus level

AM Fungal Abundance by Prevalence at a Site

The top 10 most abundant taxa across the trial by prevalence at a site, were VTX00281 (*Paraglomus laccatum* - 55 sites), VTX00065 (*Glomus caledonium* - 42 sites), VTX00143 (*Glomus MO-G20* - 40 sites), VTX00245 (*Archaeospora trappei* - 36 sites), VTX00283 (*Ambispora fennica* - 31 sites), VTX00052 (*Scutellospora MO-S2* - 28 sites), VTX00005 (*Archaeospora Other1* - 23 sites), VTX00306 (*Diversispora sp.* - 23 sites), VTX00435 (*Paraglomus MO-P4* - 20 sites), and VTX00108 (*Glomus Whitfield type 7* - 18 sites). AM fungal abundance by prevalence is displayed in Figure 2.3.

Seventeen taxa were found at one site only, and included two *Archaeospora,* one *Claroideoglomus*, 12 *Glomus*, one *Paraglomus* and one *Scutellospora.* These taxa never represented more than 3.7% of the total reads at a given site on which they were found. A further fifteen taxa were found at two sites only, and included four *Acaulospora*, ten *Glomus*, and one *Paraglomus.* VTX00130 (*Glomus MO-G8*) constituted 14.3% of reads at one site in West Berkshire growing maize. VTX00155 (*Glomus acnaGlo2*) represented 37.4% of sequence reads at one site in Worcestershire, growing mixed vegetables and salads organically. VTX00072 (*Glomus MO-G16*) constituted 33.2% of reads at an organic grass pasture in East Sussex, whereas VTX00084 (*Glomus Yamato2005 D*) was 10.3% of the total community at a conventional grass pasture on the Isles of Scilly. Finally, VTX00335 (*Paraglomus Pa 1*) made up almost a quarter (24.7%) of reads at a conventional field in Kent growing oilseed rape.

Three more AM VT were found at three sites each, and were all from the genus *Glomus*. The greatest abundance of any of these *Glomus* taxa was 15.0% of reads of VTX00105 (*Glomus intraradices*), found at the same oilseed rape field in Kent.

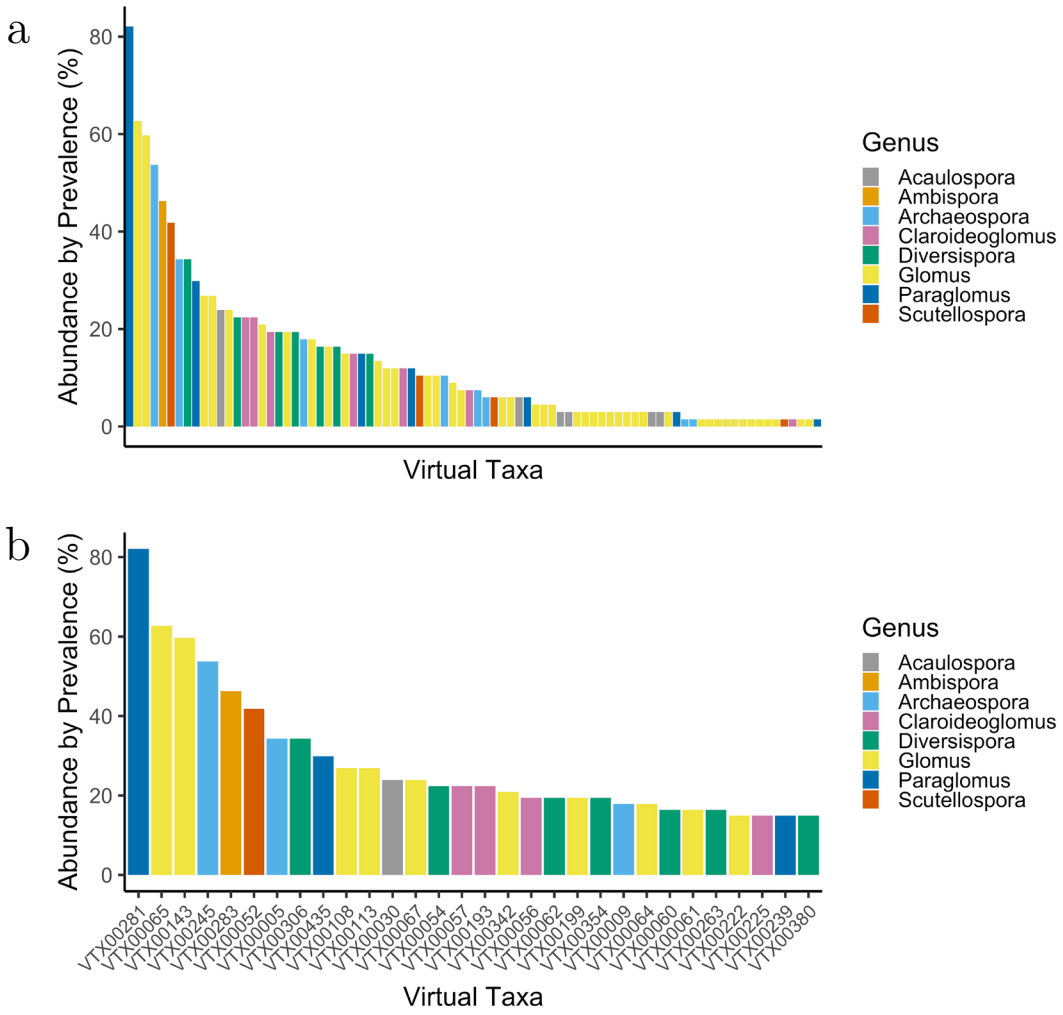

Figure 2.3 Most abundant AM fungal virtual taxa by prevalence in the FERA trial showing a) distribution of all 84 VT and b) identities of the top 30 VT
Abundance of the top AM fungal VT, expressed as a percentage of the number of times a VT appears at a site. Colours represent the genus of taxa.

AM Fungal Abundance by DNA Sequence Reads

Considering the abundance of taxa defined by the number of Illumina DNA sequence reads, VTX00281 (*Paraglomus laccatum*) dominated with 30.4% of total reads, which was seconded by VTX00065 (*Glomus caledonium*) which only constituted half as many reads (16.1%). The remaining highest abundances by DNA reads were VTX00283 (*Ambispora fennica* - 9.2%), VTX00143 (*Glomus MO-G20* - 6.9%), VTX00245 (*Archaeospora trappei* - 4.4%), VTX00052 (*Scutellospora MO-S2* - 2.8%), VTX00005 (*Archaeospora Other1* - 2.5%), VTX00067 (*Glomus mosseae* - 2.3%), VTX00306 (*Diversispora sp.* - 2.2%), and VTX00342 (*Glomus VeGlo18* - 2.2%). All of these taxa, with the exception of VTX00067 and VTX00342 were in the top 10 taxa by prevalence. VTX00067 (*Glomus mosseae*) was found at 16 sites whereas VTX00342 (*Glomus VeGlo18*) was found at 14. AM fungal abundance by abundance is displayed in Figure 2.4.

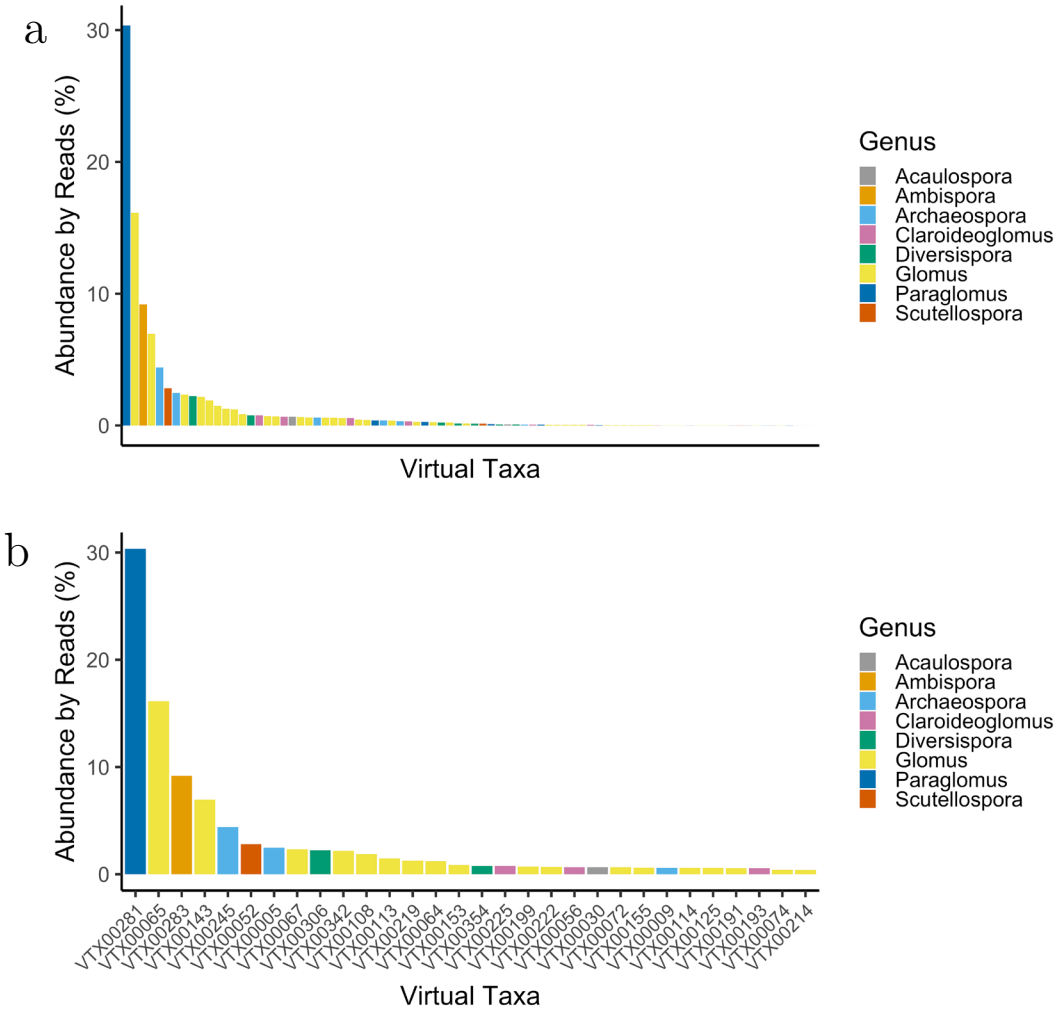

Figure 2.4 Most abundant AM fungal virtual taxa by number of DNA sequence reads in the FERA trial showing a) distribution of all 84 VT and b) identities of the top 30 VT

Abundance of the top AM fungal VT, expressed as a percentage of the number of Glomeromycotan DNA sequences amplified on the Illumina Miseq. Colours represent the genus of taxa.

VTX00435 (*Paraglomus MO-P4* - 20), and VTX00108 (*Glomus Whitfield type 7*), which were found at 20 and 18 sites respectively, did not make the top 10 taxa by DNA reads, and represented 0.26% and 1.89% of reads respectively. VTX00435, despite being the 9th most abundant my prevalence, was only the 37th most abundant by DNA reads. This AM VT was always

found at low abundance, and never constituted more than 3.5% of community composition at any given site. There was a positive correlation between the abundance, and prevalence of AM fungal taxa, however, some taxa did display high abundance but were found at very few sites (Figure 2.5).

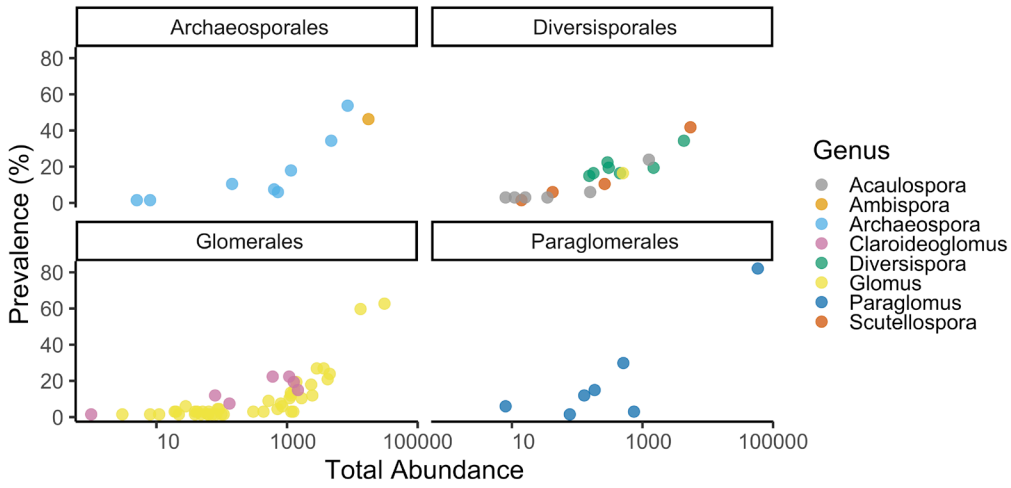

Figure 2.5 Association between total abundance of AM VT DNA reads and the prevalence of that AM VT at sites across the trial
Total abundance of DNA reads is presented on the log scale to ease interpretation. Prevalence is expressed as a percentage of the total number of sites in the trial (67).

Ordination of AM Fungal Communities in UK Agriculture

Distance-based redundancy analysis (db-RDA) is an ordination method similar to Redundancy Analysis (RDA), but it allows non-Euclidean dissimilarity indices such as Bray-Curtis distance, which is used here. Automatic forward and backward stepwise model selection was conducted, and the most parsimonious model was chosen based on model fit after 999 permutations. The full model specification included the cash crop, level of SOM, cropping system (i.e. arable/pasture), pH, whether fungicides had been applied,

whether a site was organic or conventional, soil texture, cultivation type, mean annual air temperature, and total annual precipitation. One outlier, classified as 'fruits or vines', was the only site not to classified as arable or pasture, and was subsequently removed. The site, based in Dorset, was growing raspberries but much of the other metadata was missing. The site had two observed AM VT, VTX00113 (*Glomus MO-G3*) constituted 87% of reads, with VTX00114 (*Glomus MO-G17*) proving the remainder of reads. This AM fungal community was producing significant variation in results of the below analysis, and was subsequently removed.

Impact of Cropping System on AM Fungal Communities

After 999 permutations, the optimal model found that AM fungal community composition varied by the cropping system alone (PERMANOVA: $F_{2,63}$ = 3.60, p = 0.005). To cross validate these findings, a second measure of community dissimilarity, the Analysis of Similarities (ANOSIM) between communities was conducted. This was chosen to complement PERMANOVA due to the uneven group sizes and is more robust to uneven dispersions between groups. Following 999 permutations, there was significant dissimilarity in community composition associated with the cropping system, defining whether a field was arable, pasture, or mixed (ANOSIM: R = 0.256, p = 0.004). AM fungal community composition for each cropping system is visualised in Figure 2.6.

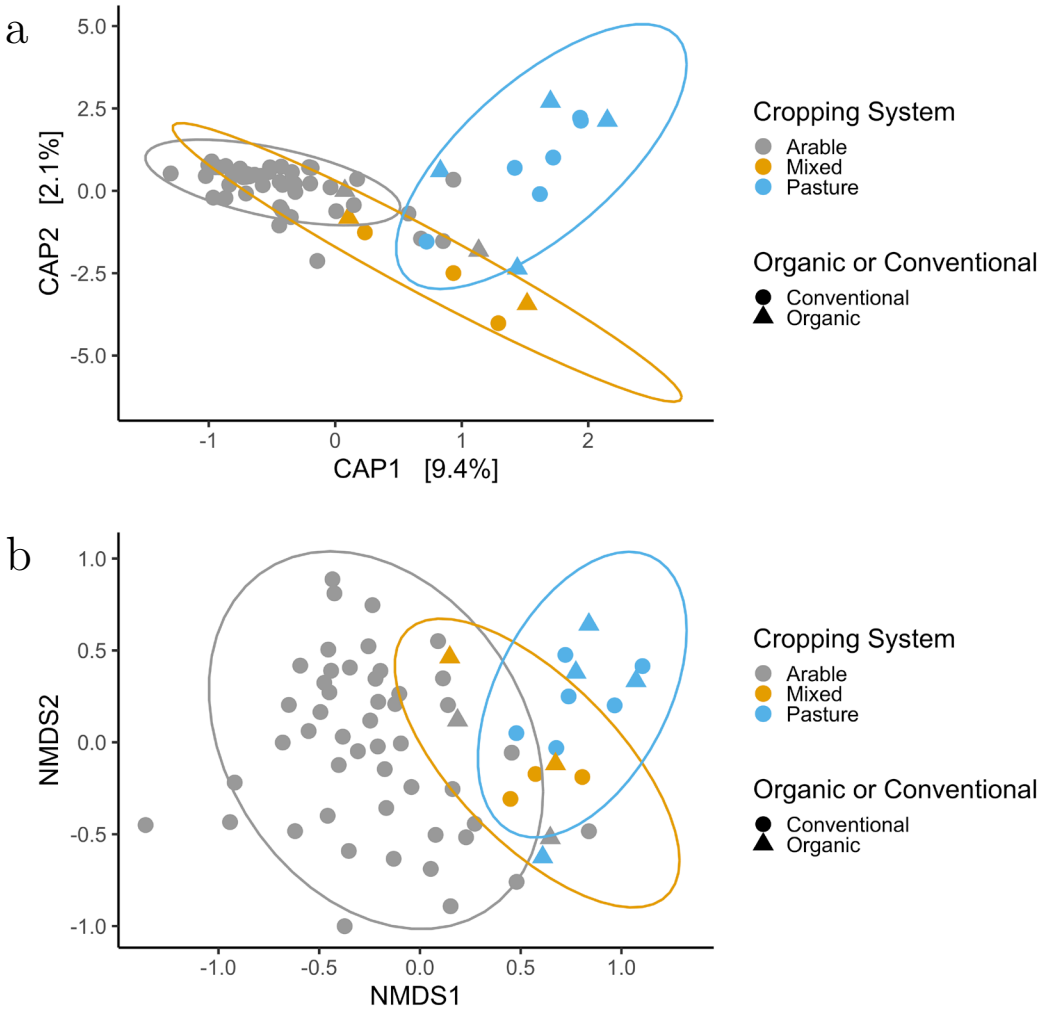

Figure 2.6 a) db-RDA ordination of Bray-Curtis distances b) NMDS ordination of Bray-Curtis distances split by the cropping system, from 66 UK agricultural sites Both distanced based RDA ($F_{2,63} = 3.60$, $p = 0.005$), and ANOSIM ($R = 0.256$, $p = 0.004$) suggested that the cropping system had a significant impact on AM fungal community composition.

Impact of Cropping System on Observed Number of AM Fungal Taxa

On average, arable sites hosted 7.7 ± 0.7 AM VT, which was lower than both the pasture (23.7 ± 2.9) and mixed cropping (19.4 ± 4.3) systems (Figure 2.7). However, the latter two groups are represented by significantly

fewer samples, so as for other mycorrhizal biodiversity observations in this chapter, care must be taken in interpretation.

The three outliers in the arable treatment were observations of 26, 20, and 19 AM VT. The greatest of these was found at Florie, a site growing wheat with non-organic methods, on a light soil texture with 2.5-5% organic matter. The site had minimal soil disturbance but used all recorded fungicides, including Chlorothalonil, Prothioconazole, Tebuconazole, Epoxiconazole, Fluxapyroxad, and Boscalid. The second largest observed number of AM taxa from an arable site was 20, recorded at a field growing non-organic barley in Aberdeenshire. Like the Florie site, the soil texture was classed as light and the organic matter levels were 2.5-5%. There was moderate soil disturbance and both Chlorothalonil and Prothioconazole fungicides were used. The third outlier of 19 AM VT was from a non-organic wheat field in Hampshire. The site had a medium soil texture with organic matter levels of 5-7.5%. There was mild soil disturbance and the farmer had used Chlorothalonil, Prothioconazole, and Tubuconazole at the site.

Similarly, the lowest recorded observed AM VT in the pasture sites was 6, at a site in Hampshire with a light soil texture and 1-2.5% SOM. No fungicides had been used at the site, but was classified as having moderate disturbance, due to the fact that the site was a restored quarry. The next lowest number of AM VT observed at a pasture site was 11.

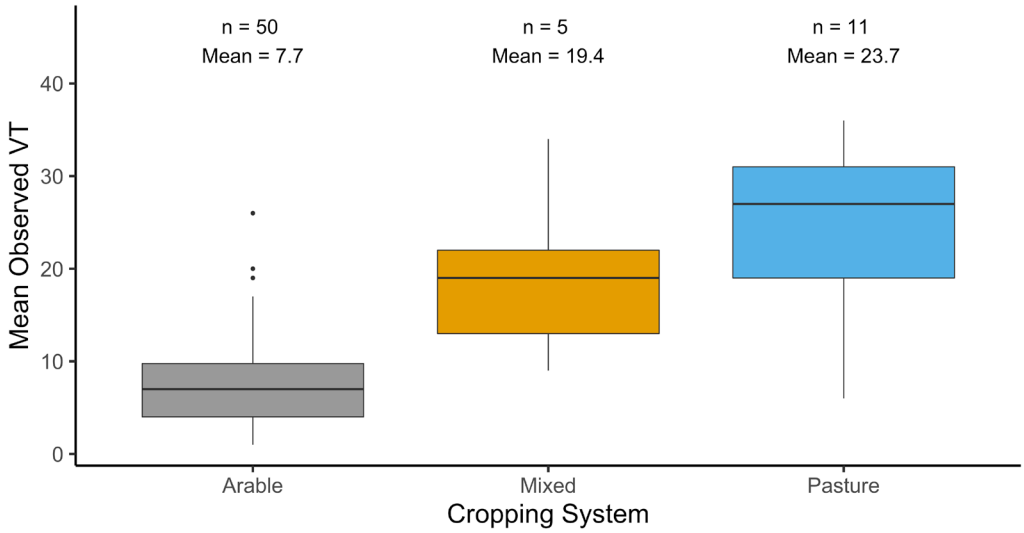

Figure 2.7 Mean observed AM fungal virtual taxa partitioned by cropping system
Mean AM fungal VT observed at the arable, pasture, or mixed sites. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Nine taxa, all belonging to the genus *Glomus* were found exclusively in arable sites (Table 2.1). A further three taxa, one *Glomus*, one *Claroideoglomus*, and one *Archaeospora* were found in the mixed cropping sites. Despite their underrepresentation compared to arable sites, the pasture sites hosted 24 taxa that were not found in the arable, or mixed systems. These included 14 taxa belonging to the *Glomus*, five from *Acaulospora*, two from *Scutellospora*, two from *Archaeospora*, and one from *Paraglomus*. Three more taxa, including two *Glomus* and one *Archaeospora* were found in both the pasture and mixed cropping sites (Figure 2.8). Five taxa were found between the arable and mixed systems, and included three *Glomus* and two *Diversispora*. Another seven taxa, including three *Glomus* and four *Paraglomus* were found in both arable and pasture sites. A further 33 AM VT were found in all three cropping systems, 11 Glomus, six *Diversispora*, two *Scutellospora*,

one *Acaulospora*, six *Claroideoglomus*, two *Paraglomus*, four *Archaeospora*, and one *Ambispora* taxa.

Table 2.1 AM Fungal VT occurring in each level of cropping system

All	Arable (n=50)	Mixed (n=5)	Pasture (n=11)	Arable & Mixed	Arable & Pasture	Mixed & Pasture
VTX00125	VTX00140	VTX00063	VTX00166	VTX00130	VTX00153	VTX00135
VTX00222	VTX00137	VTX00340	VTX00103	VTX00155	VTX00163	VTX00115
VTX00143	VTX00154	VTX00008	VTX00202	VTX00342	VTX00309	VTX00456
VTX00199	VTX00167		VTX00366	VTX00054	VTX00239	
VTX00113	VTX00148		VTX00149	VTX00354	VTX00349	
VTX00114	VTX00105		VTX00078		VTX00335	
VTX00108	VTX00295		VTX00191		VTX00444	
VTX00214	VTX00409		VTX00219			
VTX00065	VTX00293		VTX00074			
VTX00067			VTX00223			
VTX00064			VTX00072			
VTX00263			VTX00325			
VTX00062			VTX00084			
VTX00306			VTX00212			
VTX00060			VTX00049			
VTX00061			VTX00318			
VTX00380			VTX00015			
VTX00041			VTX00231			
VTX00052			VTX00230			
VTX00030			VTX00228			
VTX00057			VTX00010			
VTX00193			VTX00446			
VTX00056			VTX00007			

VTX00225	VTX00004
VTX00276	
VTX00278	
VTX00281	
VTX00435	
VTX00245	
VTX00338	
VTX00009	
VTX00005	
VTX00283	

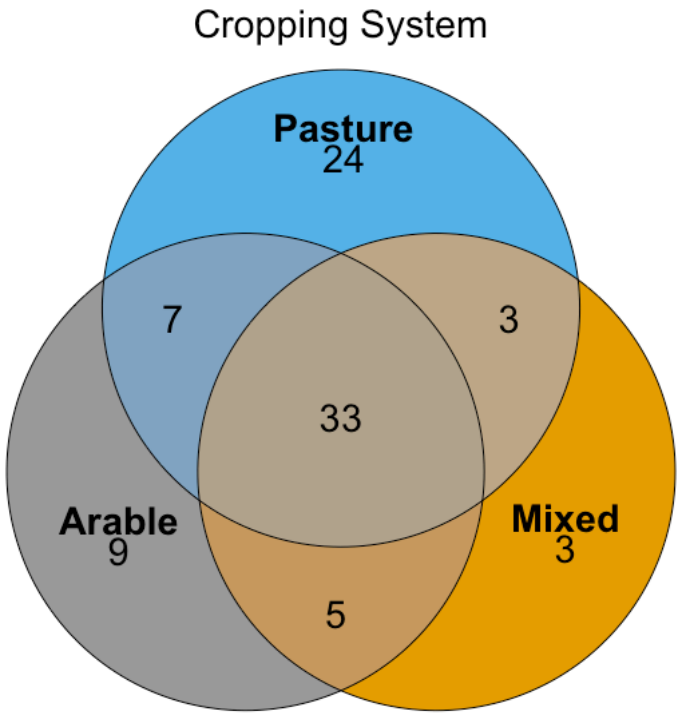

Figure 2.8 Number of AM fungal virtual observed in the different cropping systems Venn classification of AM fungal VT observed in each cropping system. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Impact of Cropping System on AM Fungal Community Composition and Analysis of Differential Abundance

Mean AM fungal community composition of the 50 arable sites consisted of 36.7% *Glomus*, 35.1% *Paraglomus*, 11.9% *Ambispora*, 7.5%, *Archaeospora*, 3.6% *Diversispora*, 3.3% *Scutellospora*, 1.3% *Claroideoglomus*, and 0.6% *Acaulospora*. In the mixed cropping systems (5 sites), *Glomus* was significantly more abundant (53.6%), whereas *Paraglomus*, *Ambispora* and *Archaeospora* were all considerably less abundant (Table 2.2). In the pasture cropping system, represented by 11 sites, *Glomus* remained as the dominant genus, at 52.3% of reads. *Paraglomus* and *Ambispora* taxa were reduced in comparison to the arable sites, with *Ambispora* only constituting 0.5% of reads. *Archaeospora*, which constituted 7.5% and 1.3% of reads in the arable and mixed samples, was increased to 15.7% of reads in the pasture samples.

Table 2.2 Composition of AM fungal genera at each level of cropping system.

Genus	Proportion of Genus in Each Cropping System (%)		
	Arable (n=50)	Mixed (n=5)	Pasture (n=11)
Acaulospora	0.6	0.9	1.4
Ambispora	11.9	3.2	0.5
Archaeospora	7.5	1.3	15.7
Claroideoglomus	1.3	8.6	4.7
Diversispora	3.6	8.8	2.5
Glomus	36.7	53.6	52.3
Paraglomus	35.1	21.6	20.6
Scutellospora	3.3	2.0	2.3

The five most abundant taxa across the arable sites were VTX00281 (34.4% - *Paraglomus laccatum*), VTX00065 (20.5% - *Glomus caledonium*), VTX00283 (11.9% - *Ambispora fennica*), VTX00245 (5.7% - *Archaeospora trappei*), and VTX00143 (5.1% - *Glomus MO-G20*). The five most abundant taxa across the pasture sites were VTX00281 (18.5% - *Paraglomus laccatum*), VTX00005 (11.6% - *Archaeospora Other1*), VTX00143 (9.9% - *Glomus MO-G20*), VTX00219 (7.7% - *Glomus MO-G5*), and VTX00108 (5.7% - *Glomus Whitfield type 7*). Finally, in the mixed cropping system, the most abundant AM VT was also VTX00281 (21.5% - *Paraglomus laccatum*), followed by VTX00143 (20.4% - *Glomus MO-G20*), VTX00108 (10.2% - *Glomus Whitfield type 7*), VTX00306 (6.7% - *Diversispora sp.*), and VTX00225 (5.6% - *Claroideoglomus Glo58*). The relative abundance of AM fungal VT, split by cropping system, is shown in Figure 2.9.

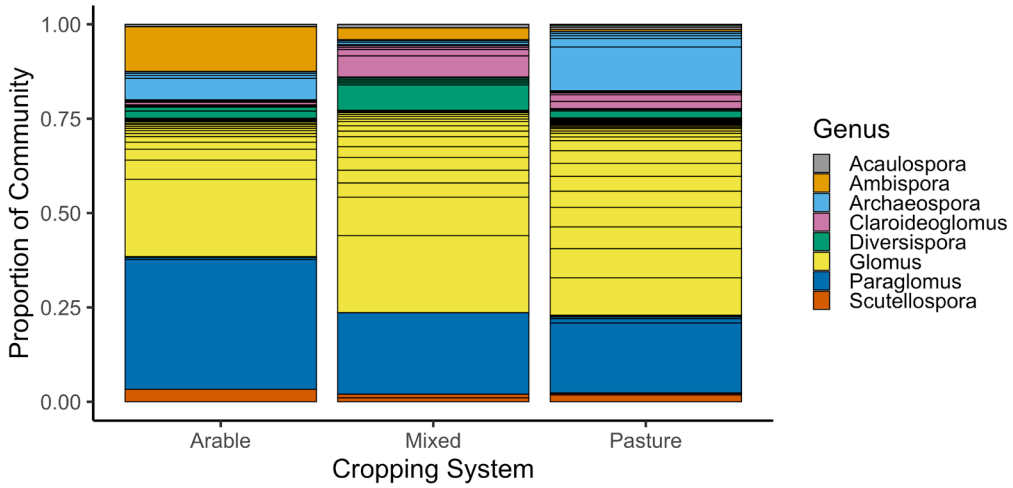

Figure 2.9 AM Fungal community composition partitioned by cropping system Mean community composition of AM fungal VT for each cropping system, expressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT.

Analysis of differential abundance of virtual taxa, using shrinkage estimation was conducted to further confirm how individual taxa had responded to different cropping systems. There were 10 differentially abundant AM VT between the arable and pasture cropping systems, three which were more abundant in arable, and seven which were more abundant in pasture sites (Figure 2.10). The three AM VT which were more abundant in the arable cropping system were VTX00342 (*Glomus* VeGlo18, $\log_2$FC = 24.85, $p <$ 0.001), VTX00354 (*Diversispora Clade3*, $\log_2$FC = 23.41, $p < 0.001$), and VTX00065 (*Glomus caledonium,* $\log_2$FC = 4.88, $p < 0.001$). The seven AM VT which were more abundant in the pasture cropping system were VTX00005 (*Archaeospora Other1,* $\log_2$FC = -4.67, $p = 0.043$), VTX00153 (*Glomus MO-G12 ,* $\log_2$FC = -8.67, $p = 0.002$), VTX00191 (*Glomus MO-G31,* $\log_2$FC = -9.62, $p < 0.001$), VTX00219 (*Glomus MO-G5,* $\log_2$FC = -

10.90, $p < 0.001$), VTX00072 (*Glomus MO-G16,* $\log_2$FC = -25.96, $p < 0.001$), VTX00004 (*Archaeospora Wirsel OTU21,* $\log_2$FC = -26.68, $p < 0.001$), and VTX00074 (*Glomus Glo3,* $\log_2$FC = -27.52, $p < 0.001$).

There was only one species which was significantly more abundant in the arable cropping system compared to a mixed cropping system, VTX00153 (*Glomus MO-G12,* $\log_2$FC = 16.58, $p = 0.003$). There were seven differentially abundant species between the pasture and mixed cropping systems. Five species, VTX00074 (*Glomus Glo3,* $\log_2$FC = 29.50, $p < 0.001$), VTX00004 (*Archaeospora Wirsel OTU21,* $\log_2$FC = 27.44, $p < 0.001$), VTX00153 (*Glomus MO-G12,* $\log_2$FC = 25.26, $p < 0.001$), VTX00072 (*Glomus MO-G16,* $\log_2$FC = 24.44, $p < 0.001$), and VTX00219 (*Glomus MO-G5,* $\log_2$FC = 10.51, $p = 0.026$) were all significantly more abundant in pasture sites, when compared to mixed cropping sites. Two taxa, VTX00354 (*Diversispora Clade-3,* $\log_2$FC = -19.43, $p < 0.001$) and VTX00342 (*Glomus VeGlo18,* $\log_2$FC = -22.53, $p < 0.001$) were significantly more abundant in the mixed cropping systems

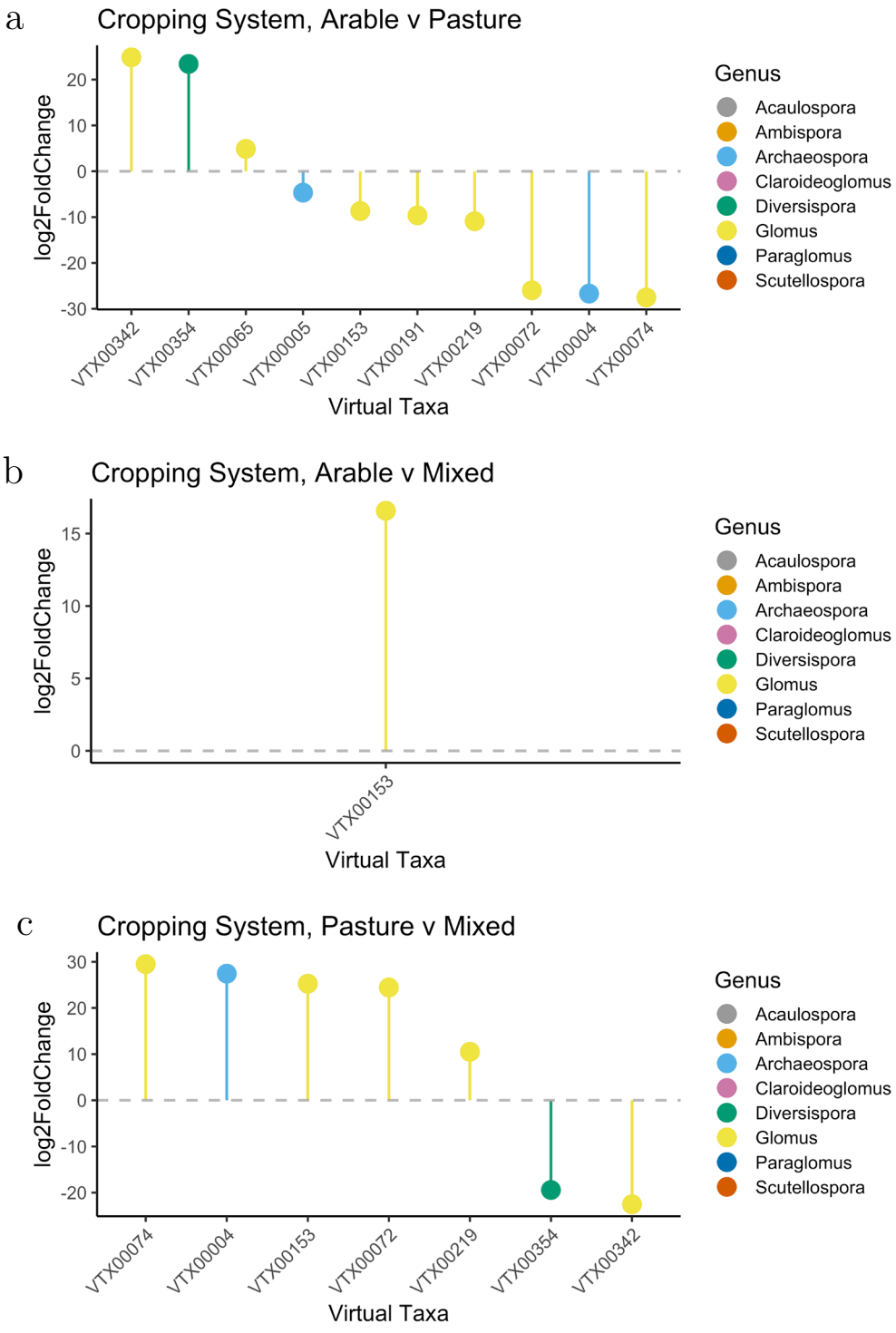

Figure 2.10 Differential abundance of AM Fungal VT by cropping system comparing a) arable and pasture sites, b) arable and mixed sites, and c) pasture and mixed sites Differential abundance of AM fungal VT comparing different cropping systems, calculated by DESeq2. AM VT were considered to be differentially abundant if their Log2FC > 1 and $p < 0.05$.

Other Factors Underpinning AM Fungal Diversity in Different Cropping Systems

To further investigate the factors which may underpin this relationship, the crop system was removed from the model, but the crop species was retained. In effect, the pasture cropping system becomes a single factor within crop species, which is retained in the model.

After 999 permutations, the optimal model (PERMANOVA: $F_{8,57} = 2.01$, $p = 0.001$) found that AM fungal communities varied by soil texture ($F_{2,57} = 1.77$, $p = 0.035$), type of cultivation ($F_{4,57} = 1.57$, $p = 0.030$), and whether fungicides had been applied to the crop ($F_{2,57} = 2.88$, $p = 0.005$). To validate these findings, a second measure of community dissimilarity, the ANOSIM was again conducted. Results of this analysis are shown below.

Impact of Cultivation Type on AM Fungal Communities

To validate these findings, a second measure of community dissimilarity, the ANOSIM was conducted. Despite being identified as a significant predictor of community composition in the db-RDA analysis, after 999 permutations, there was no significant partition in community composition between levels of the type of cultivation used (ANOSIM: $R = 0.027$, $p = 0.288$).

Table 2.3 Classification of levels of Soil Cultivation
Soil cultivation was factorised by farmers into five levels, based on the guidance and examples from FERA in the questionnaire provided to farmers.

Soil Disturbance	Description	Examples
No Soil Disturbance	Permanent pasture	Non cultivated
Minimal Disturbance	Only seeding point disturbed, light trafficking	Zero tillage
Mild Disturbance	Top 20cm disturbed, light trafficking	Minimum tillage
Moderate Disturbance	Deep ploughing, heavy trafficking	Mixed across years; annual deep plough
Heavy Disturbance	Deep ploughing, heavy trafficking, veg production (destoning, high compaction, multiple passes.	Potato/ sugar beet in rotation

Type of cultivation was partitioned into five levels, 'no soil disturbance', 'minimal disturbance', 'mild disturbance', 'moderate disturbance', and 'heavy disturbance', which was represented by 9, 9, 16, 29, and 3 sites respectively. These groupings correspond approximately to the examples given to farmers in the questionnaire, in Table 2.3. Within the 50 arable sites, three were classified as having received heavy disturbance, 22 moderate disturbance, 16 mild disturbance, 9 minimal disturbance, and zero with no disturbance. The five mixed cropping systems all fell within the moderate cultivation disturbance group. Finally, of the 11 pasture cropping sites, nine were classified as having zero soil disturbance, and two had moderate soil disturbance. Bray-Curtis distances were plotted by NMDS and db-RDA methods, to visualise clustering of AM fungal communities and shown in Figure 2.11.

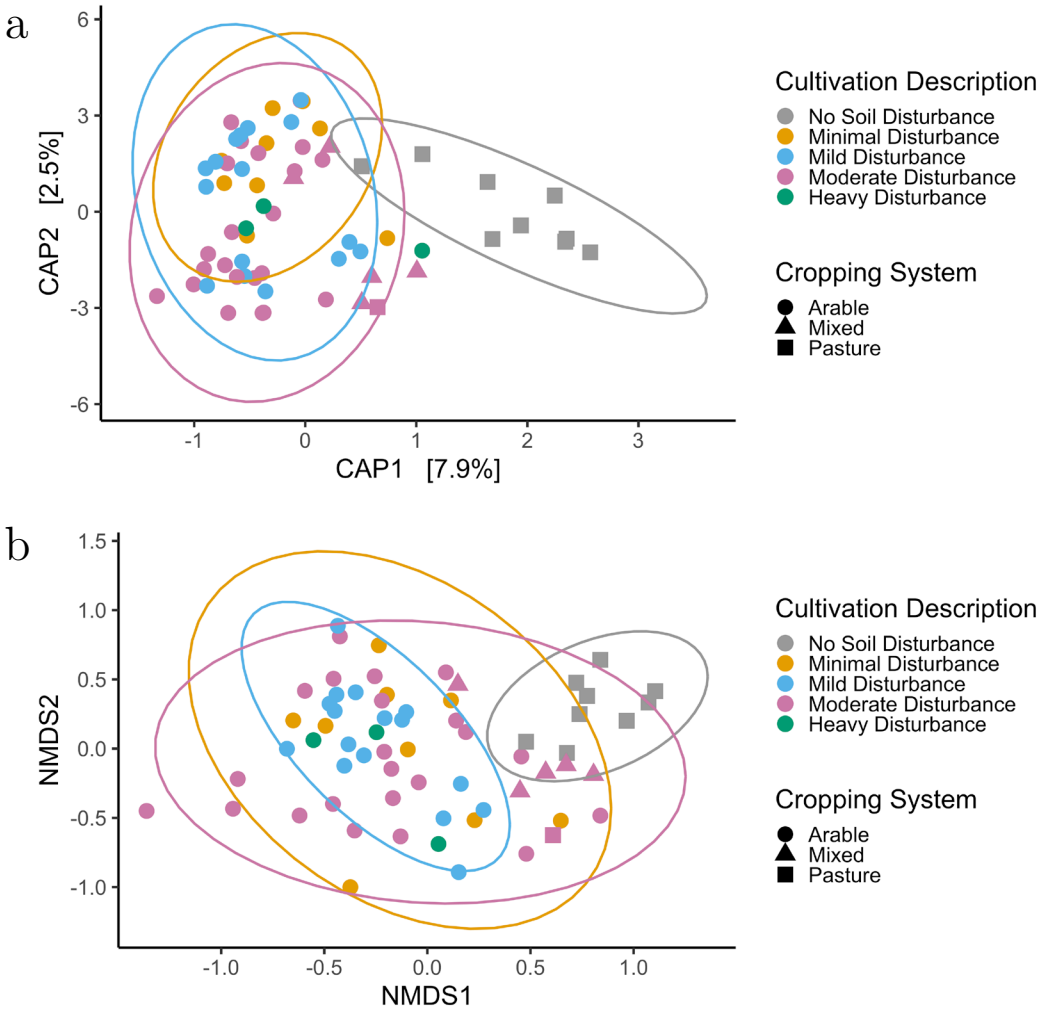

Figure 2.11 a) db-RDA ordination of Bray-Curtis distances b) NMDS ordination of Bray-Curtis distances split by the type of cultivation, from 66 UK agricultural sites Distanced based RDA ($F_{4,57} = 1.57$, $p = 0.030$), suggested that the type of cultivation had a significant impact on AM fungal community composition. However, ANOSIM found no significant dissimilarity between groups ($R = 0.027$, $p = 0.288$).

Impact of Cultivation Type on Observed Number of AM Fungal Taxa

The greatest mean number of AM fungal VT was observed at sites with no soil disturbance (27.1 ± 2.2, Figure 2.12). This was followed by moderate disturbance (9.8 ± 1.3), minimal disturbance (9.2 ± 2.3), heavy disturbance (7.0 ± 2.5), then mild disturbance (6.9 ± 1.1). The single outlier in the

minimal disturbance category was the Florie site (26 taxa) growing wheat with non-organic methods, which has previously been described. The three outliers in the mild disturbance included the previously described non-organic wheat field in Hampshire (19 taxa), an arable site in Dorset growing rye, on a medium soil texture with 2.5-5% SOM, and a site in North Yorkshire growing oilseed rape on a medium soil texture with 2.5-5% SOM. Finally, the single outlier in the moderate disturbance category was a mixed cropping site in Aberdeenshire (34 taxa), which runs a crop rotation including grass leys. The field was in grass, and had a medium soil texture with SOM of 5-7.5%. The site had not used any fungicides, but was not classed as organic.

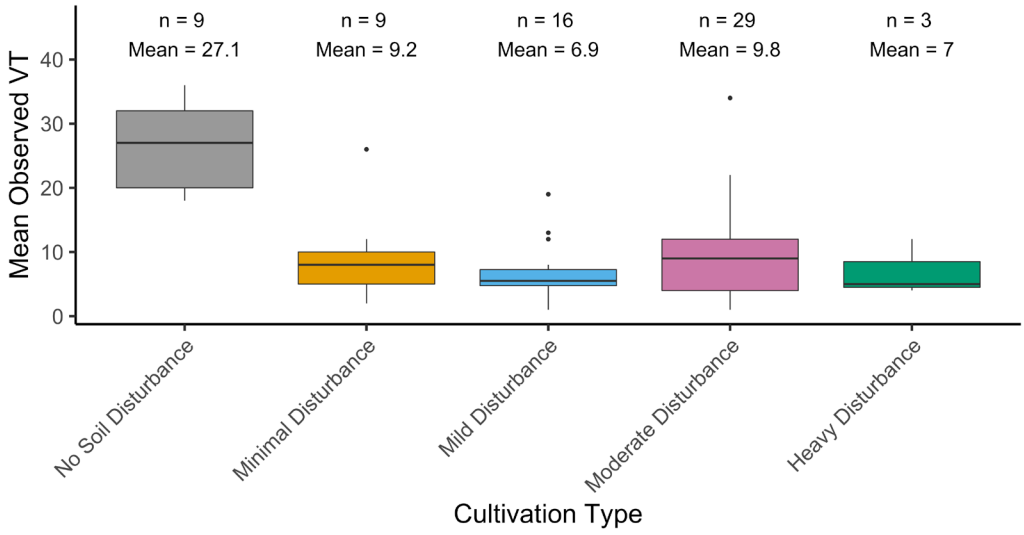

Figure 2.12 Mean observed AM fungal virtual taxa partitioned by type of cultivation

Mean AM fungal VT observed at the sites with no soil disturbance, to heavy soil disturbance. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Ten AM fungal VT were found in sites with all levels of soil disturbance, from zero to heavy disturbance (Table 2.4). These included six *Glomus*, and one of each of *Scutellospora*, *Acaulospora*, *Paraglomus*, and *Archaeospora*. Twenty-three AM VT were found exclusively in sites with no disturbance, and included 14 *Glomus*, five *Acaulospora*, two *Scutellospora*, and two *Archaeospora*, which correlated very closely with those classified as pasture in the above analysis. Four taxa were found at sites with minimal disturbance only, all from the genus *Glomus*. None of the 16 sites classified as having mild cultivation had any distinct taxa. Six taxa, including three *Glomus*, and one each of *Claroideoglomus*, *Paraglomus*, and *Archaeospora* were found exclusively at sites with moderate soil disturbance. Finally, one taxon VTX00148 (*Glomus JP5*) was found at one site in Staffordshire with a cereal-vegetable crop rotation with heavy soil disturbance. Observed numbers of taxa for each cultivation type is visualised in Figure 2.13.

Table 2.4 AM Fungal VT occurring in each level of Cultivation Type.

All	No Soil Disturbance (n=9)	Minimal Disturbance (n=9)	Mild Disturbance (n=16)	Moderate Disturbance (n=29)	Heavy Disturbance (n=3)
VTX00143	VTX00166	VTX00154		VTX00140	VTX00148
VTX00199	VTX00103	VTX00167		VTX00137	
VTX00114	VTX00202	VTX00409		VTX00063	
VTX00214	VTX00366	VTX00293		VTX00340	
VTX00065	VTX00149			VTX00446	
VTX00064	VTX00078			VTX00008	
VTX00052	VTX00191				
VTX00281	VTX00219				
VTX00005	VTX00074				
VTX00283	VTX00223				
	VTX00072				
	VTX00325				
	VTX00084				
	VTX00212				
	VTX00049				
	VTX00318				
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	VTX00004				

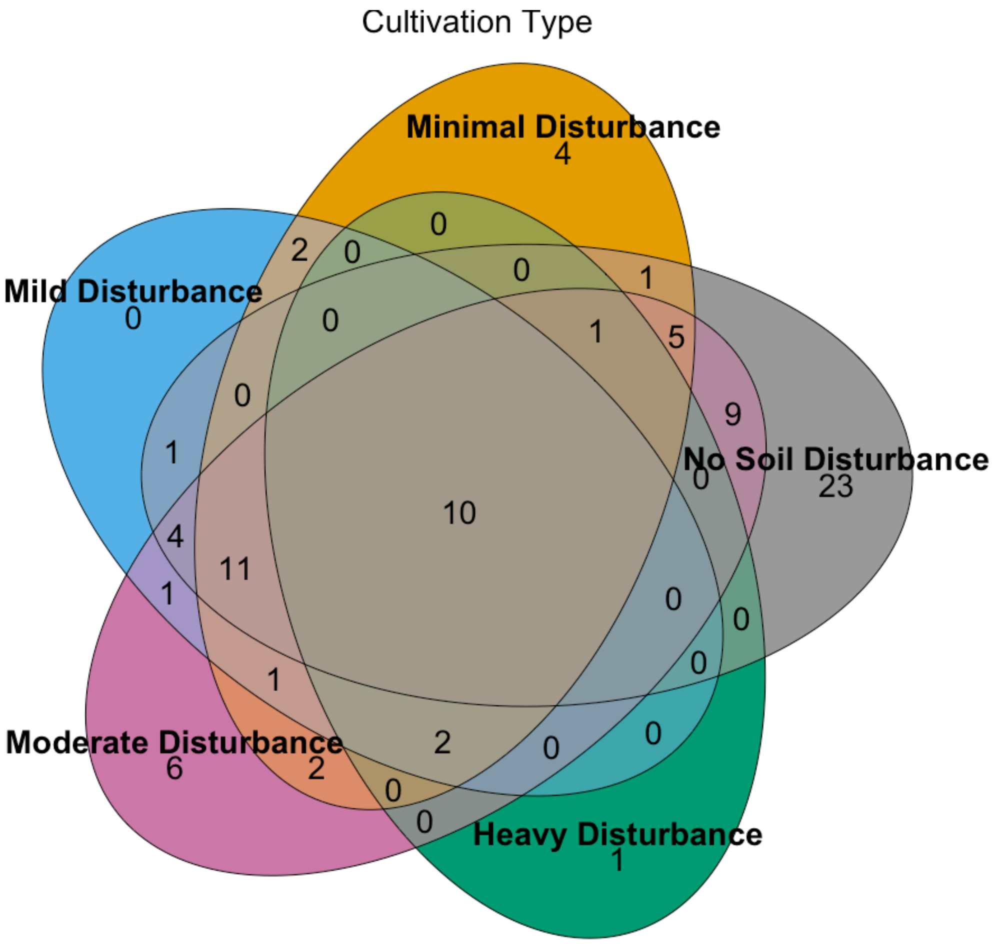


Figure 2.13 Number of AM fungal virtual observed in the different types of cultivation

Venn classification of AM fungal VT observed in each type of cultivation. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Impact of Cultivation Type on AM Fungal Community Composition and Analysis of Differential Abundance

Mean AM fungal community composition of the nine sites with zero soil disturbance was 46.2% *Glomus*, 24.8% *Paraglomus*, 18.8% *Archaeospora,* 4.8% *Claroideoglomus,* 2.2% *Scutellospora,* 1.6% *Diversispora,* 1.3% *Acaulospora*, and 0.4% *Ambispora* (Table 2.5). In the nine sites with minimal

disturbance, reads of *Archaeospora* and *Glomus* were greatly reduced, being replaced by a 27.6% increase in *Paraglomus* reads. *Paraglomus* (39.2%) remained the dominant genus in the 16 mild disturbance sites, which exhibited increased reads from Ambispora (+7.3%) and Glomus (+10.4%), compared to minimal disturbance sites. In the 29 sites with moderate disturbance, Glomus was the dominant genus, with 42.4% of reads, followed by *Paraglomus* with 24.4% and *Ambispora* with 13.0% of reads. Reads of *Diversispora* were reduced, while *Scutellospora* increased. *Acaulospora* and *Claroideoglomus* were not identified in these three samples. There was a positive relationship between the increasing level of disturbance and the abundance of *Ambispora*, increasing from 0.4 to 16.8% of reads. In a similar but less severe manner, *Scutellospora* increased in abundance at the moderate and heavy disturbance sites, although caution should be employed when considering this result as the heavy disturbance sites were only represented by three samples.

Table 2.5 Composition of AM fungal genera at each level of Cultivation Type.

	Proportion of Genus from Each Cultivation Type (%)				
Genus	No Disturbance (n=9)	Minimal Disturbance (n=9)	Mild Disturbance (n=16)	Moderate Disturbance (n=29)	Heavy Disturbance (n=3)
Acaulospora	1.3	0.1	0.1	1.3	0
Ambispora	0.4	2.7	10.0	13.0	16.8
Archaeospora	18.8	3.6	9.2	6.4	6.3
Claroideoglomus	4.8	1.2	0.9	3.2	0
Diversispora	1.6	2.7	2.8	5.7	0.2
Glomus	46.2	25.7	36.1	42.4	44.1
Paraglomus	24.8	52.4	39.2	24.4	20.3
Scutellospora	2.2	1.6	1.7	3.5	12.3

The five most abundant taxa at the nine no soil disturbance sites were VTX00281 (22.6%, *Paraglomus laccatum*), followed by VTX00005 (14.1%, *Archaeospora Other1*), VTX00143 (9.5%, *Glomus MO-G20*), VTX00219 (9.4%, *Glomus MO-G5*), and VTX00153 (5.9%, *Glomus MO-G12*). Of these, VTX00005, VTX00219, and VTX00153 were only amongst the top 5 taxa for the zero disturbance group. VTX00281 (49.5%, *Paraglomus laccatum*) then dominated reads in the minimal disturbance, followed by VTX00065 (14.5%, *Glomus caledonium*), VTX00143 (4.8%, *Glomus MO-G20*), VTX00155 (4.2%, *Glomus acnaGlo2*), and VTX00335 (2.7%, *Paraglomus Pa1*), which only made the top five taxa in this group. In the 16 sites with mild disturbance, VTX00281 (39.0%, *Paraglomus laccatum*), and

VTX00065 (20.6%, *Glomus caledonium*) remain the dominant two taxa. VTX00143 (6.3%, *Glomus MO-G20*) is still found, but two additional taxa, VTX00283 (10.0%, *Ambispora fennica*) and VTX00245 (8.7%, *Archaeospora trappei*) are within the five most abundant for this level of cultivation. In the 29 moderate disturbance sites, the dominant taxa are the previously mentioned VTX00281 (24.1%, *Paraglomus laccatum*), VTX00065 (19.3%, *Glomus caledonium*), VTX00283 (13.0%, *Ambispora fennica*), and VTX00143 (7.3%, *Glomus MO-G20*), with the addition of VTX00245 (*Archaeospora trappei*), comprising 5.2% of reads. Finally, in the heavy disturbance sites, VTX00281 (*Paraglomus laccatum*) comprised 20.3% of reads, followed by VTX00065 (19.2%, *Glomus caledonium*), VTX00283 (16.8%, *Ambispora fennica*), VTX00052 (12.3%, *Scutellospora MO-S2*), and VTX00143 (8.0%, *Glomus MO-G20*). VTX00052 (*Scutellospora MO-S2*) was not amongst the top 5 taxa in any of the other cultivation levels. The relative abundance of AM fungal VT, split by cultivation type, is shown in Figure 2.14.

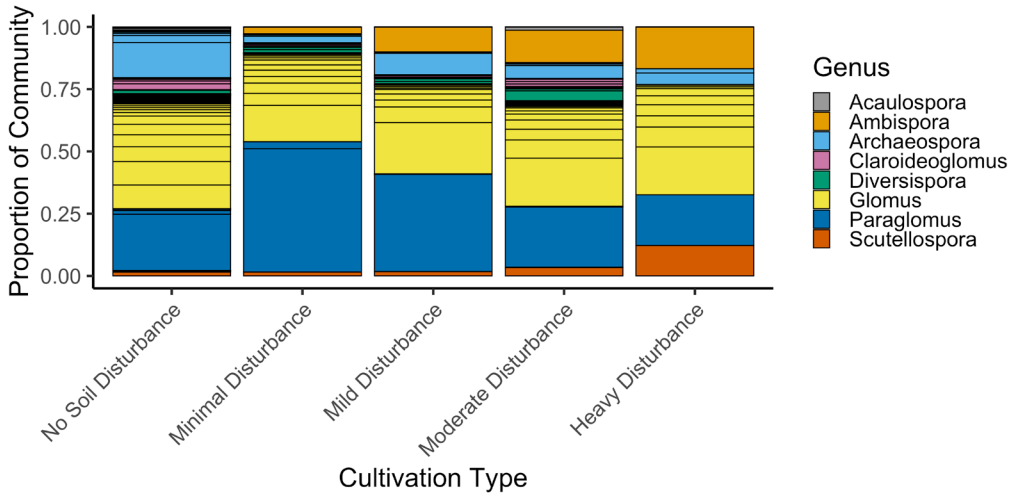


Figure 2.14 AM Fungal community composition partitioned by type of cultivation Mean community composition of AM fungal VT for each level of cultivation, ex- pressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT.

Analysis of differential abundance revealed that nine AM fungal VT were significantly more abundant in the sites with zero disturbance, compared to those with minimal disturbance (Figure 2.15). These included VTX00153 (*Glomus MO-G12*, $\log_2$FC = 28.23, $p < 0.001$), VTX00074 (*Glomus Glo3,* $\log_2$FC = 26.85, $p < 0.001$), VTX00004 (*Archaeospora Wirsel OTU21,* $\log_2$FC = 25.72, $p < 0.001$), VTX00072 (*Glomus MO-G16,* $\log_2$FC = 25.50, $p < 0.001$), VTX00225 (*Claroideoglomus Glo58,* $\log_2$FC = 25.33, $p < 0.001$), VTX00309 (*Glomus ORVIN GLO3E,* $\log_2$FC = 23.94, $p < 0.001$), VTX00219 (*Glomus MO-G5,* $\log_2$FC = 11.31, $p < 0.001$), VTX00191 (*Glomus MO-G31,* $\log_2$FC = 10.03, $p = 0.011$), and VTX00113 (*Glomus MO-G3,* $\log_2$FC = 7.62, $p = 0.009$). Three taxa, VTX00065 (*Glomus caledonium,* $\log_2$FC = 5.77, $p < 0.001$), VTX00354 (*Diversispora Clade-3,* $\log_2$FC = 20.62, $p < 0.001$), and VTX00342 (*Glomus VeGlo18,* $\log_2$FC = 22.86, $p < 0.001$)

were significantly more abundant in those sites receiving minimal soil disturbance. Comparing sites receiving mild and zero soil disturbance, nine AM VT were significantly more abundant in the zero disturbance sites. Eight out of nine of these overlapped with the previous zero vs minimal comparison, and included VTX00153 (*Glomus MO-G12,* $\log_2$FC = 28.08, $p < 0.001$), VTX00074 (*Glomus Glo3,* $\log_2$FC = 26.72, $p < 0.001$), VTX00004 (*Archaeospora Wirsel OTU21,* $\log_2$FC = 25.69, $p < 0.001$), VTX00072 (*Glomus MO-G16,* $\log_2$FC = 25.30, $p < 0.001$), VTX00309 (*Glomus ORVIN GLO3E,* $\log_2$FC = 24.22, $p < 0.001$), VTX00225 (*Claroideoglomus Glo58,* $\log_2$FC = 24.04, $p < 0.001$), VTX00219 (*Glomus MO-G5,* $\log_2$FC = 11.16, $p < 0.001$), and VTX00191 (*Glomus MO-G31,* $\log_2$FC = 9.88, $p = 0.004$). The AM taxon VTX00113 was replaced by VTX00108 (*Glomus Whitfield type 7,* $\log_2$FC = 8.69, $p < 0.001$) in this comparison. The same three AM VT were more abundant in the sites receiving a mild cultivation, and included VTX00065 (*Glomus caledonium,* $\log_2$FC = 6.62, $p < 0.001$), VTX00354 (*Diversispora Clade-3,* $\log_2$FC = 18.82, $p < 0.001$), and VTX00342 (*Glomus VeGlo18,* $\log_2$FC = 21.77, $p < 0.001$). In the comparison of moderate and zero disturbance, five VT were more abundant in the zero disturbance group, including four *glomus*: VTX00074 (*Glomus Glo3,* $\log_2$FC = 27.88, $p < 0.001$), VTX00072 (*Glomus MO-G16,* $\log_2$FC = 27.63, $p < 0.001$), VTX00219 (*Glomus MO-G5,* $\log_2$FC = 11.11, $p < 0.001$), and VTX00191 (*Glomus MO-G31,* $\log_2$FC = 9.83, $p = 0.002$), and one *Archaeospora* VTX00004 (*Archaeospora Wirsel OTU21,* $\log_2$FC = 27.37, $p < 0.001$). Once more, three taxa, VTX00065 (*Glomus caledonium,* $\log_2$FC = 6.60, $p < 0.001$), VTX00354 (*Diversispora Clade-3,* $\log_2$FC = 20.63, $p < 0.001$), and VTX00342 (*Glomus*

VeGlo18, $\log_2$FC = 19.74, $p < 0.001$) were significantly more abundant in those sites receiving moderate soil disturbance. The largest number of differentially abundant taxa were seen between the heavy and zero cultivation sites. Twelve AM fungal VT were significantly more abundant in the zero cultivation sites, compared to the heavy cultivation sites. These included VTX00225 (*Claroideoglomus Glo58,* $\log_2$FC = 29.33, $p < 0.001$), VTX00153 (*Glomus MO-G12,* $\log_2$FC = 25.74, $p < 0.001$), VTX00074 (*Glomus Glo3,* $\log_2$FC = 25.30, $p < 0.001$), VTX00004 (*Archaeospora Wirsel OTU21,* $\log_2$FC = 24.16, $p = 0.001$), VTX00072 (*Glomus MO-G16,* $\log_2$FC = 23.92, $p = 0.002$), VTX00108 (*Glomus Whitfield type 7,* $\log_2$FC = 23.73, $p < 0.001$), VTX00306 (*Diversispora sp.,* $\log_2$FC = 23.31, $p < 0.001$), VTX00245 (*Archaeospora trappei,* $\log_2$FC = 22.16, $p < 0.001$), VTX00309 (*Glomus ORVIN GLO3E,* $\log_2$FC = 22.12, $p = 0.003$), VTX00067 (*Glomus mosseae,* $\log_2$FC = 21.59, $p < 0.001$), VTX00219 (*Glomus MO-G5,* $\log_2$FC = 12.15, $p = 0.007$), and VTX00435 (*Paraglomus MO-P4,* $\log_2$FC = 9.54, $p = 0.023$). Two *Glomus* were significantly more abundant in the sites receiving heavy disturbance, and included VTX00065 (*Glomus caledonium,* $\log_2$FC = 5.76, $p = 0.018$) and VTX00342 (*Glomus VeGlo18,* $\log_2$FC = 20.49, $p < 0.001$).

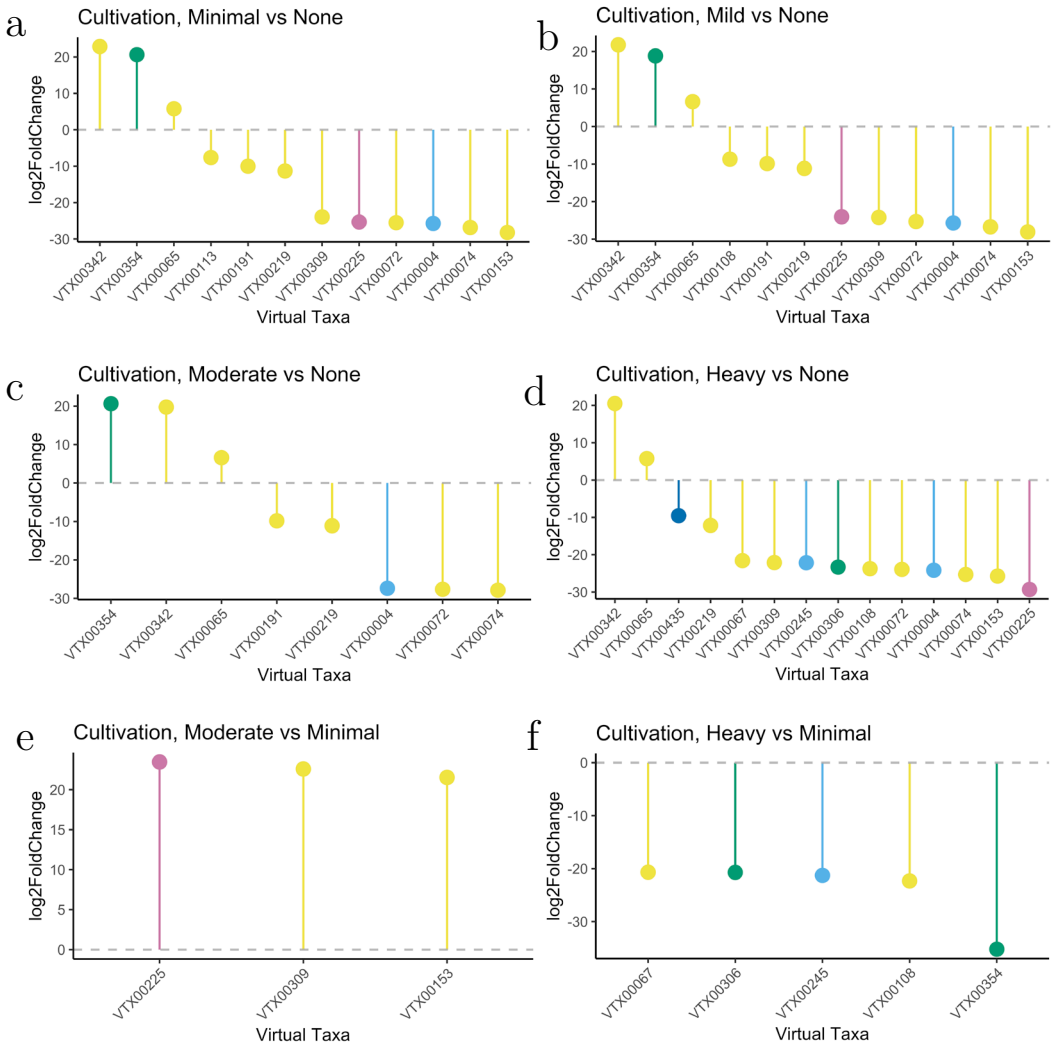
Comparing sites with minimal disturbance as the baseline, there were no significantly differentially abundant AM VT between the mild and minimal soil disturbance sites. Three AM VT were more abundant in the moderate disturbance sites, compared to minimal disturbance. These included two *Glomus,* VTX00153 (*Glomus MO-G12,* $\log_2$FC = 21.53, $p < 0.001$) and VTX00309 (*Glomus ORVIN GLO3E,* $\log_2$FC = 22.59, $p < 0.001$), and one

Claroideoglomus VTX00225 (*Claroideoglomus Glo58,* $\log_2$FC = 23.46, $p <$ 0.001). Five VT were less abundant in the heavy disturbance sites, compared to minimal disturbance. These included VTX00354 (*Diversispora Clade-3,* $\log_2$FC = 35.22, $p < 0.001$), VTX00108 (*Glomus Whitfield type 7,* $\log_2$FC = 22.33, $p < 0.001$), VTX00245 (*Archaeospora trappei,* $\log_2$FC = 21.27, $p < 0.001$), VTX00306 (*Diversispora sp.,* $\log_2$FC = 20.71, $p < 0.001$), and VTX00067 (*Glomus mosseae,* $\log_2$FC = 20.68, $p < 0.001$).

With mild disturbance as the baseline condition, four AM VT, including three *Glomus* and one *Claroideoglomus* were significantly more abundant in the moderate disturbance sites. These were VTX00108 (*Glomus Whitfield type 7,* $\log_2$FC = 9.55, $p < 0.001$), VTX00153 (*Glomus MO-G12,* $\log_2$FC = 21.38, $p < 0.001$), VTX00225 (*Claroideoglomus Glo58,* $\log_2$FC = 22.18, $p <$ 0.001), and VTX00309 (*Glomus ORVIN GLO3E,* $\log_2$FC = 22.87, $p < 0.001$). At the heavy cultivation sites, five AM VT were less abundant when compared to the milt disturbance sites, including two *Glomus,* two *Diversispora,* and one *Archaeospora.* These corresponded to VTX00108 (*Glomus Whitfield type 7,* $\log_2$FC = 15.04, $p < 0.001$), VTX00067 (*Glomus mosseae,* $\log_2$FC = 22.42, $p < 0.001$), VTX00306 (*Diversispora sp.,* $\log_2$FC = 22.83, $p < 0.001$), VTX00354 (*Diversispora Clade-3,* $\log_2$FC = 33.41, $p < 0.001$), and VTX00245 (*Archaeospora trappei,* $\log_2$FC = 25.46, $p < 0.001$).

Finally, comparing differentially abundant AM fungal VT between the moderate and heavy disturbance sites found that eight VT were less abundant in the heavy cultivation sites. These included four *Glomus*, two *Diversispora*,

one *Archaeospora*, and one *Claroideoglomus* corresponding to VTX00153 (*Glomus MO-G12,* $\log_2$FC = 19.04, $p = 0.005$), VTX00309 (*Glomus ORVIN GLO3E,* $\log_2$FC = 20.76, $p = 0.002$), VTX00067 (*Glomus mosseae,* $\log_2$FC = 23.90, $p < 0.001$), VTX00108 (*Glomus Whitfield type 7,* $\log_2$FC = 24.59, $p < 0.001$), VTX00306 (*Diversispora sp.,* $\log_2$FC = 24.13, $p < 0.001$), VTX00354 (*Diversispora Clade-3,* $\log_2$FC = 35.23, $p < 0.001$), VTX00245 (*Archaeospora trappei,* $\log_2$FC = 24.92, $p < 0.001$), and VTX00225 (*Claroideoglomus Glo58,* $\log_2$FC = 27.47, $p < 0.001$), respectively.



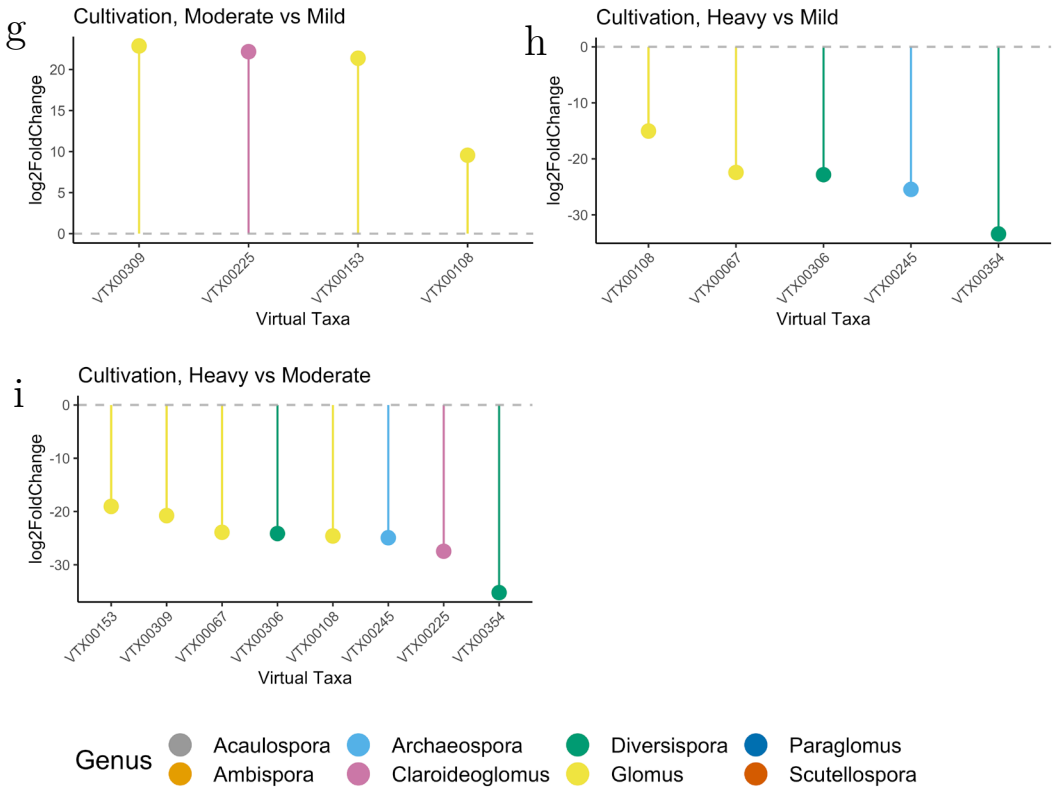


Figure 2.15 Differential abundance of AM Fungal VT by cultivation comparing sites with a) minimal and no cultivation, b) mild and no cultivation, c) moderate and no cultivation, d) heavy and no cultivation, e) mild and minimal cultivation, f) moderate and minimal cultivation, g) heavy and minimal cultivation, h) moderate and mild cultivation, i) heavy and mild cultivation, and heavy and moderate cultivation

Differential abundance of AM fungal VT comparing different types of cultivation, calculated by DESeq2. AM VT were considered to be differentially abundant if their Log2FC > 1 and $p < 0.05$.

Impact of Fungicide Application on AM Fungal Communities

As per the type of cultivation, ANOSIM was again conducted to validate the findings of the db-RDA analysis. After 999 permutations, there was significant dissimilarity in community composition due to the effect of whether

fungicides had been applied to the crop or not (ANOSIM: $R = 0.195$, $p = 0.002$).

Fungicide application was broadly categorised by the farmer responses according to the survey question: had fungicide been applied to the crop, 'yes' or 'no'? More detailed information on the type of fungicide used was collected, but as they showed very similar trends, analysis of the type of fungicide will remain qualitative. Within the fungicide application factor, 46 sites had used fungicide, and 19 had not. One site, in Cumbria used to grow conventional (not organic) barley failed to record whether fungicides had been used, so was excluded from further analysis.

Within the arable cropping system, 44 sites had used fungicide, and five had not. In the mixed approach, two sites had used fungicides, and three had not, and in the pasture cropping system, all 11 sites had not used fungicides. Bray-Curtis distances were plotted by db-RDA and NMDS methods, to visualise clustering of AM fungal communities and shown in Figure 2.16.

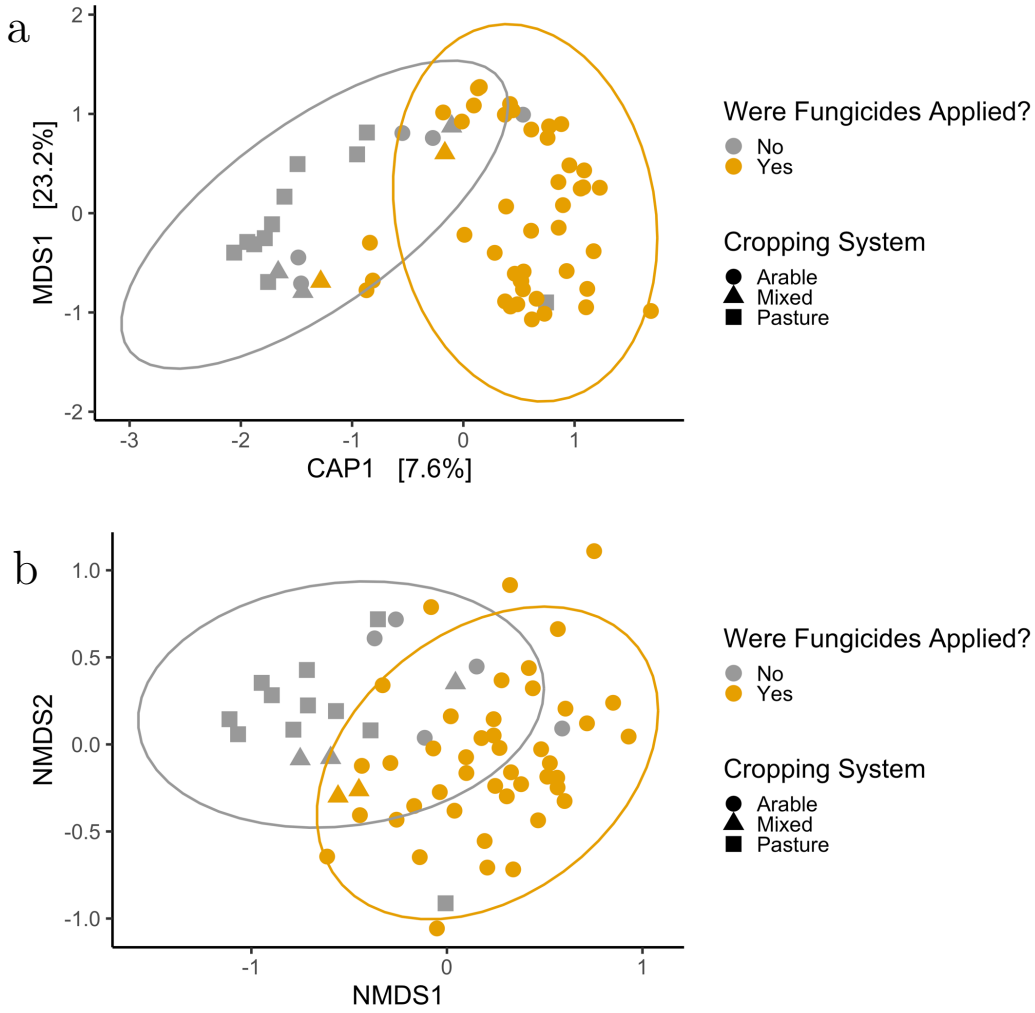


Figure 2.16 a) db-RDA ordination of Bray-Curtis distances b) NMDS ordination of Bray-Curtis distances split by whether fungicides had been applied, from 66 UK agricultural sites

Both distanced based RDA ($F_{2,57} = 2.88$, $p = 0.005$), and ANOSIM ($R = 0.195$, $p = 0.002$) suggested that the application of fungicides had a significant impact on AM fungal community composition.

Impact of Fungicide Application on Observed Number of AM Fungal Taxa

Mean number of AM VT at sites not receiving fungicides was 19.4 ± 2.4, which was considerably higher than those sites receiving fungicide application (8.0 ± 0.8). The fungicide treated sites had lower variation in AM diversity, and had two outliers with especially high diversity (Figure 2.17). These sites were one non-organic maize field in West Berkshire (22 taxa), which is part of a mixed cropping approach with leys. The site has a medium soil texture with 2.5-5% SOM, and moderate soil disturbance. The site has received fungicide, but not any of Chlorothalonil, Tebuconazole, Epoxiconazole, or Prothioconazole. The other site had 26 AM VT, and was the previously described wheat field in Florie, which had used all of the fungicides included in the questionnaire, including Chlorothalonil, Prothioconazole, Tebuconazole, and Epoxiconazole, also noting that they had used Fluxapyroxad and Boscalid.

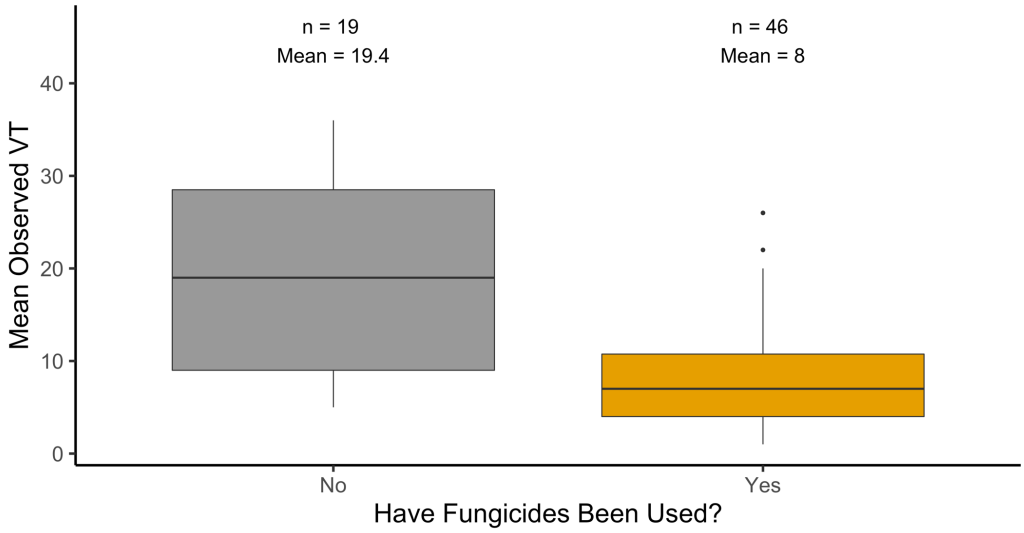


Figure 2.17 Mean observed AM fungal virtual taxa partitioned by whether fungicides had been used

Mean AM fungal VT observed at sites using, or not using fungicides. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Twenty-eight AM fungal VT were found at sites that had not received fungicide applications, which was almost three times more than sites which had, despite the fact that untreated sites accounted for less than half the total number of sites returning samples (Table 2.6). Twenty-eight AM fungal VT found at sites which had not received fungicides included 15 *Glomus*, two *Scutellospora*, five *Acaulospora*, one *Claroideoglomus*, one *Paraglomus*, and four *Archaeospora*. Ten AM VT were found exclusively at sites which had received fungicides, all of which were from the genus *Glomus*. The remaining 46 AM VT detected in this trial were found at sites which both had and had not applied fungicides, these included 18 *Glomus*, eight *Diversispora*, two *Scutellospora*, one *Acaulospora*, six *Claroideoglomus*, six *Paraglomus*, four *Archaeospora*, and one *Ambispora* taxa. Observed numbers of taxa for each fungicide regime is visualised in Figure 2.18.

Table 2.6 AM Fungal VT occurring in each level of Fungicide Application.

All		Fungicides Applied (n=46)	No Fungicides Applied (n=19)	
VTX00125	VTX00061	VTX00130	VTX00166	VTX00212
VTX00222	VTX00380	VTX00140	VTX00103	VTX00049
VTX00153	VTX00041	VTX00137	VTX00202	VTX00318
VTX00143	VTX00052	VTX00154	VTX00366	VTX00015
VTX00135	VTX00354	VTX00167	VTX00149	VTX00231
VTX00155	VTX00030	VTX00148	VTX00078	VTX00230
VTX00342	VTX00057	VTX00105	VTX00191	VTX00228
VTX00163	VTX00193	VTX00063	VTX00219	VTX00010
VTX00199	VTX00056	VTX00409	VTX00074	VTX00340
VTX00113	VTX00225	VTX00293	VTX00223	VTX00446
VTX00114	VTX00276		VTX00072	VTX00456
VTX00295	VTX00278		VTX00115	VTX00007
VTX00108	VTX00281		VTX00325	VTX00004
VTX00309	VTX00435		VTX00084	VTX00008
VTX00214	VTX00239			
VTX00065	VTX00349			
VTX00067	VTX00335			
VTX00064	VTX00444			
VTX00054	VTX00245			
VTX00263	VTX00338			
VTX00062	VTX00009			
VTX00306	VTX00005			
VTX00060	VTX00283			

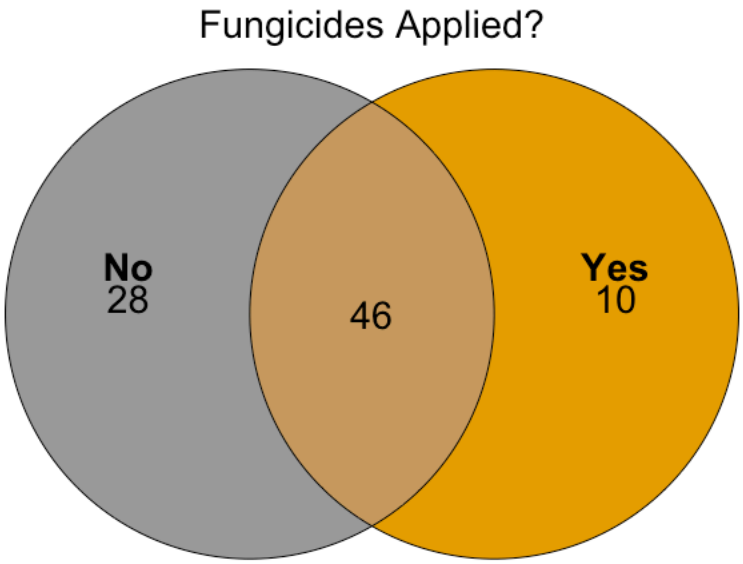


Figure 2.18 Number of AM fungal virtual observed in sites using and not using fungicides

Venn classification of AM fungal VT observed in sites using and not using fungicides. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Impact of Fungicide Application on AM Fungal Community Composition and Analysis of Differential Abundance

Mean AM fungal genera composition for the non-fungicide sites was 43.1% *Glomus*, 24.5% *Paraglomus*, 12.1% *Archaeospora*, 8.4% *Diversispora*, 6.4% *Claroideoglomus*, 2.4% *Scutellospora*, 1.9% *Ambispora*, and 1.2% *Acaulospora* (Figure 2.19). The proportion of *Glomus* (39.3%, down 3.8%), *Archaeospora* (7.0%, down 5.1%), *Diversispora* (2.0%, down 6.4%), *Claroideoglomus* (0.9%, down 5.5%), *and Acaulospora* (0.6%, down 0.5%) were all lower in the fungicide treated sites. The proportion of *Paraglomus* (34.3%, up 9.9%), *Scutellospora* (3.3%, up 0.9%), and *Ambispora* (12.6%, up 10.6%) reads were all higher in the fungicide treated sites.

The five most abundant taxa in the sites not receiving fungicides were VTX00281 (23.2% - *Paraglomus laccatum*), VTX00143 (11.2% - *Glomus MO-G20*), VTX00005 (7.2% - *Archaeospora Other1*), VTX00306 (6.6% - *Diversispora sp.*), and VTX00108 (5.2% - *Glomus Whitfield type 7*). VTX00281 (33.6% - *Paraglomus laccatum*), was also the most abundant species in the fungicide treated sites, up 10.4% compared to non-treated sites. The second most common AM fungal taxon was VTX00065 (21.1% - *Glomus caledonium*), followed by VTX00283 (12.6% - *Ambispora fennica*), VTX00143 (5.5% - *Glomus MO-G20*), and VTX00245 (5.3% - *Archaeospora trappei*).

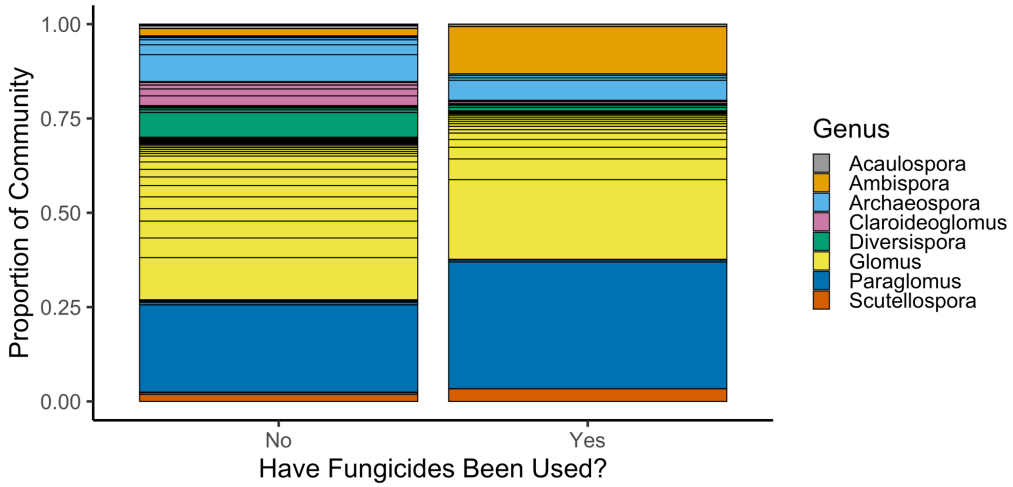


Figure 2.19 AM Fungal community composition partitioned by whether fungicides had been used

Mean community composition of AM fungal VT for sites which had or had not had fungicide application, expressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT.

Analysis of differential abundance at the $p_{adj} < 0.05$ level revealed that the abundance of 12 AM VT were significantly different between the fungicide and non-fungicide sites (Figure 2.20). Four AM VT were more abundant in the fungicide treated sites, and included VTX00354 (*Diversispora Clade-3*, $\log_2$FC = 23.75, $p < 0.001$), VTX00342 (*Glomus VeGlo18*, $\log_2$FC = 11.49, $p < 0.001$), VTX00065 (*Glomus caledonium*, $\log_2$FC = 3.94, $p < 0.001$), and VTX00283 (*Ambispora fennica*, $\log_2$FC = 3.67, $p = 0.003$). Eight VT were more abundant in the non-fungicide sites. Three of these, VTX00005 (*Archaospora Other1*, $\log_2$FC = -4.34, $p = 0.003$), VTX00456 (*Archaeospora MO-Ar5*, $\log_2$FC = -5.81, $p = 0.012$), and VTX00041 (*Scutellospora castanea*, $\log_2$FC = -6.20, $p = 0.012$) had log-fold changes of less than 10, while five taxa, VTX00004 (*Archaeospora Wirsel OTU21*, $\log_2$FC = -24.54, $p < 0.001$), VTX00072 (*Glomus MO-G16*, $\log_2$FC = -25.78, $p < 0.001$), VTX00219 (*Glomus MO-G5*, $\log_2$FC = -26.02, $p < 0.001$), VTX00153 (*Glomus MO-G12*, $\log_2$FC = -26.83, $p < 0.001$), and VTX00191 (*Glomus MO-G31*, $\log_2$FC = -27.43, $p < 0.001$) had a $\log_2$FC of ~25 or higher.

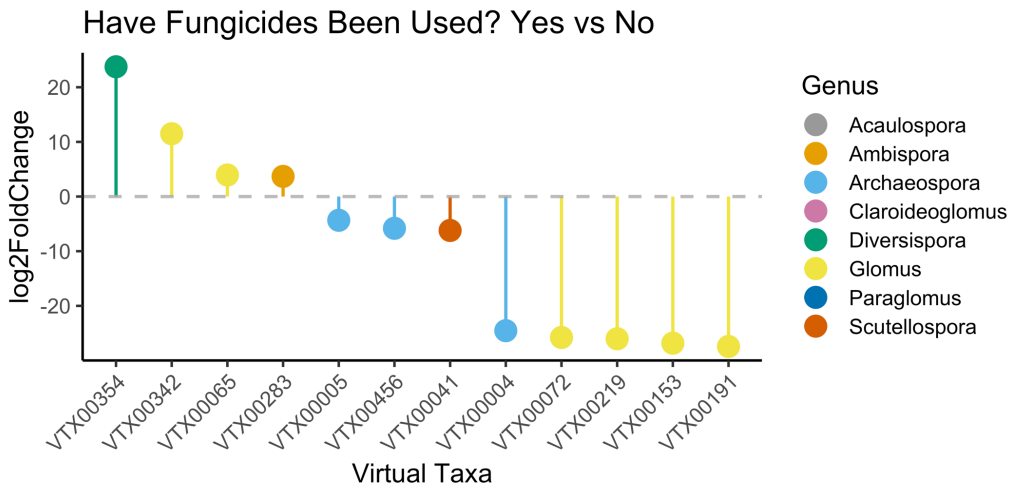


Figure 2.20 Differential abundance of AM Fungal VT at sites using, and not using fungicides

Differential abundance of AM fungal VT comparing whether fungicide had or had not been used at a site, calculated by DESeq2. AM VT were considered to be differentially abundant if their Log2FC > 1 and p < 0.05.

Impact of Soil Texture on AM Fungal Communities

To further test the contribution of soil texture on AM fungal community composition, ANOSIM was again conducted, and after 999 permutations, there was significant dissimilarity in community composition due to the effect of soil texture (ANOSIM: $R = 0.143$, $p = 0.010$). Soil texture was partitioned into three levels, 'light', 'medium', and 'heavy'. The light soil type was represented by 21 sites, medium by 40 sites, and heavy by 5 sites. Levels of soil texture were fairly well distributed amongst levels of cropping system. Within the purely arable cropping system, 13 sites had a light soil texture, 32 had medium, and all five heavy soil sites were arable. Within the mixed cropping system, two were on light soil types, and three were on medium, and in the pasture cropping system, six sites had light soil texture and five

had medium. Bray-Curtis distances were plotted by NMDS and db-RDA methods, to visualise clustering of AM fungal communities and shown in Figure 2.21.

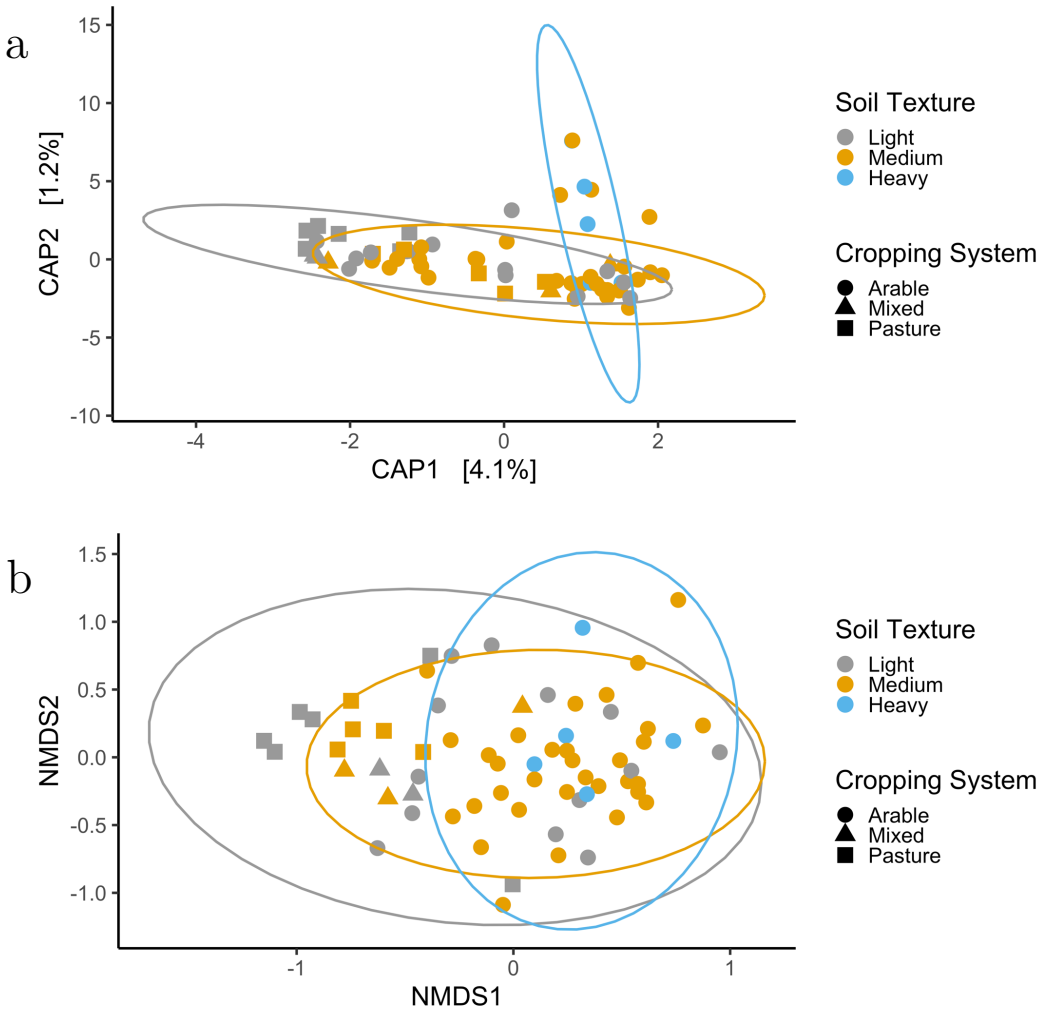


Figure 2.21 a) db-RDA ordination of Bray-Curtis distances b) NMDS ordination of Bray-Curtis distances split by soil texture, from 66 UK agricultural sites
Both distanced based RDA ($F_{2,57} = 1.77$, $p = 0.035$), and ANOSIM ($R = 0.143$, $p = 0.010$) suggested that the soil texture had a significant impact on AM fungal community composition.

Impact of Soil Texture on Observed Number of AM Fungal Taxa

The greatest mean number of AM fungal VT were observed at the 21 sites with a light soil texture (14.2 ± 2.4 taxa). This was reduced to 10.4 ± 1.3 taxa in the medium soil texture, which was represented by 40 sites (Figure 2.22). Notably, there were two outliers at the high range of the medium texture classification, of 34 and 30 observed AM VT. These observations corresponded to a previously described site in Aberdeenshire, which runs a crop rotation including grass leys, and employs cultivations which were classed as moderate. The site had not used any fungicides, but was not classed as organic. The site representing 30 AM VT corresponded to a site in Cumbria, a grass pasture classified as non-organic, although no fungicide, herbicide, or insecticides are used. The site has SOM levels of 2.5-5%, and has zero soil disturbance. Finally, the sites with heavy high clay content soil texture had the lowest number of observed AM fungal VT (5.6 ± 1.3), although this figure was based on only five sites, corresponding to non-organic arable sites growing wheat (2), oilseed rape (2), and barley. The two lowest observations were two and four AM VT, made at the oilseed rape sites. The barley site had six AM VT, whereas the wheat fields had six and 10 taxa, the latter of which was classified as an outlier. This site, in Northamptonshire, had moderate disturbance, and had used Chlorothalonil, Prothioconazole, and Tebuconazole fungicides.

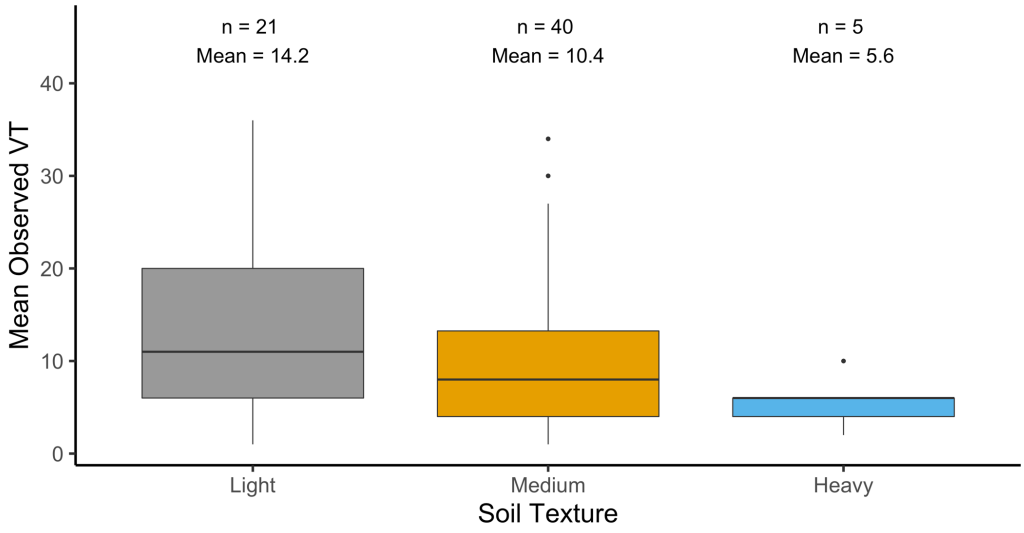


Figure 2.22 Mean observed AM fungal virtual taxa partitioned by soil texture
Mean AM fungal VT observed at the sites with light, medium, or heavy soil texture. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Fourteen AM VT were found in all three soil textures, and included four *Glomus*, three *Diversispora*, one *Scutellospora*, one *Acaulospora*, two *Paraglomus*, two *Archaeospora*, and one *Ambisopora* taxa (Table 2.7). Twenty-one AM fungal VT were found at sites with a light soil texture only. These included 14 *Glomus*, one *Scutellospora*, four *Acaulospora*, one *Paraglomus* and one *Archaeospora.* Thirteen taxa were found exclusively in the medium soil texture sites, and included eight *Glomus*, one *Claroideoglomus*, two *Paraglomus*, and two *Archaeospora* taxa. There were zero VT which were found exclusively in heavy soil types, although this group was underrepresented, with only five sites. The remaining 36 remaining AM VT were found in both the light and medium soil textures, and were represented by 17 of *Glomus*, five of *Diversispora*, two of *Scutellospora*, one of *Acaulospora*, six of *Claroideoglomus*, two of *Paraglomus*, and three of *Archaeospora* taxa.

There were no unique AM VT shared between the light and heavy, or medium and heavy soil types. Observed numbers of taxa for each soil texture is visualised in Figure 2.23.

Table 2.7 AM Fungal VT occurring in each level of Soil Texture.

All	Light (n=21)	Medium (n=40)	Heavy (n=5)	Light & Medium	
VTX00143	VTX00140	VTX00130	-	VTX00125	VTX00306
VTX00199	VTX00137	VTX00135		VTX00222	VTX00060
VTX00113	VTX00154	VTX00155		VTX00153	VTX00061
VTX00065	VTX00166	VTX00202		VTX00342	VTX00380
VTX00054	VTX00103	VTX00105		VTX00163	VTX00041
VTX00263	VTX00366	VTX00295		VTX00167	VTX00049
VTX00052	VTX00149	VTX00325		VTX00191	VTX00231
VTX00354	VTX00078	VTX00063		VTX00219	VTX00057
VTX00030	VTX00148	VTX00340		VTX00072	VTX00193
VTX00281	VTX00074	VTX00349		VTX00114	VTX00056
VTX00435	VTX00223	VTX00335		VTX00115	VTX00225
VTX00245	VTX00084	VTX00338		VTX00108	VTX00276
VTX00005	VTX00409	VTX00008		VTX00209	VTX00278
VTX00283	VTX00293			VTX00212	VTX00239
	VTX00318			VTX00214	VTX00444
	VTX00015			VTX00067	VTX00009
	VTX00230			VTX00064	VTX00456
	VTX00228			VTX00062	VTX00004
	VTX00010				
	VTX00446				
	VTX00007				

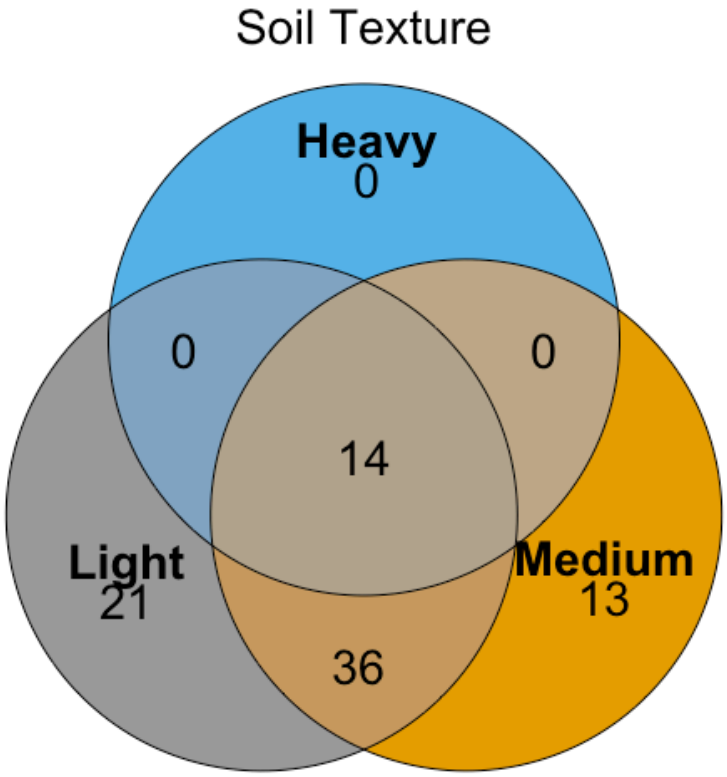


Figure 2.23 Number of AM fungal virtual observed in the different soil textures
Venn classification of AM fungal VT observed in each soil texture. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Impact of Soil Texture on AM Fungal Community Composition and Analysis of Differential Abundance

Mean AM fungal community composition at the genus level, of the light soil texture sites consisted of 58.2% *Glomus*, 19.7% *Paraglomus*, 6.1% *Archaeospora*, 5.4% *Diversispora*, 3.8% *Claroideoglomus*, 3.4% *Scutellospora*, 2.5% *Ambispora*, and 0.8% *Acaulospora* (Table 2.8). In the medium soil texture sites, *Paraglomus* becomes the dominant genus, at 37.3% of DNA reads, an absolute increase of 17.6%. Second highest is *Glomus* at 33.9% (down 24.3%),

with additional small increases in *Archaeospora* (10.1%, up 4.0%), and *Ambispora* (9.3%, up 6.82%). In the heavy soil type samples, DNA reads were dominated by *Ambispora* (37.9%), which was 28.6% higher than the medium soil texture. *Paraglomus* (37.1%) was comparable to the medium soil texture, down only 0.2% of reads. *Glomus* comprised 19.6% of reads (down 14.3%), which left only ~5% for other genera. *Claroideoglomus*, which had comprised 3.8% and 2.0% of reads in the light and medium textures, was absent from the heavy soil texture. However, care must be taken when interpreting this result, due to the low sample number for the heavy soil texture.

Table 2.8 Composition of AM fungal genera at each level of Soil Texture.

Genus	Proportion of Genus from Each Soil Texture (%)		
	Light (n=21)	Medium (n=40)	Heavy (n=5)
Acaulospora	0.8	0.9	<0.1
Ambispora	2.5	9.3	37.9
Archaeospora	6.1	10.1	3.7
Claroideoglomus	3.8	2.0	0
Diversispora	5.4	3.3	1.0
Glomus	58.2	33.9	19.6
Paraglomus	19.7	37.3	37.1
Scutellospora	3.4	3.1	0.6

The five most abundant taxa in the light soil texture grouping were VTX00281 (19.1% - *Paraglomus laccatum*), VTX00065 (15.5% - *Glomus caledonium*), VTX00143 (10.0% - *Glomus MO-G20*), VTX00067 (5.3% -

Glomus mosseae), and VTX00108 (3.9% - *Glomus Whitfield type 7*). In the medium soil texture, VTX00281 (*Paraglomus laccatum*) was still the dominant taxon, and represented over one third (36.2%) of reads. VTX00065 (*Glomus caledonium*) was also still the second most abundant taxon, and relatively unchanged in its abundance compared to the light soil texture (16.8%). The third most abundant was VTX00283 (9.3% - *Ambispora fennica*), then VTX00143 (6.1% - *Glomus MO-G20*), and VTX00245 (5.9% - *Archaeospora trappei*). In the heavy soil type, VTX00283 (*Ambispora fennica*) was the most abundant taxon, with 37.9% of reads. This taxon comprised all of the *Ambispora* reads in this group. VTX00281 (*Paraglomus laccatum*) represented 36.9% of reads, over 99% of all *Paraglomus* reads for the heavy soil type. VTX00065 (*Glomus caledonium*) constituted 17.1% of reads, which remained remarkably consistent between all three soil textures. The fourth and fifth most abundant taxa in the heavy soil texture sites were VTX00143 (2.4% - *Glomus MO-G20*), and VTX00245 (2.0% - *Archaeospora trappei*), which was the same as the medium soil texture, just with lower representation. The relative abundance of AM fungal VT, split by soil texture, is shown in Figure 2.24.

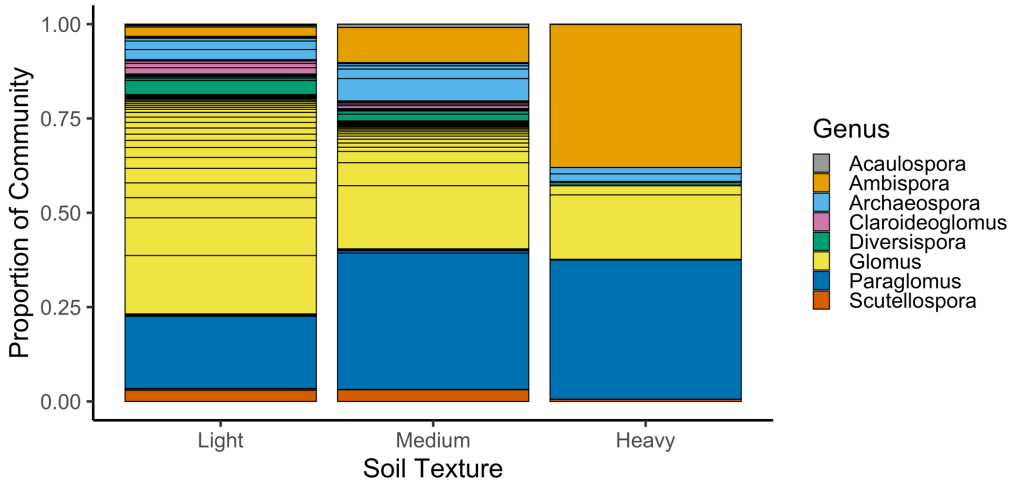


Figure 2.24 AM Fungal community composition partitioned by soil texture
Mean community composition of AM fungal VT for sites with sites of different soil texture, expressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT.

Analysis of differential abundance with DESeq2 revealed that six AM fungal VT were differentially abundant between the light and heavy soil textures (Figure 2.25). Five *Glomus* were significantly more abundant in the light soil texture samples, and included VTX00067 (*Glomus mosseae,* $\log_2$FC = 22.07, $p < 0.001$), VTX00108 (*Glomus Whitfield type 7,* $\log_2$FC = 21.85, $p < 0.001$), VTX00219 (*Glomus MO-G5,* $\log_2$FC = 21.52, $p < 0.001$), VTX00125 (*Glomus MO-G22,* $\log_2$FC = 20.02, $p < 0.001$), and VTX00342 (*Glomus VeGlo18,* $\log_2$FC = 18.29, $p < 0.001$). One *Ambispora,* VTX00283 (*Ambispora fennica,* $\log_2$FC = -9.36, $p < 0.001$) was significantly more abundant in the heavy soil samples.

Comparing the medium and heavy soil textures, the same five *Glomus* were significantly more abundant in the light soil texture samples, and included

VTX00067 (*Glomus mosseae,* $\log_2$FC = 20.10, $p < 0.001$), VTX00108 (*Glomus Whitfield type 7,* $\log_2$FC = 20.43, $p < 0.001$), VTX00219 (*Glomus MO-G5,* $\log_2$FC = 16.66, $p = 0.003$), VTX00125 (*Glomus MO-G22,* $\log_2$FC = 16.73, $p < 0.001$), and VTX00342 (*Glomus VeGlo18,* $\log_2$FC = 22.55, $p < 0.001$). The same *Ambispora* taxon, VTX00283 (*Ambispora fennica,* $\log_2$FC = -6.79, $p = 0.002$*)* was again significantly more abundant in the heavy soil samples. There were no significantly differentially abundant AM VT between the light and medium soil textures at the $p < 0.05$ level.

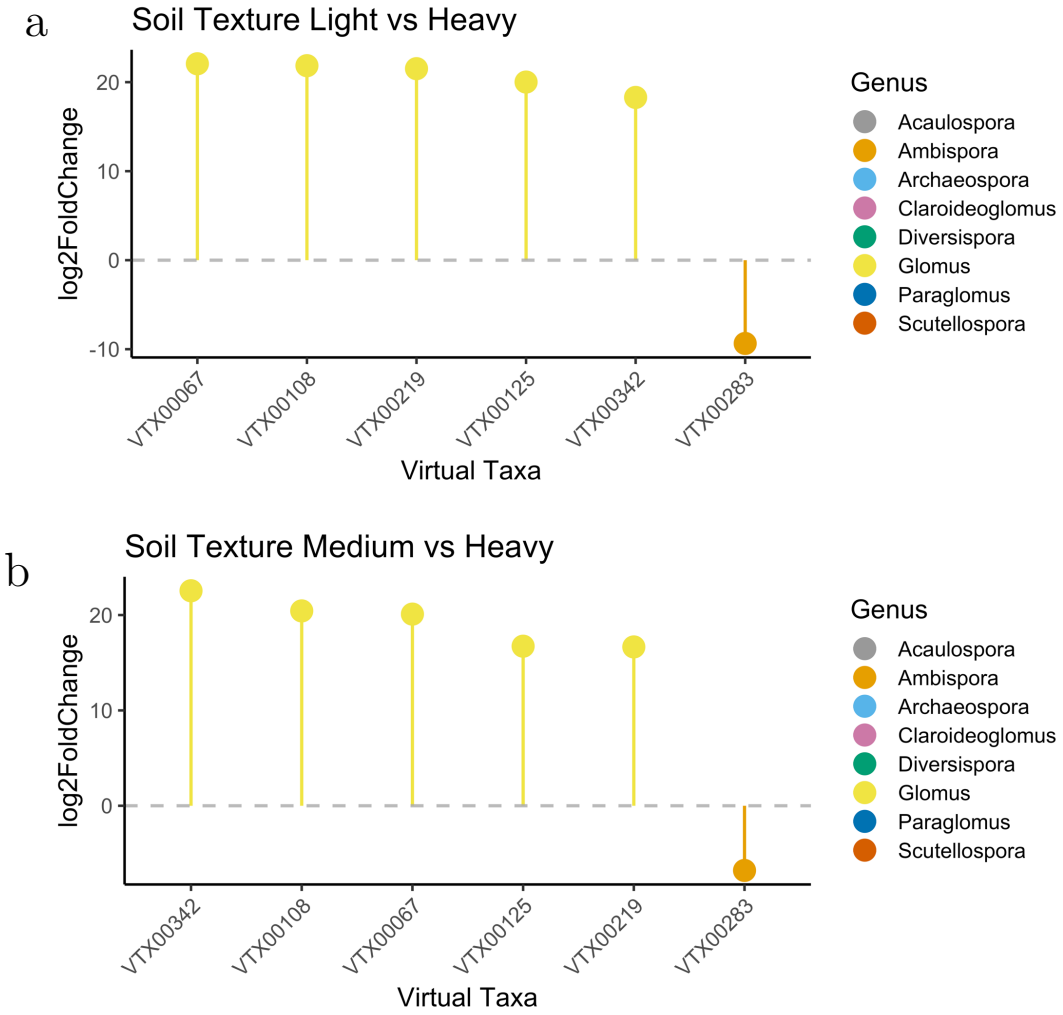


Figure 2.25 Differential abundance of AM Fungal VT by soil texture comparing a) light and heavy soil texture sites, b) medium and heavy soil texture sites

Differential abundance of AM fungal VT comparing different soil textures, calculated by DESeq2. AM VT were considered to be differentially abundant if their Log2FC $>$ 1 and $p < 0.05$. There were no There were no significantly differentially abundant AM VT between the light and medium soil textures at the $p < 0.05$ level.

2.4 Discussion

In this chapter, 84 AM fungal VT were successfully amplified from 148 samples from 67 independent agricultural sites from within the UK. This corresponds to 22% of the known diversity of AM fungi globally. The 84 recorded taxa comprised 18% of the globally recorded *Acaulospora*, 25% *Ambispora*, 62% *Archaeospora*, 44% *Claroideoglomus*, 41% *Diversispora*, 17% *Glomus*, 37% *Paraglomus*, and 30% *Scutellospora*.

As documented by other scientists in the field, extracted DNA samples had to be diluted by 10 to 100 times to enable successful amplification of the 18S rDNA region (Opik, *pers. com.).* For consistency, all samples were diluted by 100 times before amplification by PCR, resulting in successful amplification in 148 of the 150 samples. Bioinformatical analysis was conducted with customised scripts in BASH, following discussion with Alex Dumbrell, a leading author in the field of molecular ecology. Using bioinformatical programs outside of a wrapper such as QIIME2 enables increased control of analysis, and aids in understanding the data manipulations occurring. QIIME2 is an excellent end to end data processing and visualisation platform, which has been optimised for analysis of bacterial (16S) and fungal (ITS) communities, which utilise Absolute Sequence Variants (ASVs). ASVs are redundant in analysis of AM fungi, as the MaarjAM database clusters taxa into VT with a 3% error. There may be interest in the future to explore how AM fungal ASVs, which correspond to sub-species level diversity, are impacted by biotic and abiotic factors, but this analysis could comprise a large

proportion of a subsequent thesis. Stringent thresholding was chosen as to not artificially inflate AM fungal diversity. This reduced the total number of reads available for analysis, but as the AM fungi have such low overall diversity, it has been shown that only a few hundred DNA reads are required to fully capture the diversity from a single sample (Vasar et al. 2017).

The most common AM VT in UK agricultural systems was VTX00281 (*Paraglomus laccatum*). This taxon comprised over 30% of all DNA sequence reads across the 67 samples, and was found at over 80% of these sites. The next most common taxon VTX00065 (*Glomus caledonium),* comprised 16% of reads at over 60% of sites. Although abundance of AM VT by DNA sequence reads quickly declined, almost all of the top 30 taxa by prevalence were found at 20% or more of the independent sites, suggesting a low level of geographic endemism observed at the global scale by Davison et al. (2015). Of the 84 identified VT, 17 were identified at one site, 15 were identified at two sites, and three were found at only three sites. These may represent rare taxa, which require a very narrow range of biotic and abiotic factors for growth, or engage in symbiosis with a different or specific host.

Automatic forward and backward stepwise model selection of db-RDA found that the most parsimonious model included cropping system as the sole covariate. Arable sites hosted 7.7 AM VT, whilst the permanent pasture sites hosted 23.7 AM VT. The five sites utilising a mixed cropping approach experienced an intermediate number of AM VT. This is agreement with observations by Öpik et al. (2006), who found that arable sites hosted around

half the number of AM VT of grasslands. Similarly, Menéndez et al. (2001) found that continuous wheat or barley hosted significantly fewer species than nearby grassland, although this study was limited to sporulating species (Oehl et al. 2017). In this chapter, nine AM VT belonging to the genus *Glomus* were found exclusively in arable sites, which may reflect potential adaptation to high disturbance sites (Helgason et al. 1998; Sýkorová et al. 2007a; Schnoor et al. 2011). Of the 24 AM VT found exclusively in the pasture sites, there were 14 *Glomus*, two *Scutellospora*, five *Acaulospora*, one *Paraglomus*, and two *Archaeospora*. *Acaulospora* and *Scutellospora* species have previously been shown to be impacted by soil disturbance associated with arable sites. Five out of six of the recorded *Acaulospora*, and two out of three of the recorded *Scutellospora* were found in the pasture systems only, supporting the claim that these genera are impacted by soil disturbance. In addition to those species present or absent at a site, a further five *Glomus* and two *Archaeospora* were significantly less abundant in the arable sites. One *Glomus* and one *Diversispora* species were significantly more abundant in the arable sites, suggesting that AM VT within the same genus can respond differently to the biotic and abiotic factors associated with a cropping system.

To further investigate the factors which define the cropping system, the crop system was removed from the db-RDA model. After forwards and backwards selection, the most parsimonious model found that soil texture, cultivation, and fungicide application shaped AM fungal communities. It is clear that

cultivation and fungicide application will contribute two of the most considerable forms of disturbance within arable sites, so it is unsurprising that the model identified these factors as influencing AM fungal diversity. The impact of cultivation on AM fungal diversity has been well documented in the literature (Helgason et al. 1998; De La Providencia et al. 2007; Alguacil et al. 2008; Schalamuk and Cabello 2010; Verbruggen and Kiers 2010; Säle et al. 2015). In this study, there is collinearity between the zero soil disturbance and the pasture cropping system, which results in the greatest number of AM fungal VT. There is little difference between the remaining kinds of cultivation, although samples are heavily biased towards the moderate disturbance level. Interestingly, as cultivation level increases, as does the proportion of the community, which is *Ambispora*, increasing from 0.4 to 16.8% of reads in a near linear fashion. There is very limited literature on whether *Ambispora* are tolerant to soil disturbance, however, in direct contrast to the findings of this chapter, Allan (2017) speculated that the genus is sensitive to disturbance by cultivation. Clearly, further research will be required to dissect the response of this genus to soil disturbance.

There is surprisingly little literature on the impact of fungicide application on AM fungal diversity in field conditions. Studies from the field, as well as in vitro conditions are inconsistent, and suggest that fungicides with different modes of action may impact spore germination, hyphal growth, and RLC in varying amounts (Jin et al. 2013; Buysens et al. 2015). Representation of *Claroideoglomus* and *Diversispora* was considerably reduced in the fungicide treated sites, whereas there were considerably more reads for *Ambispora*.

Despite this trend, only VTX00283 (*Ambispora fennica*) was significantly more abundant following analysis of differential abundance with DESeq2. Due to the lack of background literature on the impact of specific fungicides, and unbalanced nature of this trial, further clarification of how application of fungicides impact AM fungal community composition in field conditions would be useful to quantify relative impact.

Observed numbers of AM fungal VT were highest in the light soil texture, followed by medium, then heavy soils. Light soils were dominated by *Glomus* species, which was reduced as the soil texture became heavier. The heavy soil texture was only represented by five sites, but reads were dominated by VTX00283 (*Ambispora fennica*), VTX00281 (*Paraglomus laccatum*), and VTX00065 (*Glomus caledonium*). It is possible that the soil texture, as determined by the proportion of sand, silt, and clay, as well as organic matter content, influenced water holding, aeration, and ion exchange capacity of the soil, which have been shown to influence AM fungal communities (Oehl et al. 2017). Specifically, Jansa et al. (2014) found that *Diversispora* and *Gigaspora* were negatively correlated with silt or clay content of the soil. In this study, abundance of *Diversispora* and *Scutellospora* (of the Gigasporaceae) were also reduced in the heavy clay rich soils assessed in this study.

2.5 Conclusion

In this chapter, the AM fungal community composition has been characterised for 148 samples from 67 independent sites, which had been submitted

as part of the FERA Big Soil Community. 84 AM fungal VT were identified, and it was found that the cropping system was the primary driver of AM community composition. Underpinning this, cultivation, fungicide application, and soil texture may also have contributed to the structure of communities. As sample collection relied on farmer participation and full agronomy and crop husbandry are unknown, care should be taken when considering the relative importance of these factors on AM fungal community composition. Nevertheless, this study has set the framework for AM fungal diversity within UK agricultural sites, and is a useful initial resource that could prime further replicated trials exploring the impact of farming practices on AM fungal diversity in UK agriculture.

3 Chapter Three – Cover Cropping and AM Fungal Inoculation to Improve Soil Health and Increase Crop Yield

3.1 Introduction

A significant body of work indicates that there are many benefits to plant growth attributable to associations with AM fungi, this is discussed at length in Chapter One. This chapter will consider the hypothesis that mycorrhizal benefit can be further improved by AM fungal inoculum inputs in combination with cover crops drilled in the autumn preceding a cash crop. Specifically, this study sought to determine whether populations of AM fungi can be fostered to increase crop yields in broad-acre crops, and whether crop yields could be further improved by the introduction of cover crops. There is good evidence that either a longer term ley or cover crop have increased AM colonisation of subsequent cash crops, and in some cases, increased diversity of AM species (Kabir and Koide 2002; Lehman et al. 2012; Ramos-Zapata et al. 2012; Higo et al. 2013, 2017; Bowles et al. 2017). Cover crops can also provide several additional benefits to soil health, and other ecosystem services, which were discussed in detail in Chapter One.

Since some of this benefit is thought to arise from enhanced AM fungal inoculum potential, this study considered whether introduction of AM fungal inoculum ahead of drilling could maximise this benefit. There is a large body of literature exploring inoculation in controlled or open-field conditions, with

good evidence to suggest that inoculation is effective at increasing AM colonisation, plant growth, nutrient uptake, and yield (Berruti et al. 2016), although this benefit can be inconsistent (Hart et al. 2018). There is also increasing evidence to suggest that plant-mycorrhizal associations show higher specificity than was previously envisioned, and this raises the question of whether AM inoculation can be tailored to specific crops, rotations, or field conditions to maximise plant growth and yield (McGonigle and Fitter 1990; Lekberg et al. 2007; Martínez-García and Pugnaire 2011, and in depth discussion in Chapter One).

AM Fungal Inoculums and Their Effectiveness

It is possible to inoculate crops directly with the spores of specific AM fungal species. This theoretically gives the grower increased control of the quantity and identity of symbionts being added to the system, but does not provide the other benefits that a cover crop or ley can provide to soil, such as increased organic matter content, and reduced winter soil erosion. Furthermore, products containing AM inoculums are costly, and their longevity is unproven in field conditions (Hart et al. 2018). Despite this, the ability to increase or replenish AM inoculum potential more quickly than by natural processes is an appealing way to increase plant-fungal symbiosis on the field scale for sites that may be depleted in natural inoculum potential. AM fungal inoculums, and their impact on RLC, biomass, and yield, is discussed at length in Chapter One.

When considering potential host specificity of AM fungi, it is plausible to expect that different cover crop species or mixtures may preferentially associate with different AM fungal species, thereby increasing the number of propagules of that species, and increasing the likelihood that this species of AM fungi will colonise the following crop (McGonigle and Fitter 1990; Johnson et al. 2004; Öpik et al. 2009; Martínez-García and Pugnaire 2011). This is one of the hypotheses that forms the basis of this PhD, and is described in detail in Chapter One, and explored further in Chapters Four and Five.

Selection of Commercial AM Fungal Inoculum

The inoculum tested here is a five species mixture sourced from PlantWorks Ltd (Sittingbourne, UK) a commercial producer and distributor of AM fungal, and rhizobacterial inoculants. The AM fungal mixture contains *Funneliformis mosseae, Funneliformis geosporum, Claroideoglomus claroideum, Rhizophagus irregularis, and Glomus microaggregatum*, which are all quite easy to co-culture with a compatible plant host under glasshouse conditions. Occurrence and impact of each of these species has been documented in the literature, in the context of ecology, remediation, or more fundamental studies around genetic regulation of signalling and symbiosis, in the case of the model species *Rhizophagus irregularis.* It is possible that these *r*-strategists may not be the best suited to providing plant benefit, instead prioritising their own reproduction via aggressive plant colonisation and greater spore production (Pianka 1970; Verbruggen and Kiers 2010; Grime and Pierce

2012). Despite this, it will be of interest to investigate the negative, as well as positive effects on growth and yield, resulting from application of this generalist inoculum.

Testing Efficacy of AM Inoculum in Semi-Controlled Conditions

A glasshouse experiment was set up to investigate crop response to inoculation with AM fungi (Figure 3.1), and evaluate the relative contribution of each of the five components of the AM fungal inoculum. This study complements the Bawburgh field trial which follows below. This study aims to compare the impact of inoculation with different AM taxa on root colonisation and plant growth, considering each in isolation and as a mixture. This controlled experiment enabled a comparison of mycorrhizal outcomes in a much simplified soil microbiome, and without the complicating impact of existing inoculum potential that was likely to vary across the field site, owing to the use of sterilised soil.

Spring barley (var. Laureate) was inoculated with one of each of the five components of the mixed AM inoculum drilled in the Bawburgh trial. These inoculated plants were compared to a non-inoculated control, and to plants colonised by the commercial five species inoculum. As well as looking at the impact of each individual AM taxa on plant colonisation and growth, the relative colonisation of each species in the five way mix was evaluated using molecular approaches. This aim was to determine whether each of the AM species in the commercial inoculum were able to colonise the plant, and in

what ratios. In the event of a single dominant taxon colonising barley root systems, it would then be possible to assess whether those species with the highest colonisation in the mixed inoculum were those which had the greatest growth benefit in the monoxenic inoculum, or if high levels of colonisation by certain AM fungi came at the cost of reduced plant benefit.

The glasshouse trial, was run under semi-controlled conditions, and though abiotic environmental conditions differed from the field, the study aimed to replicate many of the conditions experienced in the field at the Bawburgh site. Importantly, the same Laureate spring barley seed was used as in the field trial, to negate the impacts of varietal effects. The seed was coated in Bayer Redigo Pro fungicide treatment, containing prothioconazole and tebuconazole; although this may negatively influence AM colonisation, it was important to replicate field conditions using treatment protocols routinely used by farmers. This will therefore better reflect factors experienced in the field, and explain the patterns of colonisation observed in the field trial.

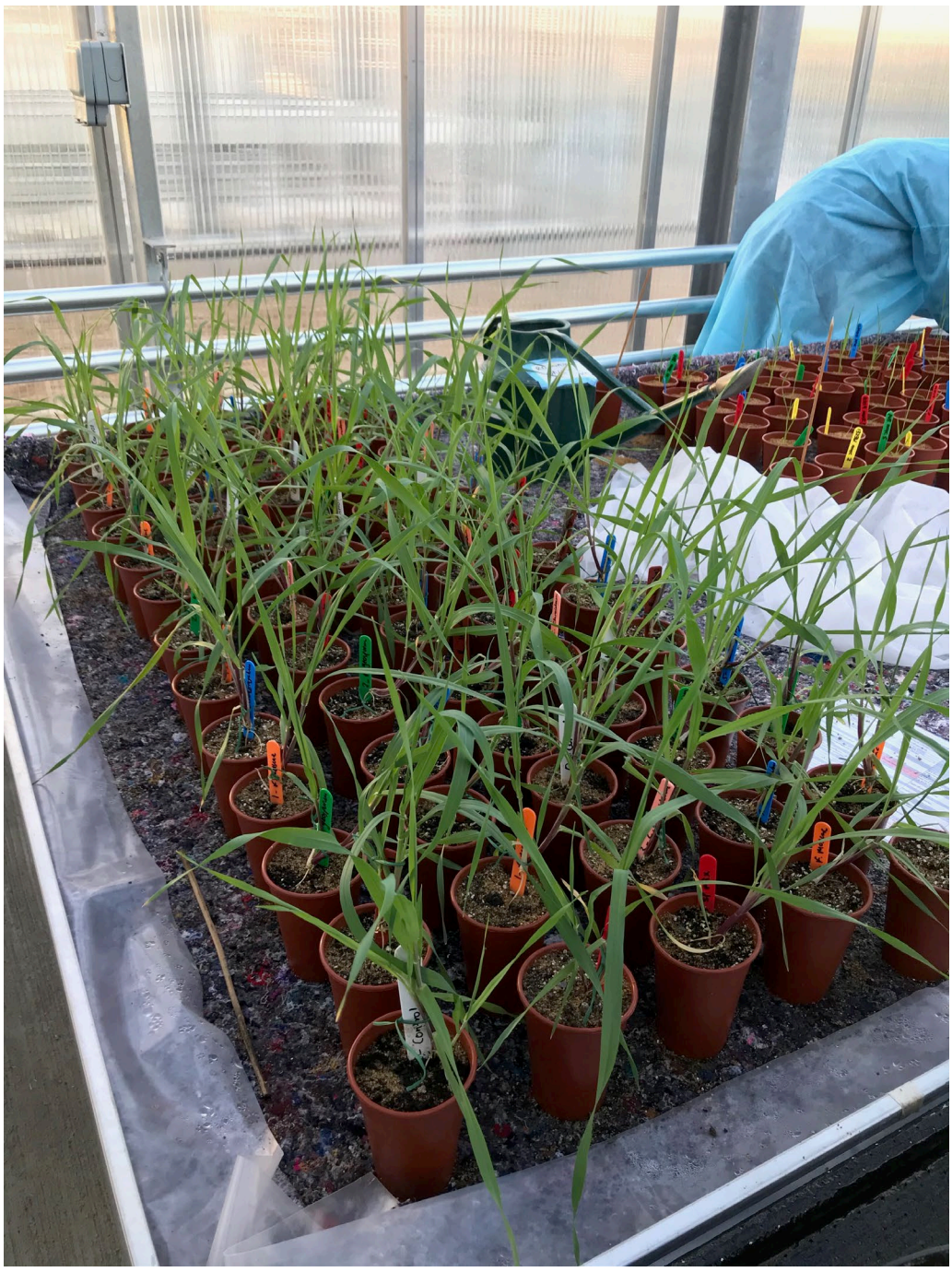


Figure 3.1 Growing spring barley var. Laureate in glasshouse conditions at NIAB Cambridge, UK.

Testing Efficacy of Cover Cropping and AM Inoculum in Field Conditions

The replicated field trial was based in Bawburgh, Norfolk, UK, at a site with a Burlingham 1 sandy loam substate and previously homogenous plant growth and yield. The site was prepared with a double cultivation to homogenise existing AM fungal populations ahead of experimentation.

The five species AM inoculum was drilled into fallow soil, or with one of three species of cover crop, grown individually, in pairs, and as a three species mix. Each of these cover crop treatments were grown with and without the AM fungi inoculum to enable comparison between natural and applied inoculum. Cover crops were desiccated (killed) using glyphosate herbicide and followed by spring barley (var. Laureate) in year one, and winter oat (var. Mascani) in year two, to assess whether the increased abundance of AM propagules colonised crops throughout the rotation, or whether plant identity had a greater effect on the AM species colonising the crop root system.

In addition to exploring the impact of cover cropping and inoculation on AM populations, plant growth, and yield, soil health characteristics such as soil structure and levels of organic matter were recorded. Both cover copping and abundance of AM fungi have been linked to soil health, so it was important to assess all potential benefits of the experimental treatments.

3.2 Methods

Glasshouse Methods

Components of the AM Fungal Inoculum in the Glasshouse Trial

AM fungal inoculum for both the glasshouse and field trials was sourced from PlantWorks Ltd (Sittingbourne, UK). Each of the five AM fungal species, as well as the five species mix was assessed. The species used were as follows:

- *Funneliformis mosseae*
- *Funneliformis geosporum*
- *Claroideoglomus claroideum*
- *Rhizophagus irregularis*
- *Glomus microaggregatum*

Spores and other infectious propagules were supplied as 'crude' inoculum, in a clay substrate, which contained spores, hyphae, and colonised root fragments. Crude inoculum of each species was added to the sand soil mixture at 10% (v/v) as recommended by the manufacturer. Unlike other trials of its kind, this experiment makes no attempt to standardise the number of infectious propagules between treatments. This is to reflect that the manufacturer also does not standardise number of propagules, and instead combines the mixed inoculum based on a fixed volume of each of the five components, once a predetermined minimum number of infectious propagules has been achieved. It was therefore considered to be more representative to

investigate the effect of the inoculum, rather than extracting and applying a set number of spores.

Soil Conditions in the Glasshouse Trial

Autoclaved John Innes Number 1 was mixed in equal volume with autoclaved yellow Garside silica sand. The sand-soil mixture was thoroughly mixed by hand before being divided into seven equal amounts of known volume, for inoculation with crude inoculum of each of the five individual species.

The resultant sand-soil mixture had the following attributes: pH 7.8, 1.8% organic matter, 81.6% dry matter, 66.67 mg/kg nitrate, 0.67 mg/kg ammonium, 252.5 kg N/ha available N, 6.8 mg/l P, 34 mg/l K, and 56 mg/l Mg.

Seed Sterilisation, Germination and Planting in the Glasshouse Trial

Spring barley (var. Laureate) was sterilised using 3% sodium hypochlorite solution, to prevent transfer of non-inoculum AM species as well as pathogens, which may have been present on the seed surface. The sodium hypochlorite solution also effectively removed some of the excess Bayer Redigo Pro seed treatment, containing Prothioconazole and Tebuconazole fungicides, which may have had a greater influence on AM fungal colonisation in the small volume of soil, compared to in field conditions.

After inverting seeds for 10 minutes in the sodium hypochlorite solution to ensure that seed was thoroughly sterile, seeds were rinsed three times with Reverse Osmosis (RO) H_2O and transferred to 0.8% bactoagar in a sterile flow cabinet. Plates were sealed, wrapped in opaque foil, and transferred to a 37°C incubator for five days.

After five days, plants were transferred to the glasshouse where 400ml/ 9cm Lily of the Valley pots had been prepared with the sand-soil mixture, and inoculated with crude inoculum if appropriate (Figure 3.2). Pre-germinated seedlings were transferred to the pots using forceps, being careful not to damage the root system. 10 plants were sown per experimental treatment, resulting in 60 inoculated, and 10 control plants. Once all seedlings were planted, pots were randomised to negate the effect of abiotic variation in the glasshouse, and covered with a fine mesh to prevent drying. The mesh was removed after seven days of growth.

Figure 3.2 Spring barley var. Laureate seedlings after being transferred to inoculated sand-soil substrate.
Plants are covered by horticultural mesh to prevent desiccation, and was removed after seven days of growth.

Glasshouse Conditions and Plant Care

Glasshouse conditions for the trial were as follows: daylength 12-14 hours, supplemented with 10000 lux sodium lamps, daytime minimum temperature 20°C, and night-time minimum temperature 18°C.

Plants were watered using RO water only for two weeks, to allow for germination and establishment of the AM symbiosis. After two weeks, plants were watered twice a week with low P Hoaglands solution, containing 10 µM P.

This amended low P solution has been optimised to maximise AM colonisation in experimental conditions. Plants continued to be watered using RO water when the soil was drying.

Figure 3.3 Selected replicates typical of plant growth from the glasshouse trial.
Selected replicates typical of plant growth from each treatment 7 weeks post inoculation. From left to right: Control, *F. mosseae, F. geosporum, C. claroideum, R. irregularis, G. microaggregatum,* 5 species commercial mix

Plant Harvest and Sample Storage

Plants were harvested at seven weeks post inoculation. Representative plants of each experimental treatment are shown in Figure 3.3, immediately before harvest. Individual plants were removed from their pots and roots washed in RO water, being careful not to damage the root system. Plant shoots were separated from roots and placed into a pre-weighed envelope, then dried and reweighed. Root systems were blotted dry and cut into 1-2cm sections and thoroughly mixed. Approximately 10 root pieces per plant

were transferred into 2ml Eppendorf tubes containing 50% ethanol, for microscopic analysis of AM colonisation. A further 10 root pieces were harvested from two plants per inoculation treatment, which were stored in 50% ethanol for staining with WGA-488, to determine morphology of individual strains of AM fungi. For the 10 plants in the mixed inoculum treatment, 100mg of root material was transferred to autoclaved 2ml Eppendorf tubes containing one steel bead, and flash frozen in liquid nitrogen. These samples were transferred to the freezer for storage at -80°C.

Staining and Quantification of Root Length Colonisation by Trypan Blue

Root samples were rinsed with ddH_2O, transferred to 10% (w/v) potassium hydroxide, and heated at 90°C for 60 minutes, replacing KOH every 20 minutes. KOH was removed and roots were rinsed three times with ddH_20, before being acidified in 0.3M hydrochloric acid (HCl) at room temperature for 60 minutes. HCl was removed, and replaced with 0.05% w/v trypan blue in lactoglycerol. Roots in the trypan blue solution were heated at 90°C for a further 10 minutes. Trypan blue solution was removed, and stained roots were washed twice with 50% lactoglycerol before immediately being mounted for microscopy.

Root length colonisation was calculated using an adapted version of the gridline intersect method, following staining with trypan blue (Gutjahr et al. 2008). Slides were viewed on a Leica DM2500 microscope, and images were produced with a Leica DFC420 microscope camera. The method makes

assessment of colonisation at 100 random intervals along the sampled roots, and at each field of view, mycorrhizal structures were counted. This included intraradical hyphae, arbuscules, and vesicles. Extraradical hyphae were omitted due to variation resulting from how the roots were washed between different experiments and seasons in this PhD. Spore counts were omitted for similar reasons, and despite the importance of spores in confirming the completion of the fungal life cycle, vesicle formation can provide information about whether the fungus is receiving adequate carbon from its host. Percentage colonisation was established for spring barley, winter oats, oat and vetch cover crops, and a representative sample of non-mycorrhizal radish, to confirm that fungal structures were absent.

Analysis of AM Fungal Phenotypic Variation by Alexa WGA-488 staining and Confocal Microscopy

Roots were removed from ethanol and gently rinsed with ddH_2O. Roots were then transferred into 20% (w/v) KOH and left for 2-3 days at room temperature. Next, KOH was removed and roots were thoroughly rinsed with ddH_2O, before being transferred to 0.1M HCl for 1-2 hours. HCl was removed and roots were again rinsed twice with ddH_2O, and once with 1x phosphate buffered saline (PBS). Then, roots were transferred into 0.2 µg/ml Wheat Germ Agglutinin Alexa Fluor™ 488 Conjugate in 1x PBS, wrapped in foil, and left for 1-2 weeks. Immediately prior to imaging, roots were rinsed twice with ddH_2O before being transferred to 10 µg/ml propidium iodide in 1x

PBS, for 3-4 minutes. Roots were rinsed twice more with ddH2O before being mounted.

Roots were visualised on a Leica SP8 confocal microscope, exciting WGA bound to AM fungal chitin at 488 nm, and propidium iodide bound to root cellulose at 561 nm. Line and frame averages were increased to 8 to increase sharpness of images.

DNA Extraction with an Adapted CTAB Protocol

Frozen root material was ground with 1 tungsten carbide bead in a tissue lyser for 2 minutes, pausing after one minute so ensure even grinding. Next, 800ul of preheated 2% CTAB buffer, containing 100mM Tris-HCl (pH 8.0), 1.4M NaCl, 20mM EDTA (pH 8.0), 1% polyvinylpyrrolidone K30, and 0.2% freshly added β-mercaptolethanol, was added to tubes containing the ground tissue. Samples were vortexed before being incubated at 60°C for 10 minutes. Next, the samples were vortexed and 500 µl chloroform:isoamyl-OH 24:1 solution was added to the tube containing CTAB buffer and root material. The tube was inverted for 20-30 seconds before being centrifuged at 12,000 X g for 5 minutes at 4°C. The aqueous layer was transferred to a clean 2ml Eppendorf tube and 600 µl 100% isopropanol was added to each tube. The tube was inverted for 20-30 seconds, then incubated for 20 minutes at room temperate (RT). Tubes were centrifuged at 12,000 X g for 3 minutes at RT, and supernatant was discarded. To the same tube, 800 µl of 75% ethanol and 10 µl 3M NaOAc was added, plates were inverted for 20-30 seconds, and

incubated at RT for 30 minutes. Next, tubes were centrifuged at 12,000 X g for 5 minutes at RT, supernatant was removed, and 800 µl 75% ethanol was added to each tube. Tubes were inverted for 20-30 seconds, incubated at RT for 30 minutes, then centrifuged at 12,000 X g for 5 minutes at RT. Supernatant was removed and tubes were left to air dry for up to 10 minutes. DNA was then eluted in 30 µl MilliQ H_2O. Prior to PCR, 5 µl of DNA was diluted 1:100 with MilliQ H_2O to improve amplification of AM fungi. Concentrated, and diluted DNA was stored at -20°C.

PCR Conditions, Sequencing, and Bioinformatics

PCR conditions, sequencing, and bioinformatics were identical to Chapter Two.

Statistical Analysis for the Glasshouse Trial

Data conformed to the Gauss-Markov assumptions for parametric analysis of variance, and was therefore analysed with the lm package in R (R Core Team 2019). Post-hoc tests were conducted with Tukey's Honest Significant Difference test using the standard threshold of p=0.05. Tukey's HSD is a post-hoc test based on the studentized range distribution, used to determine whether group means significantly differ from one another.

Analysis of sequence data followed the methodology of Chapter Two. Observations of taxa abundances lower than 0.1% were omitted from further

analysis. Additionally for the glasshouse experiment, taxa observed in three or less samples were removed from further analysis. Two taxa, VTX00307 (*Glomus sp.*) consisting of 128 reads across two samples, and VTX00344 (*Glomus Glo72*) consisting of 159 reads across 3 samples were removed from further analysis, as these were determined to have arisen from sequence error.

Field Methods

Site Selection and Preparation for the Cover Crop Inoculation Trial

The trial site was located at 52°37'37.9"N 1°11'03.5"E, approximately 7.5km west of Norwich, UK. Satellite and NDVI images were consulted to select a uniform area of the field in which to set up the trial (Figure 3.4). The soil in the experimental plots were classified as a Burlingham 1 sandy loam, with soil organic matter level of 2.3%, pH of 7.6, 6.8 kg/ha available N, 1.5 mg/kg nitrate, 0.3 mg/kg ammonium, 14.7 mg/l P, 51.2 mg/l K and 21.5 mg/l Mg. The trial site was ploughed to reduce the effect of previous compaction, and to disrupt existing networks of AM fungi. Physicochemical parameters for the glasshouse substrate and Bawburgh field soil are compared in Table 3.1.

Figure 3.4 Satellite image of the Bawburgh trial site from September 2006, showing evidence of heterogeneity potentially due to water availability.

Table 3.1 Comparison of soil physicochemical parameters for the glasshouse substrate and Bawburgh field site. Glasshouse soil was homogenised and a single sample was sent for analysis, whereas field measurements are presented as a mean of 54 plot measurements.

Soil Parameter	Glasshouse	Field
SOM (%)	1.8	2.3
pH	7.8	7.6
Available N (kgN/ha)	252.5	6.8
Nitrate (mg/kg)	66.67	1.5
Ammonium (mg/kg)	0.67	0.3
Available P (mg/l)	6.8	14.7
Available K (mg/l)	34	51.2
Available Mg (mg/l)	56	21.5

Cover Crop Choices and Drilling at the Bawburgh Site

Cover crop seed was drilled on 28th August 2018 and rolled on 31st August 2018 to further improve establishment (Figure 3.5). Three species, one oat, one vetch, and one radish were chosen as to represent a cereal, legume, and brassica cover crop. The brassica, a new variety "SMART" radish (Green-Cover, USA) has been bred specifically for the cover crop and soil health market, and boasts greater plant tillering and biomass. Seed rates of the cover crop mixtures were as follows: SMART radish (10 kg/ha), vetch (100 kg/ha), oats (60 kg/ha), SMART radish + oats (6 + 30 kg/ha), SMART radish + oats (10 + 30 kg/ha), oats + vetch (30 + 50 kg/ha), SMART radish + vetch (10 + 50 kg/ha), and SMART radish + vetch + oats (10 + 50 + 30 kg/ha). Representative images of the cover crop mixtures are shown in Figure 3.6. Cover crops and their mixtures were grown with, and without a five species AM fungal inoculum, sourced from PlantWorks (Sittingbourne, UK) at the recommended rate (10 kg/ha).

Cover crops received no fertiliser or other crop protection amendments, and were desiccated with 3.2 L/ha of Roundup Vista Plus on 21st February 2019. Trial plots received a shallow non-inversion cultivation on 21st March 2019 to create a favourable seedbed and to ensure proper establishment of the following crop.

Figure 3.5 Drilling cover crop seed with the NIAB trials team in August 2018.

Cover Crop Sampling and Storage

Cover crops were sampled on 3rd January 2019, following the method of Higo et al. (2014), to attain a suitable number of plants to accurately represent AM fungal diversity, but fitting within the time and resource constraints of the PhD. Briefly, in each plot, five plants of each species were pooled into one biological sample. three pooled samples per species were taken for each of the 54 plots, equating to 15 plants per species per plot, and a total of 630 plants across the trial. Plants were sampled evenly across the length of each plot, ensuring samples were taken at least 1m away from the plot boundary, to reduce edge effects.


a
b
c
d
e
f

Figure 3.6 Cover crop treatments on 24th January 2019, showing a) fallow, b) oat, c) vetch, d) radish, e) low seed rate radish and oat, f) high seed rate radish and oat, g) oat and vetch, h) radish and vetch, i) oat, vetch, and radish cover crops.

For each pooled biological sample, cover crop shoots were separated from roots, and dried at 60°C for 48 hours for analysis of dry weight. Roots of were gently washed of soil, cut into 1cm fragments, and representative samples were taken for DNA extraction and microscopic analyses. For DNA extraction, 100mg of root material was flash frozen in liquid nitrogen before being stored at -80°C. For microscopic analyses, approximately 10-12 1cm

fragments of root material was stored in 50% ethanol for analysis of root length colonisation by trypan blue staining. For some additional samples, a second assortment of root material was stored in 50% ethanol for staining with Wheat Germ Agglutin (WGA) 488 conjugate, for visualisation of fine scale phenotypic differences in AM fungi in the root.

Cover Crop Soil Sampling at the Bawburgh Trial

Soil sampling was carried out on 7th January 2019. Eight soil cores were collected per plot at random, to a depth of 30cm. Cores were pooled, stones and plant material was removed, and samples were sealed and placed into cool boxes. Cool boxes were sent via courier to NRM labs (Bracknell, UK) for analysis of soil nutrients.

Drilling of Laureate Spring Barley at the Bawburgh Trial

Laureate spring barley was drilled on 26th March 2019 at a seed rate of 170 kg/ha (300 seeds/m^2), following cover crop desiccation with glyphosate and a shallow non-inversion cultivation (Figure 3.7).

Although several publications have shown that minimal soil disturbance is beneficial to maintenance of networks of AM fungi, and that there may be a negative effect of glyphosate on AM fungal colonisation rates, these methods were used for the following reasons. Firstly, it was important to ensure

good and even establishment of plants in the trial, ensuring that plant establishment did not bias AM fungal communities, plant growth, or yield results. As an AHDB/AFCP funded project, it was also important to replicate normal current farm practice that is used on a large majority of arable farms with similar soil conditions and cropping. It is anticipated that if or when glyphosate is phased out of farm application, and the use of direct drilling into cover crop residues increases, it will be easier to conduct trials of this type without concern for poor establishment or crop failure once soil structure and key attributes such as organic matter content have stabilised at a higher rate.

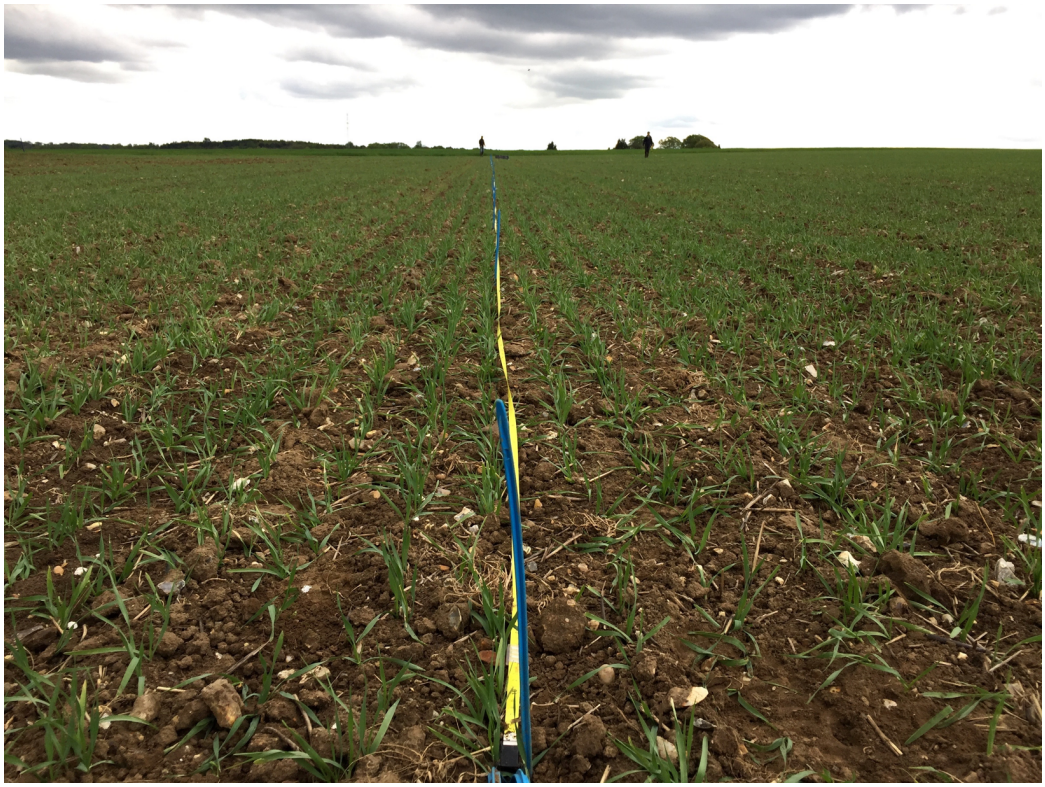


Figure 3.7 Installing plot markings for spring barley var. Laureate in late spring 2019.

2019 Application of Plant Protection and Growth Products at the Bawburgh Trial

Spring barley was sprayed with 130 L/ha N, and 24 L/ha SO_3 on 24th April 2019. On 13th May 2019 the crop was sprayed with 70 g/ha Finish SX herbicide (containing 67 g/kg metsulfuron-methyl and 333 g/kg thifensulfuron-methyl for spring weed control) 0.5 L/ha Jaunt fungicide (containing 75 g/L fluoxastrobin, 150 g/L prothioconazole and 75 g/L trifloxystrobin) and 1 L/ha Headland Multiple micronutrient foliar fertiliser (containing 84 g/L Magnesium (Mg), 110 g/L Copper (Cu), 330 g/L Manganese, and 84 g/L Zinc (Zn)).

Plant Sampling and Yield Measurement in Spring Barley at the Bawburgh Trial

Spring barley was sampled on 29th May 2019, again following the method of Higo et al. (2014), described above. This date corresponded to growth stage (GS) 38-40 on Zadoks growth scale, at the end of stem elongation but prior to booting. Three biological replicates, representing 15 individuals was sampled per plot, a total of 810 plants across the trial. Spring barley was harvested for each plot on 15th August 2019 and standardised to 15% moisture content for further analysis. Unfortunately, damage to plots 53 and 54 in the trial, meant that yield could not be estimated from these plots.

Spring Barley Soil Sampling at the Bawburgh Trial

Soil sampling was carried out on 27th May 2019, to determine changes in soil nutrients caused by the cover crop treatments. Soil cores were taken, processed, and dispatched to NRM as above. Due to sampling in very warm weather, measurements of soil nitrogen could not be accurately measured and have been omitted from further analysis.

Figure 3.8 Installing plot markings for winter oat var. Mascani in late autumn 2020.

Drilling of Mascani Winter Oat at the Bawburgh Trial

Trial plots were prepared by conducting a shallow cultivation with a Vaderstad Opus cultivator on 11th September 2019, followed by application of 0-18-36 fertiliser containing P and K. Mascani winter oat was drilled on 6th November 2019, following application of Roundup Vista Plus herbicide on 3rd October 2019 (Figure 3.8).

2020 Application of Plant Protection and Growth Products at the Bawburgh Trial

ADAMA Hurricane SC, a broadleaf herbicide, and Sumitomo Kingpin insecticide was applied to the emergent crop on 19th November 2019 at the recommended rate. The oat crop received two applications of liquid N 27 + S, on 13th March 2020 and 21st April 2020. Prior to harvest, the crop received applications of BASF Gemstone fungicide, containing epoxiconazole and pyraclostrobin, and Agrovista 3 See 750 plant growth regulator, at 0.8 L/ha and 2.0 L/ha respectively.

Plant Sampling and Yield Measurement in Winter Oat at the Bawburgh Trial

Winter oat was sampled on 11th May 2020, which corresponded to GS38-40. Like for spring barley, three biological replicates, representing 15 individuals were sampled per plot, a total of 810 plants across the trial. Winter oat was

harvested for each plot on 24th August 2020 and standardised to 15% moisture content for further analysis (Figure 3.9).

Figure 3.9 Harvesting of winter oat var. Mascani on 24th August 2020.

Measurement of Soil Physicochemical Properties

Soil physicochemical properties were assessed by NRM Laboratories (Bracknell, UK) and follow the protocols outlined below.

pH

Prior to analyses, soil samples were air dried and sieved at 2mm to remove plant material, before being mixed with water in the ratio of 1:2.5. pH was determined using a potentiometer, in a temperature-controlled environment.

Available Nitrogen

The mineral-N content of the soil is defined as the total nitrogen available to the plant, this includes nitrate-N, nitrite-N and ammonium-N. Prior to analyses, soil samples were stored between 2-8°C to restrict further N mineralisation, and stones were removed. Soil samples were then shaken with 2M Potassium Chloride to form solutions. Nitrate was reduced to nitrite by exposure to cadmium metal in the form of an open tubular cadmium reactor (OTCR). Resulting total oxidised nitrite (TON) was determined by the formation of a diazo compound between nitrite and sulphanilamide, where addition of N-1-Napthylethylenediamine dihydrochloride yielded a red azo dye. The resulting colour was measured colourimetrically at 540nm. The assay was repeated concurrently, omitting the reduction of nitrate. Subsequently, nitrate was calculated by deducting nitrite-N from TON. Ammonium-N was determined by the indophenol-blue method, and measured colourimetrically at 540nm. Precipitation of calcium and magnesium hydroxides was eliminated by the addition of a combined potassium sodium tartrate/ sodium citrate complexing reagent.

Available Phosphorus

Available phosphorus is defined at the fraction of soil phosphorus available to a plant in solution. Prior to analyses, soil samples were air dried and sieved at 2mm to remove plant material. Soil was mixed with 0.5M sodium bicarbonate solution (Olsen's reagent), and shaken at 20°C at pH 8.5 for 30 minutes. The solution was mixed with ammonium molybdate, and reduced with ascorbic acid, forming a blue colour which was measured spectrophotometrically at 880nm.

Potassium and Magnesium

Prior to analyses, soil samples were air dried and sieved at 2mm to remove plant material. Soil was then shaken with 1M Ammonium Nitrate for 30 minutes at 20°C, filtered, and concentration of potassium was determined by atomic absorption spectrometry.

Organic matter

Soil organic matter (OM) was determined by the loss on ignition method. Prior to analyses, soil samples were air dried and sieved at 2mm to remove plant material. Samples were weighed then heated to 430°C until constant weight. Samples were reweighed and reduction of mass was attributed to the loss of OM.

Measurement of Soil Penetration Resistance at the Bawburgh Trial

Measurements of soil penetration resistance were taken on 20th March 2020, following a failed attempt during summer of 2019. Five to 10 measurements were taken per plot with a soil penetrometer (Figure 3.10), aiming to maximise depth recorded. 'Clean' measurements, where the probe did not encounter a stone, were very rare at this site. Subsequently, data was manually filtered to remove hits, which are clearly visible as spikes in resistance measurements.

Figure 3.10 Taking measurements with a soil penetrometer at the Bawburgh trial on 20th March 2020.

Microscopic and Molecular Analysis of Experimental Samples

Microscopic and molecular analyses were conducted as in the glasshouse trial, outlined above.

Statistical Analysis for Field Conditions

Measures of alpha and beta diversity were calculated in the Phyloseq package (McMurdie and Holmes 2013) in R (R Core Team 2019) following the methods of Chapter Two.

Restricted Maximum likelihood (REML) estimates of the predictors of linear mixed effects models were determined with the lmer function in the package lme4 in R Studio (version 3.5.1) (Bates et al. 2015; RStudio Team 2015). AM fungal root length colonisation, and alpha diversity metrics were considered as predictor and response variables in different statistical models. Experimental block was used as a random effect to account for spatial variation in soil physiochemical parameters. Accounting for this variation with a more complex model including autoregressive correlation structures, or correlation in rows and columns did not improve either model in this trial. Histograms of predictor and response variables, and model residuals were plotted to ensure that the Gauss-Markov assumptions of linearity, non-collinearity, randomness, exogeneity, and homoscedasticity had been met. Post-hoc multiple comparisons were conducted using the contrast function in the package emmeans (Lenth et al. 2020).

3.3 Results

Glasshouse Analysis

Laureate Spring Barley Growth and Biomass in the Glasshouse Trial

All 70 pre-germinated seedlings grew successfully in the glasshouse conditions. The lowest and highest shoot weights of 0.523g and 1.269g were observed in the control treatment. There was a significant negative effect of inoculum on shoot dry weight (ANOVA: $F_{6,63} = 3.37$, $p = 0.006$) (Figure 3.11), The highest mean dry shoot weight of 0.927g was observed in the control treatment, and was significantly larger than the *R. irregularis* treatment, which was 0.688g (Tukey $p = 0.003$). *F. mosseae* (0.860g), *F. geosporum* (0.769g), *C. claroideum* (0.838g), *G. microaggregatum* (0.810g) and the five species mix (0.756g) were not significantly different to the control, or *R. irregularis* at the $p < 0.05$ level.

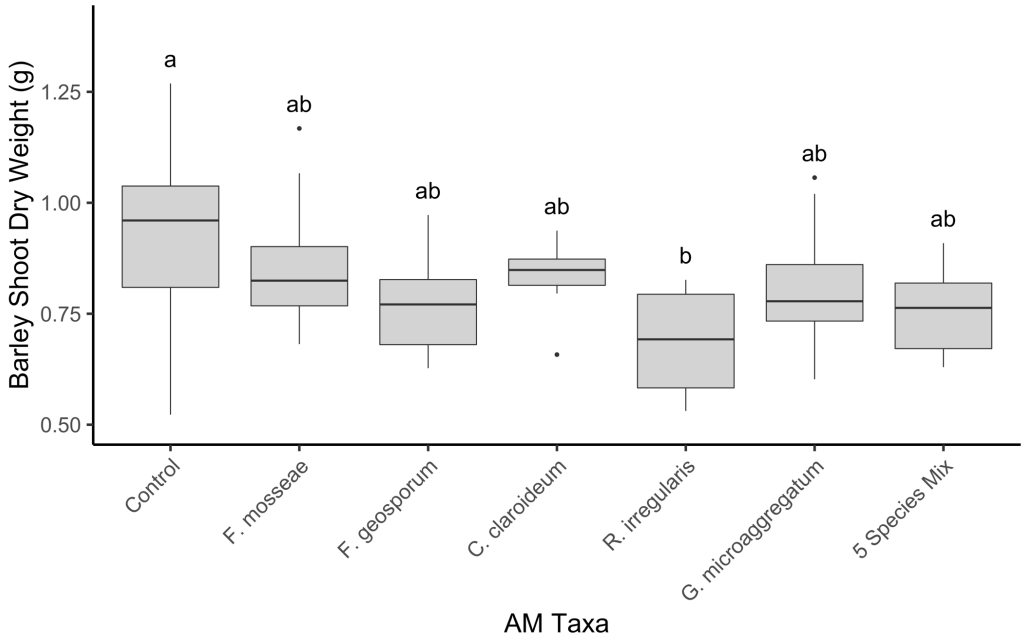


Figure 3.11 Dry weight of spring barley (var. Laureate) following inoculation of each of the five AM taxa, and the five species mix, compared to the control treatment. Means followed by the same letter did not differ significantly (Tukey test, $p<0.05$).

Spring Barley (Var. Laureate) Root Length Colonisation in the Glasshouse Trial

Barley roots were removed from 50% EtOH, cleared, and stained with trypan blue dye, which binds to fungal chitin and allows for microscopic quantification of fungal structures (Figure 3.12). In the inoculated treatments, the greatest mean root length colonisation was observed in the *R. irregularis* treatment (84.6%), which was significantly higher than all other treatments (Tukey $p < 0.001$). The five species mix exhibited the second greatest root length colonisation, at 56.7%, followed by *F. mosseae* (21.0%), *G. microaggregatum* (12.4%), *F. geosporum* (12.0%) and *C. claroideum* (10.9 %), which all exhibited significantly reduced colonisation when compared to the

R. irregularis and mixed treatments. None of the 10 plants in the control treatment were colonised by AM fungi. There was a significant effect of inoculum on barley root length colonisation (ANOVA: $F_{6,63} = 72.14$, $p<0.001$).

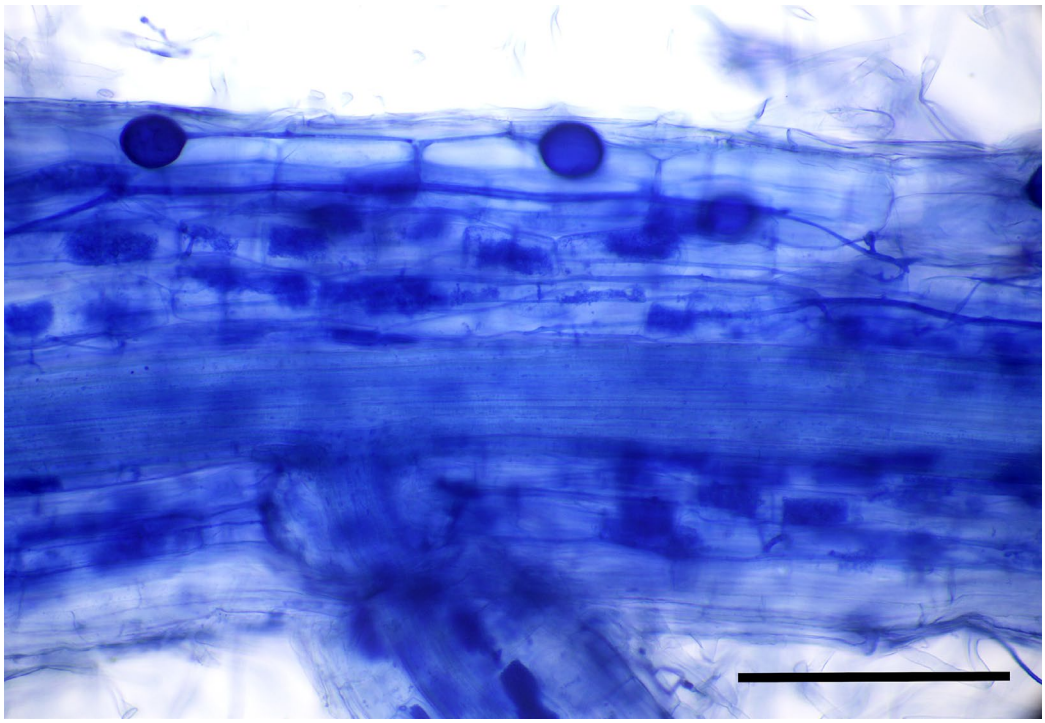


Figure 3.12 Trypan blue stained micrograph of spring barley var. Laureate from the Glasshouse trial, colonised with *Rhizophagus irregularis*. Scale bar represents 200µm.

Colonisation of the root length by arbuscules ranged from 32.4% in *R. irregularis* treated plants, to 4.4% in plants inoculated with *G. microaggregatum* (Figure 3.13). There was a significant effect of inoculum on root length colonisation by arbuscules (ANOVA: $F_{6,63} = 16.52$, $p < 0.001$). Arbuscule root length colonisation of *R. irregularis* and the five species mix was significantly higher than of *F. geosporum, G. microaggregatum, C. claroideum,* and the

control. Arbuscule root length colonisation of *F. mosseae* (7.2%), *F. geosporum* (5.9%), *C. claroideum* (5.4%), and *G. microaggregatum* (4.4%) was not significantly different from the control treatment (Tukey $p > 0.05$).

The presence of vesicles (the fungal storage structure) in colonised roots, ranged from 4.9 ± 1.4% in *F. geosporum* to 74.2 ± 2.63 in *R. irregularis*. There was a significant effect of inoculum on root length colonisation by vesicles (ANOVA: $F_{6,63} = 67.47$, $p < 0.001$). Vesicular colonisation by *R. irregularis* (74.2 ± 2.6%) was significantly higher than the five species mix inoculum (44.7 ± 5.5%), which in itself was significantly higher than *F. mosseae* (15.0 ± 2.92%). *F. mosseae* vesicle presence was not significantly higher than that of *G. microaggregatum* (7.3 ± 2.2%), and *F. geosporum* (4.9 ± 1.4), but was higher than *C. claroideum* (6.2 ± 3.7%) and the control. *G. microaggregatum* and *G. geosporum* were significantly higher than the control (0%), but not *C. claroideum*, which itself was not higher than then control.

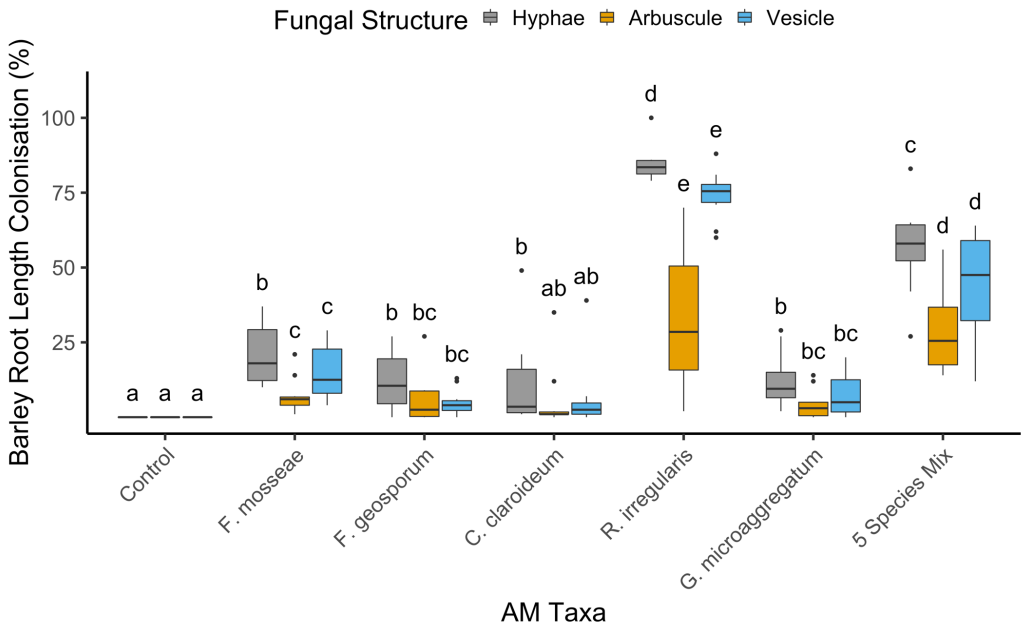


Figure 3.13 Root length colonisation (RLC) of spring barley (var. Laureate) following inoculation of each of the five AM taxa, and the five species mix, compared to the control treatment. Multiple comparisons were conducted across treatments only. Means followed by the same letter did not differ significantly (Tukey test, $p<0.05$).

Relative Colonisation of AM Taxa in the 5 Species Mix

All 10 glasshouse samples were successfully amplified. After stringent quality thresholding, 333,342 Glomeromycotinian DNA sequences remained for further analysis. Number of reads per sample averaged 33,334 and ranged from 41,267 to 6098, which was considerably lower than even the next lowest, at 17,222.

In all 10 samples, sequence reads were dominated by reads representing *R. irregularis*, which comprised almost three quarters of total reads. The next most prominent coloniser of barley roots was *F. mosseae*, with 15% of reads. *C. claroideum* and *F. geosporum* were represented by 8% and 7% of reads

respectively. Reads of *G. microaggregatum* were not identified in any sample. To ensure that VT were not being incorrectly assigned to morphospecies, the two known sequences of VTX00104/ *G. microaggregatum* were aligned to the identified taxa, and shown in red in Figure 3.14.

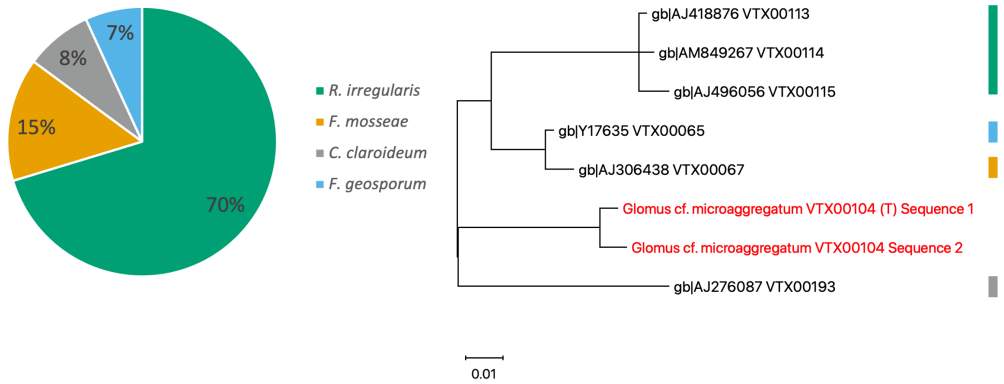


Figure 3.14 Relative colonisation of inoculated AM fungal taxa.
Proportion of the five AM species colonising spring barley (var. Laureate) in the glasshouse trial.

Field Analysis

Cover Crop Green Area Index Through the Winter Season

Early drilling of cover crops ensured a fast rate of growth through September, before showing a reduced rate of growth through the winter months. Across all experimental treatments, GAI increased from 0.24 ± 0.001 in September, to 1.34 ± 0.08 in October, which represented 69.8% of the final GAI measurement in February, of 1.92 ± 0.08 (Figure 3.15). The highest mean GAI observed in February was 2.37, and associated with the oat, vetch and radish

cover crop mix, sown both with and without AM fungal inoculum. The lowest mean GAI in February, excluding the fallow treatment, was observed following the AM fungal inoculated oats and vetch. In the control, fallow plots, mean GAI increased from 0.167 ± 0.03 in the non-inoculated plots to 0.733 ± 0.07, whereas GAI in inoculated plots increased from 0.200 in September to 0.667 ± 0.133 in February. After mean-centring data to account for bimodality, the cover crop species (ANCOVA: $F_{8,214} = 13.38$, $p < 0.001$), and the interaction between cover crop species and sampling month (ANCOVA: $F_{40,214} = 1.80$, $p < 0.004$), had a significant impact on plot GAI. Addition of AM fungal inoculum had no impact on GAI of cover crops through the growing season (ANCOVA: $F_{1,214} = 1.47$, $p = 0.227$).

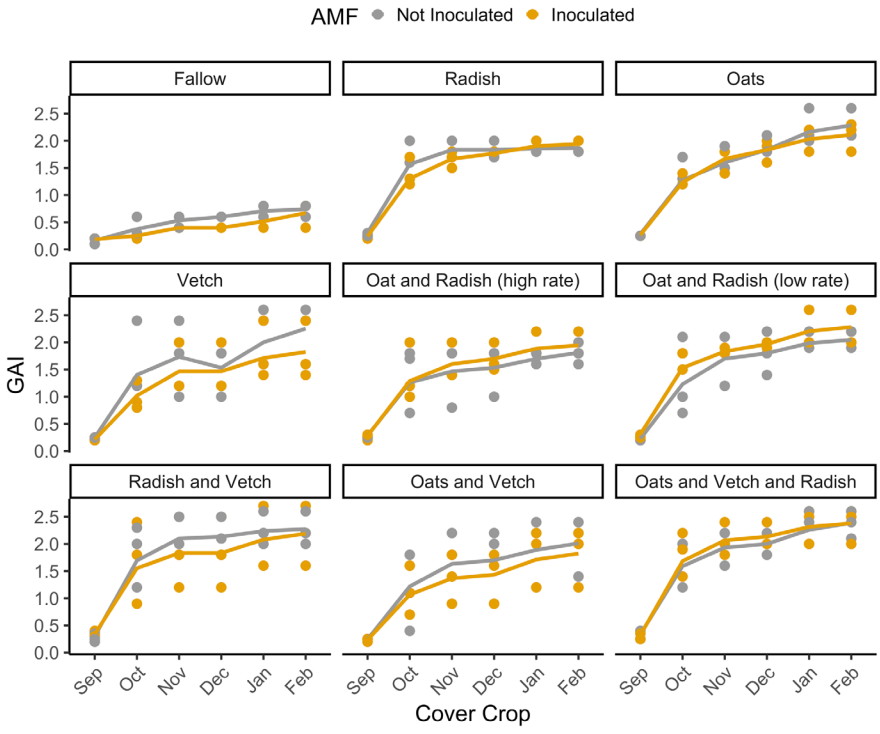


Figure 3.15 Green Area Index (GAI) of spring barley (var. Laureate).
GAI following each of the cover crop treatments, inoculated with or without an AM fungal inoculum.

Influence of Cover Crop on Soil Organic Matter at the Bawburgh Trial

The mean organic matter at the site, prior to experimental treatments was 2.3%, and ranged from 2% to 2.6%. All experimental treatments, including the control, displayed increase in SOM (Figure 3.16). The greatest increase of 0.43 ± 0.09% represented a 16.5% relative increase in SOM, and was observed in the inoculated oat and high seed rate radish plots. The lowest absolute increase in SOM of 0.03 ± 0.12% was seen in the inoculated fallow plots. Despite this, the uninoculated fallow plots displayed the second largest increase of SOM, of 0.4 ± 0.1%. Across the trial, neither the cover crop treatment (ANOVA: $F_{8,34} = 0.85$, $p = 0.563$), nor addition of AM fungal inoculum (ANOVA: $F_{1,34} = 0.43$, $p = 0.516$) influenced SOM individually, but their interaction did cause a significant effect (ANOVA: $F_{8,34} = 2.85$, $p = 0.016$). Post-hoc analysis of cover crop treatments revealed that AM fungal inoculation significantly reduced accumulation of SOM in the fallow plots (Tukey $p = 0.004$), and increased SOM in the oat and high seed rate radish plots (Tukey $p = 0.057$), although not significantly.

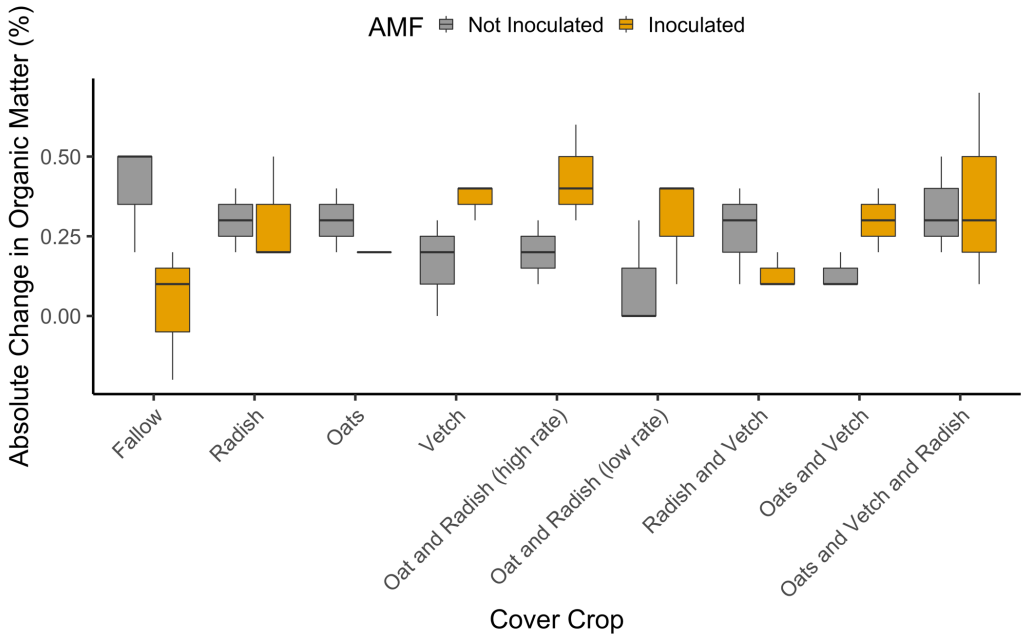


Figure 3.16 Absolute change in Soil Organic Matter (SOM) following experimental treatments

Influence of cover crop treatments, and AM fungal inoculation on the soil organic matter content of the experimental plots.

Influence of Cover Crop on Soil Penetration Resistance at the Bawburgh Trial

Five to 10 measurements of soil penetration resistance were taken with a custom-built soil penetrometer. Due to a large number of stones, there would be as many as 20 failed attempts per plot, to achieve a satisfactory number of measurements without a stone collision. There was significant variation in soil penetration resistance across the trial, resulting from the cover crop treatment (ANCOVA: $F_{8,1025} = 13.24$, $p < 0.001$), and the interaction between the cover crop treatment and AM fungal inoculation (ANCOVA: $F_{8,1025} = 3.73$, $p < 0.001$), but not AM fungal inoculation alone (ANCOVA: $F_{1,1025} = 1.91$, $p = 0.168$). Plots following a radish (Tukey $p = 0.985$), or oat,

vetch, and radish (Tukey $p = 0.998$) cover crops exhibited the lowest soil penetration resistance, although this was not significantly better than the fallow plots (Figure 3.17). Oats and vetch (Tukey $p = 0.006$), oat and radish (high rate, Tukey $p = 0.001$), and radish and vetch (Tukey $p < 0.001$) had significantly higher penetration resistance compared to the control plots.

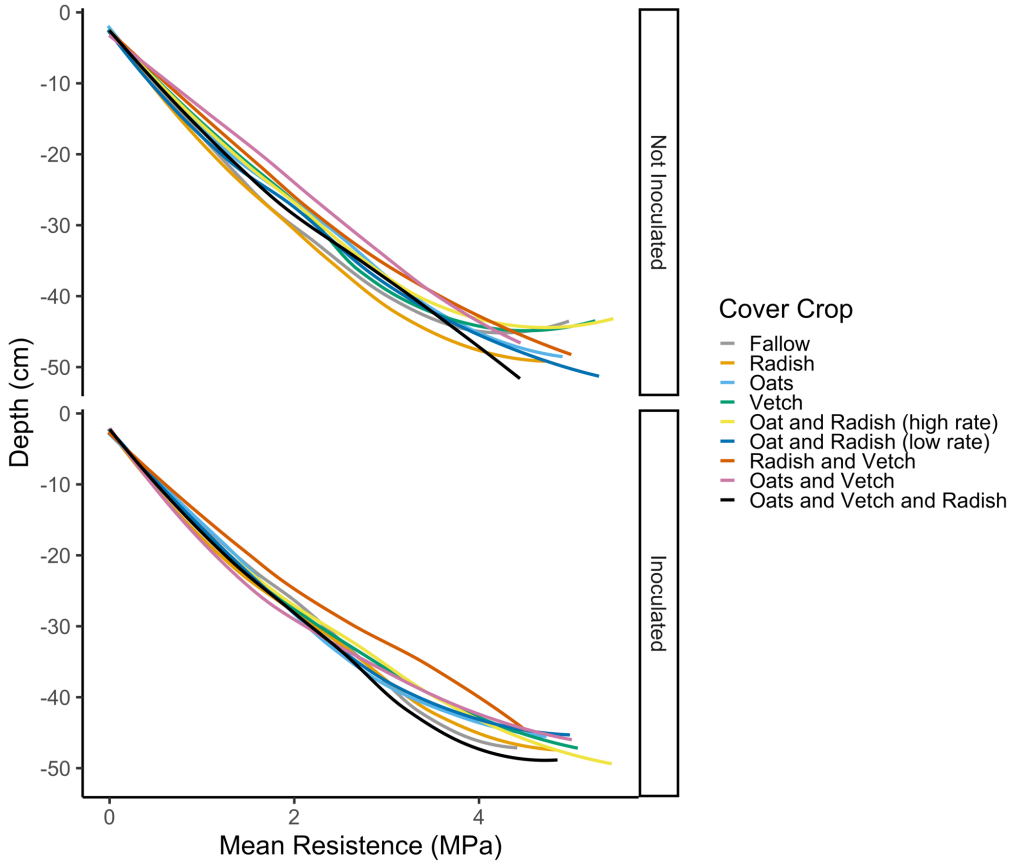


Figure 3.17 Soil penetration resistance (MPa) following experimental treatments. Influence of cover crop treatments, and AM fungal inoculation on the soil penetration resistance, measured in megapascals (MPa) of the experimental plots.

Cover Crop Biomass at the Bawburgh Trial

Cover crops were sampled on 3rd January 2019, pooling 15 individual plants into three biological replicates, to increase the number of plants sampled whilst maintaining feasible analysis. Radish cover crops were significantly larger than the oat and vetch cover crops, and therefore, due to model complexity, the cover crop biomass was modelled as individual species.

Mean oat biomass across the cover crop treatments was 6.19 ± 0.21g, and ranged from 5.19g in the uninoculated plots growing oat with a high radish seed rate, to 7.09g in the inoculated plots grown with the low seed rate radish alone (Figure 3.18). There was no significant effect of AM inoculation (ANOVA: $F_{1,18} = 0.85$, $p = 0.369$), the cover crop mix from which the oat was sampled (ANOVA: $F_{4,18} = 0.82$, $p = 0.530$) or their interaction (ANOVA: $F_{4,18} = 0.36$, $p = 0.835$) on oat biomass.

Mean vetch biomass across the cover crop treatments was 6.53 ± 0.30g, and ranged from 4.73g when inoculated and grown alone, to 8.73g when grown with oat and uninoculated. The inoculated oat and vetch mix produced the second largest vetch biomass, of 7.21g. After log transformation to conform to assumptions of normality, there was no significant effect of AM inoculation (ANOVA: $F_{1,14} = 0.83$, $p = 0.377$), the cover crop mix from which the vetch was sampled (ANOVA: $F_{3,14} = 1.54$, $p = 0.248$), or the interaction between AM inoculation and the cover crop mix from which the vetch was sampled (ANOVA: $F_{3,14} = 0.50$, $p = 0.690$), on vetch biomass.

Mean radish biomass across the experimental treatments was 15.1 ± 0.52g and ranged from 11.18g in the inoculated plot with radish and vetch, to 18.93g when inoculated, grown with oat and drilled at a low seed rate. There was no significant effect of AM inoculation (ANOVA: $F_{1,18} = 0.57$, $p = 0.461$), or the interaction between AM inoculation and the cover crop mix from which the radish was sampled (ANOVA: $F_{4,18} = 1.60$, $p = 0.218$) on radish biomass. There was a significant effect of the cover crop mix on radish shoot biomass (ANOVA: $F_{4,18} = 3.23$, $p = 0.037$), and post-hoc testing revealed that radish grown at low seed rate with oat, had significantly higher biomass than radish grown at a high seed rate, and with vetch (Tukey $p = 0.029$). This result was associated with inoculation of other species within these cover crop treatments, as the mean inoculated radish biomass was 69.3% larger in the radish and oat plots, compared to the radish and vetch. All other pairwise comparisons were not significant at the $p < 0.05$ level.

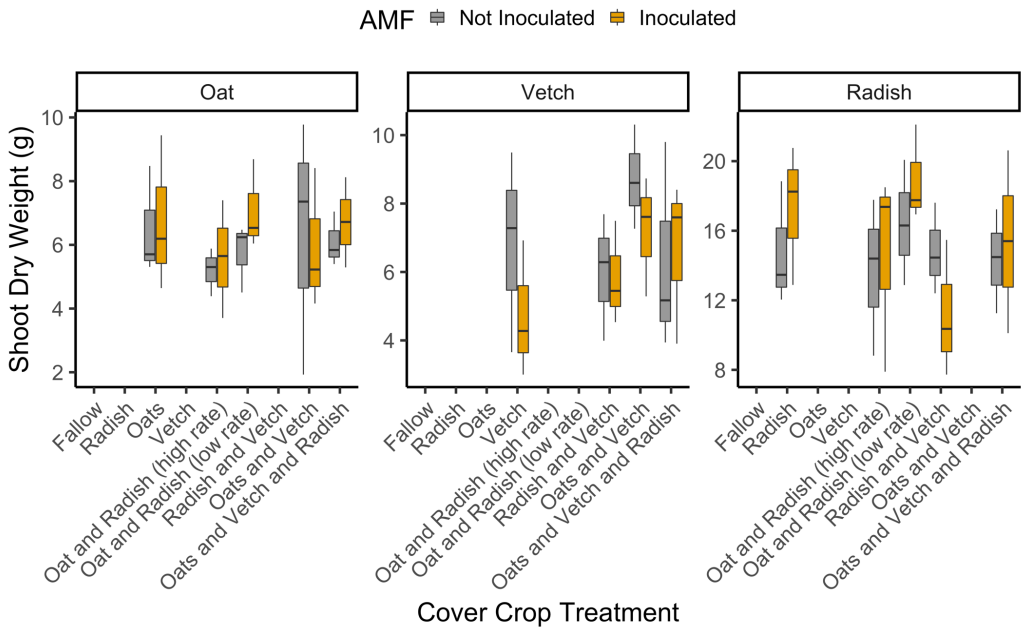


Figure 3.18 Cover crop dry shoot weight in the Bawburgh trial.
Dry shoot weights represent the green shoot of five spring barley plants, classified as one biological replicate.

Cover Crop Root Length Colonisation at the Bawburgh Trial

Cover crop roots were stored at 4°C for 24 hours prior to processing and storage in 50% ethanol. Representative radish roots from each treatment were also stained to confirm radish as a non-host of AM fungi. Mean oat RLC was 38.6% and ranged from 33.2 to 48.7%, which was significantly higher than vetch RLC which ranged from 15.3% to 29.4% (ANOVA: $F_{1,39}$ = 11.50, p = 0.002, Figure 3.19).

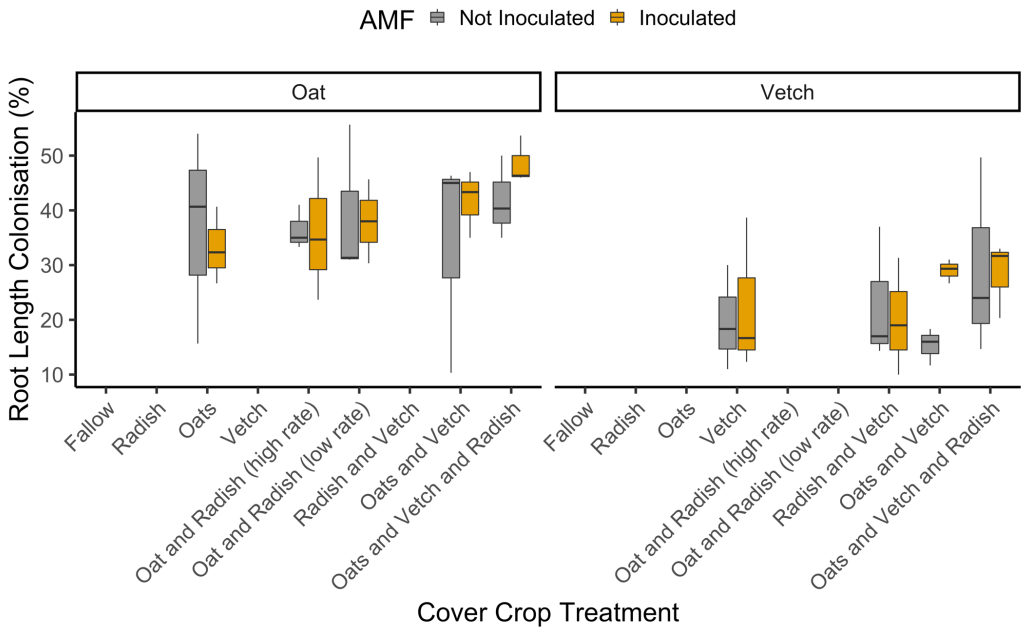


Figure 3.19 Oat and vetch cover crop root length colonisation (RLC) by trypan blue. RLC expressed as a percentage of the total root length colonised, following measurement of an adapted version of the grid intersect method.

The highest mean oat colonisation was observed in the inoculated three-way cover crop mix, while the highest vetch RLC was in the non-inoculated three-way mix. The lowest RLC for oat and vetch cover crops were observed in the inoculated single species oat, and non-inoculated oat and vetch cover crop mixtures, respectively. Variation in vetch RLC in the latter cover crop mix was lower than in other treatments, for both inoculated and non-inoculated plots. RLC of vetch from the inoculated oat and vetch cover crop plots were 89.5% higher than their non-inoculated equivalents, although this difference was not statistically significant (Tukey $p = 0.072$). Overall, there was no effect of AM inoculation (ANOVA: $F_{1,39} = 0.21$, $p = 0.648$), the cover crop mix from which the sample was taken (ANOVA: $F_{6,39} = 0.83$, $p = 0.554$), or their interaction (ANOVA: $F_{6,39} = 0.52$, $p = 0.793$).

To further evaluate the impact of cover crops on AM fungi, oat and vetch cover crop roots were stained with Alexa 488 conjugate, and visualised on the Leica SP8 microscope. AM fungal arbuscules in oat were generally more square than in vetch, and also displayed irregular shaped vesicle structures, however it was not clear whether this was due to fungal or plant root morphology (Figure 3.20).

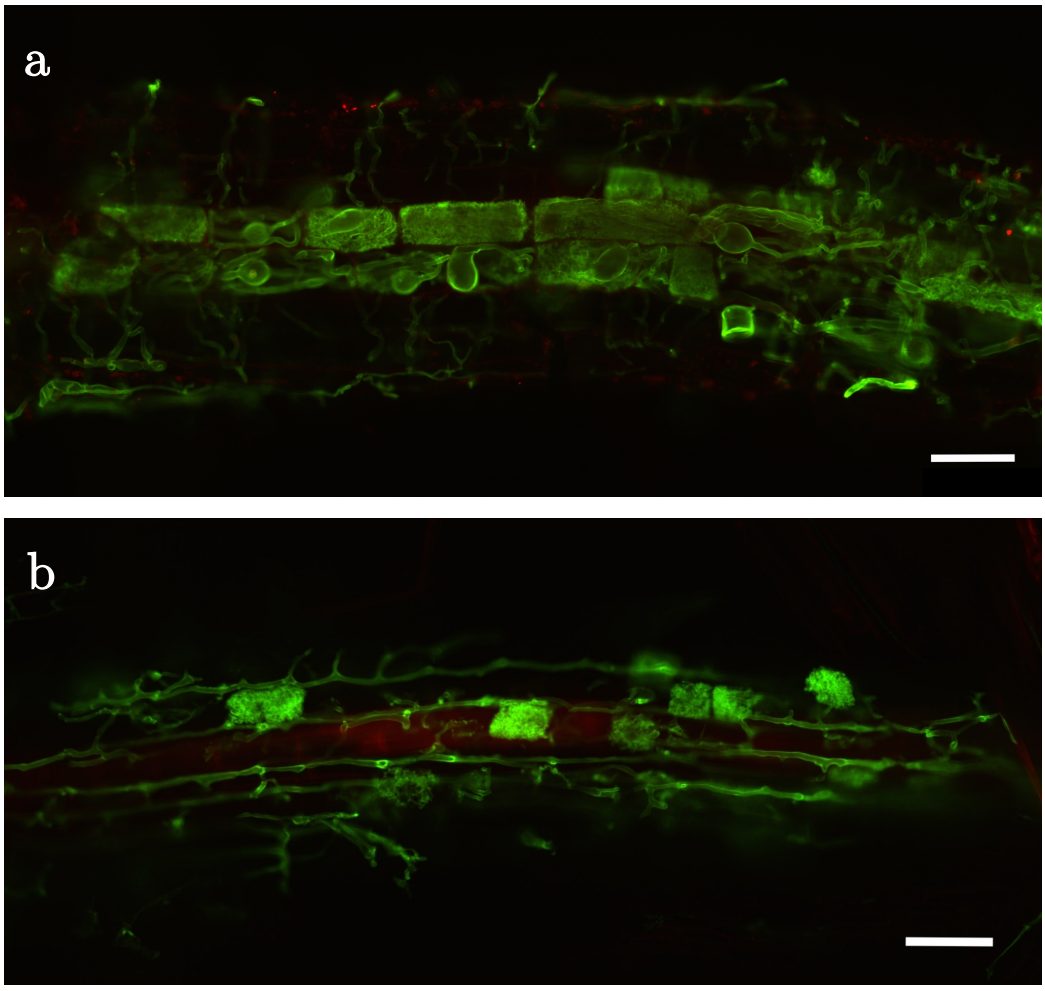


Figure 3.20 Alexa WGA-488 Confocal Micrograph showing fungal structures in a) oat and b) vetch. Images taken on Leica SP8 microscope, Scale bar represents 25µm.

Spring Barley Shoot Biomass at the Bawburgh Trial

A soil block containing intact spring barley plants were carefully removed from plots using a sharpened garden fork, which allowed plant roots to be freed from dry soil. Barley shoots were blotted dry and five shoots constituting one biological replicated were placed into a pre-dried envelope and oven dried. Mean spring barley shoot biomass at the Bawburgh site was 18.07 ± 0.21g, ranging from 17.30 ± 0.30g in the barley crop following the inoculated oats and vetch cover crop, to 19.00 ± 0.29g observed following the inoculated low-rate radish and oats treatment (Figure 3.21). Barley following the non-inoculated fallow control had the third highest shoot biomass, at 18.70 ± 0.15g, which fell to 17.53 ± 0.29g following the inoculated fallow treatment. This marked reduction in shoot weight, resulting from AM fungal inoculation was also observed following the oat and vetch, and oat, vetch and radish cover crop treatments. Spring barley following both of the radish and oat cover crop mixtures, with the low and high seed rate radish, saw notable increase in shoot weight following AM fungal inoculation. Despite these observations, there was no significant effect of AM inoculation (ANOVA: $F_{1,34} = 0.16$, $p = 0.691$), the preceding cover crop (ANOVA: $F_{8,34} = 1.07$, $p = 0.406$), or the interaction between AM inoculation and the cover crop mix (ANOVA: $F_{8,34} = 1.35$, $p = 0.252$) on barley shoot biomass.

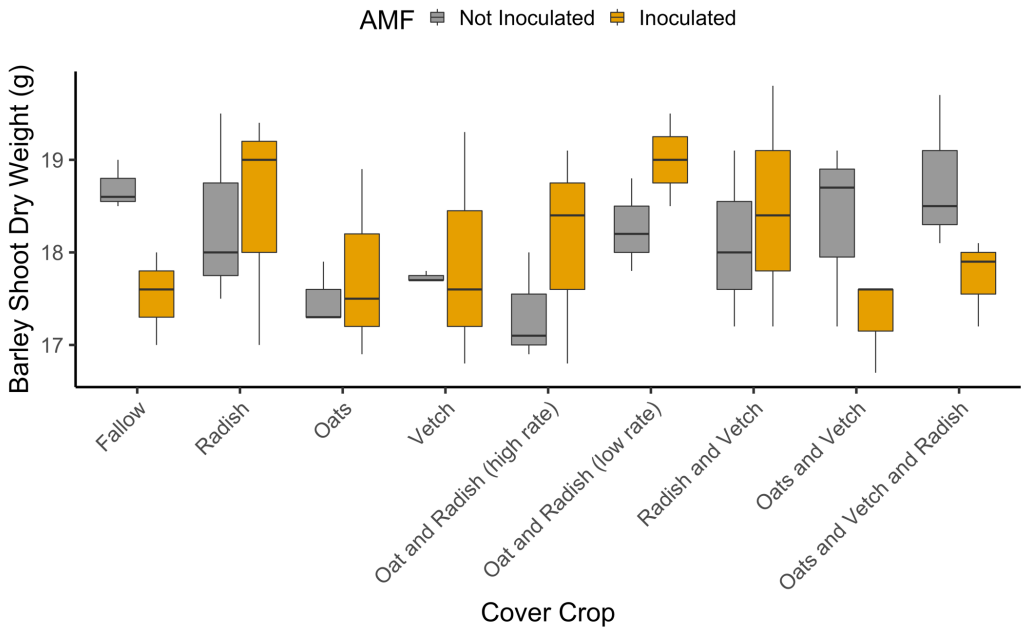


Figure 3.21 Spring barley dry shoot weight.
Dry shoot weights represent the green shoot of five spring barley plants, classified as one biological replicate.

Spring Barley RLC at the Bawburgh Trial

Mean spring barley (var. Laureate) RLC across the trial was 27.3%, and ranged from 16.3 ± 4.0% to 33.7 ± 9.4%, following the inoculated fallow treatments and non-inoculated radish and vetch cover crops, respectively (Figure 3.22). Barley following the non-inoculated fallow control had a RLC of 29.4 ± 8.8%, making it the eighth highest treatment for maximising RLC. There was no significant effect of AM inoculation (ANOVA: $F_{1,34} = 1.26$, $p = 0.270$), the preceding cover crop (ANOVA: $F_{8,34} = 0.37$, $p = 0.927$), or the interaction between AM inoculation and the cover crop mix (ANOVA: $F_{8,34} = 0.56$, $p = 0.803$) on barley RLC. Addition of the measure of available P in the soil as a covariate significantly improved model fit ($\chi^2 = 9.49$, d.f. =

1, $p = 0.002$). Available P, as measured at the time of root sampling, was negatively correlated with barley RLC (r (52) = -0.44, $p < 0.001$, Figure 3.23).

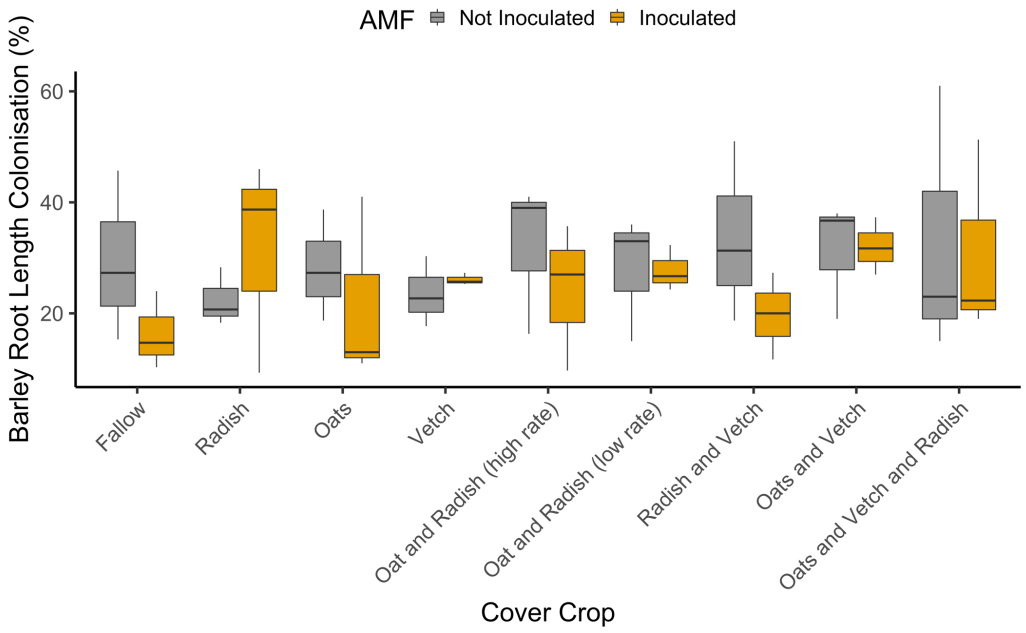


Figure 3.22 Spring barley root length colonisation (RLC) by trypan blue.
RLC expressed as a percentage of the total root length colonised, following measurement of an adapted version of the grid intersect method.

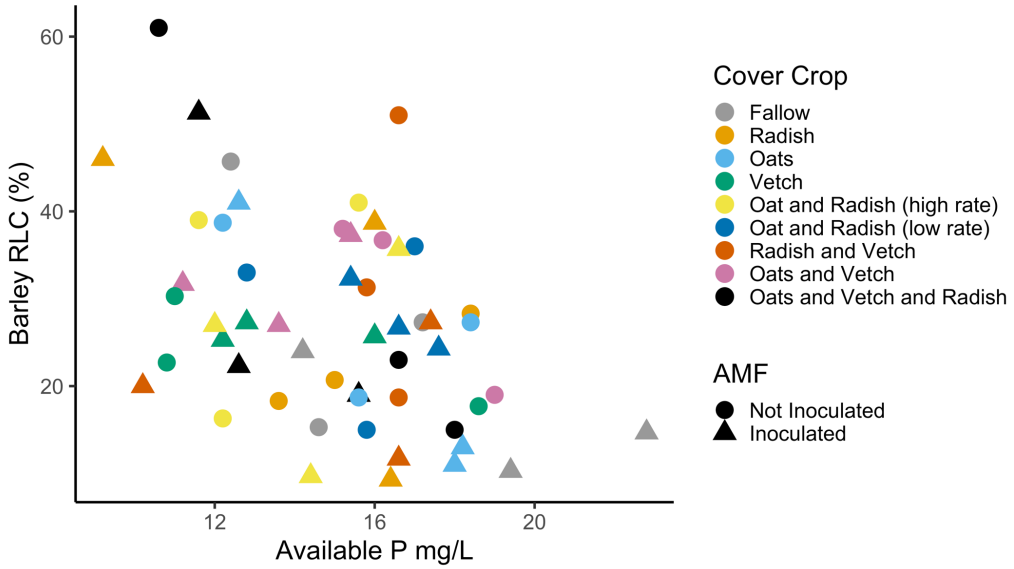


Figure 3.23 Interaction between available soil P and barley root length colonisation (RLC). Available P plotted against the respective mean RLC per plot, expressed as a percentage of the total root length colonised. Available P, as measured at the time of root sampling, was negatively correlated with barley RLC (r (52) = -0.44, $p < 0.001$).

Spring Barley Plant Counts at the Bawburgh Trial

Spring barley plant counts were made on 26th June 2019, taking an average of four measurements per plot. Fallow plots had on average 115.2 ± 7.0 and 118.4 ± 4.9 barley plants per m^2 in the non-inoculated, and inoculated plots, respectively. This value was reduced in the high rate radish and oat treatments, yielding 103.6 ± 1.1 and 110.6 ± 3.7 plants per m^2 in the inoculated and non-inoculated, respectively. Barley following the inoculated vetch (112.0 ± 10.9) and the inoculated oat and vetch (112.8 ± 4.2) cover crop had reduced plant counts compared to the non-inoculated fallow. The highest plant counts were observed following the three-way oat, vetch, and radish cover crop (127.1 ± 5.5), then oat (125.7 ± 6.3), and radish (124.8 ± 4.3). Mean plant counts are shown in Figure 3.24. The overall effect of the

preceding cover crop on spring barley plant counts was not significant (ANOVA: $F_{8,32} = 2.01$, $p = 0.078$). Furthermore, there was no effect of AM fungal inoculation alone (ANOVA: $F_{1,32} = 0.00$, $p = 0.997$), or in interaction with the preceding cover crop (ANOVA: $F_{8,32} = 0.50$, $p = 0.847$) on barley plant counts. Interestingly, addition of spring barley RLC as a covariate significantly improved model fit ($\chi^2 = 10.30$, d.f. $= 1$, $p = 0.001$), with higher levels of RLC resulting in lower numbers of plant counts ($r\,(50) = -0.33$, $p = 0.017$).

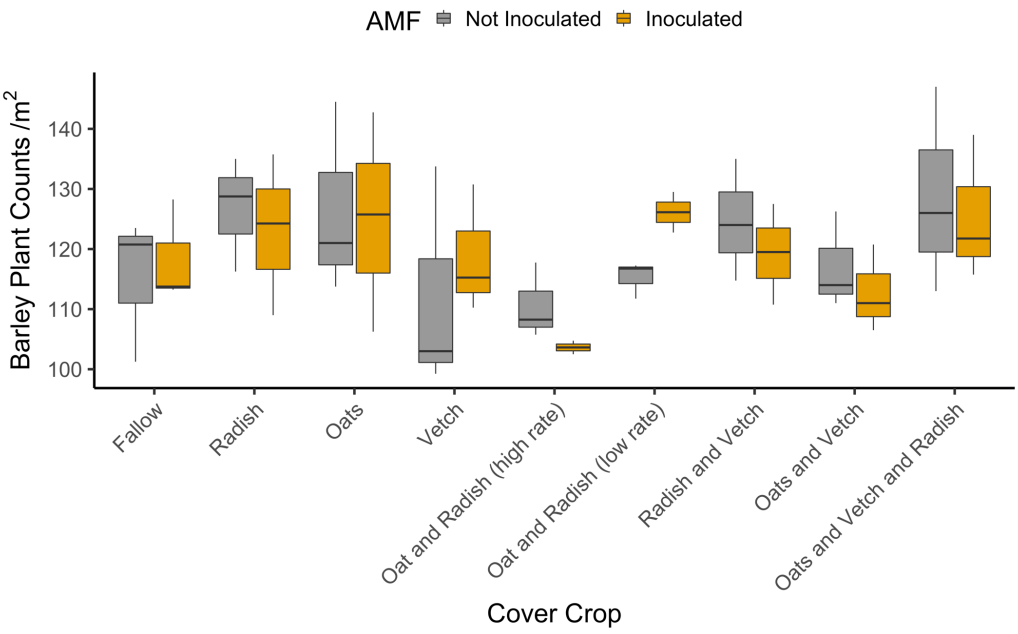


Figure 3.24 Spring barley plant counts at the Bawburgh trial.
Plant counts of spring barley (var. Laureate) expressed as a plot mean of four technical replicates with a 0.25m^2 quadrat.

Spring Barley Height at the Bawburgh Trial

Measurement of barley plant height was made on 1st July 2019, taking an average of four measurements per plot. Mean height in the non-inoculated fallow control was 72.3 ± 2.2cm, dropping to 66.8 ± 2.6cm in the inoculated comparison, which was also the shortest of all experimental treatments (Figure 3.25). The second and third shortest plants were following non-inoculated (68.1 ± 0.7cm), and inoculated (68.3 ± 2.2cm) oats. Only three treatments were taller than the non-inoculated fallow control, inoculated vetch (72.6 ± 2.4cm), inoculated oat, vetch and radish (73.6 ± 1.7cm), and the tallest barley crop was following a non inoculated vetch cover crop (73.8 ± 0.7cm).

Overall, there was no effect of the preceding cover crop (ANOVA: $F_{8,34} = 0.97$, $p = 0.472$), AM fungal inoculation (ANOVA: $F_{1,34} = 0.46$, $p = 0.503$), or their interaction (ANOVA: $F_{8,34} = 0.76$, $p = 0.636$), on spring barley height. Like for plant counts, addition of barley RLC improved model fit ($\chi^2 = 5.29$, d.f. = 1, $p = 0.022$), this time, having a positive correlation with barley height ($r\,(52) = 0.32$, $p = 0.017$).

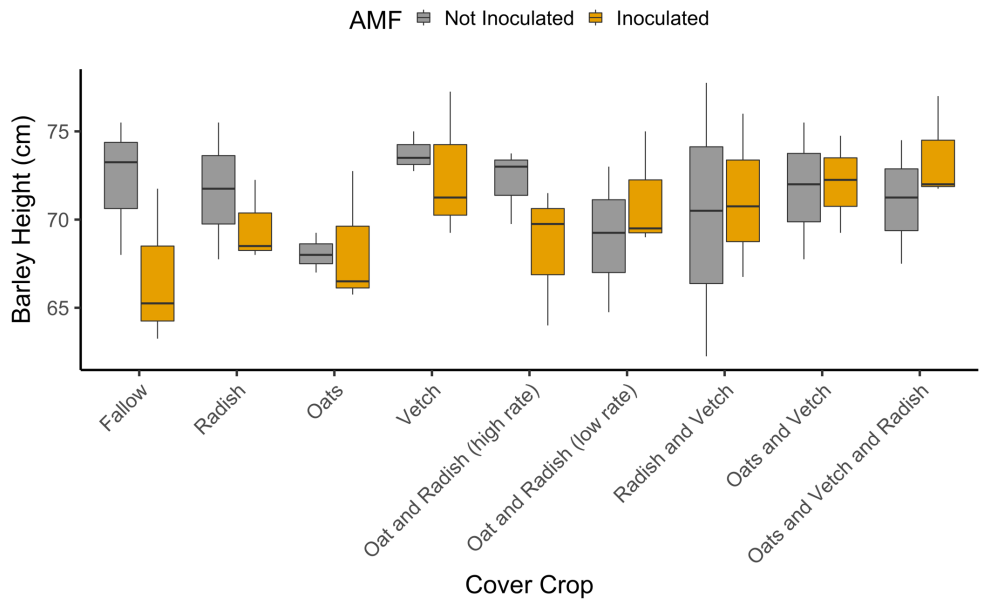


Figure 3.25 Spring barley plant height at the Bawburgh trial.
Height of spring barley (var. Laureate) expressed as a plot mean of four technical replicates with a 0.25m^2 quadrat.

Spring Barley Yield at the Bawburgh Trial

Spring barley (var. Laureate) yield was measured with a Haldrup trial combine and standardised to 15% moisture content. Grain was collected for each plot for further analysis. Mean barley yield across the trial was 5.74 ± 0.08 t/ha and ranged from 5.2 t/ha following the AM inoculated vetch plots, to 6.4 t/ha following the non-inoculated oat cover crop (Figure 3.26). The minimum yield observed in a single plot was 4.6 t/ha following the inoculated fallow treatment, and the maximum observed plot yield was 7.6 t/ha, following the AM inoculated three-way mix cover crop treatment. Barley following the non-inoculated fallow control had a mean yield of 5.5 t/ha, ranging from 5.4 - 5.7 t/ha. There appeared to be a small beneficial impact of

both radish, and oat cover crops, when growth without the AM fungal inoculum. This trend was also seen following the non-inoculated low seed rate radish and oat cover crop treatment, but was not observed at the higher radish seed rate. After log transforming yield data to conform to Gauss-Markov assumptions, there was no significant effect of AM inoculation (ANOVA: $F_{1,32} = 1.28$, $p = 0.267$), or the interaction between AM inoculation and the cover crop mix (ANOVA: $F_{8,32} = 0.62$, $p = 0.751$) on barley yield. There was an effect of the preceding cover crop on barley yield, but this was not significant at the $p < 0.05$ level (ANOVA: $F_{8,32} = 2.07$, $p = 0.069$). To test whether increased interaction with AM fungi resulted in increased yield, barley RLC was added to the model but did not improve model fit ($\chi^2 = 2.99$, d.f. = 1, $p = 0.084$, Figure 3.27). Furthermore, there was no correlation between barley RLC and yield ($r\,(50) = -0.10$, $p = 0.479$).

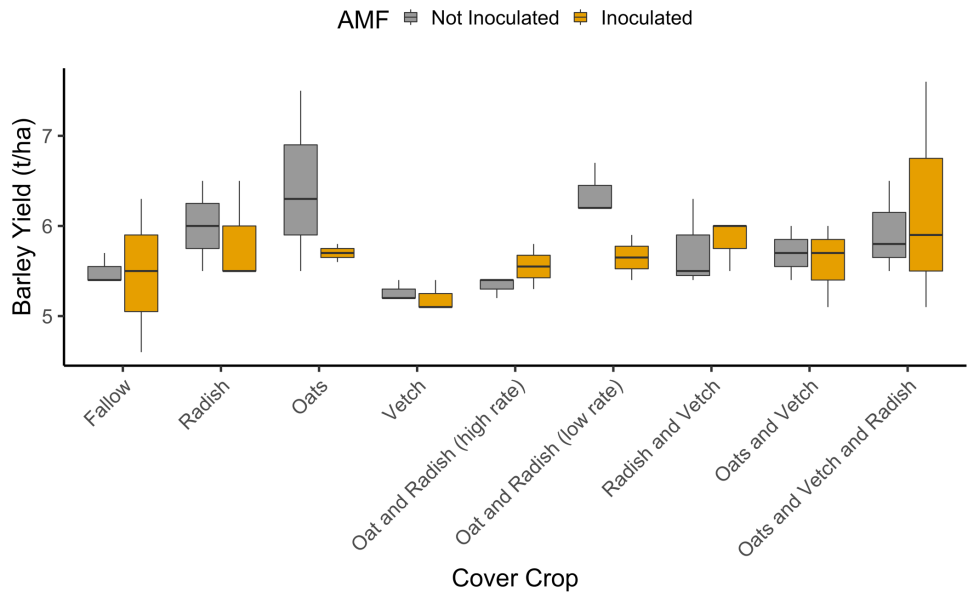


Figure 3.26 Spring barley yield at the Bawburgh trial.
Barley yield expressed in tonnes per hectare, following standardisation to 15% moisture content.

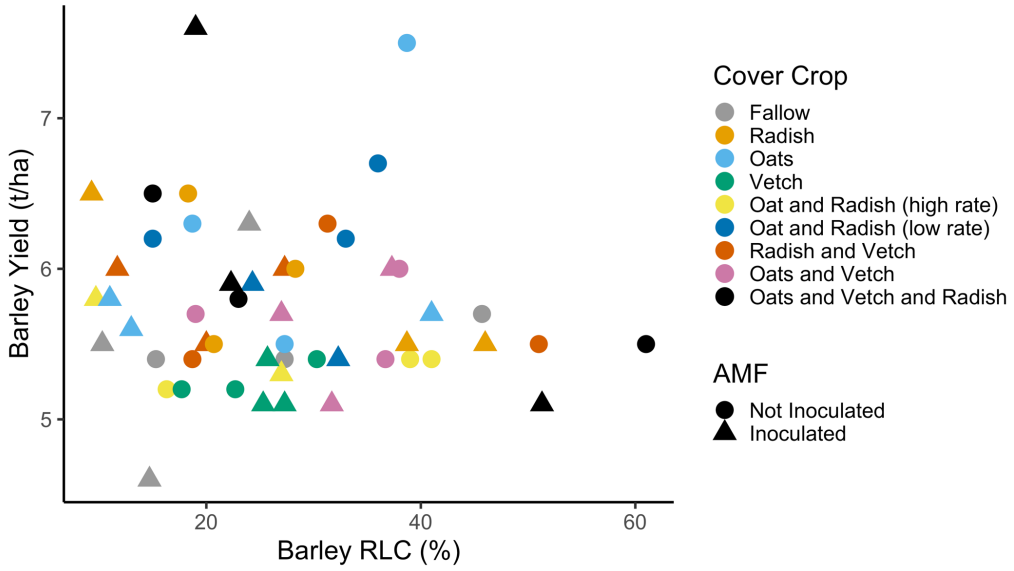


Figure 3.27 Interaction between barley root length colonisation (RLC) and yield. Mean RLC expressed as a percentage of the total root length colonised, plotted against the respective plot yield.

Spring Barley Grain Analysis from the Bawburgh Trial

Grain collected at harvest was subjected to a number of tests to determine grain quality traits which are sought after by brewers, and may increase profitability of grain.

Spring Barley Thousand Grain Weight from the Bawburgh Trial

Spring barley (var. Laureate) Thousand Grain Weight (TGW) ranged from 48.3 ± 1.3g following the non-inoculated radish and vetch treatment to 52.6 ± 0.3g following the non-inoculated high seed rate radish and oat treatment (Figure 3.28). Barley following the non-inoculated fallow control had a TGW of 50.1 ± 0.7g, which was only marginally lower than the trial mean of 50.8

± 0.25g. Barley following an oat cover crop, or an oat and radish cover crop at either seed rate appeared to have a small increase in TGW. The radish and vetch treatment appeared to cause a decrease, although the impact was more variable. There was no effect of AM fungal colonisation (ANOVA: $F_{1,34}$ = 0.06, p = 0.805), or the interaction between AM fungal colonisation and the preceding cover crop (ANOVA: $F_{8,34}$ = 0.98, p = 0.472) on barley TGW. There was a marginally significant effect of the preceding cover crop when considered alone (ANOVA: $F_{8,34}$ = 1.91, p = 0.091), but no pairwise comparisons reached the $p < 0.05$ significance threshold. Addition of barley RLC as a covariate to test the contribution of RLC on TGW did not improve model fit (χ^2 = 0.21, d.f. = 1, p = 0.642).

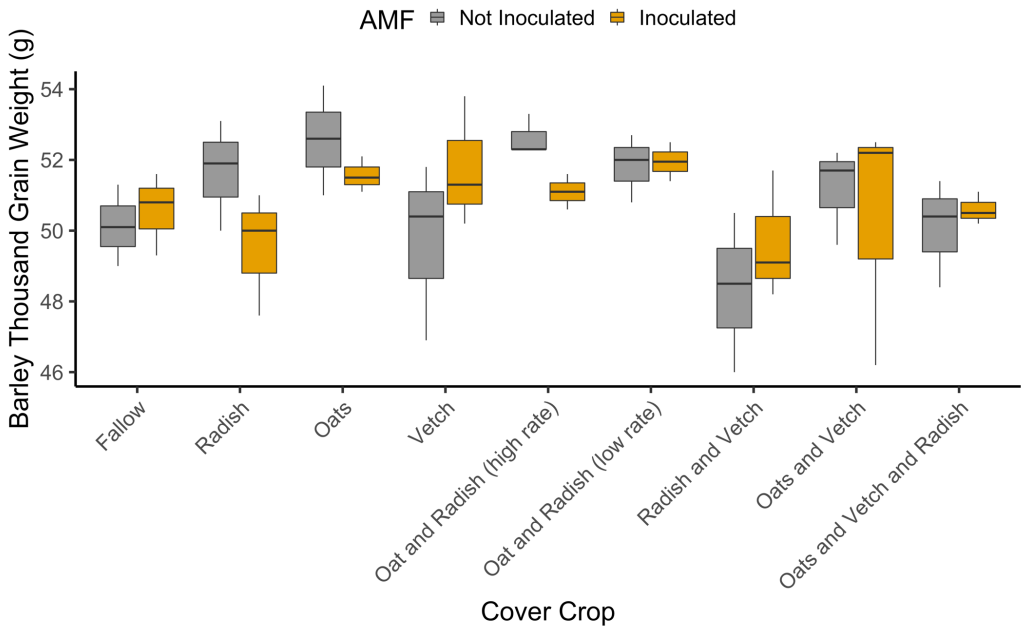


Figure 3.28 Spring barley thousand grain weight.
Thousand grain weight of spring barley (var. Laureate) from the Bawburgh trial, after standardisation for moisture content.

Spring Barley Grain Size Analysis from the Bawburgh Trial

Post-harvest analysis of grain size was conducted to determine the effects of AM fungal inoculation and cover cropping on grain size, another important brewing metric. Grain size is expressed in grams per 100g of grain and due to this methodology, the sum of various grain sizes may exceed 100g.

Across the trial, 83.2 ± 0.4g/100g of grain was greater than 2.8mm, the largest assessed grain size (Figure 3.29a). This ranged from 79.4 ± 2.8g/100g following the non-inoculated high seed rate radish and oat, to 85.9 ± 0.4g/100g following the non-inoculated three-way mix cover crop, containing oat, vetch, and radish. 82.6 ± 2.1g/100g of grain following the non-inoculated, fallow treatment plots were greater than 2.8mm.

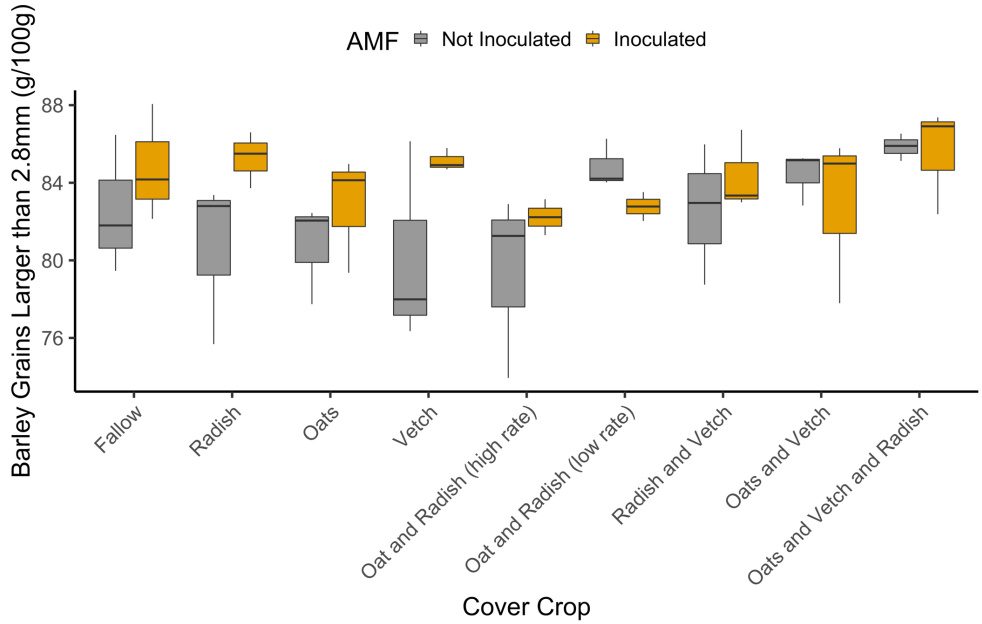
There was no effect of the identity of the preceding cover crop (ANOVA: $F_{8,34} = 1.13$, $p = 0.369$), or the interaction between AM fungal colonisation and the preceding cover crop (ANOVA: $F_{8,34} = 0.94$, $p = 0.496$) on the number of grains greater than 2.8mm. The impact of AM fungal inoculation by AM fungi was also non-significant (ANOVA: $F_{1,34} = 3.63$, $p = 0.065$), although AM inoculation increased the proportion of grains >2.8 mm following the inoculated vetch, and radish treatment plots. This positive trend was also observed following the fallow treatment, and the remaining single species oat cover crop, but not following the two or three-way mixes

Another important metric is grain which is larger than 2.5mm, which was on average 92.8 ± 0.4g/100g across the trial (Figure 3.29b). This ranged from 88.9 ± 2.5g/100g following the non-inoculated high seed rate radish and oat, to 95.4 ± 1.4g/100g following the barley following the inoculated fallow plot. 93.6 ± 2.2g/100g of grain following the non-inoculated, fallow treatment plots were greater than 2.5mm. There was no effect of AM fungal inoculation (ANOVA: $F_{1,34} = 2.17$, $p = 0.150$), the identity of the preceding cover crop (ANOVA: $F_{8,34} = 1.49$, $p = 0.196$), or the interaction between AM fungal colonisation and the preceding cover crop (ANOVA: $F_{8,34} = 0.87$, $p = 0.550$) on the number of grains greater than 2.5mm. However, the trend for the inoculated fallow, as well as the inoculated single species cover crop treatments to outperform their non-inoculated equivalents remained, although it seems that inoculation does not increase TGW compared to the control plots, but rather prevents the decrease observed in the non-inoculated plots.

The observed trend continued for grains greater than 2.2mm, although less severe (Figure 3.29c). There still appeared to be a benefit of inoculating the fallow and radish cover crop treatments, although the increase previously seen following the oat, and vetch cover crop treatments had been minimalised. The lowest number of grains greater than 2.2mm was observed following the non-inoculated high seed rate radish and oat plots, which achieved 92.5 ± 2.3g/100g. Like the analysis of grain over 2.5mm, the largest proportion of grain greater than 2.2mm was 100.6 ± 1.6g/100g in the barley following the inoculated fallow plot compared to 98.1 ± 1.9g/100g following

the non-inoculated, fallow treatment plots. There was no effect of AM fungal inoculation (ANOVA: $F_{1,34} = 1.64$, $p = 0.209$), the interaction between AM fungal inoculation and the identity of the preceding cover crop (ANOVA: $F_{8,34} = 0.92$, $p = 0.515$), however the identity of the preceding cover crop approached significance (ANOVA: $F_{8,34} = 1.92$, $p = 0.090$). Despite this putative result there were no further pairwise differences of post-hoc comparisons at the $p < 0.05$ level.

a



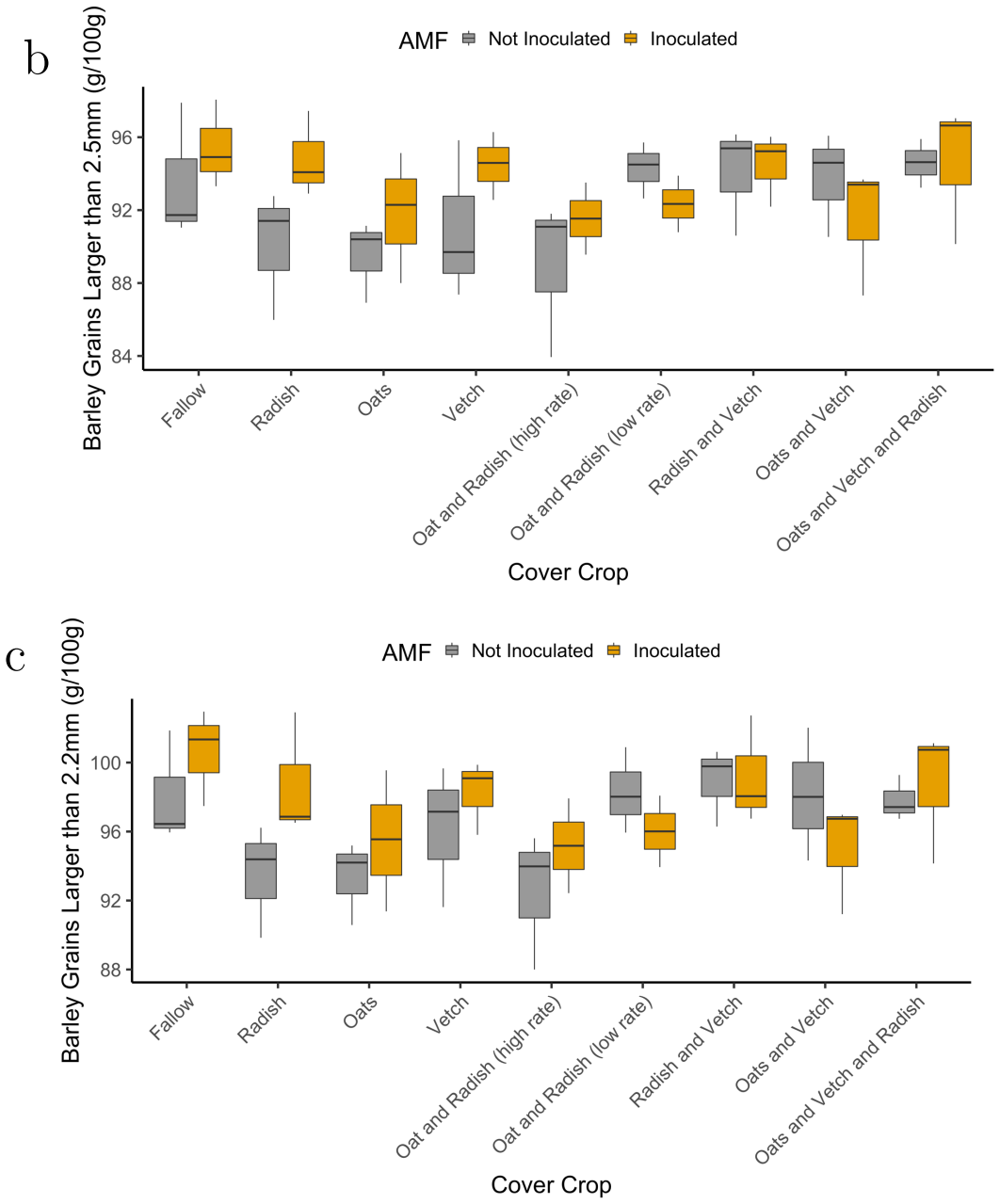


Figure 3.29 Proportion of spring barley grains greater than a) 2.8mm, b) 2.5mm, and c) 2.2mm. Great size distribution of spring barley (var. Laureate) from the Bawburgh trial.

Winter Oat Biomass at the Bawburgh Trial

Winter oat var. Mascani plants were sampled in a similar manner to spring barley (above), although weather conditions meant that this was not as much as an issue as in the previous year. Oat shoots were separated from roots, were blotted dry, and five shoots constituting one biological replicated were placed into a pre-dried envelope and oven dried. Mean oat shoot dry weight was 6.55g, with treatment means ranging from 5.2 ± 0.1g following the inoculated high seed rate radish and oat treatment, to 7.8 ± 1.7g following the inoculated fallow treatment (Figure 3.30). Winter oat in the non-inoculated fallow control exhibited the fifth highest shoot biomass, of 7.2 ± 1.5g, although oat following both the inoculated, and non-inoculated fallow treatments had a large standard error. After log transforming biomass data to conform to Gauss-Markov assumptions, there was no significant effect of AM inoculation (ANOVA: $F_{1,34} = 0.45$, $p = 0.507$), or the interaction between AM inoculation and the cover crop (ANOVA: $F_{8,34} = 1.42$, $p = 0.224$) on winter oat dry shoot biomass. There was a significant effect of the preceding cover crop (ANOVA: $F_{8,34} = 2.28$, $p = 0.045$) on oat shoot biomass. Vetch (Tukey p =0.100), and high seed rate radish and oat (Tukey p =0.118), appeared to have the most detrimental impact on oat shoot biomass (compared to fallow), but this was not observed in the low seed rate radish and oat cover crop treatment (Tukey p =1.000), which again had a high standard error but was well in the range of the oat shoot biomass following the fallow plots.

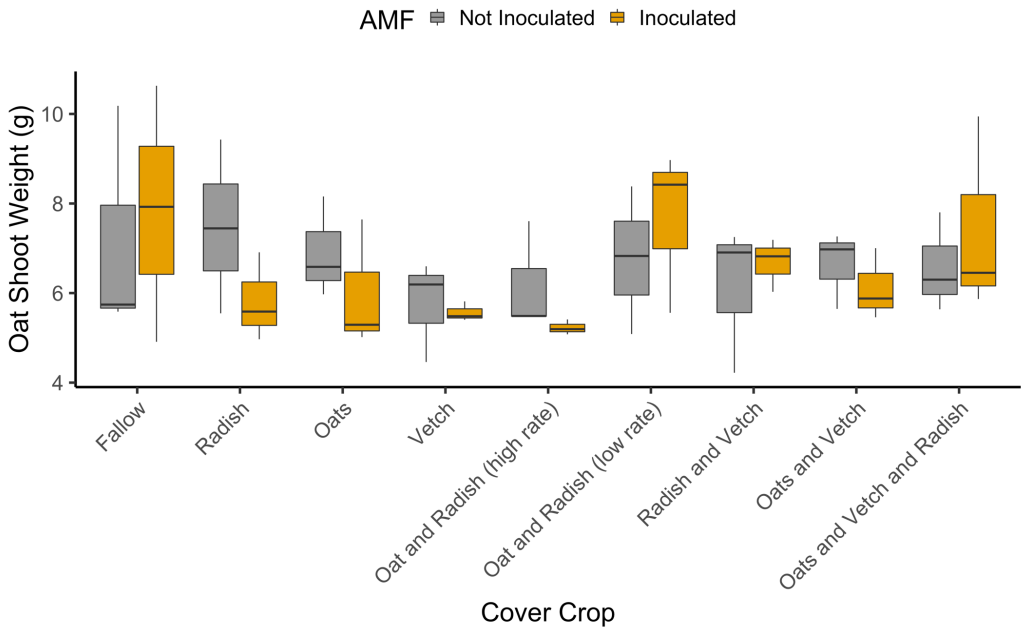


Figure 3.30 Winter oat dry shoot weight.
Dry shoot weights represent the green shoot of five winter oat plants, classified as one biological replicate.

Winter Oat RLC at the Bawburgh Trial

Winter oat RLC was quantified by the adapted gridline intersect method, as was conducted for the cover crop, and spring barley. Oat roots cleared and stained well and made for easy quantification (Figure 3.31). Mean winter oat RLC ranged from 19.3 ± 5.3% following the inoculated low seed rate radish and oat treatment, to 38.4 ± 6.1% following the non-inoculated three-way mix cover crop treatment (Figure 3.32). The inoculated comparator of the three-way mix cover crop also caused the third highest oat RLC, at 32.7 ± 5.2%. The mean oat RLC across the trial was 28.0 ± 1.1%, which was higher than that of the non-inoculated control, at 23.8 ± 2.9%, although both the inoculated and non inoculated fallow controls had large standard

errors. Comparing inoculated and non-inoculated treatments, oat RLC following the radish, and oat and vetch cover crops appeared to benefit from AM fungal inoculation, whereas the oat crop following the inoculated oat cover crop experienced a 24% reduction in RLC.

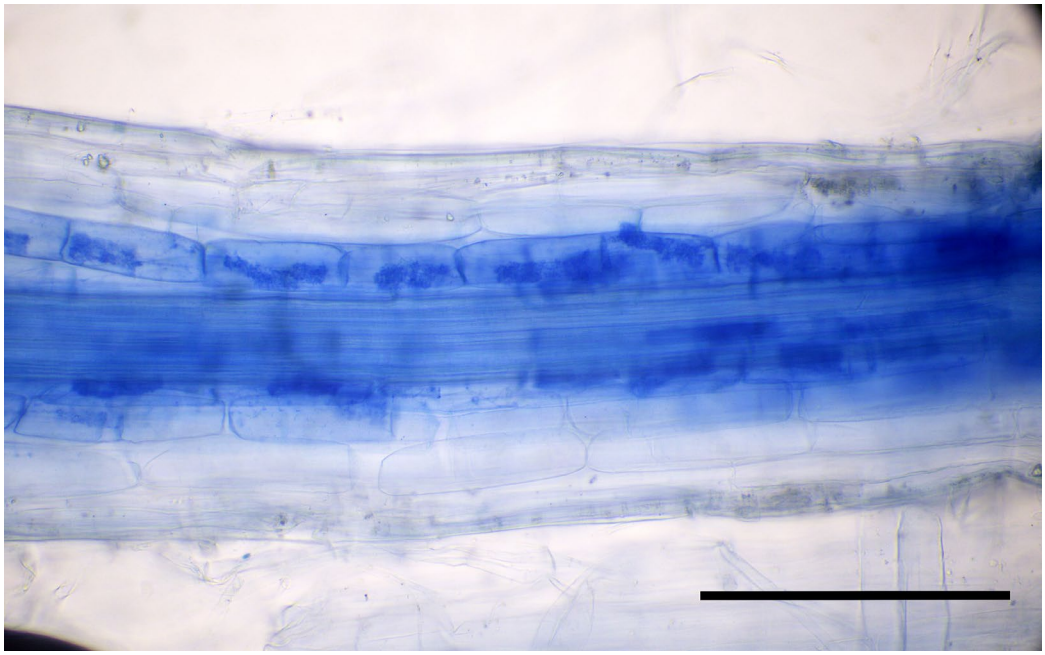


Figure 3.31 Trypan blue stained root of winter oat var. Mascani from the Bawburgh trial, displaying irregular shaped arbuscules. Scale bar represents 200µm.

After arcsin transformation of oat RLC data to conform to Gauss-Markov assumptions, there was no significant effect of AM inoculation (ANOVA: $F_{1,34} = 3.02$, $p = 0.091$), the preceding cover crop (ANOVA: $F_{8,34} = 1.21$, $p = 0.324$), or the interaction between AM inoculation and the cover crop (ANOVA: $F_{8,34} = 0.96$, $p = 0.479$) on winter oat RLC. Addition of the measure of available P (as measured during the preceding year) in the soil as a covariate improved model fit ($\chi^2 = 14.44$, d.f. = 1, $p < 0.001$). Available P, as measured at the time of barley root sampling in the previous year, was

negatively correlated with winter oat RLC ($r\,(52) = -0.29$, $p = 0.033$, Figure 3.33).

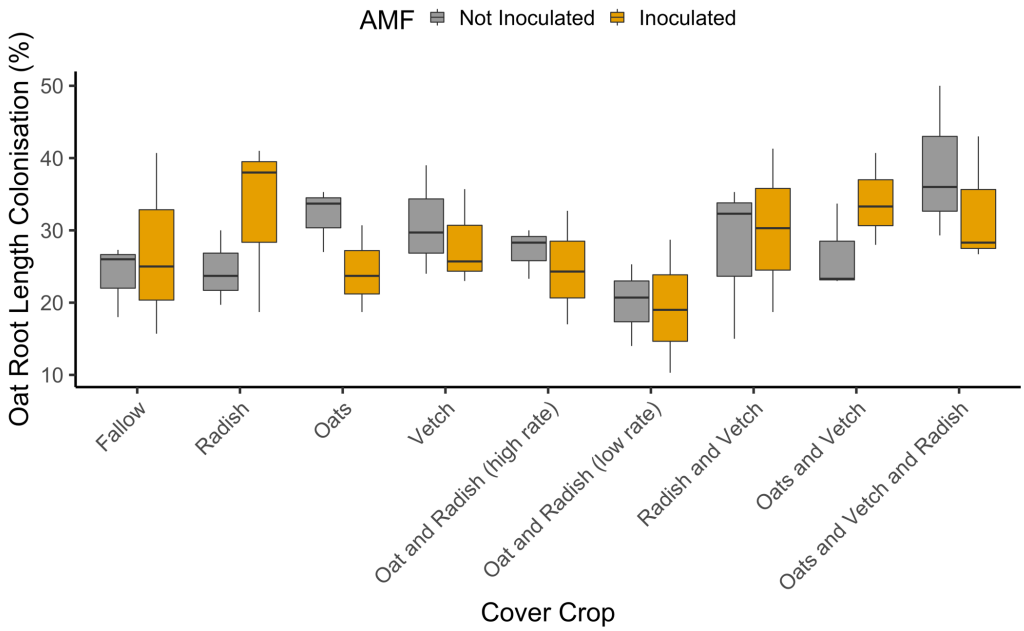


Figure 3.32 Winter oat root length colonisation (RLC) by trypan blue. RLC expressed as a percentage of the total root length colonised, following measurement of an adapted version of the grid intersect method.

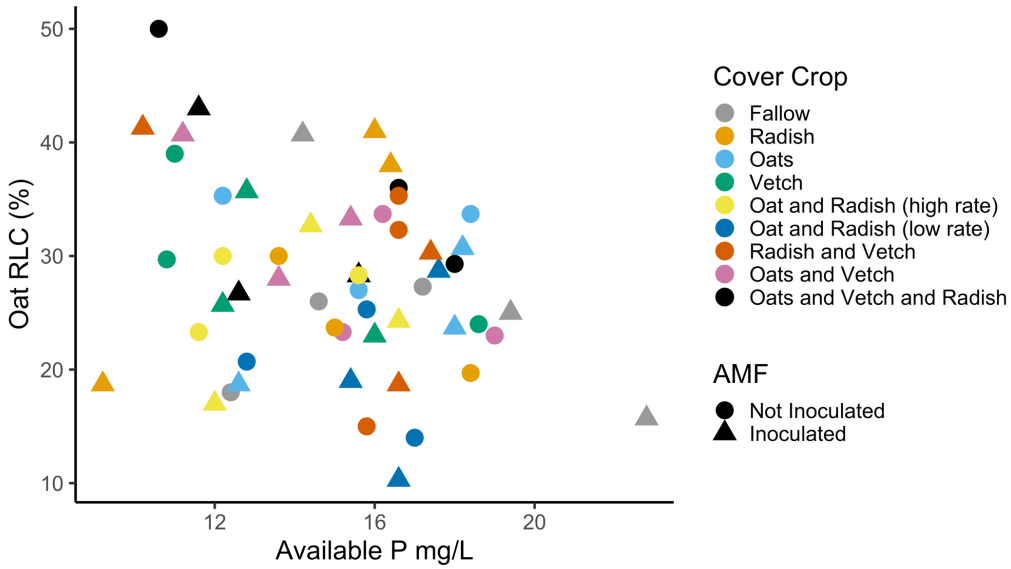


Figure 3.33 Interaction between available soil P and oat root length colonisation (RLC). Available P plotted against the respective mean RLC per plot, expressed as a percentage of the total root length colonised.

Winter Oat Plant Counts at the Bawburgh Trial

Assessment of the number of oat plants was conducted at GS22 to determine how experimental treatments had influenced the establishment of crop plants. Across the trial, the mean number of oat plants established per meter squared at GS22 was 167.2 ± 1.8. This ranged from a plot mean of 156.0 ± 7.4 following the non-inoculated oat and vetch cover crop plots, to 183.1 ± 16.8 following the non-inoculated low seed rate radish and oat cover crop treatment (Figure 3.34). The non-inoculated fallow control plots produced 169.7 ± 12.2 oat plants/ m^2, the sixth highest of the treatment means. There was no significant effect of AM inoculation (ANOVA: $F_{1,34} = 0.08$, $p = 0.779$), the preceding cover crop (ANOVA: $F_{8,34} = 0.56$, $p = 0.799$), or the interaction between AM inoculation and the cover crop mix (ANOVA: $F_{8,34} = 0.19$, $p = 0.990$) on the number of oat plants at GS22. Addition of oat RLC as a model covariate did not improve model fit ($\chi^2 = 1.58$, d.f. = 1, $p = 0.207$).

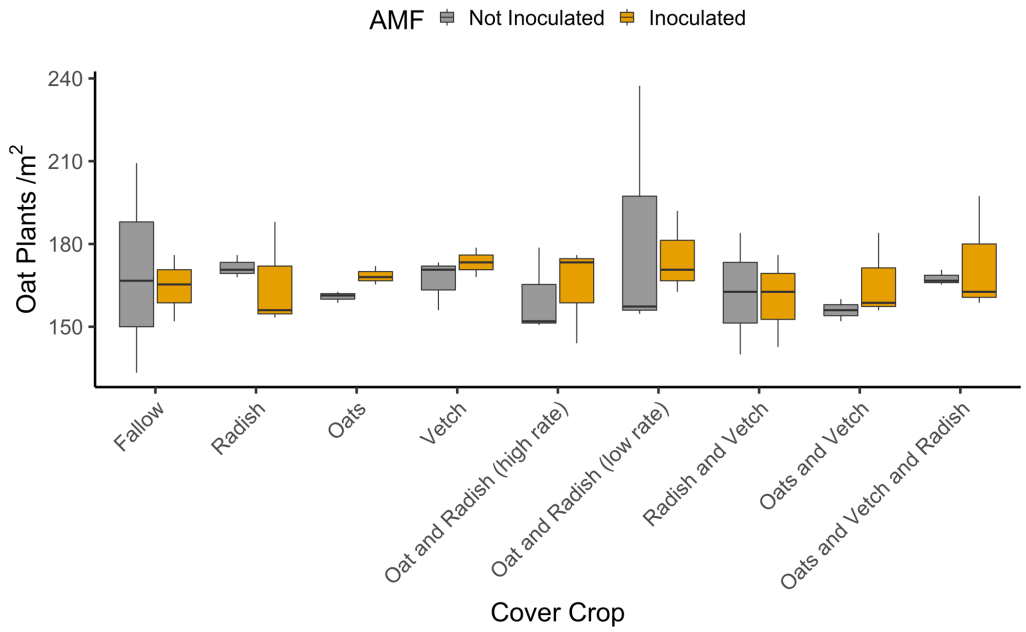


Figure 3.34 Winter oat plant counts at the Bawburgh trial.
Plant counts of winter oat (var. Mascani) expressed as a plot mean of three technical replicates with a 0.25m^2 quadrat.

Winter Oat Head Counts at the Bawburgh Trial

Assessment of the number of oat heads was conducted at GS71, on the 10th July 2020. There were 390.7 ± 16.6 heads/m^2 following the non-inoculated fallow control, dropping slightly to 368.3 ± 21.5 heads/m^2 following the inoculated fallow treatment, which was also the lowest mean of any treatment (Figure 3.35). The high seed rate radish and oat produced notably higher numbers of oat heads, at 422.7 ± 7.3 heads/m^2 and 441.7 ± 12.4 heads/m^2 in the inoculated and non-inoculated treatments respectively, the latter being the highest recorded mean in the trial. Inoculation of the oat and vetch cover crop resulted in a 15% increase in oat heads, increasing from 373.0 ± 16.8 heads/m^2 to 429.3 ± 14.8 heads/m^2, which was also the second highest

in the trial. No other cover crop treatment experienced such increases due to the effect of AM fungal inoculation.

There was no significant effect of AM inoculation (ANOVA: $F_{1,33} = 0.39$, $p = 0.538$), the preceding cover crop (ANOVA: $F_{8,33} = 1.53$, $p = 0.185$), or the interaction between AM inoculation and the cover crop mix (ANOVA: $F_{8,33} = 0.723$, $p = 0.676$) on the number of oat heads at GS71. Addition of oat RLC as a model covariate significantly improved model fit ($\chi^2 = 5.29$, d.f. = 1, $p = 0.022$), with RLC being positively correlated with the number of oat heads ($r\,(52) = 0.35$, $p = 0.009$).

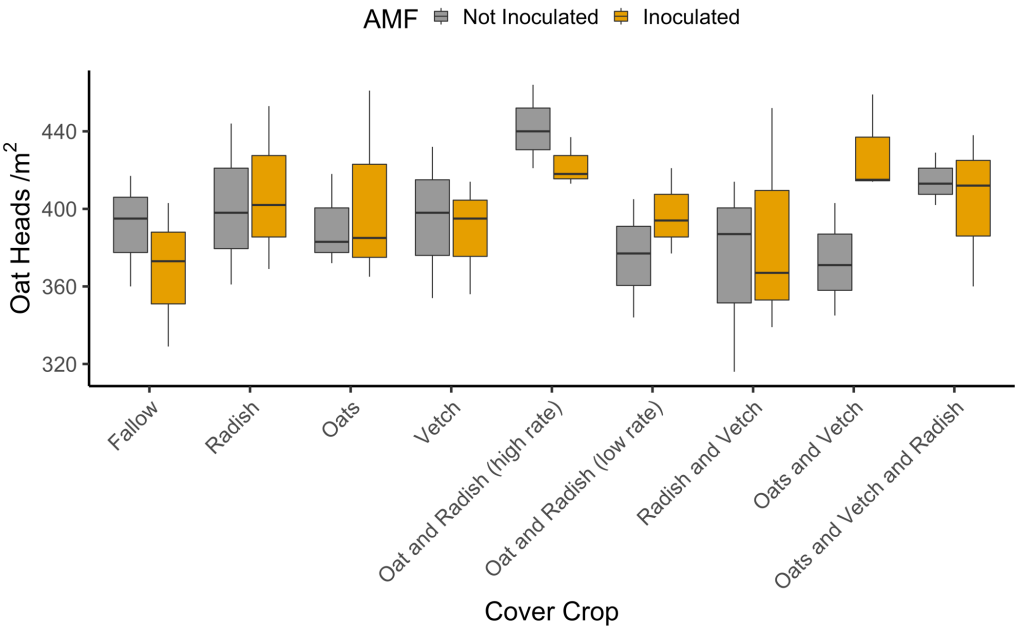


Figure 3.35 Winter oat head counts at the Bawburgh trial.
Head counts of winter oat expressed as a plot mean of three technical replicates with a 0.25m^2 quadrat.

Winter Oat Yield at the Bawburgh Trial

Winter oat yield was measured with a Haldrup trial combine and standardised to 15% moisture content. Mean winter oat (var. Mascani) yield across the trial was 7.28 ± 0.10 t/ha and ranged from 6.33 ± 0.38 t/ha following the AM inoculated fallow plots, to 8.07 ± 0.35 t/ha following the non-inoculated high seed rate radish and oat cover crop (Figure 3.36). The minimum yield observed in a single plot was 5.2 t/ha following the non-inoculated radish and vetch cover crop treatment, and the maximum observed plot yield was 8.6 t/ha, following the non-inoculated high seed rate radish and oat cover crop treatment. Oat following the non-inoculated fallow control had a mean yield of 7.6 ± 0.3 t/ha, ranging from 7.2 to 8.3 t/ha. This was 1.3 t/ha higher than its AM fungal inoculated equivalent, which only achieved 6.3 ± 0.4 t/ha. Overall, there was no significant effect of the preceding cover crop (ANOVA: $F_{8,34} = 1.21$, $p = 0.324$), or the interaction between the cover crop and AM fungal inoculation (ANOVA: $F_{8,34} = 0.96$, $p = 0.479$) on oat yield. Overall, AM inoculation decreased oat yield from 7.4 ± 0.2 t/ha to 7.1 ± 0.1 t/ha across the trial (ANOVA: $F_{1,34} = 3.02$, $p = 0.091$), however this was outside of the $p < 0.05$ threshold for significance. In contrast, addition of oat RLC as a covariate improved model fit ($\chi^2 = 14.43$, d.f. = 1, $p < 0.001$), with a significant positive correlation between oat RLC and yield ($r\,(52) = 0.36$, $p = 0.008$, Figure 3.37).

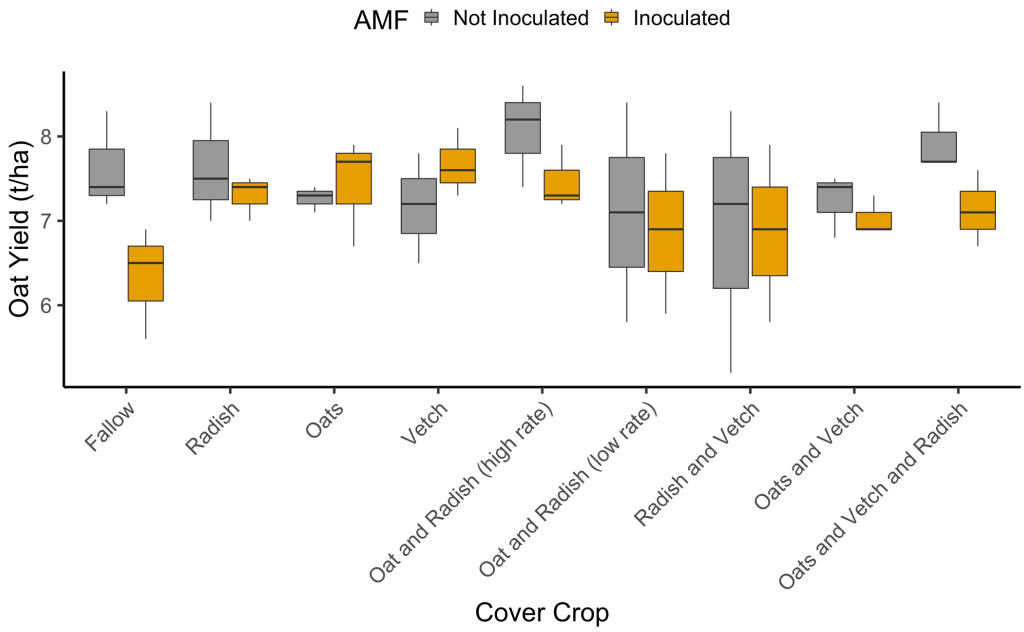


Figure 3.36 Winter oat yield at the Bawburgh trial.
Barley yield expressed in tonnes per hectare, following standardisation to 15% moisture content.

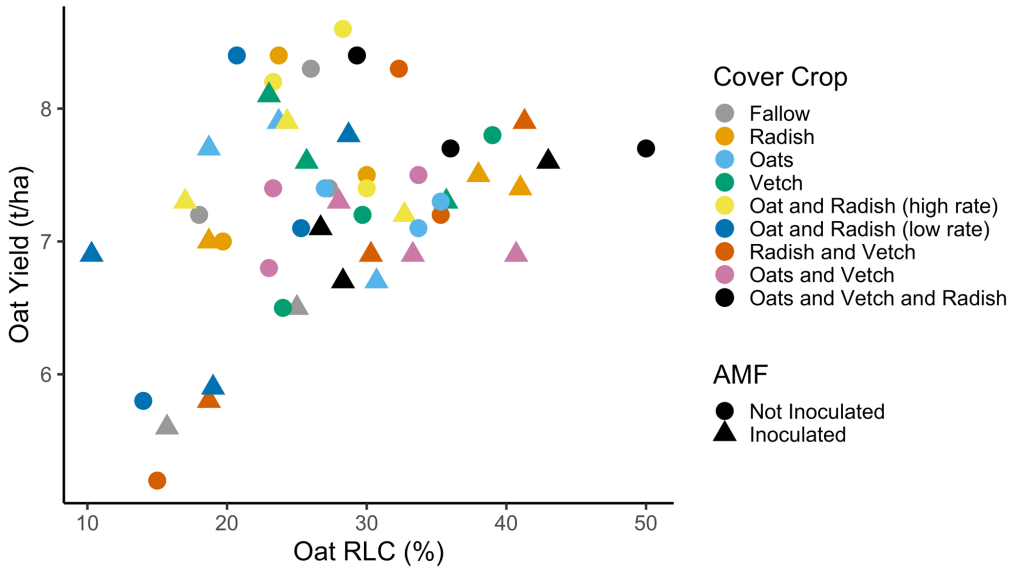


Figure 3.37 Interaction between oat root length colonisation (RLC) and yield.
Mean RLC expressed as a percentage of the total root length colonised, plotted against the respective plot yield.

AM Fungal Diversity at the Bawburgh Trial

After stringent quality thresholding, 64,4439 Glomeromycotinian DNA sequences remained for further analysis. In total, 40 Virtual Taxa were identified from across the trial, including one *Acaulospora*, one *Ambispora*, four *Archaeospora*, three *Claroideoglomus*, six *Diversispora*, 19 *Glomus*, five *Paraglomus*, and one *Scutellospora*.

The top 10 most abundant taxa across the trial were VTX00065 (37.8% - *Glomus caledonium),* VTX00245 (27.2% - *Archaeospora trappei*), VTX00283 (13.5% - *Ambispora fennica)*, VTX00342 (6.6% - *Glomus VeGlo18*), VTX00281 (3.8% - *Paraglomus laccatum),* VTX00052 (3.1% - *Scutellospora MO-S2),* VTX00354 (2.3% - *Diversispora Clade-3),* VTX00295 (0.9% - *Glomus Glo-A),* and VTX00064 (0.6% - *Glomus MO-G18*, which are shown in Figure 3.38.

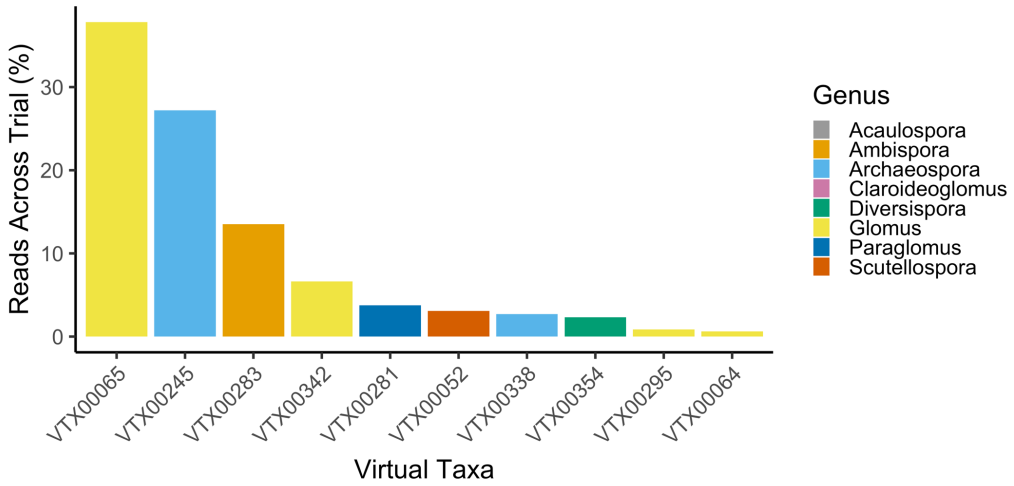
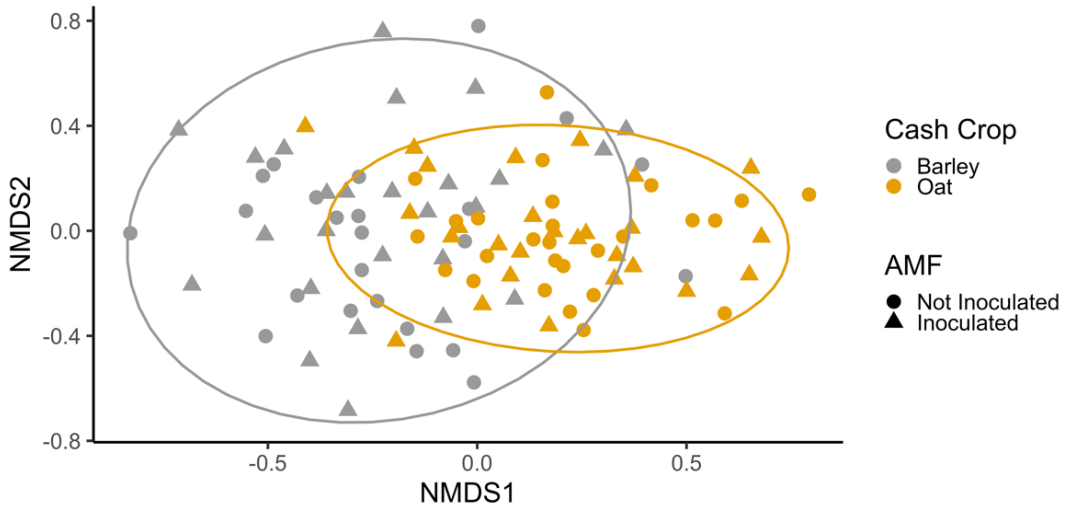


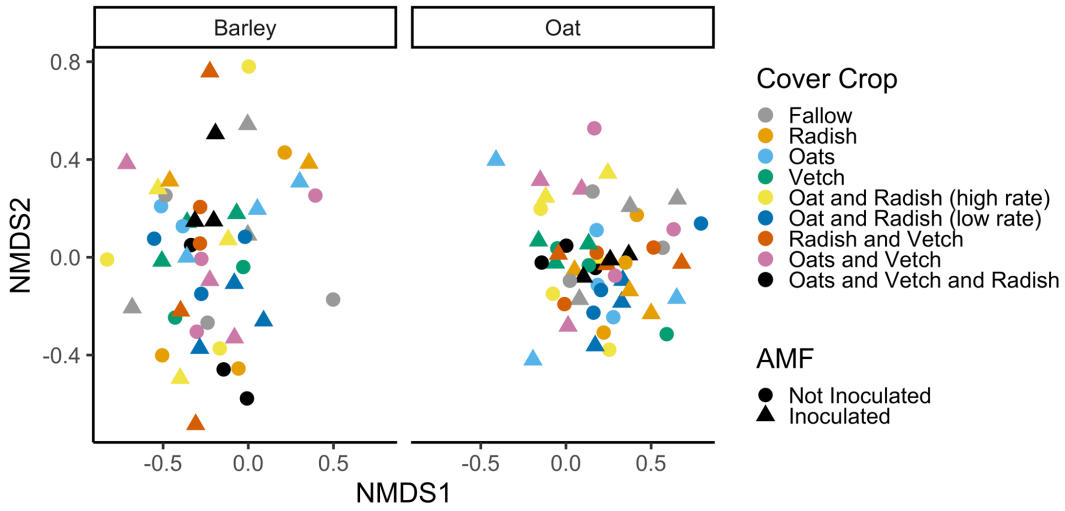
Figure 3.38 Top 10 most abundant AM fungal virtual taxa in the Bawburgh trial. Abundance of the top 10 AM fungal VT, expressed as a percentage of the number of DNA reads across the trial. Colours represent the genus of taxa.

Ordination of AM Fungal Communities at the Bawburgh Trial

Bray-Curtis dissimilarity distances were calculated and the multivariate homogeneity of groups dispersions were calculated for both the cover crop and AM fungal inoculation treatments. After 999 permutations, neither the cover crop ($F_{8,95} = 0.44$, $p = 0.896$) nor inoculation by AM fungi ($F_{1,102} = 0.01$, $p = 0.924$) treatments exhibited significantly different dispersions, however, community dispersions between the barley and oat cash crop were significantly different ($F_{1,102} = 4.12$, $p = 0.042$).

Bray-Curtis distances were plotted by NMDS and PCoA methods, to visualise clustering of communities (Figure 3.39). Analysis of similarities (ANOSIM) between communities was conducted in place of PERMANOVA due to the uneven dispersions between groups. Following 999 permutations, there was no significant dissimilarity in community composition due to the effect of the cover crop (ANOSIM: $R = $ -0.024, $p = 0.880$) or whether a plot had been inoculated by AM fungi (ANOSIM: $R = $ -0.014, $p = 0.945$). There was however, significant dissimilarity between the AM fungal communities hosted by barley and oat (ANOSIM: $R = 0.333$, $p = 0.001$).


0.8
0.4
0.0
-0.4
-0.8
NMDS2
-0.5
0.0
0.5
NMDS1
Cash Crop
Barley
Oat
AMF
Not Inoculated
Inoculated


Barley
Oat
0.8
0.4
0.0
-0.4
NMDS2
-0.5
0.0
0.5
NMDS1
Cover Crop
Fallow
Radish
Oats
Vetch
Oat and Radish (high rate)
Oat and Radish (low rate)
Radish and Vetch
Oats and Vetch
Oats and Vetch and Radish
AMF
Not Inoculated
Inoculated

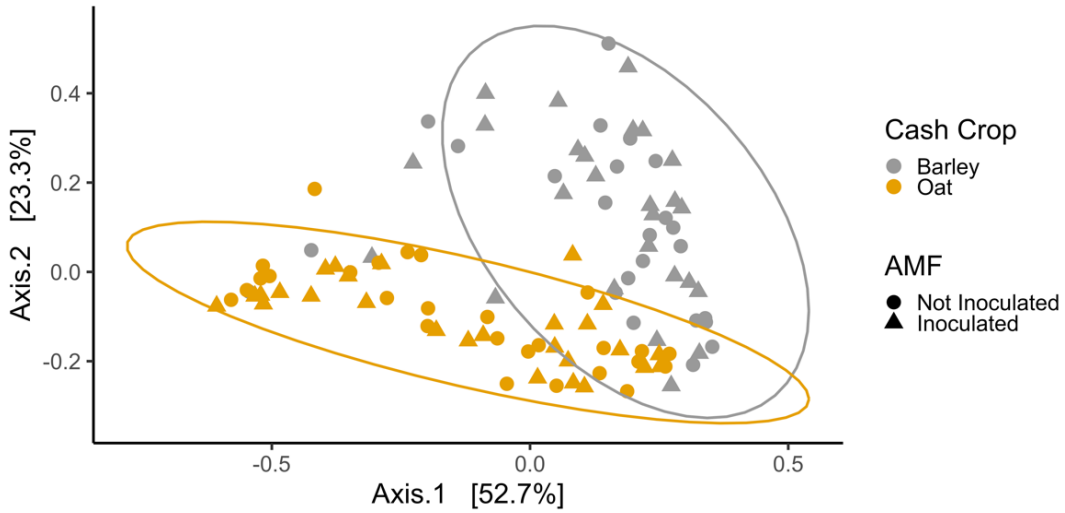

0.4
0.2
0.0
-0.2
Axis.2 [23.3%]
-0.5
0.0
0.5
Axis.1 [52.7%]
Cash Crop
Barley
Oat
AMF
Not Inoculated
Inoculated

Figure 3.39 a) NMDS ordination of Bray-Curtis distances for the NFS trial b) Principal coordinate analysis (PCoA) ordination of Bray-Curtis distances for the NFS trial. Non-metric Multi-dimensional Scaling (NMDS) plot of Bray-Curtis dissimilarities from the Bawburgh trial. Multivariate Analysis of variance by PERMANOVA found no significant dissimilarity in community composition due to the effect of the cover crop (ANOSIM: R = -0.024, p = 0.880) or whether a plot had been inoculated by AM fungi (ANOSIM: R = -0.014, p = 0.945). There was, however, significant dissimilarity between the AM fungal communities hosted by barley and oat (ANOSIM: R = 0.333, p = 0.001).

Observed Number of AM Fungal Taxa at the Bawburgh Trial

Mean observed virtual taxa across the trial was 9.3 ± 0.2; individual numbers of observed taxa are shown in Figure 3.40. Spring barley hosted 9.8 ± 0.3 taxa on average, which was significantly higher than oat, which hosted 8.7 ± 0.3 (ANOVA: $F_{1,66}$ = 7.75, p = 0.007). For spring barley, the minimum number of taxa in a single plot was five, observed following a non-inoculated oat and vetch plot. The greatest number of taxa observed in a single plot was 15, and observed in both the inoculated and non-inoculated radish and vetch plots. These treatments also had the greatest mean observed number of taxa at 12.7 ± 1.2 and 12.5 ± 2.5 for the inoculated and non-inoculated treatments, respectively. There was no effect of the preceding cover crop on the observed number of AM taxa in crop roots (ANOVA: $F_{8,66}$ = 1.69, p = 0.118), but there was a significant effect of the interaction between the cash crop and the preceding cover crop (ANOVA: $F_{8,66}$ = 2.29, p = 0.031). Ignoring the effects of AM fungal inoculation, the lowest treatment mean was 8.4 ± 0.7, following the radish cover crop treatment, then radish and oat (9.0 ± 0.7), vetch (9.4 ± 0.5), then the fallow

control at 9.5 ± 0.3 taxa. AM fungal inoculation increased the mean richness of taxa colonising barley roots from 9.5 ± 0.4 to 10.0 ± 0.4, although neither the global effect of inoculation (ANOVA: $F_{1,66} = 3.89$, $p = 0.053$), nor the pairwise comparison for barley (Tukey $p = 0.55$) was significantly different. Further, there was no interaction effect of AM inoculation and cash crop (ANOVA: $F_{1,66} = 1.21$, $p = 0.275$) or AM inoculation and cover crop (ANOVA: $F_{8,66} = 0.41$, $p = 0.909$) on AM fungal diversity. Addition of cash crop RLC as a model covariate improved model fit ($\chi^2 = 12.25$, d.f. = 1, $p < 0.001$) and there was a significant positive correlation between RLC and observed AM VT (r (102) = 0.25, $p = 0.011$).

For winter oat, the minimum number of taxa in a single plot was also five observed following a non-inoculated radish and oat cover crop treatment, which also had the lowest mean richness (7.0 ± 0.4 taxa). This was the only treatment to host fewer taxa than the fallow control, which hosted 7.7 ± 0.5 taxa, and only varied in standard error between inoculated and non-inoculated treatments (7.7 ± 0.7 and 7.7 ± 0.9 respectively). The greatest number of taxa colonising oat roots in a single plot was 14, found following an inoculated oat cover crop. An oat cash crop following an oat cover crop also hosted the greatest mean richness of any cover crop treatment (10.8 ± 0.8). The four treatments with the highest mean richness were inoculated with AM fungi. These were oats (12.3 ± 0.9), oats and vetch (11.0 ± 0.0), high-rate radish and oats (10.7 ± 0.9), and vetch (10 ± 1.2). Inoculation with AM fungi significantly increased AM richness of oat crops (Tukey $p = 0.029$), from 8.1 ± 0.4 to 9.3 ± 0.4.

To test the impact of the richness of AM fungal diversity on metrics such as crop biomass as yield, the observed number of AM fungal taxa was added to previous models as a covariate. Addition of AM diversity as a covariate did not improve model fit of spring barley shoot biomass ($\chi^2 = 0.316$, d.f. = 1, $p = 0.574$), proportion of grain over 2.8mm ($\chi^2 = 0.714$, d.f. = 1, $p = 0.398$), thousand grain weight ($\chi^2 = 1.58$, d.f. = 1, $p = 0.209$), plant counts ($\chi^2 = 0.084$, d.f. = 1, $p = 0.772$), or yield ($\chi^2 = 0.232$, d.f. = 1, $p = 0.629$). Likewise, for oat, addition of AM diversity as a covariate did not improve model fit of winter oat shoot biomass ($\chi^2 = 0.248$, d.f. = 1, $p = 0.619$), plant counts at GS20 ($\chi^2 = 0.21$, d.f. = 1, $p = 0.647$), head counts at GS71 ($\chi^2 = 2.13$, d.f. = 1, $p = 0.144$), or yield ($\chi^2 = 0.13$, d.f. = 1, $p = 0.718$).

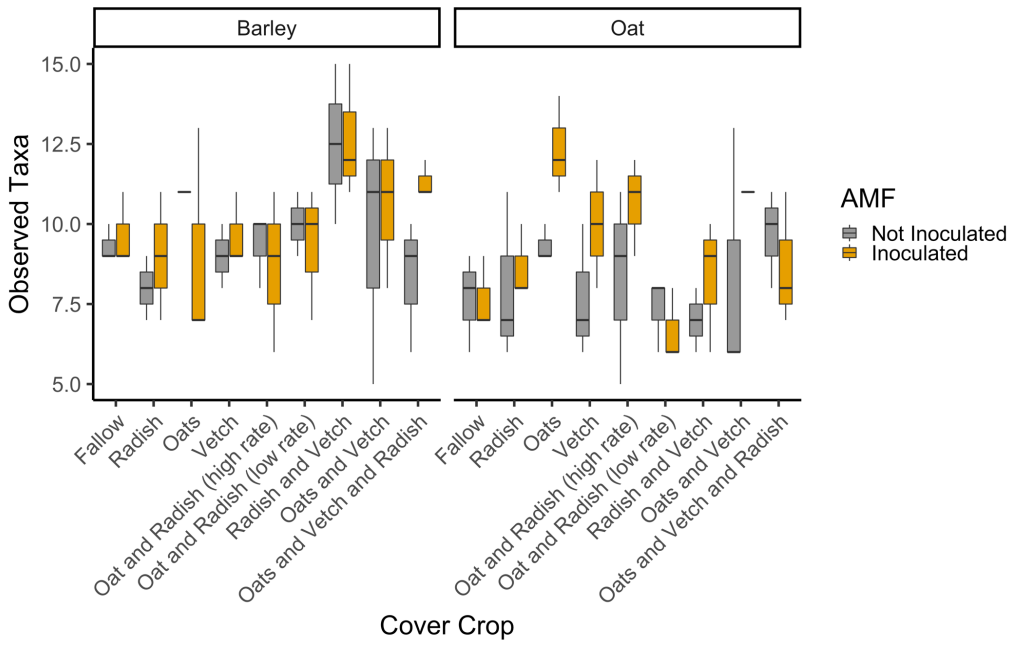


Figure 3.40 Observed AM fungal virtual taxa in the Bawburgh trial.
AM fungal VT observed in each experimental treatment. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Twenty six taxa were observed in both the barley and oat cash crop, including 13 *Glomus*, four *Archaeospora*, three *Paraglomus*, three *Diversispora*, one *Scutellospora*, one *Acaulospora*, and one *Ambispora* taxa (Table 3.2). Thirteen taxa were found exclusively in the spring barley samples including six *Glomus*, three *Diversispora*, two *Paraglomus*, and two *Claroideoglomus*. One taxon, VTX00193 (*Claroideoglomus lamellosum)* was found exclusively in the oat cash crop samples. Numbers of AM fungal taxa found in each cash crop are visualised in Figure 3.41.

Table 3.2 AM Fungal VT occurring in each cash crop species in the Bawburgh trial

Barley and Oat	Barley Only	Oat Only
VTX00153	VTX00125	VTX00193
VTX00143	VTX00222	
VTX00342	VTX00108	
VTX00199	VTX00100	
VTX00113	VTX00309	
VTX00114	VTX00307	
VTX00105	VTX00054	
VTX00295	VTX00062	
VTX00065	VTX00380	
VTX00067	VTX00225	
VTX00063	VTX00278	
VTX00409	VTX00349	
VTX00064	VTX00444	
VTX00306		
VTX00060		
VTX00052		
VTX00354		
VTX00030		
VTX00281		
VTX00435		
VTX00239		
VTX00245		
VTX00338		
VTX00009		
VTX00005		
VTX00283		

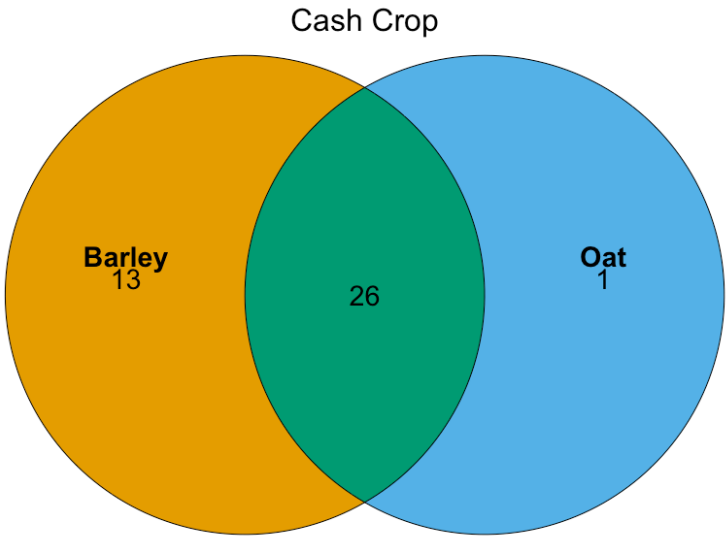


Figure 3.41 Number of AM fungal virtual observed between the two cash crops. Venn classification of AM fungal VT observed in each cash crop. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Within the barley samples, all 39 AM fungal taxa were observed in both the inoculated, and non-inoculated treatments. Within oat samples, 23/27 taxa were found in both inoculated and non-inoculated treatments, but four: VTX00143 (*Glomus MO-G20*), VTX00063 (*Glomus viscosum*), VTX00409 (*Glomus Torrecillas12b Glo-G13*), and VTX00239 (*Paraglomus brasilianum*) were found exclusively in inoculated plots.

Inverse Simpson's Diversity for Community Evenness in the Bawburgh Trial

The Inverse Simpson's Index as a measure of community evenness can be considered as a measure of the effective number of taxa in a community, after considering the relative abundance of each taxa. Perhaps unsurprisingly, the higher richness of observed taxa in barley also resulted in a significantly higher inverse Simpson's index (ANOVA: $F_{1,66} = 24.12$, $p < 0.001$). Mean inverse Simpson's index for barley was 3.16 ± 0.15, and 2.34 ± 0.09 for oat (Figure 3.42). The minimum evenness observed in a single plot was 1.26 for barley and 1.21 for oat, with larger differences between the maximum plot evenness, of 6.56 for barley and 3.71 for oat. The largest mean evenness was observed following the inoculated three-way mix cover crop (4.85 ± 0.85) for barley, and inoculated vetch (2.97 ± 0.55) for oat. The lowest evenness was observed following the inoculated radish and vetch treatment (2.26 ± 0.58) for barley, and the non-inoculated radish and oat cover crop (1.79 ± 0.09) for oat. Ignoring the impact of AM inoculation, barley and oat AM fungal evenness was lowest following the radish and vetch cover crop (2.74 ± 0.46 and 1.88 ± 0.18 respectively). The highest evenness was observed following the three-way mix for barley (3.62 ± 0.73) and vetch for oat (2.64 ± 0.33). However, the vetch cover crop did produce the second highest evenness in barley, and the three-way mix cover crop produced the second highest evenness in oat.

Despite this, statistically the preceding cover crop had no impact on inverse Simpson's index, either alone (ANOVA: $F_{8,66} = 0.97$, $p = 0.465$), or in concert with the different cash crops (ANOVA: $F_{8,66} = 0.25$, $p = 0.979$), or levels of AM fungal inoculation (ANOVA: $F_{8,66} = 1.36$, $p = 0.229$). Likewise, there was no effect of AM fungal inoculation alone (ANOVA: $F_{1,66} = 0.71$, $p = 0.401$), or in interaction with the species of cash crop (ANOVA: $F_{8,66} = 1.36$, $p = 0.229$). There was a significant effect of the three way interaction between cash crop, preceding cover crop, and AM fungal inoculation (ANOVA: $F_{8,66} = 2.31$, $p = 0.030$). This indicates that the experimental treatments may have impacted the two cash crops (barley and oat) differently.

To further investigate the significant interaction term, the data were split into cash crop species. For barley, there was no effect of AM fungal inoculation (ANOVA: $F_{1,30} = 2.61$, $p = 0.117$), or the preceding cover crop (ANOVA: $F_{8,30} = 0.38$, $p = 0.925$), on inverse Simpson's diversity. The interaction term between AM inoculation and the cover crop was not significant (ANOVA: $F_{8,30} = 2.14$, $p = 0.063$), and no post-hoc comparisons achieved the $p < 0.05$ cut off. For oat, there was no significant effect of AM fungal inoculation (ANOVA: $F_{1,34} = 0.49$, $p = 0.488$), the preceding cover crop (ANOVA: $F_{8,34} = 1.07$, $p = 0.408$), or their interaction (ANOVA: $F_{8,34} = 1.60$, $p = 0.160$) on inverse Simpson's diversity.

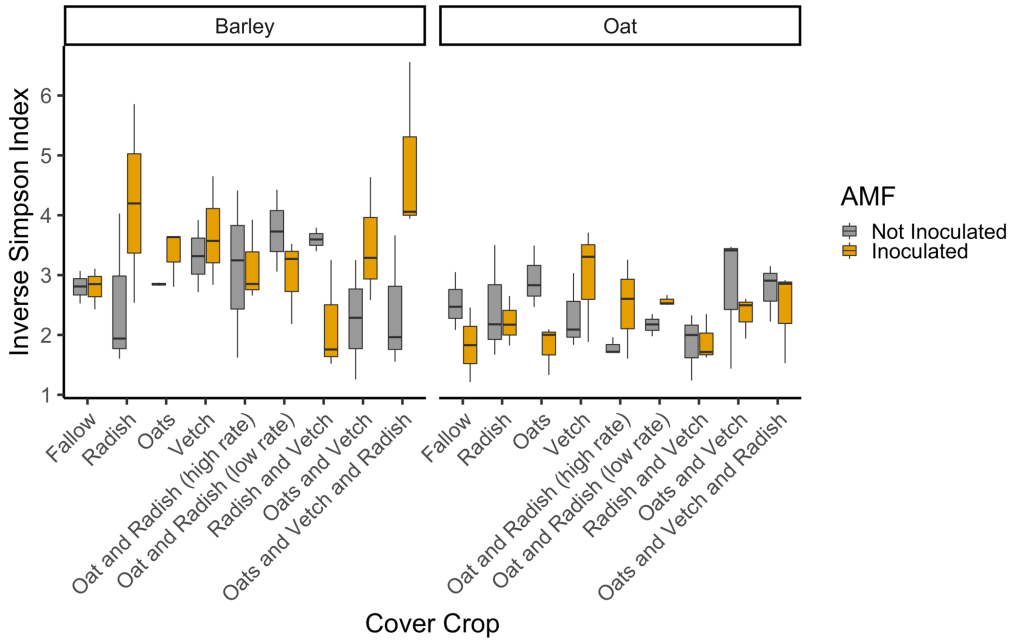


Figure 3.42 Inverse Simpson's Index for AM fungal community evenness.
Inverse Simpson's index of AM fungal communities observed in each experimental treatment, in both the barley and oat cash crop. Inverse Simpson's index represents the 'effective' diversity and is bounded by 1 and the number of observed VT per treatment.

AM Fungal Community Composition and Analysis of Differential Abundance at the Bawburgh Trial

AM fungal community composition in the spring barley (var. Laureate) plots consisted of 54.4% *Glomus*, 20.1% *Ambispora*, 12.5% *Archaeospora*, 4.7% *Paraglomus*, 4.2% *Diversispora*, 3.9% *Scutellospora,* 0.1% *Acaulospora*, and <0.1% *Claroideoglomus.* In the winter oat cash crop, the previously dominant *Glomus* represented 40.4% of reads (-14% compared to barley), and *Archaeospora* became the dominant genus at 46.1% (+33.6%). *Ambispora* was the third most dominant genus in oat, at 7.0% (-13.1%). The remaining genus abundances were 2.3% *Paraglomus* (-2.4%), 2.3% *Scutellospora* (-

1.6%), 1.0% *Diversispora* (-3.2%), 0.2% *Acaulospora* (+0.1%), and 0.1% *Claroideoglomus* (+<0.1%).

Differences in genus composition between levels of AM fungal inoculation was remarkably low. For barley, non-inoculated genus composition was 56.8% *Glomus*, 17.4% *Ambispora*, 12.9% *Archaeospora*, 5.0% *Paraglomus*, 4.0% *Diversispora*, 3.8% *Scutellospora*, <0.1% *Acaulospora* and *Claroideoglomus* was not detected. Barley genus composition in inoculated plots was 52.0% *Glomus* (-4.8%), 22.8% *Ambispora* (+2.7%), 12.2% *Archaeospora* (-0.3%), 4.3% *Paraglomus* (-0.4%), 4.3% *Diversispora* (+0.1%), 4.1% *Scutellospora* (+0.2%), 0.2% *Acaulospora* (+0.1%), and <0.1% *Claroideoglomus* (+<0.1%). For oat, non-inoculated genus composition was 45.2% *Archaeospora*, 40.0% *Glomus*, 7.8% *Ambispora*, 3.3% *Paraglomus*, 2.4% *Scutellospora*, 1.0% *Diversispora*, 0.1% *Claroideoglomus*, and 0.1% *Acaulospora*. Oat genus composition in inoculated plots was 47.0% *Archaeospora* (+1.8%), 40.7% *Glomus* (+0.7%), 6.2% *Ambispora* (-1.6%), 2.5% *Paraglomus* (-0.8%), 2.1% *Scutellospora* (-0.3%), 1.1% *Diversispora* (+0.1%), 0.3% *Acaulospora* (+0.2%) and 0.1% *Claroideoglomus* (no change).

The five most abundant taxa in the barley plots were VTX00065 (*Glomus caledonium*, 37.6%) VTX00283 (*Ambispora fennica*, 20.6%), VTX00342 (*Glomus VeGlo18*, 12.8%), VTX00245 (*Archaeospora trappei*, 11.3%) and VTX00281 (*Paraglomus laccatum*, 4.7%). This was the case for both inoculated and non-inoculated plots. The five most abundant taxa in the oat plots

were VTX00245 (*Archaeospora trappei*, 41.9%), VTX00065 (*Glomus caledonium*, 38.1%), VTX00283 (*Ambispora fennica*, 7.0%), VTX00338 (*Archaeospora Aca*, 4.1%), and VTX00281 (*Paraglomus laccatum*, 2.9%). VTX00342 (*Glomus VeGlo18*), which was the third most abundant taxon in the barley crop was only the seventh most abundant in oat, constituting 0.7% of reads (down from 12.8%). VTX00245 (*Archaeospora trappei)*, the fourth most abundant in barley dominated reads of oat roots, and increased from 11.3% to 41.9% of total reads. Finally, VTX00338 (*Archaeospora Aca*) was the fourth most abundant in oat (4.1% of reads), but was only the ninth most abundant in barley (1.2% of reads). Relative abundances of AM VT for both barley and oat is shown in Figure 3.43.

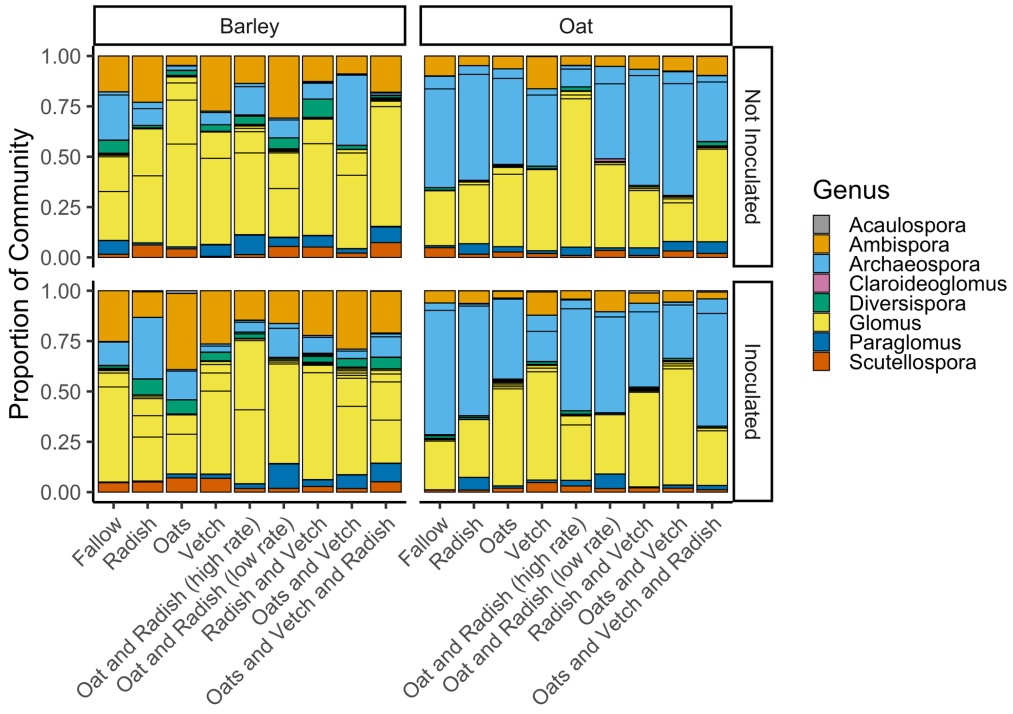


Figure 3.43 AM Fungal community composition at the Bawburgh trial.
Mean community composition of AM fungal VT for each experimental treatment, expressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT.

Analysis of differential abundance of virtual taxa, using shrinkage estimation was conducted to further confirm how individual taxa had responded to experimental treatments. Six taxa were differentially abundant between the barley and oat cash crop (Figure 3.44). Two taxa were significantly more abundant in the oat crop, VTX00338 (*Archaeospora Aca,* $\log_2$FC = 2.62, p < 0.001) and VTX00245 (*Archaeospora trappei,* $\log_2$FC = 2.57, p < 0.001). The remaining four taxa were significantly more abundant in the barley crop, and included VTX00283 (*Ambispora fennica,* $\log_2$FC = -0.97, p < 0.001), VTX00354 (*Diversispora Clade-3,* $\log_2$FC = -1.47, p = 0.001), VTX00342 (*Glomus VeGlo18,* $\log_2$FC = -4.21, p < 0.001), and VTX00295 (*Glomus Glo-*

A, $\log_2$FC = -0.97, p = 0.009). There were no differentially abundant taxa between levels of AM fungal inoculation, either at the level of the whole trial, or individual cover crop treatments.

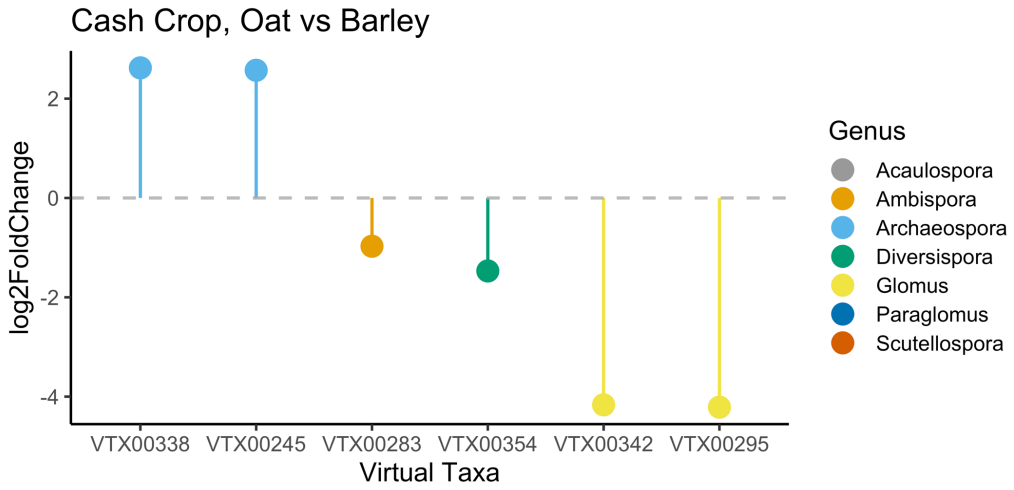


Figure 3.44 Differential abundance of AM Fungal VT comparing the oat and barley cash crops. Differential abundance of AM fungal VT comparing oat and barley cash crops, calculated by DESeq2. AM VT were considered to be differentially abundant if their $\text{Log}_2\text{FC} > 1$ and $p < 0.05$.

3.4 Discussion

In this chapter, the efficiency and longevity of a commercial AM fungal was tested, in glasshouse and field conditions. The inoculum, sourced from Plant-Works Ltd (Sittingbourne, UK) was first dissected in glasshouse conditions, experimenting how the five components of the AM fungal inoculum impacted barley RLC and biomass, when grown individually and as the commercial five species mix. *R. irregularis* was the most prolific coloniser of

spring barley roots, out of the five individual taxa, which resulted in the only significant decrease in shoot biomass when compared to the control.

Sequencing the AM fungal community colonising the mixed inoculum spring barley roots showed that *R. irregularis* comprised of almost three quarters of sequence reads, and subsequently, the RLC and biomass of the mixed inoculum was similar to that of the pure *R. irregularis* inoculum. This dominance by a single species has been shown by Gosling et al. (2016), and is possibly due to the limited numbers of stressors in glasshouse conditions. However, a meta-analysis by Yang et al. (2017) found that taxa within the Glomeraceae and Claroideoglomeraceae were associated with the highest P uptake, a likely stressor in this trial due to the addition of half concentration of P in the adapted Hoagland solution. It is surprising that the other AM fungal taxa were only able to colonise roots at very low levels. It is possible that the number of infectious propagules per gram of inoculum was low compared to *Rhizophagus*, or that this result was due to the slow growing nature of these taxa.

Experimental findings are in contrast to Berruti et al. (2016), who found that multispecies inocula increased plant biomass compared to single species. Results from this chapter show that shoot biomass following inoculation by a five species mix, is lower than the control, and some other single taxa. This may have been as a result of the five components of the inoculum being from only a limited number of closely related families, leading to functional

redundancy, and was shown in a similar manner for species of *Acaulospora* in Crossay et al. (2019), and in part by a meta-analysis by Yang et al. (2017).

In the field trial, cover crops established well, quickly accumulating biomass in September and early October, highlighting the importance of early cover crop establishment. GAI of radish plateaued in October, whereas vetch and especially oat, continued to accrue biomass throughout the winter period. Overall, there was no significant impact of experimental treatments on cover crop biomass, and very little impact on soil physicochemical parameters, including SOM.

Barley and oat plants were sampled following the method of Higo et al. (2014), who pooled 15 root samples into three biological replicates, each representing five plants. Given the resources, this was an effective method of sampling large numbers of plants to effectively capture plot level effects including AM fungal diversity. This method loses resolution, when looking at the consistently of treatments within plots, as standard errors within biological replicates cannot be ascertained. The method is far from perfect, but does make large scale harvest and analysis of plant samples possible by one single researcher, and adapted methods have been utilised by others (Kabir and Koide 2000; White and Weil 2010; Turrini et al. 2016). DNA extraction and sequencing of barley and oat root samples was successful, with oat plants yielding higher numbers of reads than barley. This may be due to the increased fungal biomass observed inside oat roots, when compared to barley. Addition of PhiX was reduced in this sequence run, from

40% as recommended by Alex Dumbrell, to 20%. This was potentially too low for this amplicon, and reduced the abundance of Q30 reads of the reverse strand. If reads were a limiting factor in future studies, 30% PhiX would be recommended for NS31-AML2 amplicons. If sample numbers are low, 40% PhiX would be optimal.

There was no effect of the experimental treatments on oat or vetch biomass, but there was a significant effect of the cover crop mix from which a radish was sampled, on radish shoot biomass. Radish grown at a low seed rate with oat was nearly 70% larger than radish grown at a high seed rate with vetch. This may suggest that a lower radish seed rate is able to produce the same benefits as smaller plants at a higher seed rate, although this result would have to be experimentally validated at other sites/ soil conditions.

Oat cover crop RLC was significantly higher than vetch, an unexpected result given the findings by Bowles et al. (2017), however, there was no impact on cover crop RLC of either the cover crop mix, or AM fungal inoculation. Unfortunately, cover crop root DNA was not sequenced for assessment of diversity of AM fungi, as it seems likely that even at this early stage, the AM fungal inoculum was unable to establish in the soil, a result also shown by Berruti et al. (2017).

In the spring barley crop, there was no impact of the preceding cover crop, or inoculation by AM fungi on barley RLC. Adding soil P to the statistical model significantly improved model fit, with higher levels of soil phosphate

resulting in lower levels of RLC. This trend is commonly shown in controlled conditions, but can be less robust in field conditions (Thomson et al. 1986; Bruce et al. 1994; Balzergue et al. 2011). Similarly, there was no impact of the preceding cover crop, or AM fungal inoculation on the AM fungal community, further proving that even if the inoculum had established in the cover crop, it was not detectable during the key growth period for the barley crop. Overall, there was no impact of AM fungal inoculation, or the preceding cover crop on spring barley yield, however the latter approached the commonly used significance threshold of $p < 0.05$ and may warrant further investigation. Barley yield following non-inoculated radish, oat, or low seed rate radish and oat cover crops was increased when compared to the control, although not significantly. Neither RLC nor AM fungal diversity was a significant predictor of barley yield in this study. In support of this result, Ryan & Graham (2018) review why farmers should be speculative for the role of the AM symbiosis in agriculture, suggesting that the literature is overly optimistic in its view of AM benefit, owing to flawed experimental methodology. However, in response to Ryan & Graham (2018), Rillig et al. (2019) criticise the overly simplified view of Ryan and Graham, noting that they have focussed on cereal crops, and especially wheat which are known to respond poorly to AM fungi. Furthermore, Rillig et al. suggest that Ryan and Graham ignore the impact of AM fungi in whole system performance and sustainability. Further to this, two meta-analyses by Van Geel et al. (2016) and Lekberg and Koide (2005) suggest that AM fungal inoculation increased crop growth and yield by 35 and 23% respectively. However, these

figures do include studies from controlled and semi-controlled conditions, a criticism of Ryan & Graham (2018).

In year two, there was a significant effect of the preceding cover crop on winter oat biomass. Vetch, and the high seed rate radish and oat cover crops elicited a significantly increased shoot biomass, but this trend was not observed following the low seed rate radish and oat, or vetch and radish cover crop. Because of this inconsistency, it is difficult to hypothesise any possible mechanisms which may have influenced this effect, however the most parsimonious hypothesis would have to include a weak, but reduced disease pressure on oat, following a cover crop of vetch or radish. The cereal cover crop may have provided a green bridge for cereal pests and diseases, which were detrimental to growth and yield in those plots following the cereal cover crop (Stobart and Gosling 2015).

Like for barley, there was no impact of the preceding cover crop, or AM fungal inoculation on oat RLC. Addition of soil available P as a covariate improved model fit, demonstrating a reduction in oat RLC as soil P increased suggesting that the AM fungal taxa found in the trial may be impacted by high levels of P. Camenzind et al. (2014) showed that the Glomerales were found to be significantly less abundant in high P soils, which were found at relatively high abundance at the Bawburgh experimental site.

AM fungal inoculation appeared to increase AM fungal richness in oat samples, but this was not in direct response to inoculation, as none of the five

inoculated taxa were found in higher abundance in the inoculated barley, or oat samples. A similar phenomenon was observed by (Berruti et al. (2017), who attributed the indirect increase in diversity to the reduction in dominance by one or a few taxa, although they do not postulate the mechanism underpinning this effect.

There was no impact of the preceding cover crop, or AM fungal inoculation on oat yield, although a yield penalty resulting from inoculation approached the $p < 0.05$ threshold for significance and may require further experimentation. Interestingly, despite this negative impact, there was a positive correlation between oat RLC and yield. This suggests that unlike other cereal crops which do not receive much benefit from the AM symbiosis, oat may have a predisposition to benefitting from the AM symbiosis. A similar benefit was shown in petroleum contaminated soils growing oat in Heilongjiang Province, China (Xun et al. 2015).

Sequencing of AM fungal communities at the Bawburgh site identified 40 distinct AM fungal taxa across four orders and eight families. VTX00065 (*Glomus caledonium*) and VTX00245 (*Archaeospora trappei*) comprised over 65% of reads across the two years of the trial. VTX00065 (*Glomus caledonium)* was found to be more highly abundant following a barley cover crop, in comparison to vetch or fallow, in a study by Hontoria et al. (2019). Similarly, Vályi et al. (2015) found *Glomus caledonium* was associated with three perennial grasses across a land use gradient of German grasslands. This suggests some level of host specificity for *Glomus caledonium,* however

further experimentation will have to be required to determine whether any benefit has arisen from this interaction. VTX00245 (*Archaeospora trappei*) has been shown to be found in greater abundance following drought episodes, which was experienced at the site the previous year (Lumini et al. 2014).

Despite the lack of evidence for experimental perturbation of AM fungal communities resulting from cover cropping or AM fungal inoculation, spring barley and winter oat communities were significantly different in the 2019 and 2020 seasons. In total, 26 AM fungal taxa were shared between the two seasons, with the barley crop hosting an additional 13 taxa, including six *Glomus*, three *Diversispora*, two *Claroideoglomus*, and two *Paraglomus*, whereas the oat hosted only one additional taxon, VTX00193 (*Claroideoglomus lamellosum*). In addition to these, two *Glomus*, one *Diversispora* and one *Ambispora* were significantly more abundant in the barley, whereas two *Archaeospora* were significantly more abundant in oat, as assessed by differential abundance analysis in DESeq2 (Love et al. 2014). Although it is tempting to speculate that these differences may have arisen from a plant-fungal specificity, the impact of abiotic influences cannot be ignored. For example, the increased abundance of VTX00245 (*Archaeospora trappei*) may be associated with the temporal proximity of the barley crop to the drought event of 2018, with the communities returning to their pre-drought composition in the following year or years (Lumini et al. 2014).

In addition to the impact on individual taxa, spring barley VT richness was significantly higher in barley than oat, with barley samples hosting over 10%

more taxa. This suggests that despite having similar values of RLC, winter oat is only able to host a subset of the AM fungal community that can barley. This observation, has not been experimentally tested in the literature, although it is known that there is considerable inter- and intraspecific variation in RLC and mycorrhizal benefit between crop species, and within varieties of single species. Therefore, it is not impossible to hypothesise that similar mechanisms, though largely unknown, may also influence the diversity of AM fungi that a plant may interact with. One alternative hypothesis is that the abiotic conditions for the 2019 barley crop were more conducive to the dispersal of the additional 13 taxa, which were less suited to conditions in 2020. If this were the case, it would pose an argument for a lack of phylogenetic conservatism within AM fungi to whichever abiotic factor or factors were causing the selection at the Bawburgh site. Other studies in the literature also indicate that abiotic environmental factors may have greater influence on AM fungal communities than plant identity, or impacts due to cover crops or other biotic factors (Njeru et al. 2014a; Higo et al. 2014, 2015).

Finally, the addition of AM fungal richness to biomass and yield models did not improve model fit, suggesting that increased diversity *per se* is not linked to increased crop performance. This is in line with findings by Verbruggen et al. (2012), who reported that maize inoculated with soil AM fungal communities hosting greater numbers of AM taxa negatively impacted productivity. However, this is in contrast to other literature, which states that increased diversity can result in increased productivity, although forbs,

woody plants, and C4 grasses responded more positively than N-fixing legumes and C3 grasses, and plant responses are most positive when P limited.

3.5 Conclusion

In this chapter, glasshouse and field trials were conducted to test the efficiency and longevity of a commercial AM fungal inoculum. It was shown that species within the AM fungal inoculum colonised spring barley to differing extents in the glasshouse trial, but failed to establish in field conditions. Additionally, cover crops had a limited impact on soil physicochemical parameters, and no impact on AM fungal communities. Interestingly, there was a significant difference in AM fungal communities between the barley and oat cash crops, grown in 2019 and 2020, although it is not possible to draw conclusions on whether this was caused through biotic or abiotic mechanisms through this trial alone. Despite this, this finding poses an interesting result which would benefit from further experimentation.

4 Chapter Four – Influence of Nitrogen Application on AM fungal communities, and benefit of symbiosis

4.1 Introduction

AM fungi are often acknowledged for their importance in P_i uptake by plants, both from a plant evolution and crop nutrition perspective. However, there is increasing evidence for a role of AM fungi in plant N uptake, which has largely been overlooked in the vast body of literature, and may play a key role in plant growth, and environmental N cycling more generally (Govindarajulu et al. 2005; Kobae et al. 2010; Breuillin-Sessoms et al. 2015; Wang et al. 2020). Evidence for a role of AM fungi in symbiotic N uptake is discussed at length in Chapter One.

Nitrogen Application for Crop Growth in Arable Farming

N is required by plants for normal growth, in huge amount for the purine nucleotides adenine and guanine, and for protein biosynthesis, including vital enzymes, active transporters, and passive channels associated with homeostasis, cellular signalling, and growth. N amendments are routinely required for increasing growth and yield of crop plants. N is applied in a number of forms, including commercially produced urea, ammonium nitrate, animal manure, green waste, or digestate from anaerobic digestion; the latter

three usually being sourced locally to reduce costs, and increase profitability. 'Bagged' and organic nitrogen each has its own pros and cons. Reactive nitrogen (N_r) sources, such as ammonium nitrate, produced by the Haber-Bosch process are readily available and give farmers high levels of control over the amount of N being applied to a crop. However, the Haber-Bosch process of producing these fertilisers is energy intensive, expensive and inefficient (Erisman et al. 2008). Organic fertiliser, whether based on animal manures, green waste, or digestate can also provide high levels of N, and may be sourced at relatively low cost to a farmer. Unfortunately, these amendments are often in short supply or may be seasonal in nature. Furthermore, they contain variable levels of N and therefore require testing prior to every application to conform with local and national N use regulations; such as legislation associated with Nitrate Vulnerable Zones (NVZs) (DEFRA and Environment Agency 2018).

Approximately 80% of total N_r fixed by the Haber-Bosch process is used in agricultural fertilisers, with an efficiency of 17% being consumed by humans as plant, dairy, or meat (UNEP and WHRC 2007; Galloway et al. 2008). Both organic and inorganic sources of N exhibit these inefficiencies, and once applied, are prone to loss through leaching, such as nitrate (NO_3) pollution of groundwater (Galloway et al. 2008; Erisman et al. 2013). Eutrophication of inland and coastal waters is greatly increased by leaching of agricultural N_r. Algal blooms cause oxygen depletion, and result in trophic collapse of biodiversity. Eutrophication also impacts quality of drinking water, and is

economically damaging for tourism due to visible damage to the environment and smell, and impacts waterside property prices (Pretty et al. 2003; Withers et al. 2014). Applied sources of N_r can also be converted to ammonia (NH_3), nitrogen oxides (NO_x), and nitrous oxide (N_2O) by denitrifying bacteria, which is released into the atmosphere by volatilisation; resulting in greenhouse gas emissions which negatively impact air quality, and contribute to climate change.

Contribution of AM Fungi to Plant N Nutrition and Cycling

Considering the inefficiencies of N uptake and utilisation by the crop, allied to pollution and global climate change, it would be desirable to decrease application of N on farm, whilst maintaining sufficient plant availability for high yields. There is a considerable body of literature demonstrating the ability of AM fungi to take up and deliver P to its plant host, both at the whole plant, and cellular level, which is covered in depth in Chapter One of this thesis. AM fungi have also been shown to contribute to N uptake, however experimental evidence is intractable, and inconsistent (Tobar et al. 1994; Leigh et al. 2009; Hodge and Fitter 2010; Hodge and Storer 2014).

Impact of Nitrogen Application on AM Fungal Diversity and Abundance

There is limited evidence to suggest that N availability can influence the degree to which plant roots become colonised by AM fungi, however, Camenzind et al. (2014) showed that N fertilisation, rather than P (which

is documented to be the primary modulating influence on colonisation rates, Sally E. Smith & Smith, 2011), decreased RLC of plants found in a tropical montane rainforest in Southern Ecuador. The authors also found that fertilisation with either P or N, or a combination of both, decreased AM fungal species richness, mostly impacting rare species. Interestingly, it was shown that fertilisation with N or P impacted different AM phylogenetic groups; with Diversisporales abundance being mostly reduced by N additions, whereas Glomerales abundance was more strongly effected by additions of P. This further suggests a role for functional specificity of certain AM fungi, that are more specialised in N scavenging and uptake, as opposed to their classically documented role being limited to P acquisition. These AM species may have previously been underestimated in terms of their efficiency as symbionts, because they were less effective at plant nutrient provision nourishment through provision of P_i, in controlled conditions.

Experimental Overview

Established in autumn 2007, the New Farming Systems trial at Morley, Norfolk, is a series of long-term experiments, considering cover copping, soil amendments, and cultivation approaches to maximise sustainability and yield (Figure 4.1). The soil is classified as sandy loam, and experiments aim to replicate on-farm conditions by the use of typical agricultural machinery and techniques (Stobart and Morris 2014). The Fertility Building Rotation trial has been set up to consider the impact of a number of rotational, cover crop, and nitrogen dose treatments in a large fully replicated trial. Cash

crops are grown at 100%, 50% or 0% of the standard N dose for that crop, which is applied as ammonium nitrate. Two cover crop mixtures of fodder radish and oat, or a five species legume mix containing crimson clover, red clover, black medic, lucerne, and vetch are grown to scavenge residual nutrients, which then become available to the following cash crop following their decomposition. It is also thought that the symbiosis between leguminous species and rhizobia will fix additional atmospheric N, especially in low N application plots (Peoples et al. 1995).

Figure 4.1 The NFS Fertility Building Rotations trial under snow in January 2018.

The Fertility Building Rotation trial is set on a sandy clay loam, which is abundant in P (Table 4.1), it is therefore of interest to consider the effect of N application on AM fungal colonisation of crop roots, and whether the

diversity of fungal symbionts is altered by N dose (Camenzind et al. 2014). Due to the large scale and rotational nature of the trial, a subset of the plots was sampled, omitting the winter sown, and long-term clover ley plots, and focussed on spring sown barley following two cover crop regimes and a fallow control. It is hypothesised that barley following cover crops will have increased AM fungal colonisation compared to fallow, and this effect may be influenced by the amount of N fertilisation. Further, it is anticipated that the plots with reduced N inputs will host distinct AM fungal species, which may be attributed to the scavenging, uptake, and subsequent transfer of N to the host plant.

4.2 Methods

Experimental Site and Sampling

The Fertility Building Rotation experiment is an incomplete factorial design with four replicates of four cover crop systems, three rotation systems, and three N treatments. The trial is located at Morley, Norfolk, on a sandy clay loam with the physicochemical parameters outlined in Table 4.1.

Table 4.1– Analysis of soil physicochemical parameters at the NFS Fertility Building Rotations Trial.

Soil Parameter	
SOM (%)	2.2
pH	6.8
Mineral N 0-30cm (kgN/ha)	9.1
Mineral N 30-60cm (kgN/ha)	8.8
Available P (mg/l)	16.8
Available K (mg/l)	94
Available Mg (mg/l)	36

Plots within the cover crop experiment are 12m × 36m, which includes the three nitrogen treatments for ease of N application (Figure 4.2). Plots are separated by grass paths which reduces proximity effects and reduces damage when accessing and sampling plots. Crops are established in accordance with local best practice, and drilling dates for this experiment are outlined below. The experiment uses shallow non-inversion establishment techniques, at a depth of around 10-15cm using disc and/or tine-based approaches.

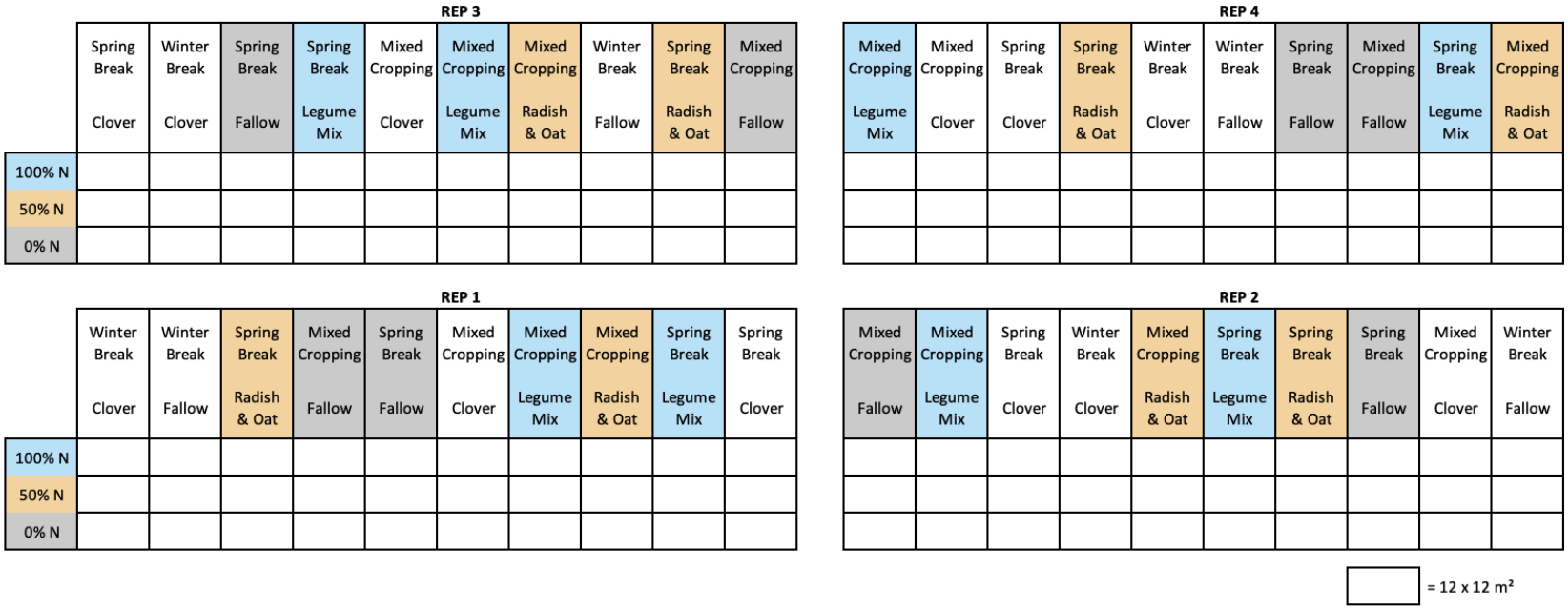


Figure 4.2 Experimental layout of the NFS Fertility Building Rotations trial.
Highlighted plots are included in this study. Clover ley and winter sown barley were omitted for ease of comparison. Each experimental plot was 36 x 12 m, split into three N doses of $12m^2$.

Selection of Rotations in the NFS Trial

The three crop rotation approaches are based around winter wheat, with different break crop approaches (Table 4.2). Winter break represents the conventional approach, spring break maximises the use of spring crops for use of cover crops in the rotation, and the mixed cropping approach utilises both spring and winter varieties. This chapter will consider only the spring break and mixed cropping approaches, as the winter break had been drilled with winter barley, and the clover cover crop was still being established.

Table 4.2 Rotational layout of the NFS Fertility Building Rotations trial.
Cover crops were grown before every spring sown crop in the spring break and mixed cropping approaches, resulting in five cover crops for each of the rotational approaches. Crop species include SOSR (spring oilseed rape), WWT (winter wheat), SBNS (spring beans), WBNS (winter beans), SBLY (spring barley), WOSR (winter oilseed rape), and SOAT (spring oat).

	Cropping and Harvest Year														
Rotation	**2008** (Year 1)	**2009** (Year 2)	**2010** (Year 3)	**2011** (Year 4)	**2012** (Year 5)	**2013** (Year 6)	**2014** (Year 7)	**2015** (Year 8)	**2016** (Year 9)	**2017** (Year 10)	**2018** (Year 11)	**2019** (Year 12)	**2020** (Year 13)	**2021** (Year 14)	**Comments**
Winter Break	WWT	WOSR	WWT	WBNS	WWT	WBLY	WOSR	WWT	WOAT	WWT	WBLY	WOSR	WWT	WBLY	Conventional approach (Benchmark for current systems)
Spring Break	WWT	SOSR	WWT	SBNS	WWT	SBLY	WOSR	WWT	SOAT	WWT	SBLY	WOSR	WWT	SBLY	Maximising spring crops for cover crop use in the systems
Mixed Croppir	WWT	SOSR	WWT	WBNS	WWT	SBLY	WOSR	WWT	SOAT	WWT	SBLY	WOSR	WWT	SBLY	A mixed rotation with spring and winter cropping

Cover Crop Species, Establishment, and Desiccation

Cover crops were drilled on 16th August 2017 at a rate of 20 kg/ha, following a shallow non inversion cultivation. Cropping systems include:

- Legume mix: Crimson clover (*Trifolium incarnatum*), red clover (Trifolium pratense), black medick (*Medicago lupulina*), lucerne (*Medicago sativa*), and vetch (*Vicia sativa)*
- Fodder radish (*Raphinus sativus*) and black oat (*Avena strigosa*) (Figure 4.3)
- Fallow

Cover crops in the spring break and mixed cropping rotation were desiccated on 30th January 2018, with 3.5 L/ha glyphosate and incorporated prior to drilling spring barley var. Laureate on 23rd April 2018 at a rate of 160 kg/ha, following a particularly cold and wet spring season.

Figure 4.3 A cover crop of radish and oat in October 2017 at the NFS trial.

Nitrogen Dose Application for Laureate Spring Barley

Each 12m × 12m section within a cover crop plot (Figure 4.4) received one of the following nitrogen treatments:

- 100% of standard dose for the crop being grown
- 50% of standard dose for the crop being grown
- No nitrogen application

The 100% dose for Laureate spring barley equated to 120 kg N/ha and 60 kg N/ha for the 50% application. Nitrogen was applied as ammonium nitrate with sulphur on 10th May 2018.

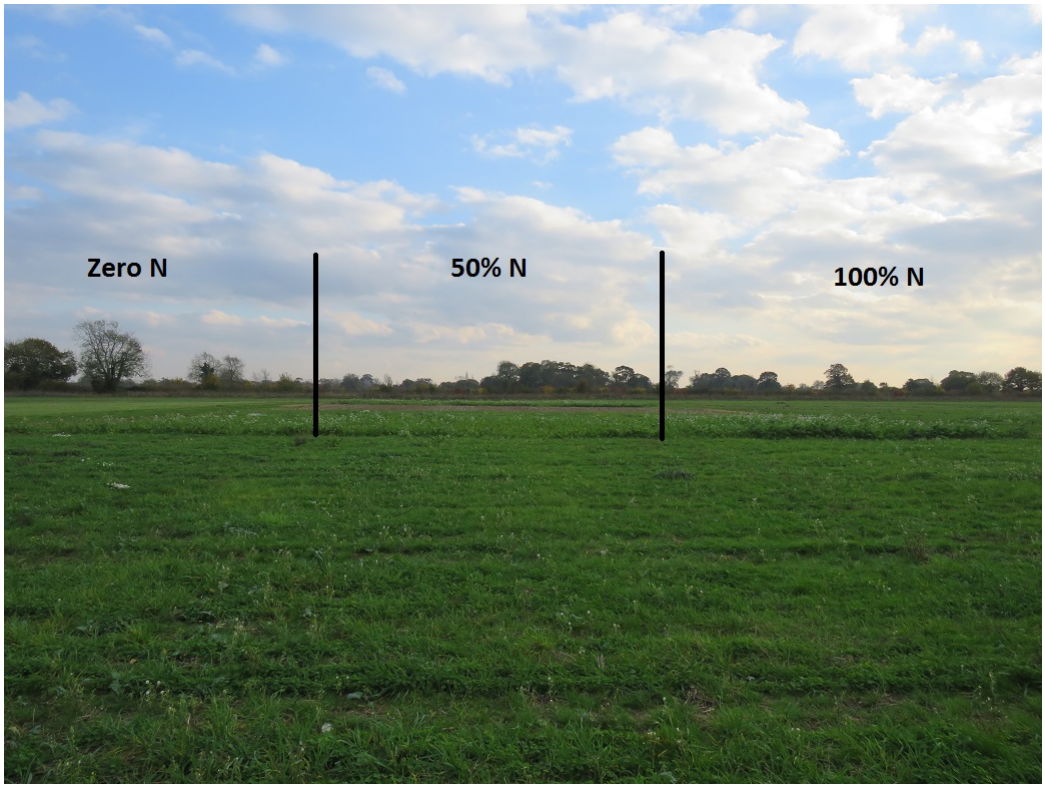


Figure 4.4 Layout of N fertilisation in November 2017 at the NFS trial.

Application of Plant Protection Products in the NFS Trial

Bayer Jaunt fungicide, containing prothioconazole, fluoxastrobin, and trifloxystrobin was sprayed at a rate of 0.4 L/ha on the 25th May 2018, with 1 kg/ha Clayton-Groove Pyrethroid insecticide, and 70 g/ha Finish SX broad leaf herbicide. Jaunt was applied again on the 15th June 2018. This second application was conducted in conjunction with 1 L/ha of Syngenta Bravo 500, containing chlorothalonil as the active ingredient.

Plant Sampling and Yield Measurement in Spring Barley

Spring barley was sampled on 24th June 2018, following the method of Higo et al. (2014), described in Chapter Three. This date corresponded to growth stage 38-40 on Zadok's growth scale, at the end of stem elongation but prior to booting. Three biological replicates, representing 15 individuals was sampled per plot, resulting in a total of 1080 plants across the trial. Each plant was taken from between 1.5m and 3m of the North facing side of each plot (Figure 4.5). This was done to negate edge effects, and preserve a 6m undisturbed strip for yield analysis. Plants were carefully removed from the soil in order not to damage roots, and gently shaken to remove loose soil. Plants were stored in sealable polythene bags, transferred back to the lab in cool boxes, and stored at 4°C prior to analysis. Spring barley was subsequently harvested for each plot on 3rd August 2018.

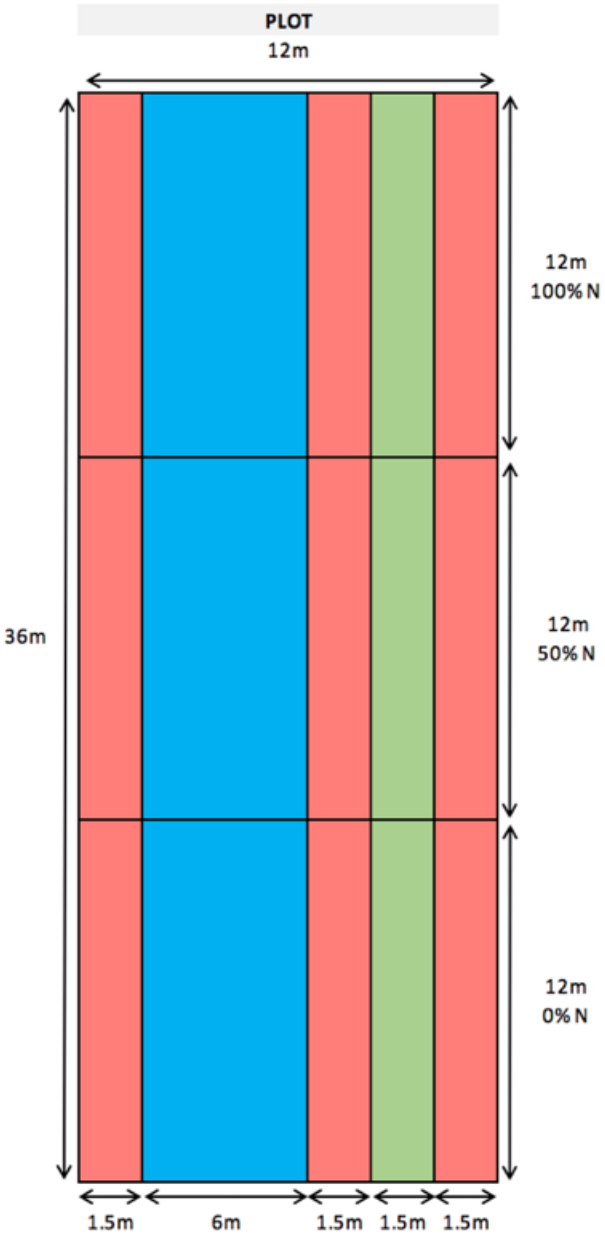


Figure 4.5 Experimental plot layout at the NFS Fertility Building Rotations trial. A single 36 x 12 m^2 plot was further broken down into three 12 x 12 m^2 split plots which were treated with a 0%, 50% or 100% nitrogen dose, for the crop being grown. Plants were sampled from the area shown in green, to preserve a 6m strip (blue) for yield analysis.

Staining and Quantification of Root Length Colonisation by Trypan Blue

Root staining and quantification using an adapted version of the gridline intersect method, was conducted as described in Chapter Three (Gutjahr et al. 2008)

DNA Extraction, PCR Conditions, Sequencing, and Bioinformatics

Methods for DNA extraction, amplification by PCR, and sequencing were conducted as in Chapter Three.

Statistical Analyses in the NFS Fertility Building Rotations Trial

As in Chapters Two and Three, data was rarefied with replacement to the median number of DNA reads before calculating measures of alpha and beta diversity in the package phyloseq (McMurdie and Holmes 2013) and vegan (Oksanen et al. 2013) in R (R Core Team 2019).

Restricted maximum likelihood (REML) estimates of the predictors of linear mixed effects models were determined with the lmer function in the package lmerTest. The experimental block was used as a random effect to account for in-field variability. Post-hoc multiple comparisons were conducted in the function emmeans in the package emmeans (Lenth et al. 2020).

Measurements of beta diversity, including PERMANOVA were conducted in the R package vegan (Oksanen et al. 2013) and differential abundance of AM virtual taxa was conducted in the DESeq2 package (Love et al. 2014). Detailed statistical methodology, including the use of these packages is described in Chapter Two.

4.3 Results

Spring Barley Shoot Weight in the NFS Trial

Mean barley shoot dry weight was lowest following the unfertilised fallow treatment, at 8.05 ± 0.28g. This was increased to 9.33 ± 0.30g and 9.14 ± 0.28g following the radish and oat, and legume mix cover crops, respectively (Figure 4.6). At the 50% N level, shoot weights were indistinguishable between cover crop regimes. Following the 50% N fallow plots, barley mean shoot weight was 9.99 ± 0.42g. Shoot weights following the radish and oat and legume mix cover crops were 10.1 ± 0.61g and 9.59 ± 0.44g respectively. At the 100% N level, barley following the fallow plots again had the lowest shoot dry weight, at 9.68 ± 0.37g. Like in the unfertilised plots, this was increased by 8.5% following both the radish and oat 10.5 ± 0.26g, and legume mix 10.5 ± 0.34g cover crop. Despite these differences there was no overall effect of cover crop treatment on barley shoot weight (ANOVA: $F_{2,21}$ = 2.28, p = 0.127). There was a strong effect of nitrogen dose on shoot weight (ANOVA: $F_{2,42}$ = 12.47, $p < 0.001$), with significant differences between the 0% vs 50% treatment (Tukey p=0.002) and 0% vs 100% treatment

(Tukey $p < 0.001$). The mean shoot weight between the 50% and 100% treatments was not significantly different (Tukey $p = 0.469$). The interaction term between N dose and cover crop treatment was also not significant (ANOVA: $F_{4,42} = 1.42$, $p = 0.246$). Addition of spring barley RLC as a model covariate did not significantly improve model fit ($\chi^2 = 1.256$, d.f. = 1, $p = 0.262$).

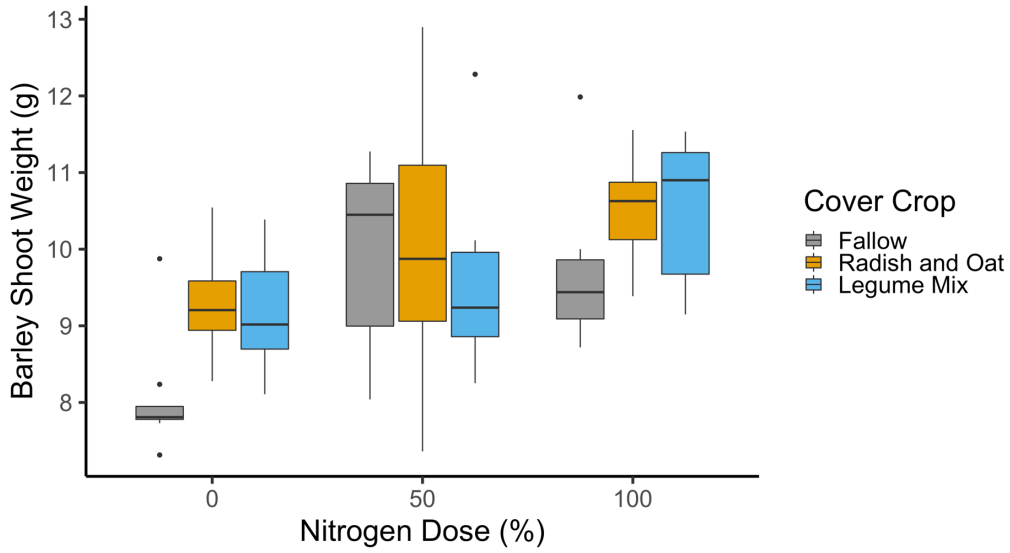


Figure 4.6 Spring barley dry shoot weight at varying nitrogen doses in the NFS trial.
Dry shoot weights represent the green shoot of five spring barley plants, classified as one biological replicate.

Barley Root Length Colonisation

Total RLC varied from 18.7% following the 100% N fallow treatment, to 72.3% observed following the 50% N legume mix treatment (Figure 4.7). The lowest mean RLC of 32.2% was observed following the 0% N fallow

treatment, which was significantly lower than the 0% N legume mix treatment at 47.5% (Tukey $p = 0.005$) when analysing N treatments separately. The radish and oat cover crop treatment was not significantly different from fallow (Tukey $p = 0.327$) or the legume mix (Tukey p=0.166). In the 50% N plots, barley following the fallow treatment had a RLC of 33.4%. This was significantly increased to 45.4% (Tukey $p = 0.033$) following the radish and oat cover crop treatment, and 51.8% (Tukey $p < 0.001$) following the legume mix, which had the highest mean RLC in the trial. This treatment also had the highest individual measurement of barley RLC, at 72.3%. In the 100% N plots, barley RLC following the fallow treatment was 34.1%. Only the legume mix cover crop induced significantly higher barley RLC than the fallow treatment, exhibiting a mean RLC of 45.5% (Tukey $p = 0.045$). The 100% N radish and oat treatment resulted in a barley RLC of 38%, and was not significantly different to the fallow (Tukey $p = 0.697$) or legume mix (Tukey $p = 0.234$) cover crop treatments.

Overall, the cover crop treatment had a significant impact on barley root length colonisation (ANOVA: $F_{2,18} = 9.88$, $p = 0.001$), only the legume mix cover crop was significantly different from the fallow plots (Tukey $p < 0.001$), although the fallow vs radish and oat (Tukey $p = 0.095$), and legume mix vs radish and oat (Tukey $p = 0.094$) approached significance. Neither the nitrogen dose (ANOVA: $F_{2,42} = 2.27$, p =0.116) nor the interaction between cover crop treatment and N dose (ANOVA: $F_{4,42} = 0.67$, $p = 0.614$) was significant.

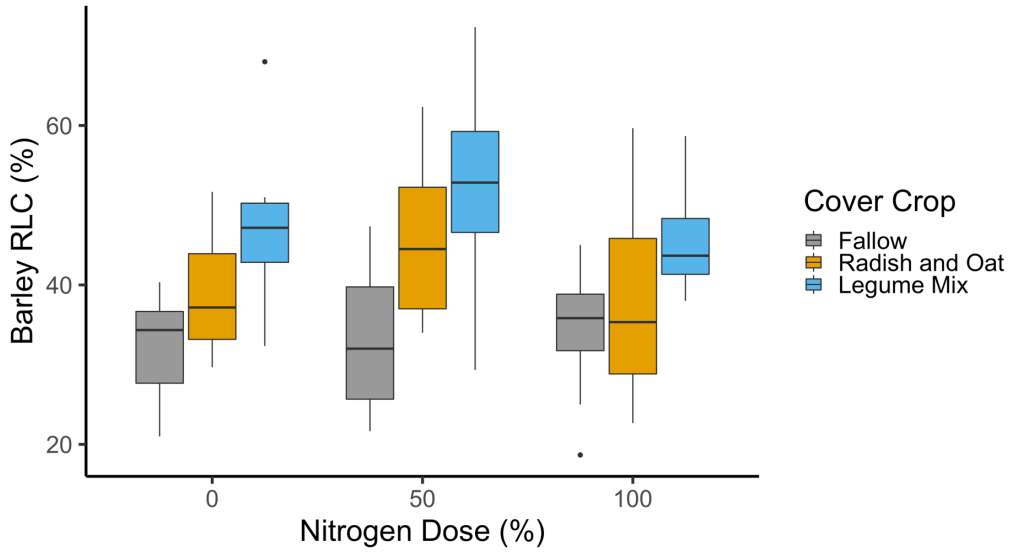


Figure 4.7 Spring barley root length colonisation (RLC) at different N doses, stained using trypan blue.
RLC expressed as a percentage of the total root length colonised, following measurement of an adapted version of the grid intersect method.

Spring Barley Yield in the NFS Trial

Spring barley yield ranged from 2.92 t/ha following the 0% N radish and oat treatment, to 6.96 t/ha following the 100% N fallow treatment (Figure 4.8). Nitrogen application had the most profound impact on yield, increasing the mean of the 0% N plots from 3.58 ± 0.09 t/ha to 5.70 ± 0.10 t/ha in the 50% N plots, and 6.09 ± 0.10 t/ha in the 100% N plots.

Mean yield following the unfertilised fallow control was 3.54 ± 0.12 t/ha, which was increased by the leguminous cover crop (4.02 ± 0.11 t/ha) and decreased by the radish and oat cover crop (3.19 ± 0.09 t/ha). There was a marked increase in yield between the 0% and 50% N treatments (Tukey p

< 0.001), with yield following fallow achieving 6.05 ± 0.23 t/ha. This was reduced in barley following both the radish and oat (5.33 ± 0.11 t/ha), and legume mix (5.73 ± 0.07 t/ha) cover crops. There were more modest increases between the 50% and 100% N doses (Tukey $p = 0.005$), where again, the radish and oat (5.87 ± 0.14 t/ha) and legume mix (5.86 ± 0.12 t/ha) cover crop treatments achieved lower yields than the fallow (6.54 ± 0.15 t/ha).

After mean centring data to account for bimodality, there was significant effect of N regime (ANOVA: $F_{2,63} = 47.18$, $p < 0.001$), the preceding cover crop (ANOVA: $F_{2,63} = 17.40$, $p < 0.001$), and their interaction (ANOVA: $F_{4,63} = 9.51$, $p < 0.001$) on spring barley yield. Barley yield following a period of fallow was significantly higher than when following radish and oat (Tukey $p < 0.001$) and legume mix (Tukey $p < 0.001$) cover crops, ignoring the effect of N regime (Tukey $p = 0.140$). Addition of spring barley RLC as a model covariate did not significantly improve model fit ($\chi^2 = 0$, d.f. = 1, $p = 0.996$).

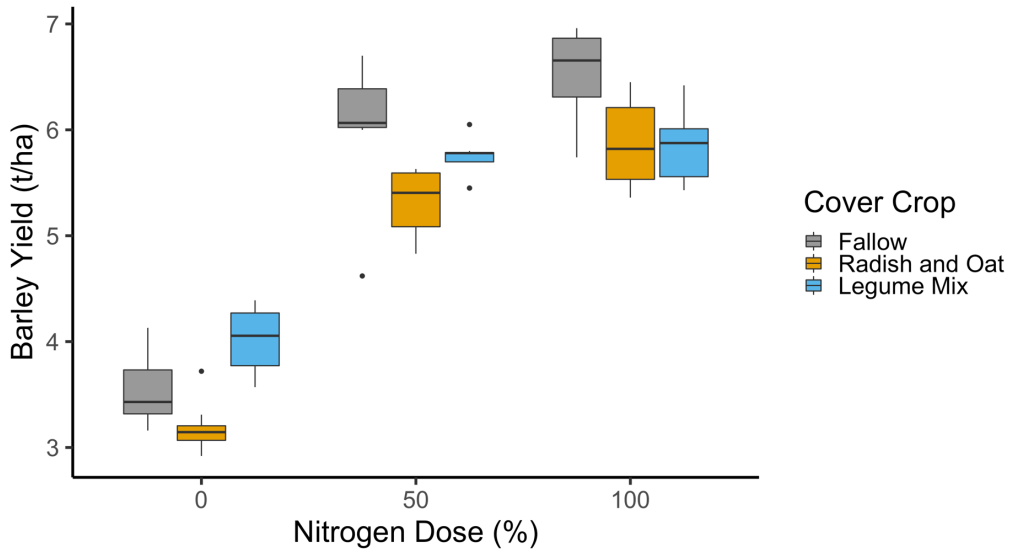


Figure 4.8 Spring barley yield at varying N doses, following each cover crop regime in the NFS trial.
Barley yield expressed in tonnes per hectare, following standardisation to 15% moisture content

Historical Yield Data at the NFS Trial

To demonstrate how cover crop treatments impacted crop yields across other crops and seasons, all historic yield data for the NFS Fertility Building Rotations trial is presented in Figure 4.9. Spring barley was first drilled in 2013, experiencing very slight yield increases due to cover cropping in the 0% and 50% N regime, and no effect following the 100% N dose. The next occurrence of this crop was in 2018 (Figure 4.10), for which this data is described in detail above. Spring barley was grown for a third time in 2020. Cover cropping, and especially the radish and oat cover crop mixture, increased spring barley yield at all three nitrogen regimes, increasing yield by 0.5 t/ha in the 0% N treatment.

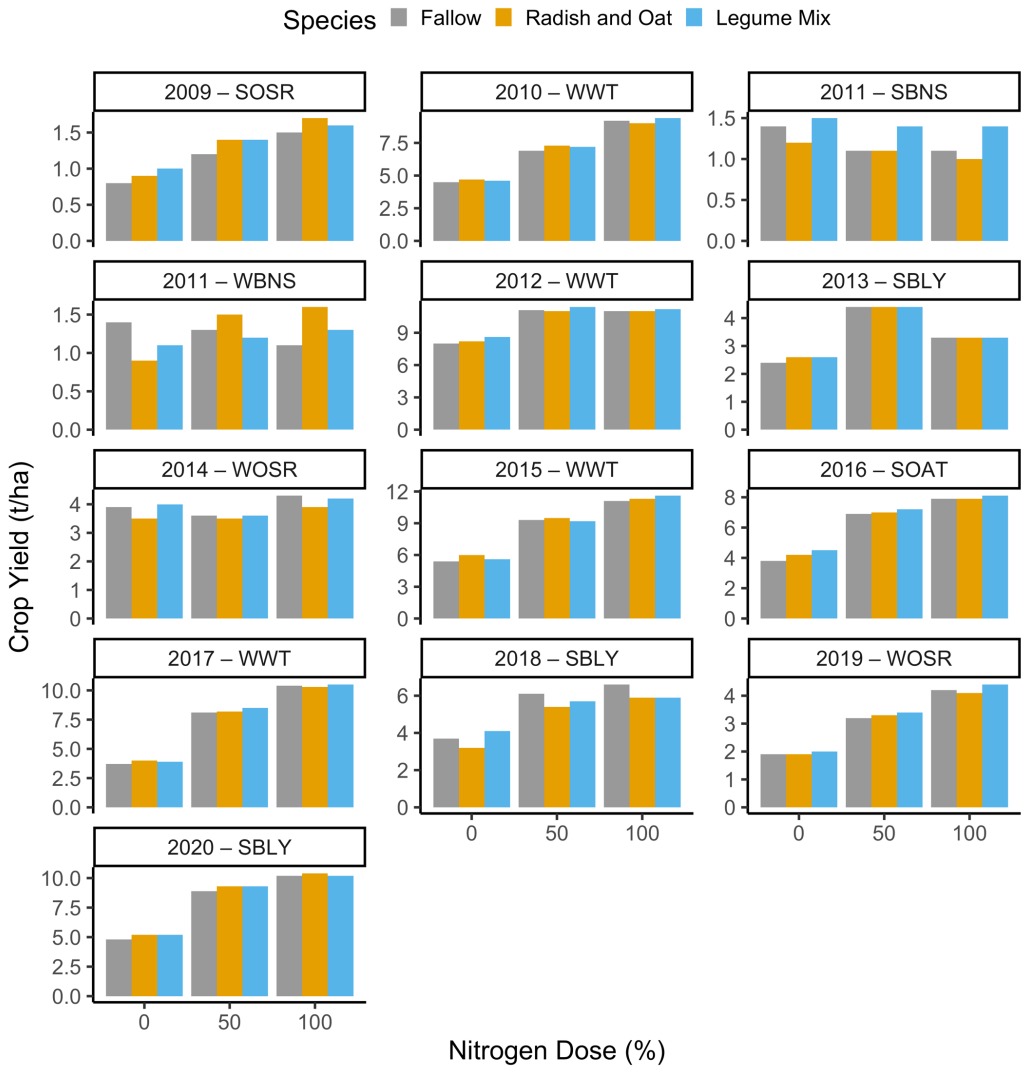


Figure 4.9 Crop yields from other experimental years in the NFS Fertility Building Rotations Trial.

Crop yield is expressed in t/ha on variable axes. Crop species include SOSR (spring oilseed rape), WWT (winter wheat), SBNS (spring beans), WBNS (winter beans), SBLY (spring barley), WOSR (winter oilseed rape), and SOAT (spring oat). The spring break and mixed cropping approaches varied in 2011 only, where the spring crop was spring beans and the mixed cropping approach cultivated winter beans.

In other experimental years and crops, cover crops exhibited a range of effects on crop yields. In the 2009 spring oilseed rape crop, both radish and oat, and the legume mix cover crops increased yield compared to the fallow control, in all three N regimes. This trend for small positive yield increases was seen to a lesser extent in all four years of winter wheat (2010, 2012, 2015, 2017), as well as the 2016 spring oat, 2019 oilseed rape, and 2020 spring barley. In the 2011 spring beans, growth of a radish and oat cover crop prior to drilling reduced bean yield. However, drilling of the mixed legume cover crop increased yields in all N regimes, compared to the fallow control. In the winter beans of the same year, both cover crops treatments reduced yield in the 0% N regime, and increased yields in the 100% N regime. At the 50% N level, radish and oat exhibited a yield increase whereas the legume mix exhibited a small yield reduction. In the 2014 winter oilseed rape, the fallow and legume mix cover crop treatments yielded similarly across N regimes, but was reduced in each case from growth of a radish and oat cover crop.

Figure 4.10 Adjacent plots of spring and winter barley in June 2018 at the NFS trial.

AM Fungal Diversity at the NFS Trial

After stringent quality thresholding, 268,452 Glomeromycotinian DNA sequences remained for further analysis. In total, 27 Virtual Taxa were identified from across the trial, including one *Acaulospora*, one *Ambispora*, two *Archaeospora*, three *Diversispora*, 14 *Glomus*, five *Paraglomus*, and one *Scutellospora*.

The top 10 most abundant taxa across the trial were VTX00281 (45.4% - *Paraglomus laccatum),* VTX00283 (20.5% - *Ambispora fennica)*, VTX00245 (13.2% - *Archaeospora trappei*), VTX00065 (6.4% - *Glomus caledonium*), VTX00108 (6% - *Glomus whitfield type 7*), VTX00342 (3.9% - *Glomus*

VeGlo18), VTX00030 (2% - *Acaulospora Acau2*), VTX00199 (0.6% - *Glomus MO-G7*), VTX00114 (0.6% - *Glomus MO-G17*), and VTX00064 (0.3% - *Glomus MO-G18*), and shown in Figure 4.11.

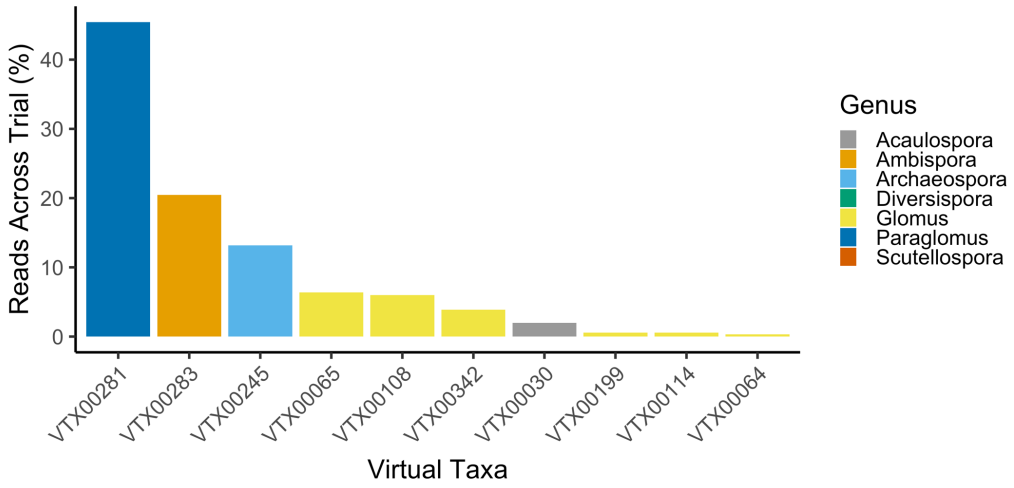


Figure 4.11 Top 10 most abundant AM fungal virtual taxa in the NFS trial. Abundance of the top 10 AM fungal VT, expressed as a percentage of the number of DNA reads across the trial. Colours represent the genus of taxa.

Multivariate Ordination of AM Fungal Community Composition in the NFS Trial

Bray-Curtis dissimilarity distances were calculated, and the multivariate homogeneity of groups dispersions were calculated for both the cover crop and nitrogen dose treatments. After 1000 permutations, neither the cover crop ($F_{2,67} = 0.81$, $p = 0.447$) nor nitrogen dose ($F_{2,67} = 1.37$, $p = 0.255$) treatments exhibited significantly different dispersions.

Bray-Curtis distances were plotted by NMDS and PCoA methods, to visualise clustering of communities (Figure 4.12). Multivariate Analysis of variance by PERMANOVA found that nitrogen dose (PERMANOVA: $R^2 = 0.065$, $F_{2,65} = 2.33$, $p = 0.013$) but not the cover crop treatment (PERMANOVA: $R^2 = 0.030$, $F_{2,65} = 1.06$, $p = 0.407$) resulted in significant partitioning of sums of squares from the Bray-Curtis distances. Subsequent pairwise multilevel comparisons of the nitrogen dose treatments showed significant differences in community distance between the 0% N and 100% N treatments (PERMANOVA: $R^2 = 0.069$, $F = 3.24$, $p = 0.033$). Distances between the 50% and 100% N treatment communities approached a significant difference (PERMANOVA: $R^2 = 0.060$, $F = 2.80$, $p = 0.084$), and there was no difference between the 0% N and 50% N treatment communities (PERMANOVA: $R^2 = 0.025$, $F = 1.20$, $p = 0.909$), suggesting that these communities are more similar to the 100% N treatment.

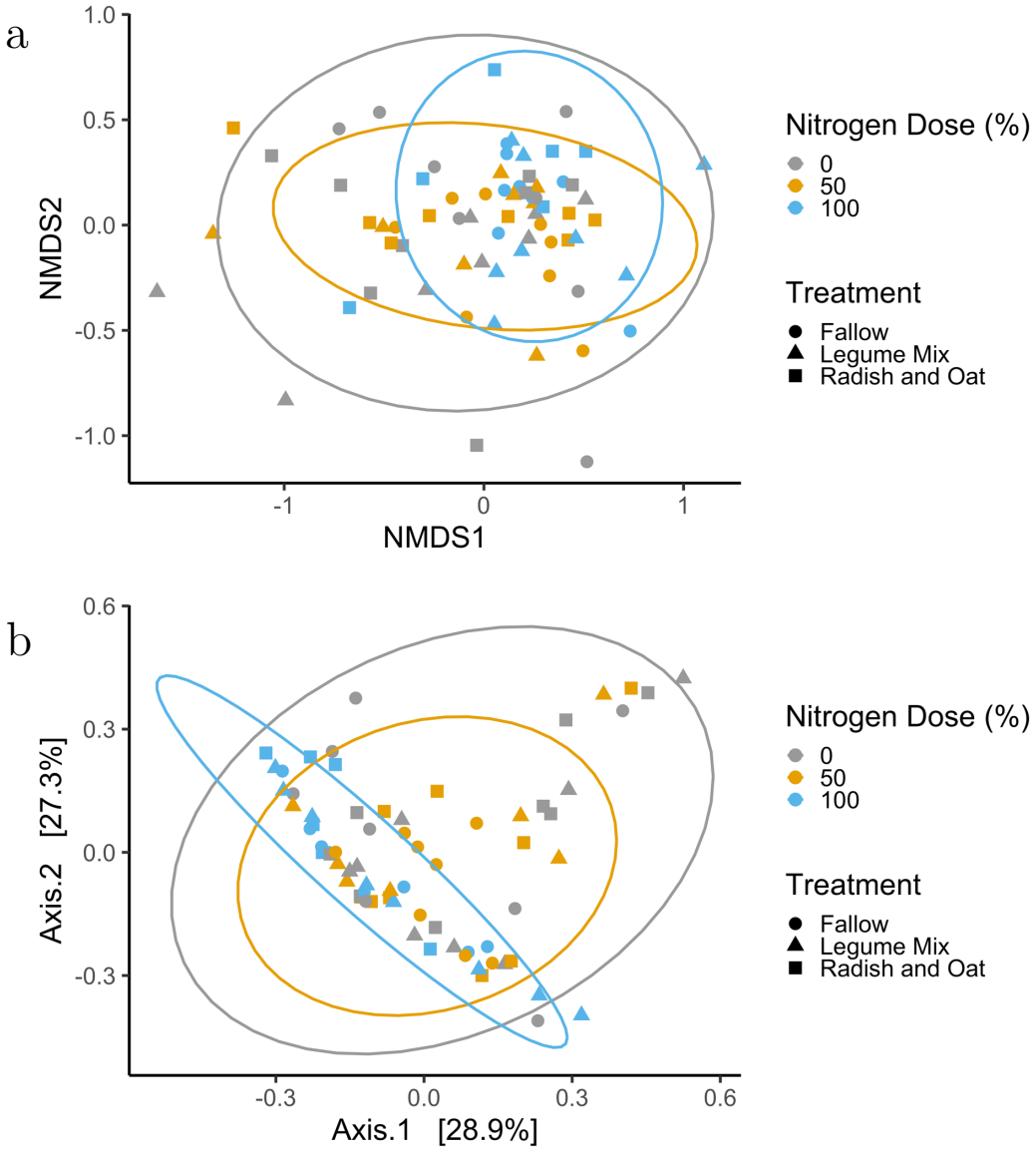


Figure 4.12 a) NMDS ordination of Bray-Curtis distances for the NFS trial b) Principal coordinate analysis (PCoA) ordination of Bray-Curtis distances for the NFS trial.

Non-metric Multi-dimensional Scaling (NMDS) plot of Bray-Curtis dissimilarities from the NFS trial. Multivariate Analysis of variance by PERMANOVA found that nitrogen dose (PERMANOVA: $R^2 = 0.065$, $F_{2,65} = 2.33$, $p = 0.013$) but not the cover crop treatment (PERMANOVA: $R^2 = 0.030$, $F_{2,65} = 1.06$, $p = 0.407$) resulted in significant partitioning.

Observed Number of AM Fungal Taxa

Mean observed AM virtual taxa across the trial was 6.3 ± 0.2. The minimum number of taxa in a single plot was three, observed following the 0% N radish and oat treatment, and the 0% N legume treatment. The greatest number of observed taxa in a single plot was 13, and was observed following the 0% and 50% N legume mix treatments (Figure 4.13). Overall, there was no significant effect of cover crop (ANOVA: $F_{2,18} = 1.13$, $p = 0.127$), nitrogen treatment (ANOVA: $F_{2,40} = 2.65$, $p = 0.08$) or their interaction (ANOVA: $F_{4,40} = 1.81$, $p = 0.146$) on the observed number of AM fungal taxa. Addition of AM diversity as a covariate did not improve model fit of spring barley shoot biomass ($\chi^2 = 0.079$, d.f. = 1, $p = 0.779$), or yield ($\chi^2 = 0.065$, d.f. = 1, $p = 0.798$).

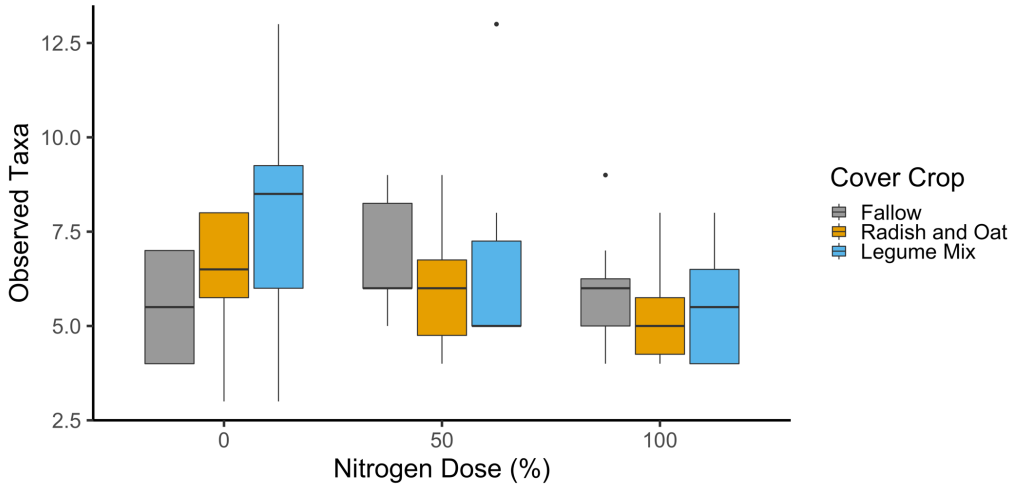


Figure 4.13 Observed AM fungal virtual taxa in the NFS trial.
AM fungal VT observed in each experimental treatment. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Fifteen taxa including seven *Glomus*, four *Paraglomus*, one *Ambispora*, one, *Acaulospora*, one *Archaeospora*, and one *Scutellospora* were observed in at least one plot of each of the three N treatments (Table 4.3). Four taxa, all from the genus *Glomus* were observed exclusively in 0% N plots, with one further taxon belonging to the *Acaulospora* being observed in both the 0% and 100% N treatments. One *Diversispora* taxon was found exclusively in the 50% N treatment, with a further five taxa being observed between the 0% and 50% treatments, including two further *Diversispora*, two *Glomus*, and one *Paraglomus*. One final taxon, belonging to the *Diversispora* was found in both 50% N and 100% N plots, with zero distinct species being identified in 100% N plots.

Table 4.3 AM Fungal VT occurring in each nitrogen regime in the NFS trial

All	0% N	50% N	100% N	0% N & 50% N	0% N & 100% N	50% N & 100% N
VTX00435	VTX00153	VTX00060		VTX00061	VTX00338	VTX00354
VTX00444	VTX00186			VTX00349		
VTX00065	VTX00163			VTX00307		
VTX00199	VTX00063			VTX00222		
VTX00283				VTX00380		
VTX00030						
VTX00114						
VTX00281						
VTX00108						
VTX00342						
VTX00245						
VTX00125						
VTX00064						
VTX00052						
VTX00239						

Segmenting the observed taxa by cover crop treatment (Table 4.4), 13 taxa were observed in at least one plot following each of the three cover crop treatments, including five *Glomus*, four *Paraglomus*, one *Ambispora*, one *Acaulospora*, one *Archaeospora*, and one *Diversispora*. Two *Glomus* and one *Diversispora* were found exclusively following the fallow plots, and five taxa, including four *Glomus* and one *Diversispora* were observed exclusively in the plots following the legume mix cover crop. There were zero AM fungal taxa

found in only the plots following the radish and oat cover crop. Two taxa, one belonging to the genus *Glomus*, and the other *Paraglomus*, were found following both the legume mix, and radish and oat cover crop mixtures. A further three taxa were found following both the legume mix and fallow plots, one *Glomus*, one *Diversispora*, and one *Scutellospora*. One further *Archaeospora* taxon, was found following at least one fallow and radish and oat cover crop plot. Observed numbers of AM fungal taxa are visualised in Figure 4.14.

Table 4.4 AM Fungal VT occurring following each cover crop mixture in the NFS trial

All	Fallow	Legume Mix	Radish and Oat	Fallow & Legume	Fallow & Radish	Legume & Radish
VTX00435	VTX00222	VTX00153		VTX00199	VTX00338	VTX00163
VTX00444	VTX00125	VTX00186		VTX00354		VTX00349
VTX00065	VTX00060	VTX00307		VTX00052		
VTX00283		VTX00063				
VTX00061		VTX00380				
VTX00030						
VTX00114						
VTX00108						
VTX00281						
VTX00342						
VTX00245						
VTX00064						
VTX00239						

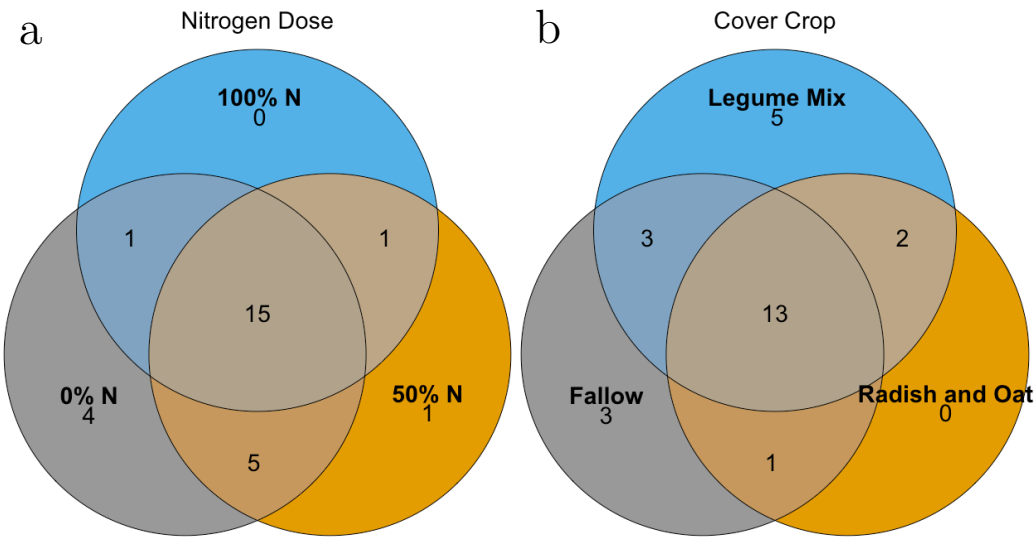


Figure 4.14 Number of AM fungal virtual observed in the a) nitrogen treatment and b) cover crop treatments.
Venn classification of AM fungal VT observed in each experimental treatment. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Inverse Simpson's Diversity for Community Evenness

The Inverse Simpson's Index as a measure of community evenness can be considered as a measure of the effective number of taxa in a community, after considering the relative abundance of each taxon. The mean Inverse Simpson's diversity across the trial was 2.37 ± 0.09, ranging from 1.07 following the 100% N radish and oat treatment, to 4.09 following the 0% N legume mix treatment, although this value was greater than 1.5 times the interquartile range of this treatment and considered an outlier (Figure 4.15).

There was a significant effect of nitrogen dose on the Inverse Simpson's Index (ANOVA: $F_{2,61} = 6.36$, $p = 0.003$). Neither the cover crop (ANOVA: $F_{2,61} = 0.38$, $p = 0.684$), nor the interaction between the cover crop and nitrogen dose (ANOVA: $F_{4,61} = 1.51$, $p = 0.210$) were significant. Mean Inverse Simpson's index for the 100% N plots was 1.99 ± 0.18, which was significantly lower than the 50% N plots at 2.68 ± 0.12 (Tukey $p = 0.003$). The mean Inverse Simpson's Index for the 0% N plots was 2.41 ± 0.15, which was not significantly different to either the 50% N (Tukey $p = 0.354$) or 100% N (Tukey $p = 0.085$) treatments.

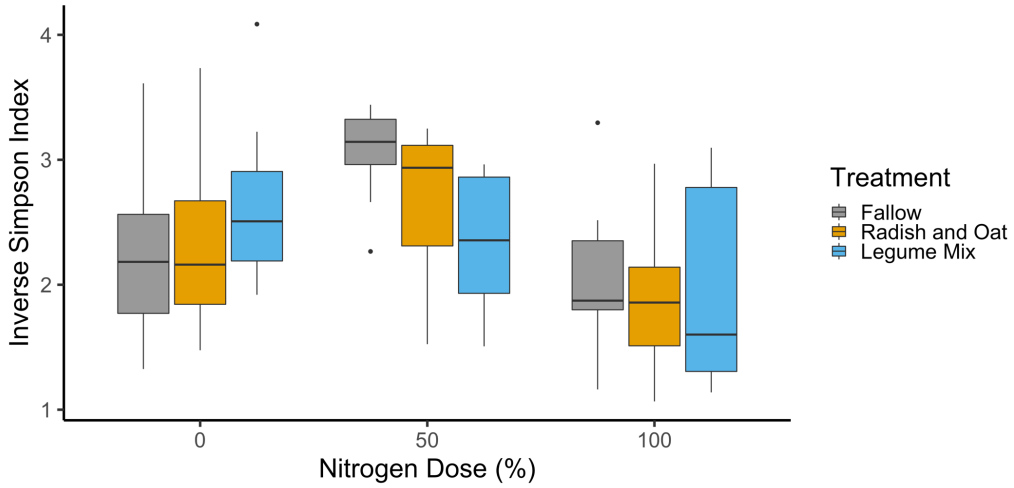


Figure 4.15 Inverse Simpson's Index for AM fungal evenness.
Inverse Simpson's index of AM fungal communities observed in each experimental treatment. Inverse Simpson's index represents the 'effective' diversity and is bounded by 1 and the number of observed VT per treatment.

AM Fungal Community Composition and Analysis of Differential Abundance

AM fungal community composition in the 0% nitrogen plots consisted of 40.7% *Paraglomus*, 26.7% *Glomus*, 22% *Ambispora*, 6.8% *Archaeospora*, 3.9% *Acaulospora*, 0.05% *Scutellospora*, and 0.003% *Diversispora* (Figure 4.16). In the 50% N treatment, genus composition was 40.8% (+0.1% compared to 0% N) *Paraglomus*, 22.6% (-4.1%) *Glomus*, 19.9% (-2.1%) *Ambispora*, 16.1% (+9.3%) *Archaeospora*, 1.1% (-2.8%) *Acaulospora*, 0.02% (-0.03%) *Scutellospora*, and 0.02% (+0.017%) *Diversispora.* In the 100% N treatment, composition shifted to 58.1% (+17.4% compared to 0%) *Paraglomus*, 4.9% (-21.8%) *Glomus*, 19.1% (-2.9%) *Ambispora*, 17% (+10.2%) *Archaeospora*, 0.8% (-0.3%) *Acaulospora*, 0.01% (-0.01%) *Scutellospora*, and 0.06% (+0.04%) *Diversispora.*

The five most abundant taxa in the 0% N treatment were VTX00281 (40.3% - *Paraglomus laccatum*), VTX00283 (22% - *Ambispora fennica*), VTX00108 (11.4% - *Glomus Whitfield type 7*), VTX00065 (8.2% - *Glomus caledonium*), and VTX00245 (6.2% - *Archaeospora trappei*). In the 50% N treatment, the five most abundant taxa only changed in their abundances. VTX00281 (*Paraglomus laccatum*) represented 40.2% of sequences, VTX00283 (*Ambispora fennica)* 19.5%, VTX00108 (*Glomus Whitfield type 7)* 6.1%, VTX00065 (*Glomus caledonium)* 9.5%, and VTX00245 (*Archaeospora trappei)* 16.1%. The 100% N treatment was dominated by three virtual taxa, VTX00281 (*Paraglomus laccatum*), VTX00283 (*Ambispora fennica)*, and

VTX00245 (*Archaeospora trappei)*, which consisted of 56.8%, 19.9% and 17.5% of the reads respectively. VTX00342 (*Glomus VeGlo18)* and VTX00114 (*Glomus MO-G17*) contributed a further 1.9% and 1.2% of reads.

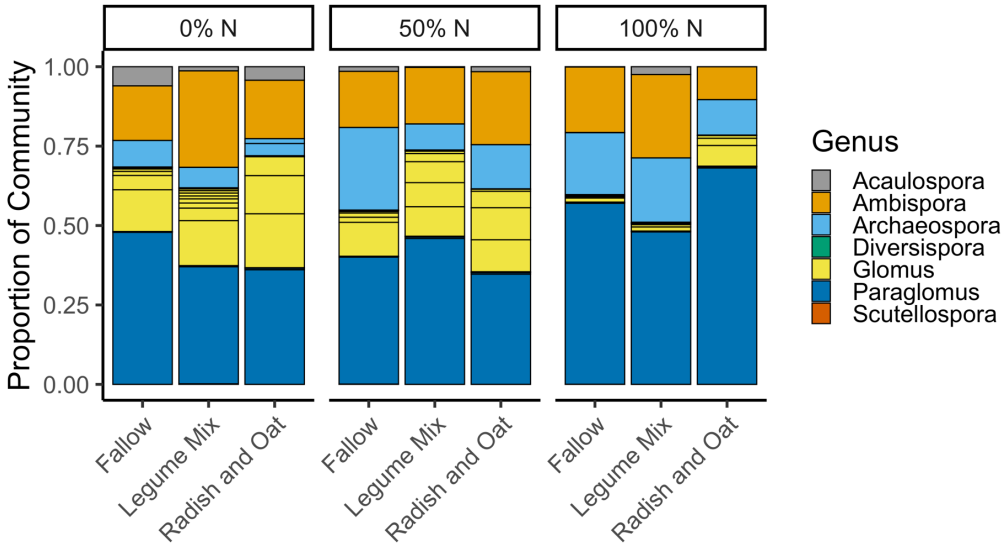


Figure 4.16 AM Fungal community composition at the NFS trial.
Mean community composition of AM fungal VT for each experimental treatment, expressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT.

Analysis of differential abundance of virtual taxa, using shrinkage estimation was conducted to further confirm how individual taxa had responded to experimental treatments. There were zero differentially abundant species between the 0% N and 50% N treatments at the adjusted $p < 0.05$ level. Three virtual taxa were differentially abundant between the 0% and 100% treatments (Figure 4.17a). VTX00108 (*Glomus Whitfield type 7)* was significantly less abundant in the 100% treatment plots ($\log_2$FC = 9.18, $p = 0.006$), as was VTX00030 (*Acaulospora Acau2,* $\log_2$FC = 4.79, $p = 0.007$).

One AM VT, VTX00245 (*Archaeospora trappei*) was significantly more abundant in the 100% N plots, when compared to the 0% N treatment ($\log_2$FC = 1.55, $p = 0.041$)

Two virtual taxa were differentially expressed when comparing the 50% and 100% N treatments (Figure 4.17b). Two taxa were significantly less abundant in the 100% N plots, including VTX00108 (*Glomus Whitfield type 7,* $\log_2$FC = 8.20, $p = 0.026$), and VTX00030 (*Acaulospora Acau2,* $\log_2$FC = 4.13, $p = 0.036$).

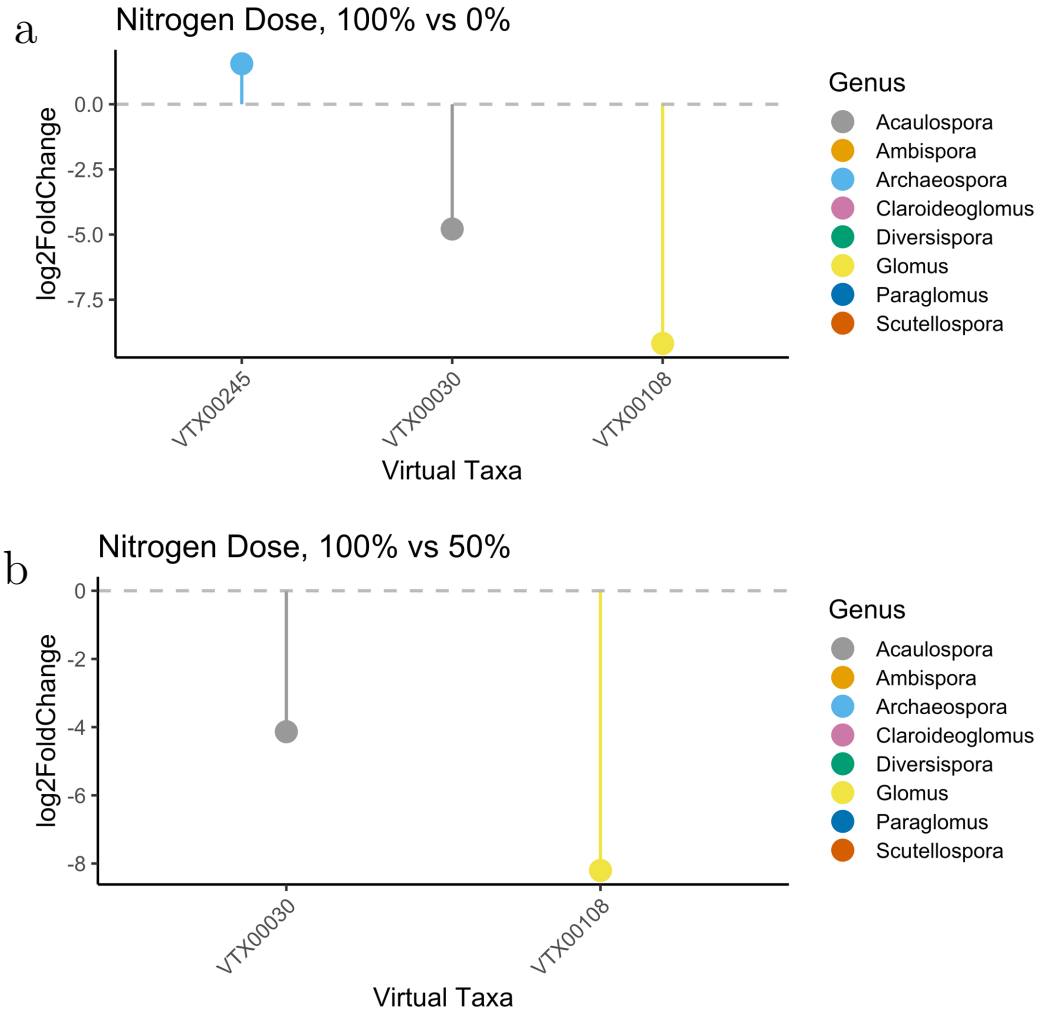


Figure 4.17 Differential abundance of AM Fungal VT by nitrogen dose comparing a) 100% vs 0% N treatments and b) 100% vs 50% treatments.
Differential abundance of AM fungal VT comparing levels of nitrogen application, calculated by DESeq2. AM VT were considered to be differentially abundant if their $\text{Log}_2\text{FC} > 1$ and $p < 0.05$.

Considering the impact of the cover crop treatment on AM fungal diversity, only the radish and oat cover crop significantly increased the abundance of one virtual taxon, VTX00342 (*Glomus VeGlo18,* $\text{Log}_2\text{FC} = 11.69$, $p < 0.001$), when compared to the fallow treatment (Figure 4.18). There were no other differentially abundant AM VT between any of the other cover crop comparisons.

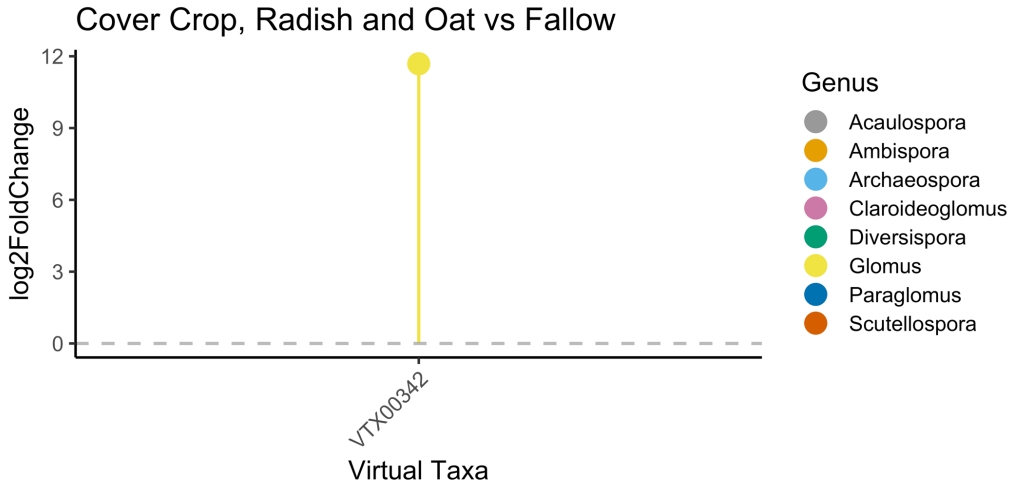


Figure 4.18 Differential abundance of AM Fungal VT by cover crop treatment. Differential abundance of AM fungal VT comparing differential abundance of taxa in the radish and oat vs fallow cover crop treatments. Differential abundances were calculated with DESeq2. AM VT were considered to be differentially abundant if their $Log_2FC > 1$ and $p < 0.05$.

4.4 Discussion

In this chapter, the long-running New Farming Systems Fertility Building Rotations trial was used as an experimental platform to address a number of experimental hypotheses. The trial is based in Morley, Norfolk on a sandy clay loam soil and has been running since 2008, with the inclusion of five cover crops in the spring break rotation with winter wheat, spring oilseed rape, spring beans, spring barley, winter oilseed rape, and a spring oat.

Crop establishment for the 2018 spring barley was good, although growth through the coming months was postulated to be impacted by a very wet spring, followed by a hot and dry summer. Many other farmers in the East of England experienced lower than average yields because of these weather

extremes. There was an unusual and unexpected interaction between nitrogen amendment and cover crop, at the resulting impact on barley shoot biomass. At the 0% and 100% N regime, both the radish and oat, and legume mix cover crops increased barley shoot biomass. This trend was not observed in the 50% dose, which may be as a result of nitrogen lock up due to the application of green manure (Pinck et al. 1948). This is well demonstrated in the literature, and will be an important consideration for farmers adopting cover crops or long term leys as part of the Government's Sustainable Farming Incentive (DEFRA 2021).

Yield of the spring barley benefitted from succeeding a leguminous cover crop at the 0% N level, however at the same N addition, the radish and oat cover crop was detrimental to yield. Neither of the cover crop mixtures at either the 50% or 100% N level were beneficial to yield in this year, suggesting that any benefit from the cover crop is less profound than the benefit from increasing N supply. Indeed, it is likely that the factor contributing to the increased yield following the 0% N legume mix, is the increase in soil N as a result from N fixation by rhizobacteria, hosted in leguminous root nodules, as opposed to other benefits of cover crops, such as improved soil structure and rooting. This result may in part be due to a particularly hot and dry growing season, and assessing spring barley yields from the same trial, in other cropping years, suggests that cover cropping can be effective at promoting moderate increases in yield. Similarly, small yield increases were observed in other crops, such as spring oilseed rape, winter wheat, and spring oat. Cover cropping had a more variable impact on the 2011 spring and

winter beans. Spring beans benefitted from following a leguminous cover crop at all N regimes. Winter beans exhibited a yield decline following either cover crop treatment in the 0% N regime, a yield increase following either cover crop treatment in the 100% N regime, and a yield increase following radish and oat, and decrease following the legume mix in the 50% N treatment. The mechanism for this result is uncertain, but may be attributable to: interplay between rhizobial N fixation and N fertilisation; cover crop biomass impacting crop drilling and establishment; or different tolerances of these species to climatic conditions.

There was a striking impact of the preceding cover crop on spring barley RLC, as quantified by trypan blue staining and the adapted grid intersect method. At all nitrogen doses, both of the cover crops, but especially the leguminous cover crop mixture, increased barley RLC, which was also observed by Bowles et al. (2017). This effect was more pronounced in the 0% and 50% N treatments, with reduced prominence in the 100% N treatment plots. This suggests that local availability of N, as well as P may impact recruitment of AM fungal symbionts at the field scale. This is in agreement with findings from Treseder (2004), who found that across a number of studies, N fertilisation reduced AM fungal abundance (including RLC, spore counts, and measurements of hyphal length) by 15%, and RLC alone by 5.8%. Treseder also notes that higher N doses had a greater impact on AM fungal abundance, which is in line with the results from this study.

In contrast, analysis of AM fungal community composition by PERMANOVA found that the AM fungal community varied by N dose, but not the preceding cover crop. Subsequent pairwise multilevel comparisons found significant differences in community composition between the 0% and 100% N doses, and further evidence for differences in community composition between the 50% and 100% N doses. This shows that the diversity and abundance of AM fungi are able to be influenced independently of one another.

Overall, there was no significant difference between the observed number of taxa in a treatment, although the impact of N addition approached the $p < 0.05$ threshold for significance. At the 0% N treatment level, both cover crop treatments increased the observed number of taxa. Observed numbers of taxa was decreased in the 50% and 100% N doses, into the range of the fallow control in the 0% N plots. Similarly, Egerton-Warburton et al. (2007) showed that N fertilisation of perennial grasslands reduced AM fungal richness, in soils abundant in P. This trend was reversed in low P sites. In this trial, there is some evidence that the interplay between the cover crop and nitrogen amendments can influence the total number of AM fungal symbionts a plant will engage with. This may have repercussions for N uptake and cycling at the plant and field scale, and warrants further experimentation.

In total, 27 AM fungal taxa were identified in the trial. Of these, 15 were found in all levels of N amendment, four *Glomus* species were found in the 0% N plots only, one *Archaeospora* was found in the 50% N plots only, and a further five, including *Glomus*, *Diversispora*, and *Paraglomus* species were

found in the 0% and 50%, but not 100% N plots. There were zero taxa found exclusively in the 100% N plots. Abundance of *Ambispora* and *Archaeospora* remained fairly constant between levels of nitrogen amendments. At the 100% N level, abundance of *Glomus* species was reduced from 20-25%, to 5% of total DNA reads, suggesting that this genus is sensitive to addition of N fertiliser, both in terms of abundance and diversity. VTX00108 (*Glomus Whitfield type 7)* was especially sensitive to N addition, decreasing in abundance at both the 50% and 100% N treatments, when compared to the 0% N level. In addition, VTX00030 (*Acaulospora Acau2)* was significantly less abundant in the 50% and 100% N levels. This is in direct contrast to Egerton-Warburton et al. (2007), who found that N fertilisation of high P grasslands increased the abundance of *Glomus* species at the expense of other rare taxa. At a tropical montane rainforest in southern Ecuador, Camenzind et al. (2014) showed a similar trend, that additions of N fertiliser had a negative impact on the abundance of Diversporales (*Acaulospora, Diversispora, Scutellospora),* whereas Glomerales (*Glomus, Claroideoglomus*) were solely affected by additions of P. This trial is in concurrence with Camenzind, in that abundance of one *Acaulospora,* VTX00030 (*Acaulospora Acau2*) was negatively impacted by N fertilisation. However, this trial suggests that the abundance of *Glomus* species, including VTX00108 (*Glomus Whitfield type 7)* and five additional *Glomus* found exclusively in the 0% N treatment plots, are strongly impacted by N fertilisation. Nitrogen fertilisation has been shown to impact rare taxa, however VTX00108 represented over 10% of sequence reads in the 0% N treatment, and was the third most abundant taxon in this treatment overall.

In place of the reduced *Glomus and Acaulospora* reads, abundance of *Paraglomus* increased at the 100% level. There is only limited research on the life history of *Paraglomus* species, and even less on VTX00281 (*Paraglomus laccatum*), however, the taxon is associated with being a generalist, similar to *Rhizophagus irregularis* or *Glomus mosseae* (Berruti et al. 2016). It is possible that this taxon was able to fill the abandoned niche left by those AM taxa which were not suited to habituating a high N environment.

Interestingly, Lumini et al. (2014) showed that VTX00281 (*Paraglomus laccatum*) was significantly less abundant in experimentally induced drought conditions in a grassland field trial. Although not directly measured, it is likely that the cover crops grown in high N plots would have greater biomass, resulting in higher levels of respiration and therefore translocation, subsequently drying the subsoil to a greater extent. If this hypothesis were true, it would be expected that that abundance of *Paraglomus* species would be reduced in the high N plots, yet the opposite is observed in this trial. Although still not addressing why there was such a drastic reduction in *Glomus* species at the 100% N level, it seems likely that the niche-filling hypothesis is the more robust of the two, to explain the increase in *Paraglomus* at high levels of N.

In addition to the increased abundance of *Paraglomus* species, VTX00245 (*Archaeospora trappei)* was significantly more abundant in the 100% N plots, when compared to the 0% N treatment. From the same study, Lumini et al. (2014) found that following a drought event, abundance of *Archaeospora*

trappei was significantly higher than in the control. This would provide some evidence for the possibility that the larger cover crops in the fertilised plots had produced drier soil conditions. More generally, taxa from the Archaeosporaceae, including *Archaeospora trappei* have been shown to be indicators of disturbed habitats, of which the 100% N treatment would have been most severe (Moora et al. 2014). Interestingly, *Archaeospora trappei* was demonstrated to promote growth of *Plantago lancelota* in controlled conditions, by improving herbivory tolerance from the specialist lepidopteran herbivore, *Junonia coenia.* It is tempting to speculate that when nutrient resources are not limiting, plants may preferentially engage with AM fungal taxa which provide benefits unrelated to nutrient uptake, such as increased resistance from pests and disease.

AM fungal communities were most even at the 50% N level, decreasing at both the 0% and 100% N levels. This suggests that at this level, the availability of N is not a driving force for dominance by one or a few AM fungal taxa. At the 0% N level, it is possible that colonisation is dominated by AM fungal taxa adapted to N uptake, and at the 100% level, colonisation is dominated by AM taxa that are adapted to proving the plant with the next most limiting resource, for example water or another nutrient. The sandy clay loam at the Morley site is abundant in P, the other main nutrient commonly associated with uptake via the symbiotic path, but this does not exclude the possibility that other macro- or micronutrients may be in limited supply in the rhizosphere. The other hypothesis explaining the reduced number of AM fungal taxa at the 100% N level, is that the 17 taxa found in the

100% N plots are adapted to high levels of N, and may have a minor role in nutrient uptake, at least in terms of N. Of these 17, there was representation from *Paraglomus*, *Glomus*, *Ambispora*, *Acaulospora*, *Archaeospora*, *Scutellospora*, and *Diversispora.* Of course, it is possible that functional traits could span beyond the phylogenetic relationships between AM fungal genera, but this is founded purely on speculation and would require considerable experimental validation.

4.5 Conclusion

In this chapter, the New Farming Systems Fertility Building Rotations trial, provides the test site to explore the impact of cover crops under varying nitrogen regimes. In their fifth iteration, the five species leguminous cover crop was able to increase crop yields when no additional N fertiliser was applied. Cover crops grown at 50% or 100% of the standard N rate, as well as the radish and oat cover crop in the 0% N treatment experienced small yield declines. High throughput sequencing of spring barley roots identified 27 AM fungal VT, with VTX00281 (*Paraglomus laccatum*) accounting for almost half of sequence reads. The nitrogen regime had a significant impact on AM fungal communities, with the 100% N dose plots being predominated by VTX00281 (*Paraglomus laccatum*), at the expense of *Glomus* and *Acaulospora* species. Despite having no impact on community composition, the preceding cover crop increased RLC of the following spring barley, at all N regimes, with the legume mix cover crop having the most prominent effect.

The New Farming Systems Trials are funded by The Morley Agricultural Foundation (TMAF) and The JC Mann Trust, and are an invaluable resource for a range of farmer relevant, experimental studies.

5 Chapter Five – Use of Cover Crops, and Soil Amendments on the Farm Scale

5.1 Introduction

Previous chapters have shown the potential of cover crops, and other amendments to alter soil conditions, including the abundance and diversity of AM fungi. It is, however, important to validate these findings on the farm scale, and test whether they are viable as part of a wider farming system. This includes factors such as cost, time constraints, disease pressure, soil-type variation, and the impact of local weather.

To test findings at the farm scale, a collaboration with six farmers was established, as part of an Innovative Farmers Field Lab. Innovative Farmers was set up in 2015 with the aim of promoting robust farmer led research, which could be easily shared and disseminated, and not lost in specialist academic journals. Research is guided by academics, who assist with experimental design, and may provide access to more complex analyses. Farmer groups, or 'Field Labs' are groups of farmers working on a specific problem, tool, or technique, and meet every few months to discuss how the project is progressing, and issues that have been encountered. The Innovative Farmers Field Lab scheme is co-ordinated by the Soil Association with financial inputs from several sources, including the AHDB and Duchy Estates.

This chapter focusses on results from one Field Lab project entitled 'Increasing Nutrient Efficiency from Anaerobic Digestate', which was set up to investigate the impact of using a secondary product following anaerobic digestion as a soil amendment, using cover crops to stabilise excess nitrogen and further improve soil health.

Anaerobic digestion of farm waste is becoming more commonplace in the UK, and it enables farmers to convert various forms of biomass into biogas and the by-product Anaerobic Digestate (AD). Biogas production from locally sourced biomass feedstocks can provide a farmer with substantial income. During the anaerobic digestion process, large amounts of organic feedstock are converted into biogas, which is burned to produce electricity through combustion in an engine-generator, which can be sold to the national grid for profit. Furthermore, excess heat produced from the digestion process can be used to heat the digester, or local farm buildings. Therefore, anaerobic digestion can utilise and valorise farm waste streams, which also reduces greenhouse gas emissions and leachate production at landfill, if such waste is disposed in this way (Liu et al. 2012).

Anaerobic Digestate as a By-Product of Anaerobic Digestion

The by-product of anaerobic digestion, AD, the solid or liquid fraction of digestion which is unable to be broken down by anaerobes, is composed of partially degraded OM, is nutrient rich, and has been shown to contain a number of microorganisms; some of which could be beneficial to agricultural

crops or soil health (Alburquerque et al. 2012; Coelho et al. 2020). In this project the farmers were especially keen to see whether the combination of OM and AD would improve soil health. Proper storage and utilisation is paramount to the success of AD as an economic and environmentally sustainable soil amendment, and the aim of this Innovative Farmers Field Lab was to investigate various means of maximising its potential benefit at the farm scale.

Utilisation of Anaerobic Digestate on Farm

Due to its nutritional composition, AD provides farmers with a possible alternative or supplement to chemical fertilisers, which are becoming increasingly expensive due to their finite nature and high energy cost of production (Walsh et al. 2012). Furthermore, when compared to other non-chemical alternatives such as farm yard manure (FYM), the process of anaerobic digestion increases the proportion of plant available N in the form of ammonium (NH_4^+) (Gutser et al. 2005). The gradual reduction in OM content of arable soils is also of increasing concern to farmers, which is important for good soil texture, structural stability, and water retention, and can be incrementally restored by application of AD (Albiach et al. 2001). The role and importance of OM is explained further in Chapter One.

Application of N in the form of AD, particularly in its liquid fraction, is subject to similar inefficiencies as bagged, inorganic N, in terms of leaching

and volatilisation. The use of cover crops is one way to stabilise the N applied in the form of AD, and may reduce pollution from volatilisation of N and leachate (Smil 1999; Bennett et al. 2001; Tilman et al. 2002). Cover cropping has also been associated with an array of other benefits to soil health, including improvements in soil structure, organic matter, and the promotion and maintenance of AM fungi (Williams and Weil 2004; Snapp et al. 2005; Cossio et al. 2007; Chen and Weil 2010; Abdollahi and Munkholm 2014; Stobart and Gosling 2015; Bowles et al. 2017). The many benefits of cover cropping and AM fungi on farm are outlined in the general introduction in Chapter One.

Influence of Anaerobic Digestate on AM fungi

There has been limited research on how application of AD impacts populations of AM fungi. Caruso et al. (2018) found that application of solid AD increased RLC of triticale, when compared to mineral, and no fertilisation. The authors attribute this to the presence of struvite in the solid AD, a form of plant unavailable phosphate formed during the digestion process. Application of the liquid fraction of the AD did not elicit the same response in AM fungal colonisation in this experiment.

Coelho et al. (2020) investigated the microbial composition of four types of AD, using 16S/ ITS sequencing, and qPCR. They found organisms with important agronomic roles, including nitrogen fixing bacteria, plant growth

promoting rhizobacteria (PGPR), AM fungi, and cellulolytic microbes, albeit at low abundance. One species of AM fungi (*Acaulospora sp.*) and methanogens were found in higher abundance in the AD sample. It is, however likely, that the measurement of *Acaulospora* includes multiple species of AM fungi due to the sequence conservation of the ITS region. Coelho et al. found that application of AD had no influence on soil microbial diversity, and the abundant taxa found in AD were unable to establish or were found at only very low abundance in the soil.

Experimental Overview

This Innovative Farmers experiment is replicated on seven fields at six farms, in the East and North East of England. A cover crop mix of fodder radish, black oat, vetch, and buckwheat was selected, due to their popularity as cover crop components, and complimentary functionality in terms of soil improvement (Figure 5.1). Five sites followed the cover crop mixture with maize (*Zea mays*), to be returned to the digester, one site drilled leek (*Allium ampeloprasum L*) and the last sugar beet (*Beta vulgaris*).

Figure 5.1 Cover crop mixture at Euston Farm in January 2018.

The experiment was designed to assess the impact of cover crops, AD, and their interaction on measures of soil health and yield, with farmers being particularly interested in the uptake and stabilisation of N to conform to NVZ regulations (DEFRA and Environment Agency 2018). The experiment is complimented by the inclusion of a fifth treatment at the Allpress site hosting two fields, the cover crop mix drilled with the addition of a five

species AM fungal inoculum (see Chapter Three for details of content). Results detailed in Chapter Three indicated that the AM fungal inoculum did not increase colonisation of spring barley but may have influenced colonisation of an oat cash crop succeeding the barley crop. Furthermore, application of the AM fungal inoculum had little influence on AM fungal community composition, with very mild increases in the abundance of VT corresponding to *R. irregularis*, and indirectly increasing community evenness. These additional treatments will allow for assessment of AM fungal inoculums at the field scale, to assess whether larger plots have an impact on mycorrhizal benefit.

This experiment is a split field trial, conducted on highly varied soil types, and was established and maintained using common farm practice and machinery. The aim of the experiment is therefore to not only draw conclusions on the relationships between cover cropping, AD, and AM fungi, but to establish possible factors such as soil physicochemical parameters, farm practice, and weather conditions that influence cover crops, AM fungi, and the feasibility of their application on farm.

5.2 Methods

Sites and Crop Establishment

The study made use of the ongoing Innovative Farmers project: 'Increasing Nutrient Efficiency from Anaerobic Digestate', in which six farmers have

contributed a field of eight hectares or larger, to investigate the interaction between cover crops and AD. Each site was split into four equal plots, an untreated control, cover crop only, AD only, or cover crop and AD, where the control and digestate only treatments were left fallow (Figure 5.2). The cover crop treatments included a predetermined mixture of black oat (*Avena strigosa var. lapar61*), common vetch (*Vicia sativa var. Early English*), fodder radish (*Raphanus sativus var. Contra)* and buckwheat (*Fagopyrum esculentum*). Experimental treatments were followed by a maize *(Zea mays*) cash crop at five of the seven sites, and leek (*Allium ampeloprasum*) and sugar beet (*Beta vulgaris)*, were cash crops at the remaining two sites. One maize site (Boxworth) failed to establish cover crops.

Figure 5.2 Arrangement of experimental treatments at the Holkham field site.

The four remaining field sites with maize as the cash crop were chosen for this study. These field sites (Figure 5.3) include Holkham Estate (52°55'53.3"N 0°53'57.5"E), an eight-hectare field of sandy loam over chalk, approximately 3km from the North Norfolk coast. Euston Estate (52°21'48.3"N 0°49'09.5"E), a ten-hectare field of sandy loam over chalk. North Moor Farm (53°31'17"N 0°45'45"E), an eight-hectare field of a silty clay loam, considerably further north than the other sites. Finally, Allpress Farm field H27 (52°27'44.5"N 0°06'55.9"E), is an eight-hectare field on Fenland peat soil, which included an additional mycorrhizal inoculant treatment. The Allpress site also hosted a second field with leek as the cash crop. This site was included in analysis as comparison to the maize trial at the Allpress site. Drilling, sampling, and desiccation dates for these sites are shown in Table 5.1.

Figure 5.3 Geographical arrangement of sites in the East of England.

Mycorrhizal Inoculant for Allpress Farm Cover Crop and AMF Treatment

Like in Chapter Three, mycorrhizal inoculum for this experiment was sourced from PlantWorks Ltd (Sittingbourne, UK). The inoculum mixture contains *Funneliformis mosseae, Funneliformis geosporum, Claroideoglomus claroideum, Rhizophagus irregularis*, and *Glomus microaggregatum* in equal quantities. Inoculum was hand broadcast over the cover crop and AMF plot at the two Allpress Farm sites after difficulty drilling cover crop seed and inoculum concurrently.

Table 5.1 Drill, sampling, and desiccation dates for cover crops and maize at each site.

Site	Cover Crop Drill Date	Cover Crop Sampling Date	Cover Crop Destruction Date	Maize Drill Date	Maize Sampling Date
Allpress H27	25/08/2017	26/02/2018	10/04/2018	24/05/2018	24/07/2018
Euston	07/08/2017	19/03/2018	15/04/2018	07/05/2018	30/07/2018
Holkham	25/08/2017	06/02/2018	23/04/2018	15/05/2018	03/08/2018
North Moor	01/09/2017	22/02/2018	20/04/2018	10/05/2018	07/08/2018

Soil Sampling for Physicochemical Analysis

Soil samples were collected between December 2017 and February 2018. Seven soil samples were taken at random per plot, and thoroughly mixed to attain a representative sample. Soil samples for bulk density were taken at three points across the field using a 15cm Dutch auger.

Sampling for the analysis of soil N was undertaken at 0-30cm, 30-60cm, and 60-90cm using a Dutch auger. The 60-90cm horizon was omitted from the Holkham site, as the auger could not penetrate the chalk bedrock (Figure 5.4). Seven repeats per plot were pooled by each depth and were thoroughly mixed before saving a subsample of approximately 300g for laboratory analysis.

Figure 5.4 Chalk bedrock in the 90cm Dutch Auger at the Holkham site.

Sampling for soil nutrients followed the same methodology as for soil N, however only the top 15cm of soil was assessed. Like the sampling of soil N, seven samples were pooled and mixed thoroughly before a representative subsample was saved. Soil samples were transported in a cool box before being transferred to a cold store at 4°C.

Soil structure was evaluated in-field by the Visual Evaluation of Soil Structure (VESS) method, using a 30cm spade to dig three sides of a cubic section of soil. The soil block (Figure 5.5) was then gently split into soil horizons, measured, and soil structure was determined using the SRUC Visual Evaluation of Soil Structure score chart, where a score of one is good structure, and five is poor structure (Supplementary Figure S5.1). VESS is a fast and easily accessible method of measuring soil structure, and measurements of VESS have been shown to correlate strongly with other, more quantitative methods of determining compaction, such as use of a soil penetrometer (Guimarães et al. 2013).

Figure 5.5 30cm soil block from the Holkham site used for VESS analysis.
Cover crop roots are clearly visible down to the 25-30cm horizon.

Plant Sampling for Biomass and Colonisation Assessment

Ten plants of each cover crop species were randomly sampled per treatment (Figure 5.6). Plants were carefully removed from soil in order not to damage the roots, and gently shaken to remove loose soil. Plants were stored in sealable polythene bags, transferred back to the lab in cool boxes, and stored at 4°C prior to analysis.

Plant samples were removed from storage and washed to remove any remaining soil, divided into root and shoot, and blotted dry using a paper

towel. The root was weighed, and cut into approximately 1-2cm lengths, before being thoroughly mixed. Approximately 100mg of root material was selected at random and stored at -80°C for molecular analyses, omitting the non-mycorrhizal radish from this step. Approximately 10 1cm (~100mg) fragments of root material was taken for microscopic analysis, and stored in 10% (w/v) KOH at 4°C. Only five out of ten radish were processed for microscopic analysis, to confirm the absence of mycorrhizal structures.

Figure 5.6 Boundary between stubble and cover crop mixture at Euston Farm in January 2018

Soil Physicochemical Properties

Soil physicochemical properties were assessed by NRM Laboratories, Bracknell, UK and follow the protocols outlined in Chapter Three.

Staining and Microscopy of AM Fungal Structures in Maize and Leek

Assessment of root length colonisation by AM fungi, as well as confocal microscopy was conducted as in Chapter Three (Figure 5.7).

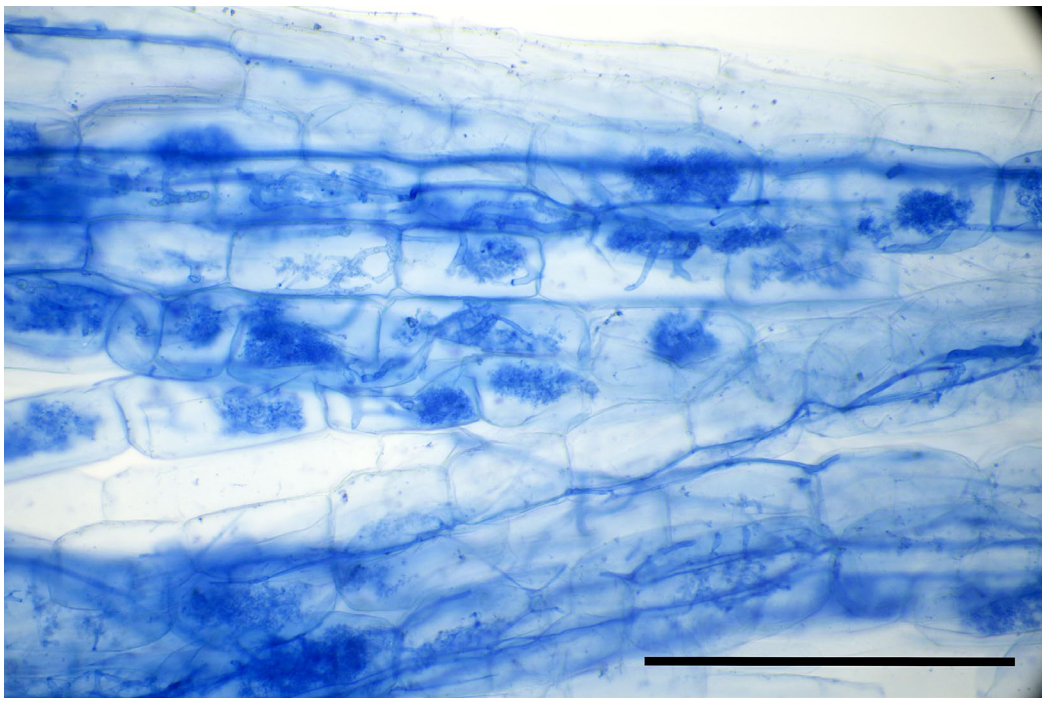


Figure 5.7 Trypan blue stained micrograph of maize from the Allpress site.
Scale bar represents 200µm.

DNA Extraction, Amplification and Sequencing of Maize and Leek

Protocols for DNA extraction, PCR amplification, DNA sequencing, and bioinformatics were conducted as described in Chapter Two.

Statistical Analysis of Innovative Farmers Trial

Maximum likelihood (ML) estimates of the predictors of linear mixed effects models were determined with the lm function in the stats package in R Studio (version 3.5.1) (RStudio Team 2015). Histograms of predictor and response variables, and model residuals were plotted to ensure that the Gauss-Markov assumptions of linearity, non-collinearity, randomness, exogeneity, and homoscedasticity had been met. Post-hoc multiple comparisons were conducted using the contrast function in the package emmeans (Lenth et al. 2020). Due to the low number of replications between sites, and complete lack of replication within sites, care must be taken when generalising results to other sites. However, experimentation at the field scale remains an invaluable validation step to confirm whether findings from experimental setups are robust in real world situations.

5.3 Results

Soil Physicochemical Parameters

There were inconsistent effects of cover crop use on measurements of the VESS. At the North Moor Farm site, use of cover crops increased the VESS score (lower scores are better) from 1.92 in the control plot, to 2.59 and 2.72 in the Cover Crop and Cover Crop and AD treatments respectively. The experimental plot with application of AD, without cover crop remained at 1.95.

At the Allpress Farm site, the Cover Crop, AD Only, and Cover Crop and AD treatments increased VESS scores by 0.67, 0.44, and 0.37 respectively, whereas the Cover Crop and AMF treatment only increased by 0.03. At the Holkham Farm site, application of AD, either alone or with a cover crop, increased the VESS score from 1.22 to 1.38. The cover crop only treatment had the same VESS score as the control. The Euston Farm site was the only site to have an experimental treatment with a positive impact on VESS. When compared to the fallow plot (1.72), the Cover Crop and AD treatment improved VESS by a modest 0.05. Both the Cover Crop only and AD only treatments increased VESS score by 0.04. VESS scores are visualised in Figure 5.8.

Overall, there was a significant impact of site (ANOVA: $F_{3,36} = 24.70$, $p < 0.001$) and treatment (ANOVA: $F_{3,36} = 4.16$, $p = 0.013$), but not their interaction (ANOVA: $F_{9,36} = 1.93$, $p = 0.079$) on the VESS. Both the cover crop (Tukey $p = 0.024$), and cover crop and AD (Tukey $p = 0.029$) treatments had significantly higher VESS scores than the control. The AD treatment was not significantly different to the cover cropped, or control treatments at the $p < 0.05$ level. A summary of soil physicochemical parameters is shown in Table S5.1.

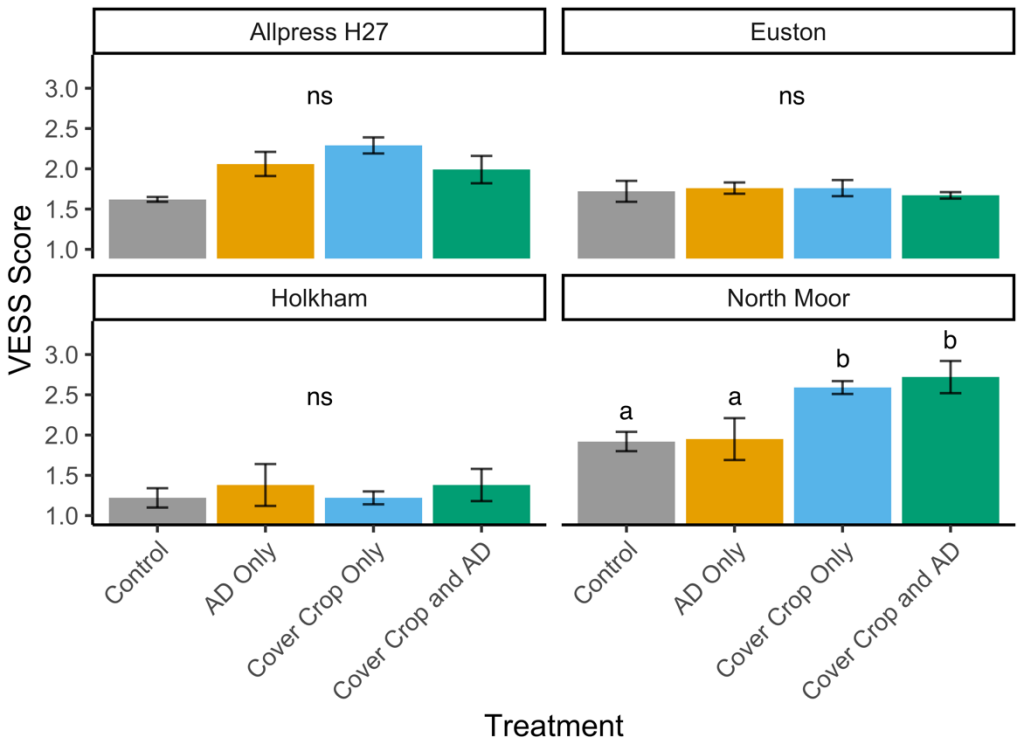


Figure 5.8 Visual Evaluation of Soil Structure (VESS) of the IF trial.
Bars represent mean score ± standard error per each experimental treatment, rated on a scale of one to five, where one indicates optimal soil structure and five poor soil structure.

There were small and inconsistent effects of cover crops and AD on SOM. North Moor Farm showed the greatest variation in SOM, with a 71%, 40%, and 19% increase in the Cover Crop only, AD Only, and Cover Crop and AD treatments respectively. The cover crop and AD treatment at Allpress Farm H27 yielded an 11% increase in SOM, with the Cover Crop only and Cover Crop and AMF treatments exhibiting a 2% increase. The SOM in the AD Only treatment increased by 4%, up from 10.5% to 10.9%.

The two sites on sandy soils showed neutral or negative responses to SOM resulting from cover crops or AD application. SOM at the Euston Farm site was 2.4%, which decreased by 4% to 2.3% in AD Only treatment. The level of SOM in the two cover crop treatments were unchanged from the control level. At the Holkham site, application of AD had no influence on levels of SOM, and remained at the control level of 2.7%. The Cover Crop treatment reduced SOM by 11% compared to the control, whereas the Cover Crop and AD treatment reduced SOM by 7%. Levels of SOM are visualised in Figure 5.9.

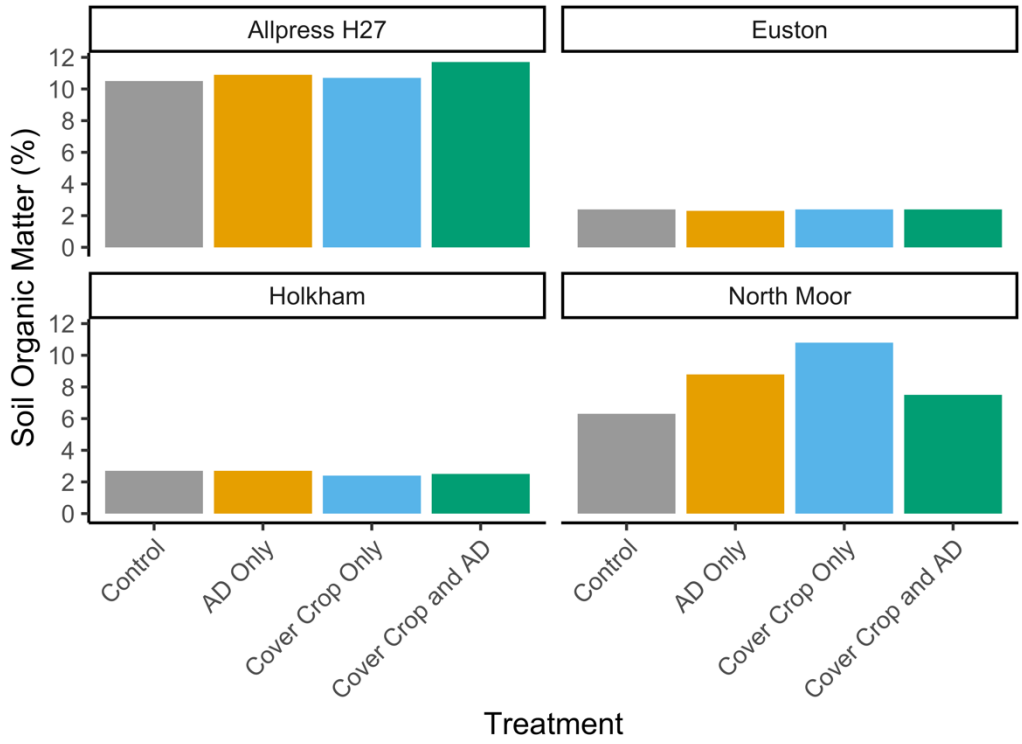


Figure 5.9 Levels of Soil organic matter at the IF trial.
Soil organic matter levels per experimental treatment per site, as determined by loss on ignition.

Available Nitrogen in the Soil Profile

Excluding the North Moor Farm site, AD was effective at increasing soil N, but large amounts of this nutrient were leached to the 30-60cm, or 60-90cm depths, with little effect to the 0-30 level (Figure 5.10). The cover crop only treatment increased soil N at the 0-30cm level compared to the control plots, and two out of three of the sites displayed lower N at the 30-60cm, and 60-90cm levels. Application of AD to cover crops resulted in the largest increase in N at the 0-30cm level for all three of these sites, reducing leaching to lower horizons compared to the AD only treatment.

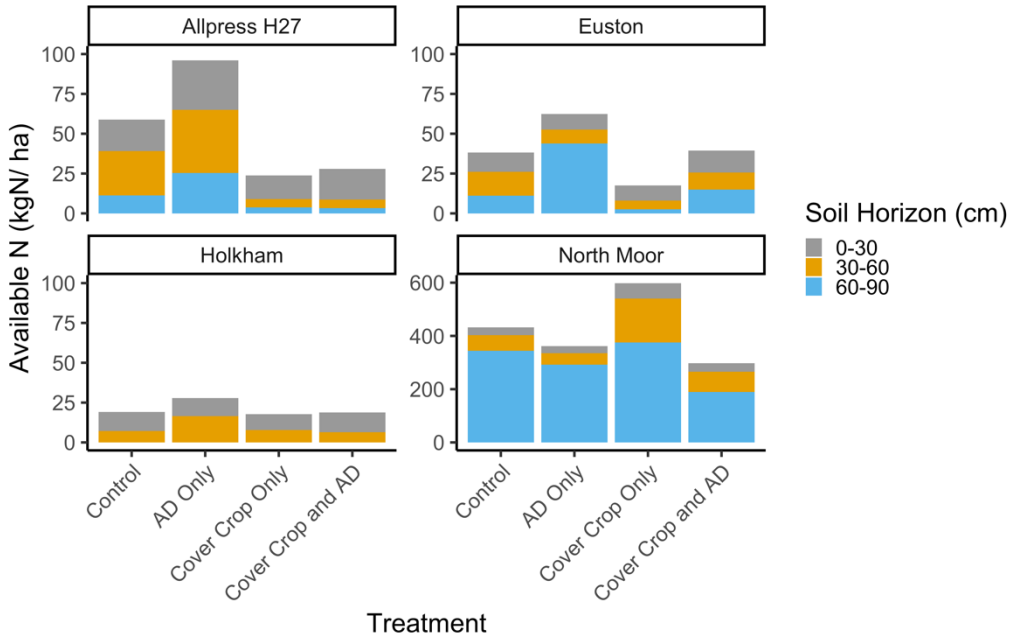


Figure 5.10 Soil available nitrogen in the soil profile.
Accumulation of available N in each treatment, at depths of 0-30cm, 30-60cm, and 60-90cm. The Holkham sites omits the 60-90cm depth, as the soil profile was not this deep. The four sites are plotted on variable axis, due to the large differences in available N between sites.

North Moor Farm showed available N levels approximately 20 times higher than the other sites, with large quantities stored in the 60-90cm horizon. Cover crops, both alone and with AD, increased soil N to the upper horizons, but interestingly, the AD only plot had the lowest total available N, and the cover crop only plot had the highest available N. This was likely influenced by the field being saturated with water and is shown in Figure 5.11.

Figure 5.11 Water infiltration at the North Moor site, after removal of a soil block for VESS analysis.

Cover Crop RLC by AM Fungi

Cover crop colonisation ranged from 0-65% for oat, and 0-89% for vetch (Figure 5.12). After arcsin transformation, there was a significant impact of site (ANOVA: $F_{3,134} = 10.90$, $p < 0.001$), cover crop species (ANOVA: $F_{1,134} = 25.28$, $p < 0.001$), and whether AD had been applied (ANOVA: $F_{1,134} = 6.35$, $p = 0.013$), on cover crop RLC. Cover crop RLC was highest at the north Moor site, followed by Euston, Holkham, then Allpress Farms. Across sites, mean colonisation of vetch (35.6%) was significantly higher than oat (20.8%), and application of AD reduced overall cover crop RLC by over 20%. Interestingly, there was no correlation between oat and vetch colonisation across sites and treatments (Pearson's correlation: r=0.014, df= 6, p=0.974).

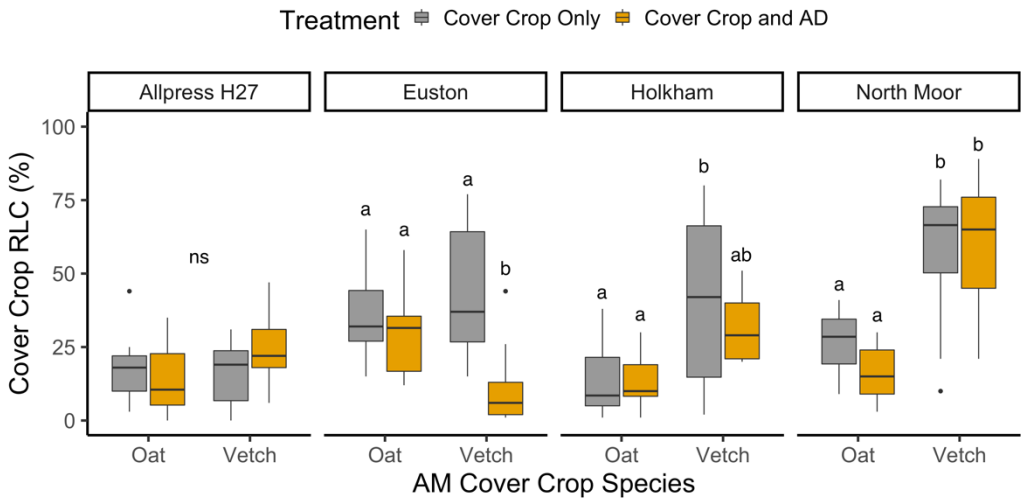


Figure 5.12 Cover crop root length colonisation by AM fungi.
Oat and vetch cover crop RLC as determined by trypan blue staining and an adapted version of the grid line intersect method for determining total colonisation. Radish cover crops belonging to the Brassicaceae were confirmed to be non-hosts of AM fungi and omitted from mycorrhizal analysis.

Cover Crop Biomass

Across the trial, radish cover crops exhibited the largest shoot biomass (3.98 ± 0.48g), compared to oat (1.43 ± 0.13g), and vetch (1.15 ± 0.14g). Radish cover crops consistently had the greatest shoot biomass across the four sites, oat was the next largest species at three out of four of the sites, excluding Euston, where the vetch was on average 0.06g larger than the oat. Addition of AD increased overall cover crop shoot biomass at three out of four of the sites. Euston, Holkham, and North Moor experienced a 39%, 6%, and 15% increase in mean shoot biomass, whereas the Allpress site displayed a 2% reduction in mean biomass. Cover crop biomass is visualised in Figure 5.13.

Across the trial, there was a significant effect of site (ANOVA: $F_{3,226} = 69.33$, $p < 0.001$) and cover crop species (ANOVA: $F_{2,226} = 76.96$, $p < 0.001$) on cover crop shoot biomass. Euston Farm had the cover crops with the greatest overall shoot biomass, followed by Allpress, North Moor, and Holkham. Radish cover crops were significantly larger than oat, which were themselves significantly larger than vetch at the $p < 0.05$ level. There was no significant effect of AD application alone (ANOVA: $F_{1,226} = 2.56$, $p = 0.111$), but there was a significant interaction between AD application and cover crop species (ANOVA: $F_{2,226} = 3.34$, $p = 0.037$). Oat and radish cover crop biomass was increased by 65% and 18% resulting from the application of AD, but vetch was decreased by 12%.

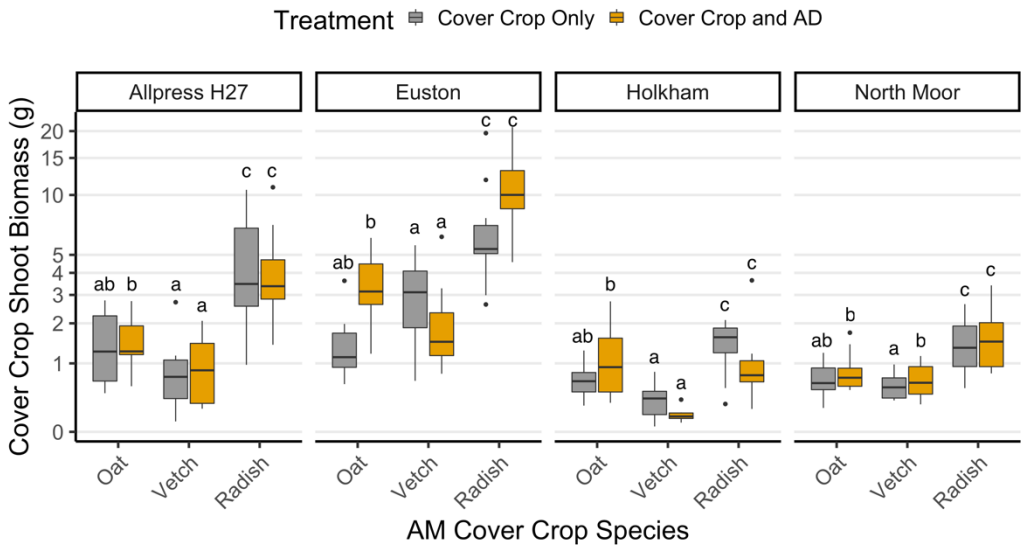


Figure 5.13 Cover crop shoot dry weight in the IF trial.
Dry weights of oat, vetch, and radish cover crops at each site, with and without the addition of AD (orange boxes).

Maize RLC by AM Fungi

Maize colonisation by AM fungi ranged from 2% in the Euston control to 99% in the Allpress cover crop and AD treatment (Figure 5.14). The North Moor site had the highest mean colonisation, at 59.7%, closely followed by the Allpress site at 59%. The two sandy sites, Euston and Holkham had mean RLC values of 44% and 40.1% respectively. Across the trials, control plots had a mean RLC of 48.7%, which decreased to 48.3% in the cover crop only treatment. Both the AD only, and cover crop and AD treatments increased the mean maize RLC across the trial, to 53.8% and 52.3% respectively. Overall, there was a significant impact of site on maize RLC (ANOVA: $F_{3,134} = 8.14$, $p < 0.001$), but neither the experimental treatment (ANOVA: $F_{3,134} = 0.69$, $p = 0.559$) nor the interaction between site and treatment (ANOVA: $F_{9,134} = 1.32$, $p = 0.234$) yielded a significant result.

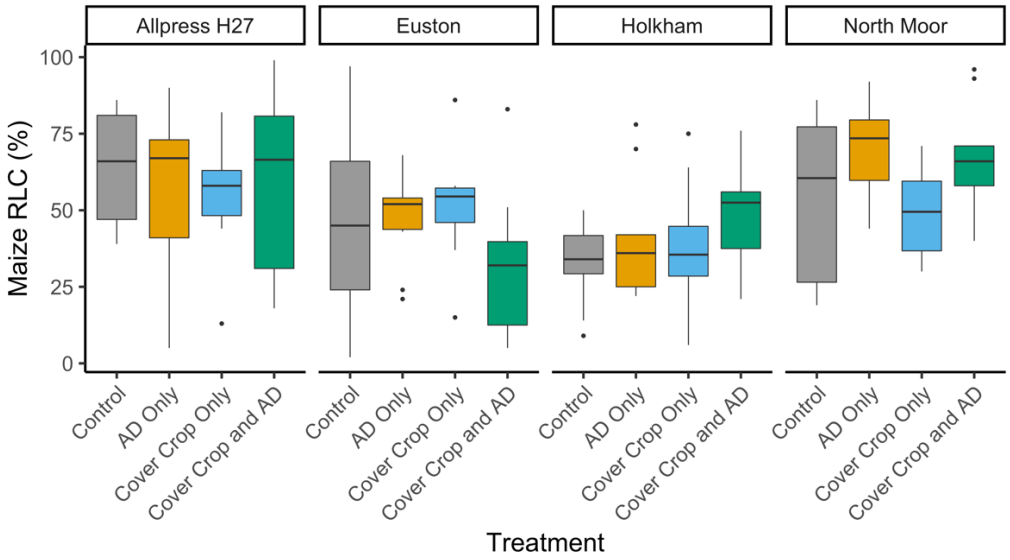


Figure 5.14 Colonisation of maize by AM fungi, in the IF trial.
Boxes display mean, interquartile range, minimum, and maximum colonisation of each plot.

Maize Biomass

Mean maize shoot biomass ranged from 34.7g at the Allpress site, following the cover crop only treatment, to 87.8g at the North Moor site, following the cover crop and AD treatment (Figure 5.15). The North Moor site also exhibited the greatest biomass across treatments, with a mean biomass of 78.3 ± 4.6g. The next largest mean shoot biomass was seen at the Euston site (54.6 ± 3.0g), then Allpress (40.4 ± 2.3g), and finally Holkham (37.8 ± 1.6g).

Across the trial, growing cover crops without digestate, or applying digestate alone reduced maize biomass by 8.5g (-15.6%), and 2.5g (-4.6%), when compared to the control 54.8g ± 3.9g. Only cover crops grown with the addition of AD increased maize shoot biomass, to 57.9 ± 4.7g (+5.8%). This effect was observed at three out of four of the trial sites, with the exception of the Holkham site, in which the cover crop and AD treatment had the second lowest shoot biomass (35.7 ± 3.8g). There was a significant effect of site on maize shoot biomass (ANOVA: $F_{3,144} = 29.97$, $p < 0.001$), and experimental treatment narrowly missed the $p < 0.05$ threshold (ANOVA: $F_{3,144} = 2.32$, $p = 0.078$), although this effect if any, was small. The interaction between site and experimental treatment was also non-significant (ANOVA: $F_{9,144} = 1.11$, $p = 0.357$, Figure 5.16), as were pairwise effects within sites (Tukey $p = 0.21$). Addition of the maize RLC as a model covariate did not significantly improve model fit ($\chi^2 = 1.72$, d.f. = 1, $p = 0.192$).

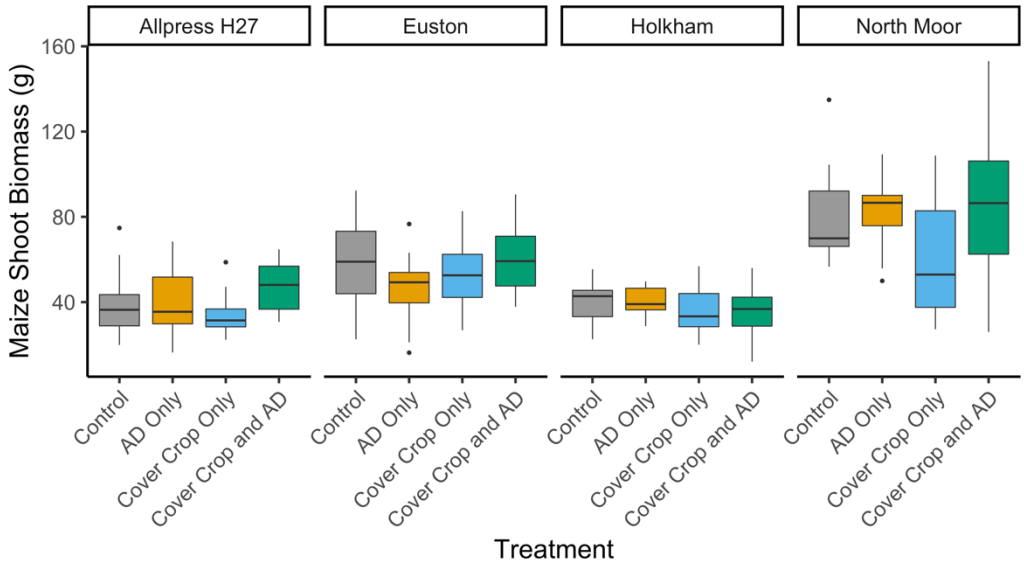


Figure 5.15 Maize dry shoot weight in the IF trial.
Dry shoot weight of maize at each site following the four experimental treatments

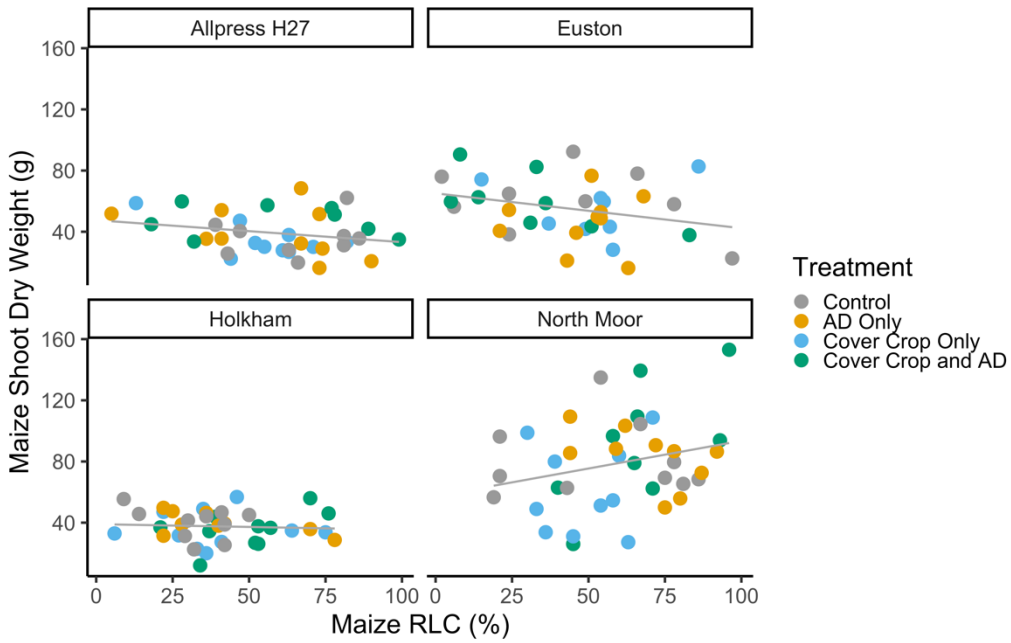


Figure 5.16 Interaction between maize RLC and shoot dry weight .
Maize RLC plotted against the relative dry weights at each site, following the four experimental treatments.

AM Fungal Inoculation of Maize and Leek at the Allpress Farm Site

Available Nitrogen in the Soil Profile at the Allpress Farm Site

At the Allpress H27 field growing maize, the fifth treatment of a cover crop inoculated with the PlantWorks AM fungal inoculum further reduced the total available N in the soil profile (total 11.6 kgN/ha). The cover crop and AMF treatment also exhibited the lowest available N in each of the soil horizons, containing 7.2, 1.8, and 2.6 kgN/ha in the 0-30, 30-60, and 60-90 horizons respectively. This corresponded to a 63%, 94%, and 77% reduction in available N at each soil horizon, respectively, compared to the cover crop only treatment.

At the Allpress H48 field growing leek, total available N in the control plot was 370.5 kgN/ha, 15.5 times higher than the nearby field H27. All treatments in field H48 resulted in higher total available N in the soil profile, including AD only (577.5 kgN/ha), cover crop only (567.2 kgN/ha), and cover crop and AMF (573.1 kgN/ha), which all showed similar values of soil N. The cover crop and AD treatment had intermediate levels of available N (465.9 kgN/ha), but this still represented an increased compared to the control. Available N at the two Allpress field sites are visualised in Figure 5.17.

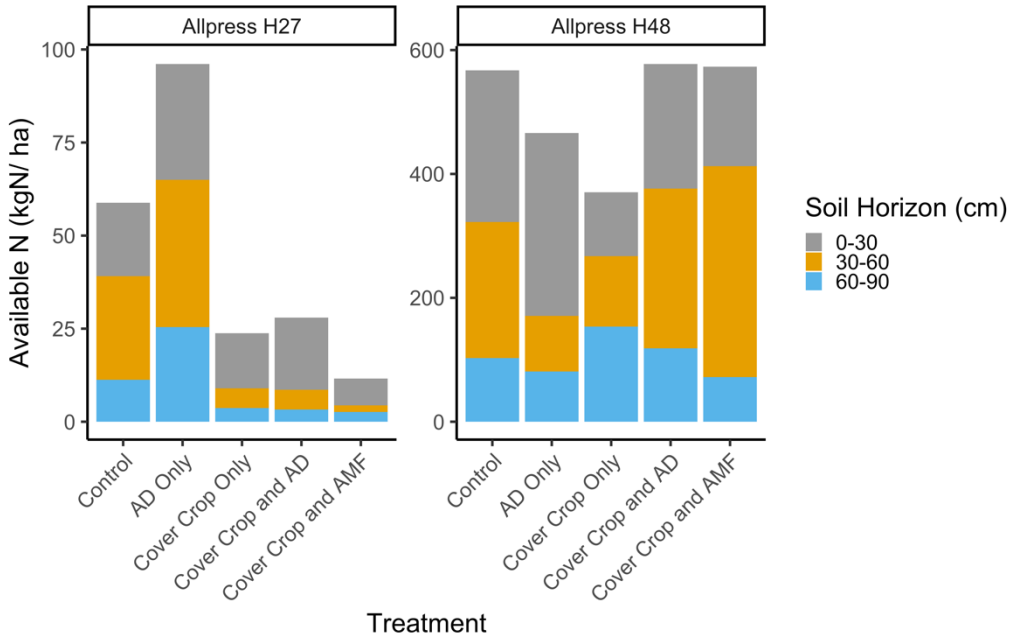


Figure 5.17 Soil available nitrogen in the soil profile at the Allpress Farm site. Accumulation of available N in each treatment, at depths of 0-30cm, 30-60cm, and 60-90cm, including the addition of a fifth treatment of a cover crop inoculated with AM fungi. The two sites are plotted on variable axis, due to the large differences in available N between fields.

Soil Physicochemical Parameters at the Allpress Farm Site

Soil organic matter at the Allpress maize field (10.9%) was considerably lower than at the adjacent leek crop (38.2%). In maize, addition of the AM fungal inoculum increased SOM by 0.2% (vs the control) to 10.7%. This was not different to the cover crop grown alone, and 1% lower than when the cover crop was grown with AD (Figure 5.18).

In the leek crop, SOM in the control plot was 43.4%, decreasing to 36.6%, 25.1% and 40.3% in the AD, cover crop, and cover crop and AD treatments, respectively. Addition of the AM fungal inoculum to the cover crop increased

SOM by 2.1% and 20.4% compared to the control, and cover crop only treatments respectively, to 45.5%.

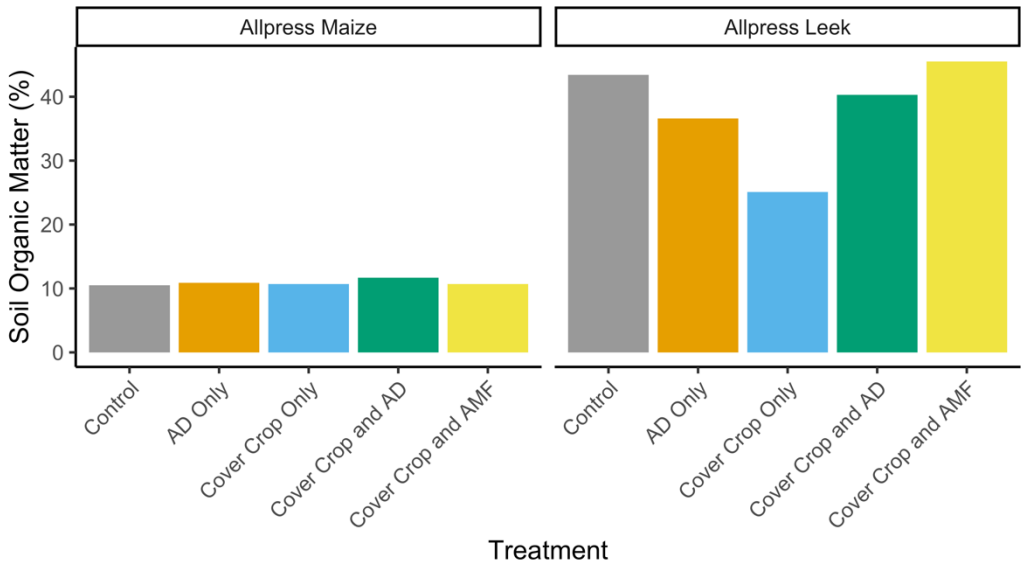


Figure 5.18 Levels of soil Organic Matter (OM) at the Allpress Farm Site.
Soil organic matter levels at the Allpress Farm site, including the addition of a fifth treatment of a cover crop inoculated with AM fungi. Soil OM was determined by the loss on ignition method.

VESS of the cover crop and AMF plot in the maize field had a mean value of 1.65 ± 0.20, which was virtually unchanged to the control (1.62 ± 0.03), and considerably better than the cover crop only treatment (2.28 ± 0.10).

In the leek crop, VESS of control plots was 1.58±0.29, which was virtually unchanged when compared to the maize crop (Figure 5.19). In this field, the AD only and cover crop only treatments did not exhibit the same negative impact on VESS, registering mean scores of 1.69 ± 0.15 and 1.68 ± 0.15 respectively. The cover crop and AD treatment saw small improvements in soil structure (1.47 ± 0.17), and the best structure was under the cover crop

and AM fungal inoculated treatment, at 1.26±0.08. Across the trial, there was a significant effect of site (ANOVA: $F_{1,20} = 14.60$, $p = 0.001$) and treatment (ANOVA: $F_{4,20} = 3.42$, $p = 0.028$), but not their interaction (ANOVA: $F_{4,20} = 0.92$, $p = 0.474$). Post-hoc comparisons show that inoculated cover crops have significantly improved soil structure, compared to when grown without inoculum (Tukey $p = 0.028$), but aren't significantly better than fallow plots (Tukey $p = 0.897$). Due to the small sample size, there were no significant pairwise comparisons within each field site.

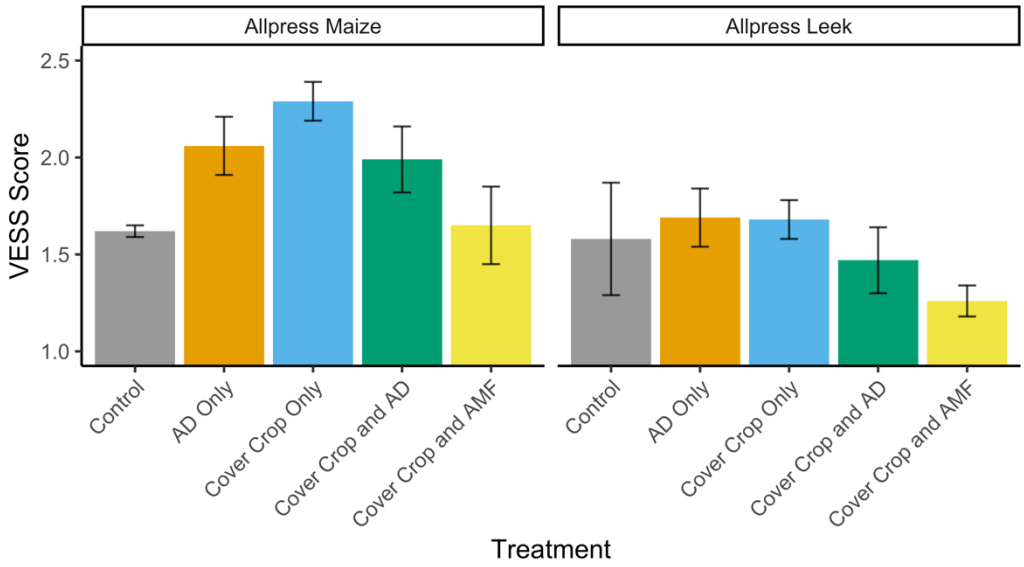


Figure 5.19 Visual Evaluation of Soil Structure (VESS) at the Allpress Farm site. Bars represent mean score ± standard error per each experimental treatment, including the addition of a fifth treatment of a cover crop inoculated with AM fungi.

Cover Crop RLC at the Allpress Farm Site

In the maize field, there was a significant effect of treatment (ANOVA: $F_{2,48} = 3.92$, $p = 0.027$), cover crop species (ANOVA: $F_{1,48} = 6.15$, $p = 0.017$),

and their interaction (ANOVA: $F_{2,48} = 3.55$, $p = 0.036$) on cover crop RLC (Figure 5.20). Vetch RLC (25.8%) was significantly higher than oat (17.2%) considering all treatments. Across both oat and vetch, RLC of the inoculated cover crop (29.9%) was significantly higher than the cover crop alone (17.4%, Tukey $p = 0.014$) or when grown with AD (18.8%, Tukey $p = 0.020$). The untreated cover crop and cover crop treated with AD did not differ in their RLC (Tukey $p = 0.984$). Within each cover crop species, there were no pairwise differences due to experimental treatment in oat, but in vetch, the RLC of the inoculated crop was significantly higher (+27.9%) than the cover crop grown alone (Tukey $p = 0.002$). Pairwise differences between the AD treated, and inoculated cover crop narrowly missed the $p < 0.05$ threshold for significance (Tukey $p = 0.059$).

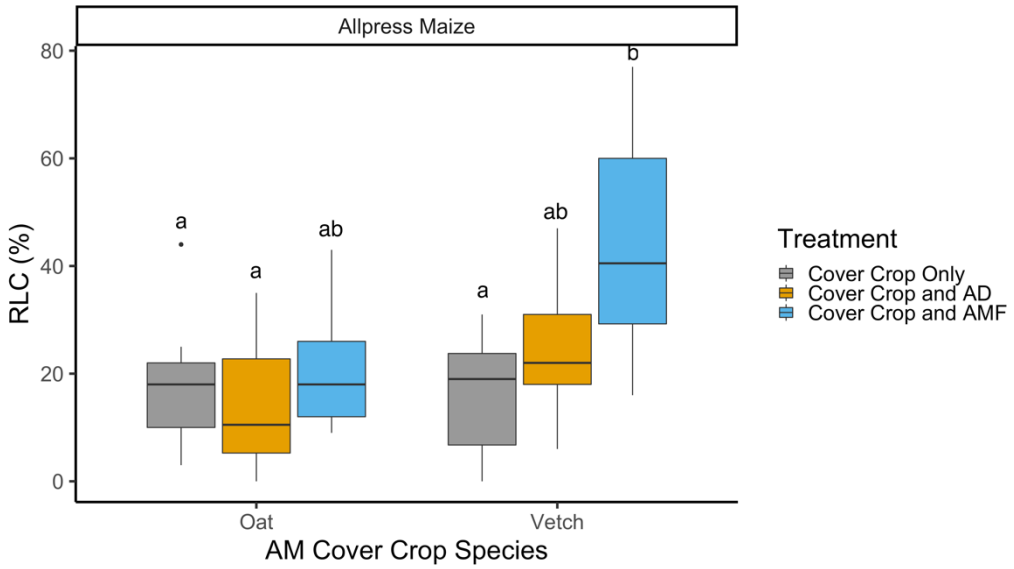


Figure 5.20 Cover crop root length colonisation by AM fungi at the Allpress site. Oat and vetch RLC observed in the two cover crop plots, including a third plot inoculum with AM fungal inoculum (blue). Cover crops in the Leek field (Field H48) were not assessed for RLC.

Cover Crop Shoot Biomass at the Allpress Farm Site

Across both sites, vetch cover crops had the lowest shoot biomass, at 0.90 ± 0.07g, followed by oat (1.43 ± 0.08g), then radish (3.74 ± 0.32g, ANOVA: $F_{2,161} = 87.17$, $p < 0.001$, Figure 5.21). Neither the experimental field (ANOVA: $F_{1,161} = 0.06$, $p = 0.815$) nor experimental treatment (ANOVA: $F_{2,161} = 0.50$, $p = 0.606$), impacted shoot biomass, with the most pronounced impact being on the radish cover crop in the maize H27 field, which had slightly reduced biomass in the AM inoculated treatment, although not significantly (Tukey $p = 0.721$).

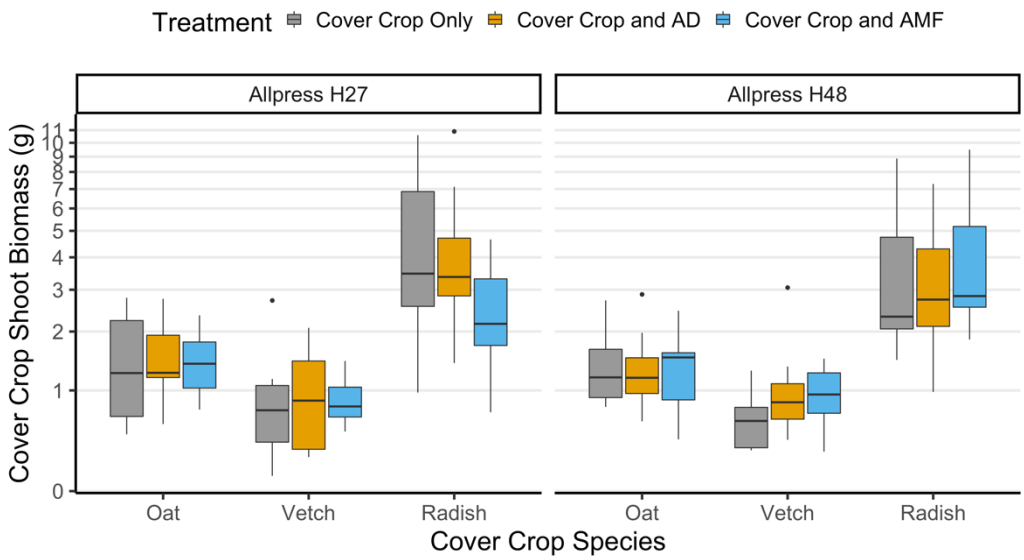


Figure 5.21 Cover crop shoot dry weight at the Allpress site.
Dry weights of oat, vetch, and radish cover crops at each site, with the addition of AD (orange boxes) or an AM fungal inoculum (blue) compared to cover crop grown without an amendment (grey).

Maize and Leek RLC at the Allpress Farm Site

Maize RLC following the AM inoculated cover crop treatment was 65.8 ± 5.5%, which was the highest observed value of maize RLC, and 10.7% higher the non-inoculated cover crop treatment. However, this value corresponded to a mere 0.5% increase compared to maize following a fallow treatment.

Leek RLC (95.3 ± 1.0%) was considerably higher than maize (60.4 ± 3.2%). Leek RLC in the control plot was 99.4 ± 0.4%, which was reduced to 97.5 ± 1.5%, 87.2 ± 3.0%, and 93.0 ± 1.8% in AD only, cover crop only, and cover crop and AD plots, respectively. Leek RLC was restored to fallow levels by the addition of AM fungal inoculum, to 99.5 ± 0.2%. Statistically, the cash crop identity was found to be the primary influencer of model RLC (ANOVA: $F_{1,87} = 162.68$, $p < 0.001$), with a minor effect of experimental treatment (ANOVA: $F_{4,87} = 3.86$, $p = 0.006$). RLC following a cover crop was significantly lower than fallow plots (Tukey $p = 0.015$), but inoculation of the cover crop resulted in a significant increase in RLC (Tukey $p = 0.012$). There was no effect of the interaction between the cash crop, and experimental treatment (ANOVA: $F_{4,87} = 1.14$, $p = 0.345$). Maize and leek RLC is visualised in Figure 5.22.

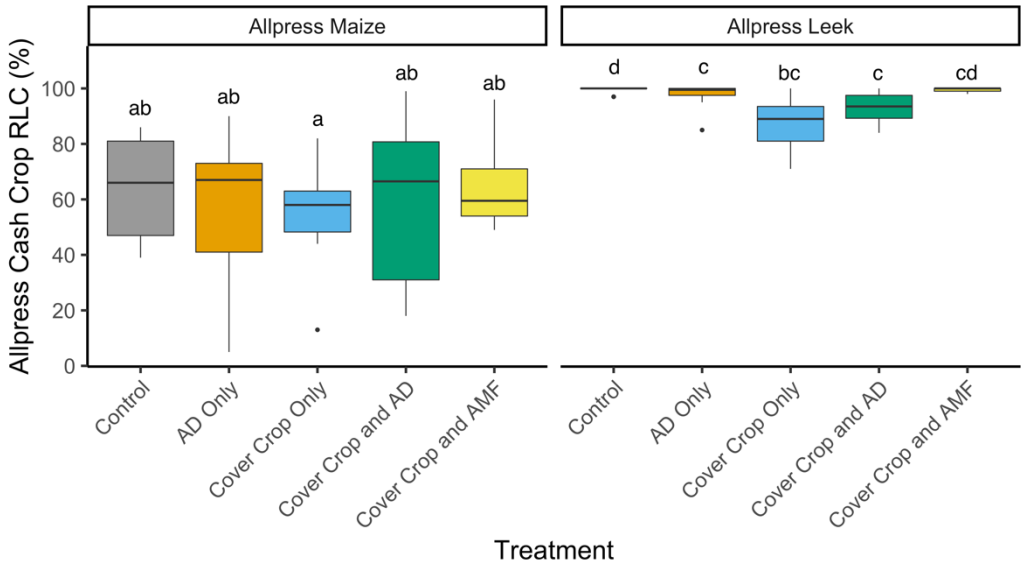


Figure 5.22 Maize and Leek dry shoot weight at the Allpress site.
Dry shoot weight of maize and leek at each of the Allpress Farm field sites following the four experimental treatments, plus a fifth treatment of cover crop and AM fungal inoculum.

Leek Vesicle Colonisation by AM Fungi at the Allpress Farm Site

Reliable vesicle RLC was recorded for leek only, and ranged from 4% following the cover crop and AD treatment, to 100% in the control (Figure 5.23). Following an arcsin transformation to conform to Gauss-Markov assumptions, there was a significant effect of experimental treatment on leek vesicle RLC (ANOVA: $F_{4,45} = 18.13$, $p < 0.001$). Leek following the fallow control ($71.2 \pm 5.5\%$), or the AM fungal inoculated cover crop ($75.2 \pm 4.0\%$) had significantly higher vesicle RLC than the AD ($24.1 \pm 4.4\%$), cover crop ($20.1 \pm 5.5\%$), and cover crop and AD ($37.7 \pm 8.2\%$) treatments, which themselves were not significantly different at the $p < 0.05$ level.

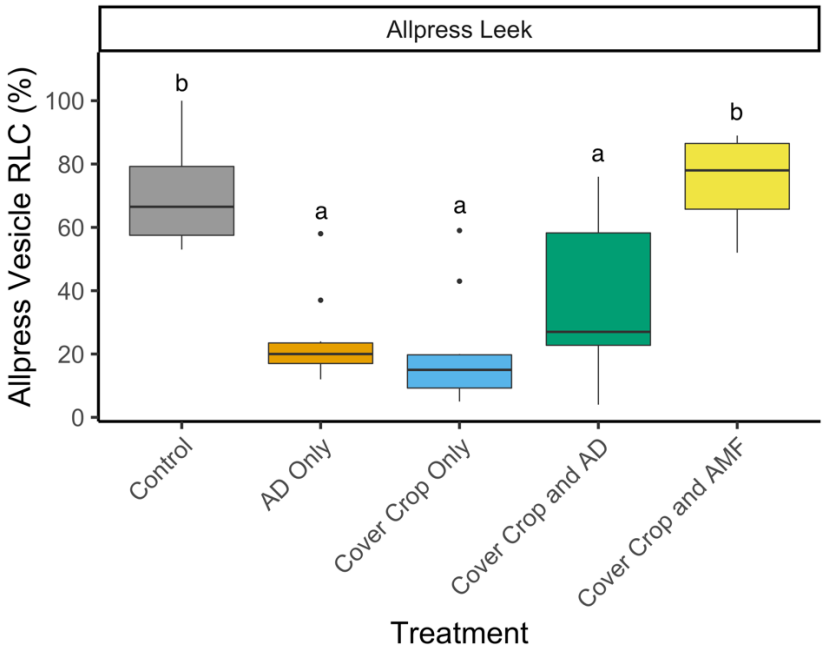


Figure 5.23 Vesicular colonisation of leek by AM fungi, at the Allpress site.
Boxes display mean, interquartile range, minimum, and maximum amount of the root length colonised by AM fungal vesicles, as determined by trypan blue staining. Due to very low abundance of vesicles in the maize crop, only data for leek is shown.

Maize and Leek Biomass at the Allpress Farm Site

In the Allpress maize crop, addition of the AM fungal inoculum increased shoot biomass from 40.0 ± 5.4g in the control to 42.8 ± 4.7g, although this was still lower than the cover crop and AD treatment, which yielded a mean maize biomass of 47.5 ± 3.8g (Figure 5.24). In the Allpress Farm leek crop, the control plot yielded 16.4 ± 2.9g, which was reduced to 12.4 ± 2.0g and 12.8 ± 1.1g following the AD only and cover crop only treatments. Leek shoot biomass was increased following the cover crop and AD treatment (18.0 ± 3.3g) and was highest following the AM fungal inoculated cover crop (22.5 ± 3.8g). After a log transformation, there was a significant effect of

treatment on cash crop biomass (ANOVA: $F_{4,90} = 2.78$, $p = 0.031$). There were no significant pairwise comparisons in the maize field, but in the leek field, the control (Tukey $p = 0.012$) and inoculated cover crop (Tukey $p = 0.016$) plots yielded significantly higher leek shoot biomass than the leeks following a cover crop with no other amendments.

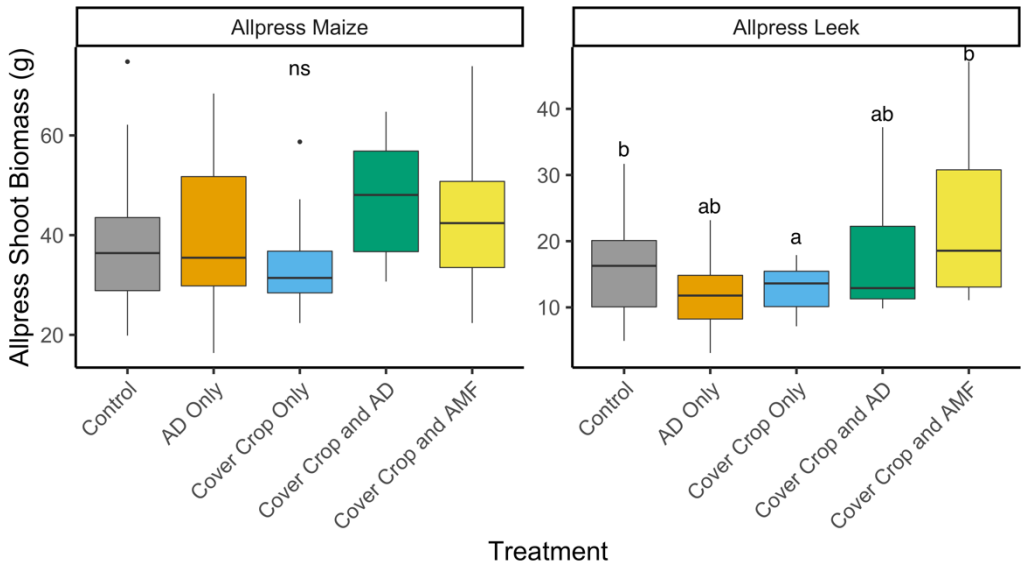


Figure 5.24 Maize and Leek dry shoot weight at the Allpress site.
Dry shoot weight of maize and leek at each site following the five experimental treatments, including addition of the cover crop and AM fungal inoculum treatment.

AM Fungal Diversity at the IF Trial

After stringent quality thresholding, 294,652 Glomeromycotinian DNA sequences remained for further analysis. In total, 31 Virtual Taxa were identified from across the trial, including one *Ambispora*, two *Archaeospora*, three *Claroideoglomus*, six *Diversispora*, 15 *Glomus*, three *Paraglomus*, and one *Scutellospora*.

Ordination of AM Fungal Communities

Bray-Curtis dissimilarity distances were calculated and the multivariate homogeneity of groups dispersions were calculated for both the site, and cover crop and AD treatments. After 1000 permutations, neither the site ($F_{4,15} = 2.00$, $p = 0.139$) nor cover crop treatment ($F_{4,15} = 0.16$, $p = 0.979$) exhibited significantly different dispersions.

Bray-Curtis distances were plotted by NMDS and PCoA methods, to visualise clustering of communities (Figure 5.25). Multivariate Analysis of variance by PERMANOVA found that site (PERMANOVA: $R^2 = 0.410$, $F_{4,11} = 2.52$, $p = 0.015$) but not the cover crop treatment (PERMANOVA: $R^2 = 0.142$, $F_{4,11} = 0.87$, $p = 0.578$) resulted in significant partitioning of sums of squares from the Bray-Curtis distances. Subsequent pairwise multilevel comparisons of the experimental site showed no significant differences in community distance following Bonferroni adjustment, likely due to the small sample size. Continuing the analysis on non-adjusted p-values, there were significant differences between the North Moor and Euston sites (PERMANOVA: $R^2 = 0.508$, $F = 5.17$, $p = 0.039$*), the North Moor and Allpress H48 leek sites (PERMANOVA: $R^2 = 0.386$, $F = 4.40$, $p = 0.025$*), and the Euston and Allpress H27 maize sites (PERMANOVA: $R^2 = 0.382$, $F = 3.71$, $p = 0.040$*). Care must be taken when considering these results, as significance levels are likely to be spurious.

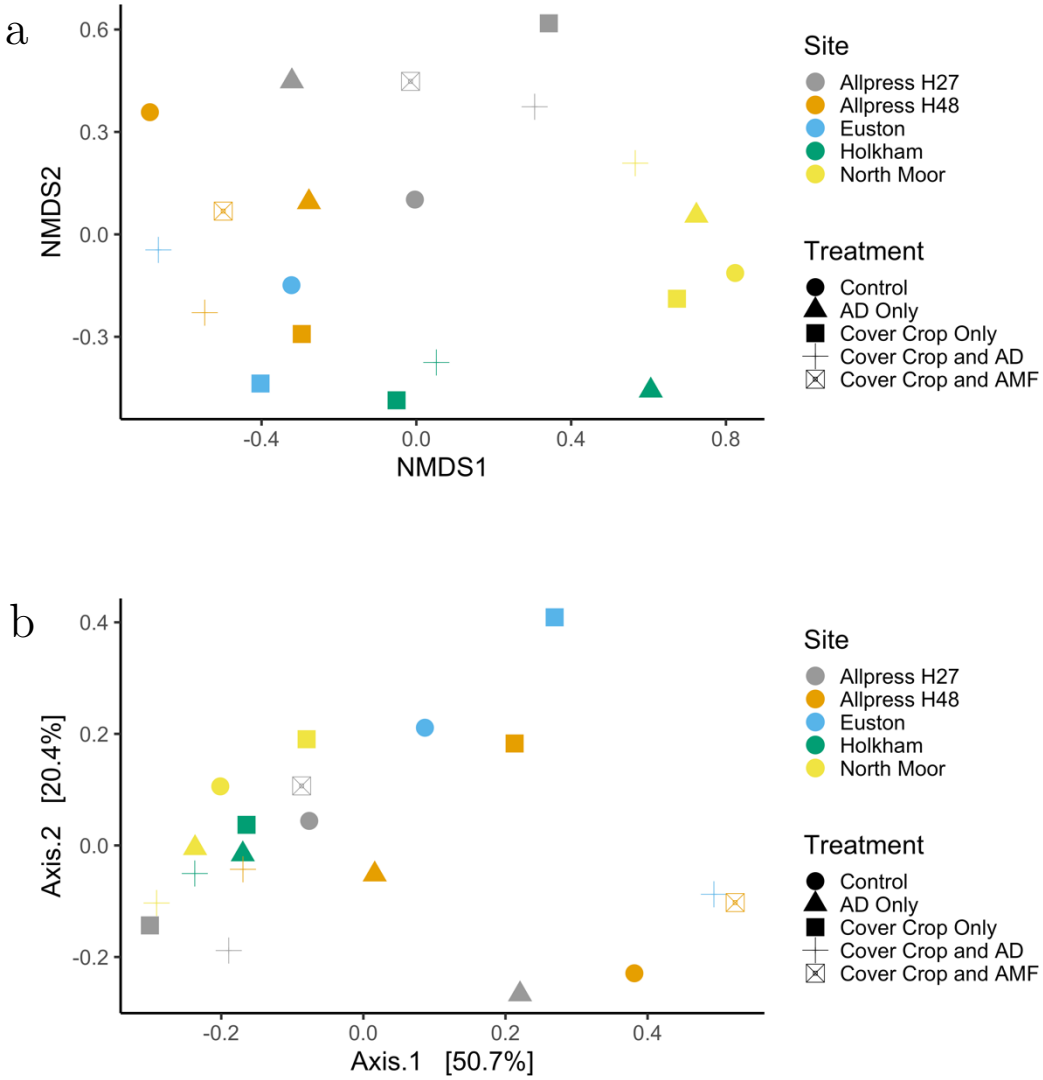


Figure 5.25 a) NMDS ordination of Bray-Curtis distances for the NFS trial b) Principal coordinate analysis (PCoA) ordination of Bray-Curtis distances for the IF trial.

Non-metric Multi-dimensional Scaling (NMDS) plot of Bray-Curtis dissimilarities from the NFS trial. Multivariate Analysis of variance by PERMANOVA found that site (PERMANOVA: $R^2 = 0.410$, $F_{4,11} = 2.52$, $p = 0.011$) but not the cover crop treatment (PERMANOVA: $R^2 = 0.142$, $F_{4,11} = 0.87$, $p = 0.570$) resulted in significant partitioning.

Observed Number of AM Fungal Taxa

Mean observed taxa across the IF trial was 11.35 ± 1.01, ranging from five taxa in the control treatment at the North Moor site, to 21 taxa observed in the cover crop and AD treatment at the Allpress H48 trial growing leek (Figure 5.26a). The highest observed number of taxa colonising maize was 13, and observed in the control treatment at the Euston site, and the cover crop only treatment at Holkham.

The highest mean overserved number of taxa at a site was 17 ± 1.8, at the Allpress H48 site growing leek. The site with the second highest mean diversity and the highest observed maize diversity was Euston, with 12 ± 0.6 taxa. The other Allpress site, H27 had a mean diversity across treatments of 10.2 ± 0.8, whilst Holkham had 9.7 ± 2.0, and North Moor observed the lowest, at 6.5 ± 0.9. Observed taxa per site is visualised in Figure 5.26b.

When considering the effect of treatment across sites, the cover crop and AMF treatment had the highest mean number of observed taxa (14.0 ± 4.0), although this result is misleading due to the relative weighting of the leek field, compared to the less diverse maize. In fact, the cover crop and AMF treatment had two fewer taxa than their relative controls, in both of the Allpress sites. The next highest mean observed taxa was found following the control plots (12.5 ± 3.1), then cover crop and AD (12.0 ± 2.3), cover crop only (10.0 ± 1.3), and the lowest mean treatment diversity was observed in

the AD only plots, at 9.8 ± 2.3 taxa. There was a significant effect of experimental site on the observed number of taxa ($\chi^2_{4,15} = 23.50$, $p < 0.001$) following a poisson regression with a log link function. Post-hoc comparisons showed that the Allpress H48 leek field had a significantly higher observed number of taxa than the Allpress H27 maize field (Tukey $p = 0.032$) and the North Moor farm site (Tukey $p < 0.001$). There was no effect of experimental treatment on the observed number of taxa ($\chi^2_{4,11} = 1.86$, $p = 0.761$).

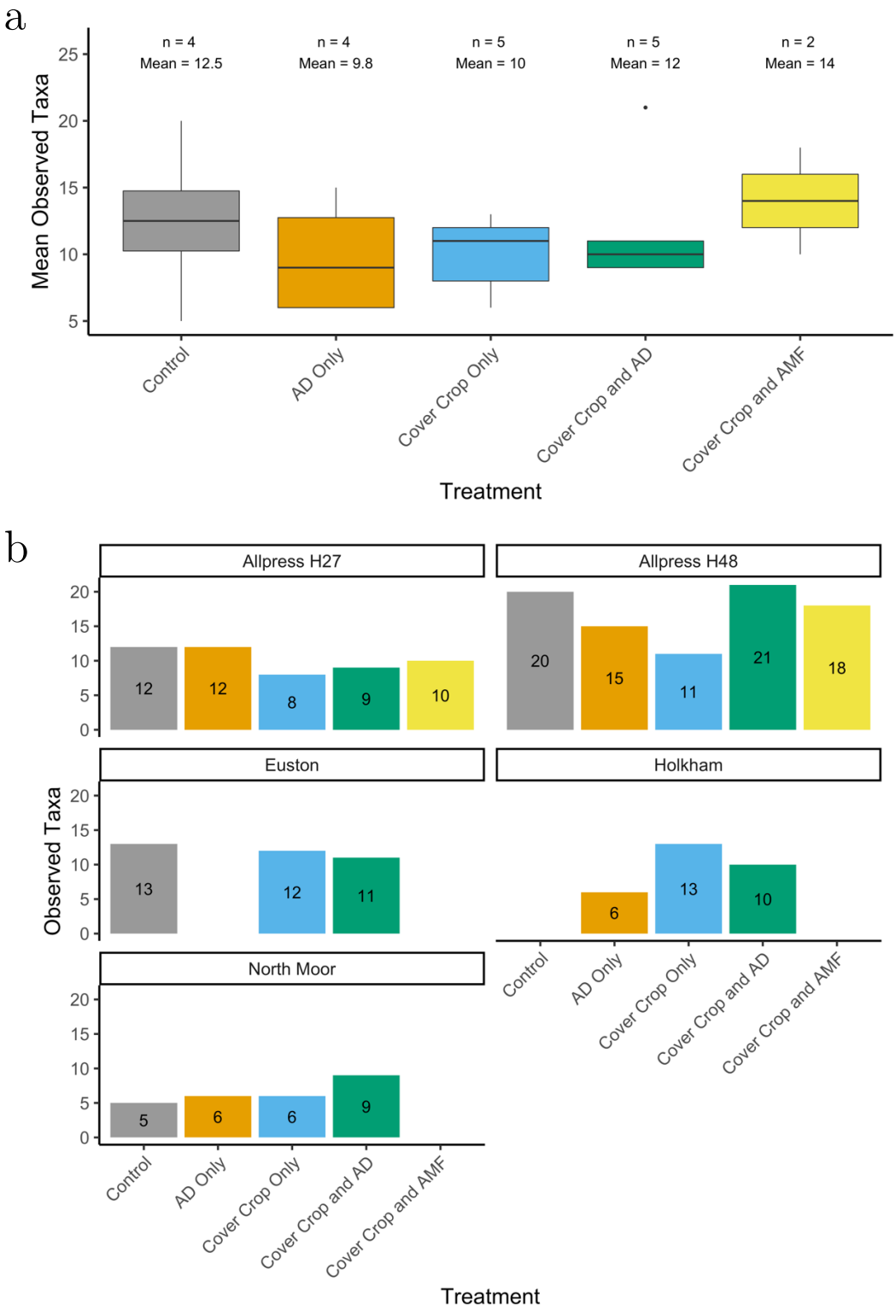


Figure 5.26 Observed AM fungal virtual taxa in the IF trial a) across all sites, b) at individual sites. AM fungal VT observed in each experimental treatment per site. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Inverse Simpson's Diversity for Community Evenness

The Inverse Simpson's Index as a measure of community evenness can be considered as a measure of the effective number of taxa in a community, after considering the relative abundance of each taxon. The mean Inverse Simpson's diversity across the trial was 3.05 ± 0.29, ranging from 1.23 in the cover crop only treatment at the Allpress H27 maize field, to 5.61 in the cover crop only treatment at the Allpress H48 leek field (Figure 5.27a). The highest value of Inverse Simpson's diversity for a maize crop was observed in the control plot of the Euston site at 3.98 (Figure 5.27b).

There was a significant effect of experimental field site on the Inverse Simpson's Index (ANOVA: $F_{4,15} = 4.58$, $p = 0.020$) following a gamma regression using the default inverse link function. Post-hoc comparisons showed that the Allpress H48 leek field had an Inverse Simpson's index of 4.44 ± 0.65 which was significantly higher than the Allpress H27 maize field (2.47 ± 0.39, Tukey $p = 0.031$) and the North Moor farm site (2.15 ± 0.34, Tukey $p = 0.024$). There was no effect of experimental treatment on Inverse Simpsons Index (ANOVA: $F_{4,11} = 0.70$, $p = 0.161$).

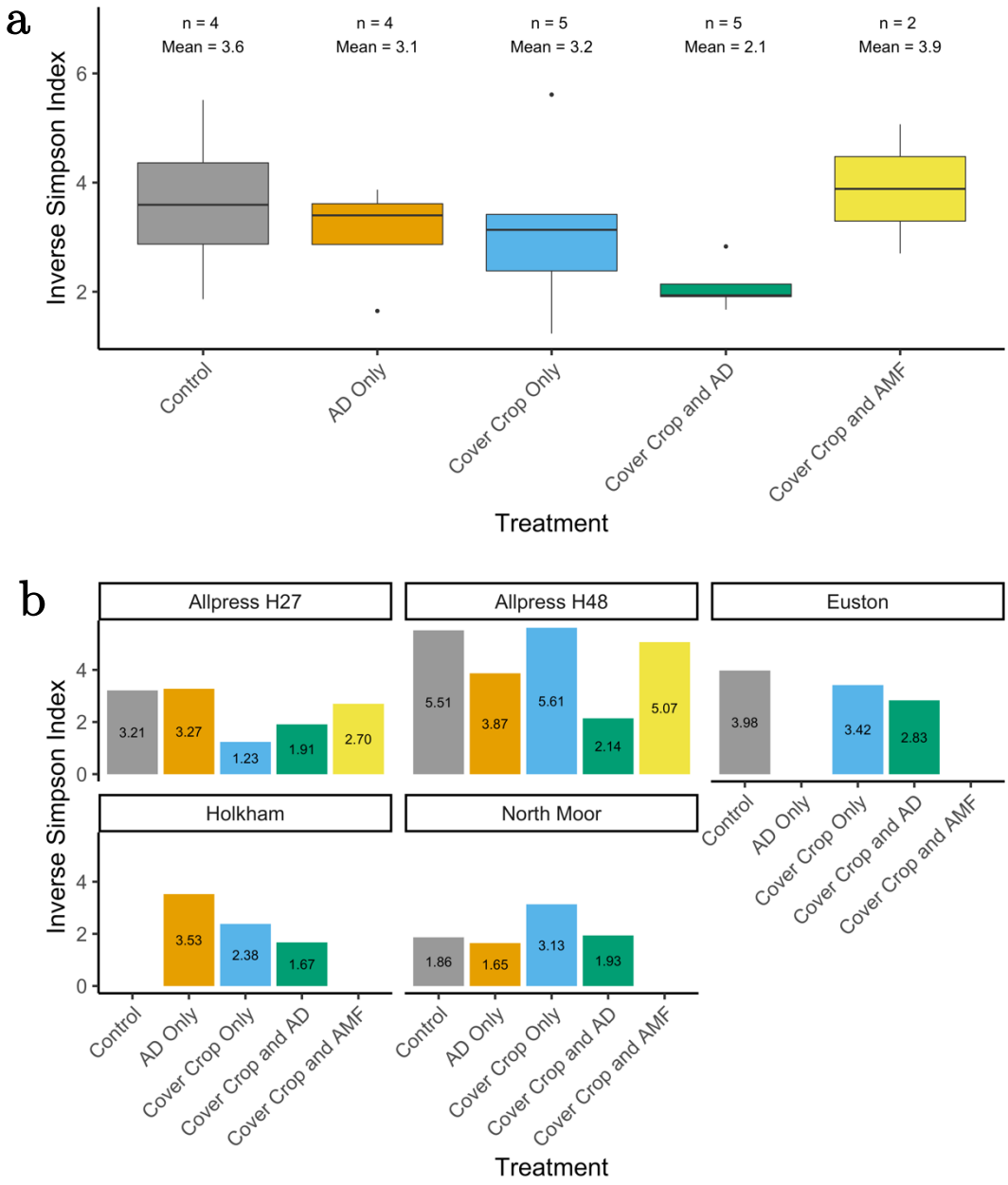


Figure 5.27 Inverse Simpson's Index for AM fungal evenness in the IF trial a) across all sites, b) at individual sites. Inverse Simpson's index of AM fungal communities observed in each experimental treatment. Inverse Simpson's index represents the 'effective' diversity and is bounded by 1 and the number of observed VT per treatment.

Most Abundant Taxa at Each IF Trial Site

The five most abundant AM fungal VT at the Allpress H27 site growing maize was VTX00065 (60.4% - *Glomus caledonium)*, VTX00113 (12.2% - *Glomus MO-G3)*, VTX00281 (10.4% - *Paraglomus laccatum*), VTX00283 (2.7% - *Ambispora fennica*), and VTX00115 (2.5% - *Glomus MO-G13*). The other Allpress site, field H48 growing leek also had VTX00065 (33%), VTX00113 (15.3%) and VTX00281 (10.2%) as the top three most abundant taxa, although VTX00065 was less dominant. The fourth most abundant taxon at this site was VTX00114 (7.9% - *Glomus MO-G17*), and the fifth, VTX00105 (6.6% - *Glomus intraradicies).* The Euston site shared a similar subset of taxa, with the four most abundant: VTX00281 (31.6%), VTX00065 (21.5%), VTX00113 (19.8%) and VTX00105 (7.7%) also being present at one or both of the Allpress sites. The fifth most abundant taxon at this site was VTX00295 (6.4% - *Glomus Glo-A*). The Holkham site's top two most abundant taxa were also shared with the other sites. VTX00065 made up of 61.8% of reads from the site, whereas VTX00281 comprised a further 10.7%. VTX00153 (*Glomus MO-G12*) represented 10.2% of reads, VTX00067 (*Glomus mosseae*) 6.3%, and VTX00342 (*Glomus VeGlo18*) made up 4% of reads across the site. Like the Holkham site, the North moor field site also had VTX00065 and VTX00281 as its most dominant taxa, with 65.1% and 19% of reads, respectively. VTX00064 (*Glomus MO-G18*) represented 5.8% of reads, VTX00342 comprised 3.8%, and VTX00052 (*Scutellospora MO-S2*) made up another 2.5% of reads. The five most abundant taxa at each site are visualised in Figure 5.28.

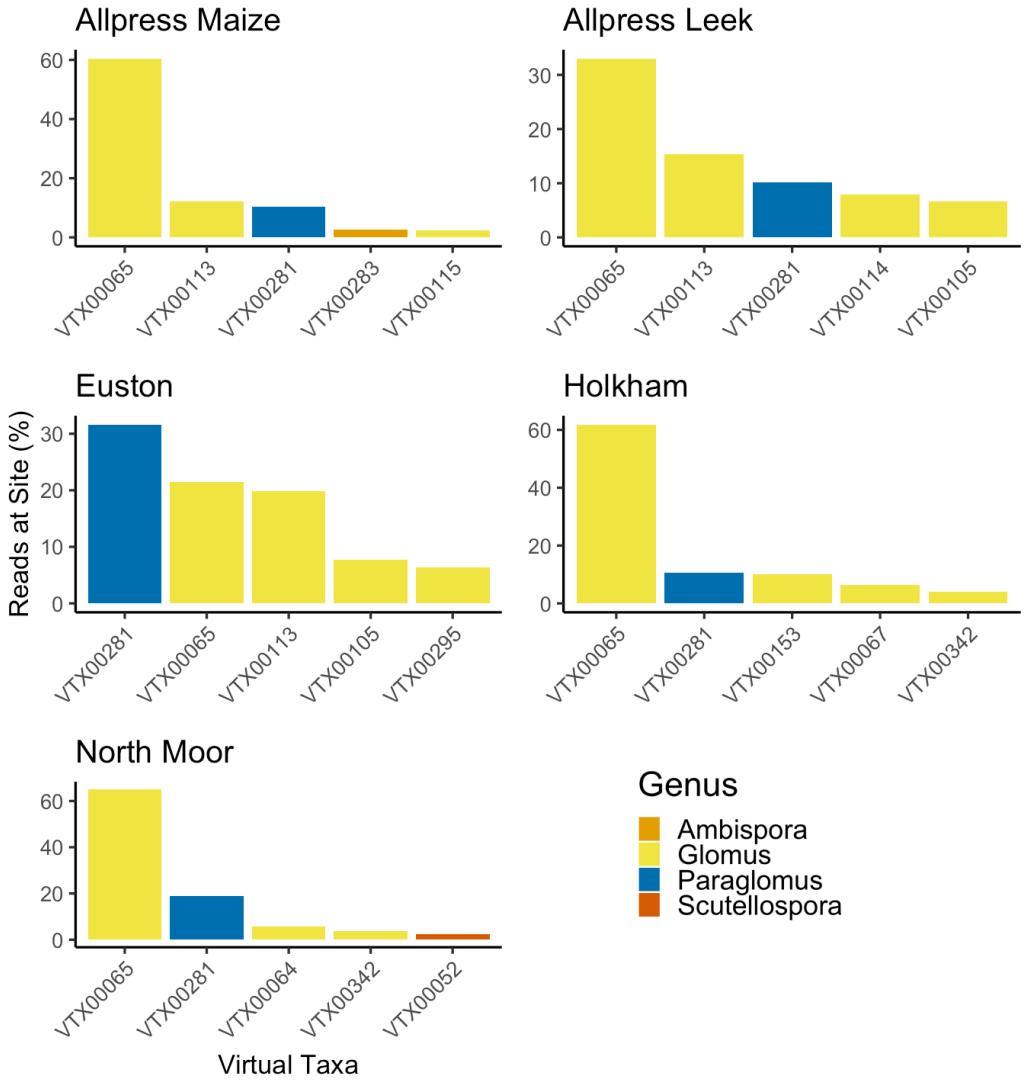


Figure 5.28 Top five taxa by abundance across experimental treatments at each IF site. Top five AM fungal taxa at each site, expressed as a percentage of the total reads at that site.

AM Fungal Community Composition at Each IF Site

Five species were observed in all treatments at the Allpress H27 site growing maize, including two *Glomus*, one *Paraglomus*, one *Archaeospora*, and one *Ambispora* (Table 5.2). Two taxa, one each of *Glomus* and *Claroideoglomus* were observed exclusively in the control plot. Two taxa, both belonging to *Glomus* were observed exclusively in the experimental plot receiving addition of AD. One *Diversispora* was observed exclusively in the cover crop only plot, and there were no AM fungal taxa found only in the cover crop and AD plot. The three taxa found exclusively in the plot receiving an AM fungal inoculum included two *Glomus* and one *Claroideoglomus.* VTX00067 (*Glomus mosseae)* was found in both the control and cover crop and AD plots, whilst VTX00342 (*Glomus VeGlo18)* was found in the two plots receiving application of AD. VTX00061 (*Diversispora epigaea*) and VTX00435 (*Paraglomus MO-P4*) were found in the control, AD only, and cover crop and AMF plots, while VTX00052 (*Scutellospora MO-S2*) was found in the control, cover crop only and cover crop and AD plots. Finally, VTX00108 (*Glomus Whitfield type7*) was found in the AD only, cover crop only, and cover crop and AD plots.

Table 5.2 AM Fungal VT found in each treatment at the Allpress H27 site growing maize. VT found exclusively in that treatment are in black, with taxa shared between treatments shown in grey.

Control	AD Only	Cover Crop Only	Cover Crop and AD	Cover Crop and AMF
VTX00064	**VTX00115**	**VTX00354**	VTX00113	**VTX00143**
VTX00057	**VTX00307**	VTX00113	VTX00065	**VTX00114**
VTX00113	VTX00113	VTX00065	VTX00281	**VTX00278**
VTX00065	VTX00065	VTX00281	VTX00245	VTX00113
VTX00281	VTX00281	VTX00245	VTX00283	VTX00065
VTX00245	VTX00245	VTX00283	VTX00052	VTX00281
VTX00283	VTX00283	VTX00052	VTX00108	VTX00245
VTX00061	VTX00061	VTX00108	VTX00067	VTX00283
VTX00435	VTX00435		VTX00342	VTX00061
VTX00052	VTX00108			VTX00435
VTX00295	VTX00295			
VTX00067	VTX00342			

In the Allpress H48 site growing leek, eight taxa, including five *Glomus*, two *Paraglomus*, and one *Claroideoglomus* were observed in all plots (Table 5.3). Two taxa, one each of *Glomus* and *Diversispora* were observed exclusively in the control plot, and a further three taxa, one *Glomus*, one *Paraglomus*, and one *Diversispora* were observed exclusively in the cover crop and AD plot. There were no taxa found exclusively in the AD only, cover crop only, or cover crop and AMF plots. VTX00340 (*Claroideoglomus Glo G8)* was found in both the control and AD only plots, VTX00306 (*Diversispora sp.)* in the control and cover crop and AD plots, and VTX00342 *(Glomus VeGlo18)* was found in both plots receiving addition of AD. Two taxa,

VTX00052 (*Scutellospora MO-S2*) and VTX00338 (*Archaeospora Aca*) were found in the control, AD only, and cover crop and AMF plots, three taxa, VTX00114 (*Glomus MO-G17*), VTX00108 (*Glomus Whitfield type7*), and VTX00307 (*Glomus sp.*) were found in the control, cover crop and AD, and cover crop and AMF plots, and VTX00064 (*Glomus MO-G18*) was found in the three plots which followed a cover crop. VTX00105 (*Glomus intraradicies)* was found in all plots except the control, VTX00245 (*Archaeospora trappei)* and VTX00283 (*Ambispora fennica)* were found in all plots except the cover crop only plot, and VTX00115 (*Glomus MO-G13)* was observed in all plots except the AD only plot.

Table 5.3 AM Fungal VT found in each treatment at the Allpress H48 site growing leek. VT found exclusively in that treatment are in black, with taxa shared between treatments shown in grey.

Control	AD Only	Cover Crop Only	Cover Crop and AD	Cover Crop and AMF
VTX00309	VTX00143	VTX00143	**VTX00153**	VTX00143
VTX00354	VTX00113	VTX00113	**VTX00380**	VTX00113
VTX00143	VTX00295	VTX00295	**VTX00349**	VTX00295
VTX00113	VTX00065	VTX00065	VTX00143	VTX00065
VTX00295	VTX00067	VTX00067	VTX00113	VTX00067
VTX00065	VTX00057	VTX00057	VTX00295	VTX00057
VTX00067	VTX00281	VTX00281	VTX00065	VTX00281
VTX00057	VTX00435	VTX00435	VTX00067	VTX00435
VTX00281	VTX00245	VTX00115	VTX00057	VTX00245
VTX00435	VTX00283	VTX00105	VTX00281	VTX00283
VTX00245	VTX00105	VTX00064	VTX00435	VTX00115
VTX00283	VTX00052		VTX00245	VTX00105
VTX00115	VTX00338		VTX00283	VTX00052
VTX00052	VTX00340		VTX00115	VTX00338
VTX00338	VTX00342		VTX00105	VTX00114
VTX00114			VTX00114	VTX00108
VTX00108			VTX00108	VTX00307
VTX00307			VTX00307	VTX00064
VTX00340			VTX00064	
VTX00306			VTX00306	
			VTX00342	

At the Euston site, nine taxa were observed in all three of the sequenced plots (excluding AD only) including five *Glomus*, two *Paraglomus*, one *Diversispora*, and one *Archaeospora* (Table 5.4). One *Diversispora* taxon was found in the control plot only, with two further *Diversispora* taxa observed in the plot growing cover crop without addition of AD. There were no unique taxa were observed in the cover crop and AD plot. One taxon, VTX00067 (*Glomus mosseae)* was found in the control and cover crop only plots, while two taxa VTX00114 (*Glomus MO-G17*) and VTX00307 (*Glomus sp.*) were shared between the control and cover crop and AD plots.

Table 5.4 AM Fungal VT found in each treatment at the Euston Site. VT found exclusively in that treatment are in black, with taxa shared between treatments shown in grey.

Control	Cover Crop Only	Cover Crop and AD
VTX00060	**VTX00061**	VTX00143
VTX00143	**VTX00380**	VTX00113
VTX00113	VTX00143	VTX00105
VTX00105	VTX00113	VTX00295
VTX00295	VTX00105	VTX00065
VTX00065	VTX00295	VTX00306
VTX00306	VTX00065	VTX00281
VTX00281	VTX00306	VTX00435
VTX00435	VTX00281	VTX00245
VTX00245	VTX00435	VTX00114
VTX00067	VTX00245	VTX00307
VTX00114	VTX00067	
VTX00307		

At the Holkham site the three sequenced treatments, AD only, cover crop only, and cover crop and AD shared six taxa, including four *Glomus*, and one of each of *Paraglomus* and *Ambispora* (Table 5.5). The plot growing the cover crop only had four further uniquely observed taxa, including two *Glomus*, one *Diversispora*, and one *Archaeospora*. One Diversispora taxon was found exclusively in the plot growing cover crops and receiving an AD application, and there were no unique taxa found in the AD only treatment plot. Three taxa, VTX00105 (*Glomus intraradicies*), VTX00295 (*Glomus Glo-A*), and VTX00435 (*Paraglomus MO-P4*) were found in both cover cropped plots grown with and without the addition of AD.

Table 5.5 AM Fungal VT found in each treatment at the Holkham Site. VT found exclusively in that treatment are in black, with taxa shared between treatments shown in grey.

All	AD Only	Cover Crop Only	Cover Crop and AD
VTX00105	VTX00153	**VTX00222**	**VTX00306**
VTX00295	VTX00342	**VTX00114**	VTX00153
VTX00435	VTX00065	**VTX00060**	VTX00342
	VTX00067	**VTX00245**	VTX00065
	VTX00281	VTX00153	VTX00067
	VTX00283	VTX00342	VTX00281
		VTX00065	VTX00283
		VTX00067	VTX00105
		VTX00281	VTX00295
		VTX00283	VTX00435
		VTX00105	
		VTX00295	
		VTX00435	

At the North Moor site, four taxa were shared between all treatments, including three *Glomus* and one *Paraglomus* (Table 5.6). Three further taxa were present in the treatment receiving cover cropping and AD application, including two of *Glomus* and one *Ambispora* taxon. The plots receiving AD only, or the cover crop only, hosted one unique *Glomus* taxon each. One final taxon VTX00064 (*Glomus MO-G18)* was found in all plots except the cover crop only treatment. Numbers of observed taxa in each treatment are visualised in Figure 5.29.

Table 5.6 AM Fungal VT found in each treatment at the North Moor Site. VT found exclusively in that treatment are in black, with taxa shared between treatments shown in grey.

All	Control	AD Only	Cover Crop Only	Cover Crop and AD
VTX00052	VTX00143	**VTX00113**	**VTX00067**	**VTX00295**
	VTX00342	VTX00143	VTX00143	**VTX00354**
	VTX00065	VTX00342	VTX00342	**VTX00283**
	VTX00281	VTX00065	VTX00065	VTX00143
	VTX00064	VTX00281	VTX00281	VTX00342
		VTX00064	VTX00052	VTX00065
				VTX00281
				VTX00064
				VTX00052

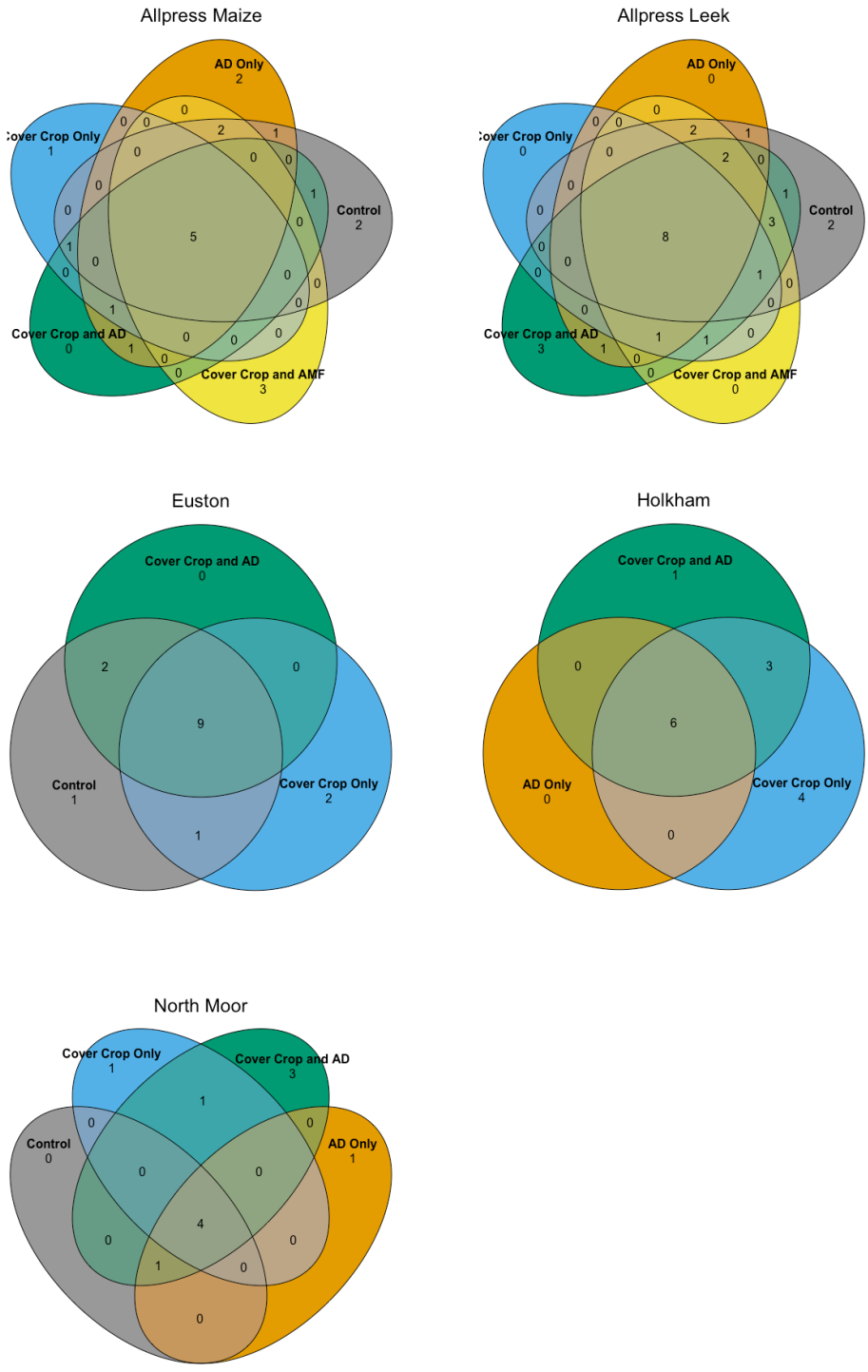


Figure 5.29 Number of AM fungal virtual observed in each experimental treatment in the IF trial. Venn classification of AM fungal VT observed in each experimental treatment. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT.

Differential Abundance of AM fungal taxa in the IF trial

Analysis of differential abundance of virtual taxa, using shrinkage estimation was conducted to further confirm how individual taxa had responded to experimental treatments, across the trial. One taxon, VTX00153 (*Glomus MO-G12)* was significantly higher abundance in the treatment following the cover crop and AD treatment, when compared to the control ($\log_2$FC = 24.6, $p < 0.001$, Figure 5.30a). One taxon, VTX00114 (*Glomus MO-G17)* was significantly higher abundance in the treatment cover crop and AD, when compared to the treatment receiving only AD ($\log_2$FC = 23.9, $p < 0.001$, Figure 5.30b). There were no differentially abundant taxa between the other plot treatments at the adjusted $p < 0.05$ level, though care should be taken when assessing global differential abundance, due to the small sample size.

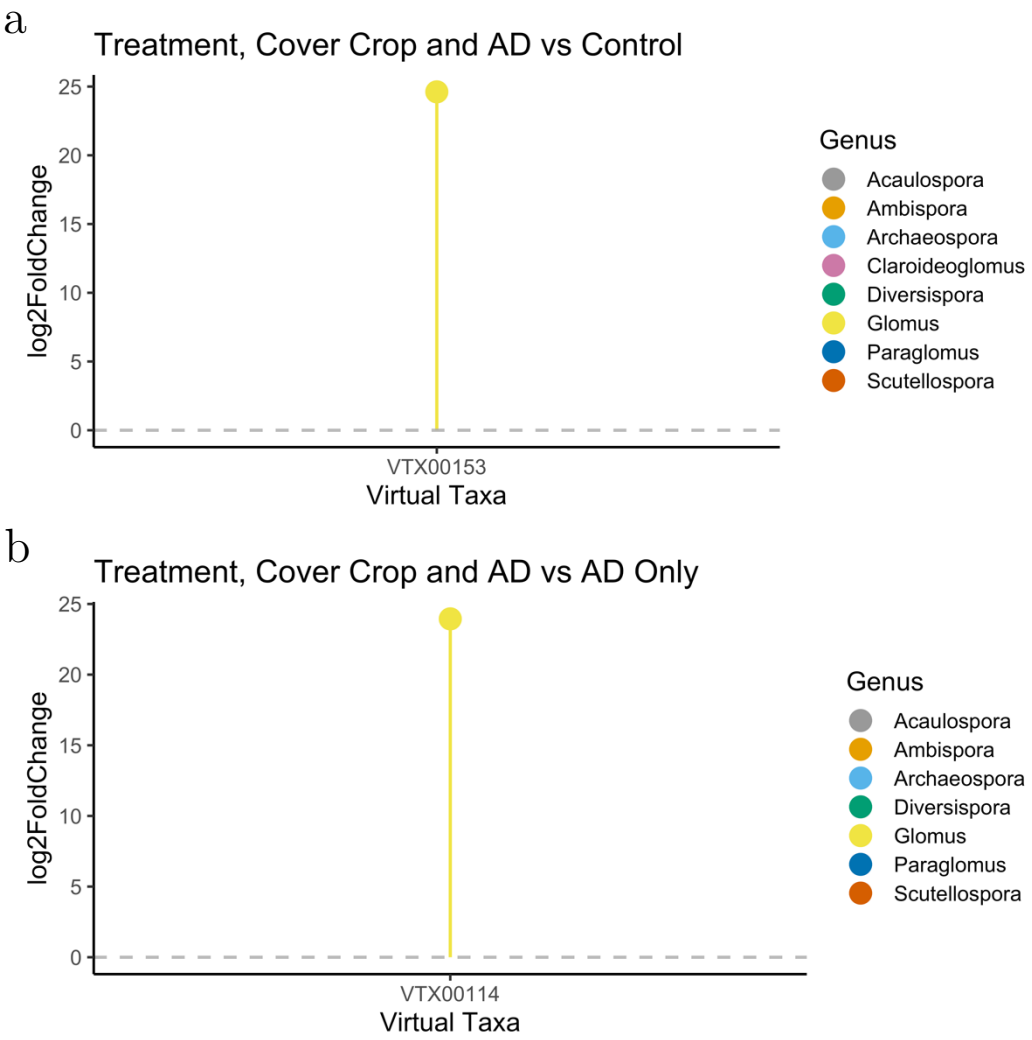


Figure 5.30 Differential abundance of AM Fungal VT comparing a) cover crop and AD vs control, and b) cover crop and AD vs AD only. Differential abundance of AM fungal VT comparing experimental treatments, calculated by DESeq2. AM VT were considered to be differentially abundant if their $Log_2FC > 1$ and $p < 0.05$.

Within individual sites, at the Allpress H27 site growing maize, VTX00113 (*Glomus MO-G3*) was considerably more abundant in the AD only treatment, comprising 41.9% of reads, compared to 4.8% (range 1.5-9.9%) for the other treatments. VTX00065 (*Glomus caledonium*) was considerably less abundant in this treatment, comprising 33.2% of reads. This taxon represented 67.2% (52.1-89.9%) of reads across the other treatments at this site,

being most abundant in the plot receiving the cover crop only. VTX00281 (*Paraglomus laccatum*) comprised 22.6% and 15.6% of reads in the cover crop and AMF and control plots respectively, which decreased to 3.2%, 6.3%, and 4% in the cover crop, AD only, and cover crop and AD treatment plots. VTX00283 (*Ambispora fennica*) was found in the highest abundance in the control plot (8.9% reads), dropping to 1.2% (0.6-2.3%) in the other treatments. AM fungal inoculation did not seem to have a considerable impact on community composition. VTX00113 (corresponding to *Rhizophagus irregularis)* comprised 3.8% of reads in the inoculated plot, but ranged from 1.5-41.9% in other treatments. VTX00114 (corresponding to *Rhizophagus irregularis)* was only found in the inoculated plot, but at very low abundance (1.7%). On the contrary, VTX00115 (corresponding to *Rhizophagus irregularis)* was only found in the AD only treatment plot, where it comprised 12.3% of reads. VTX00065 (corresponding to *Funneliformis geosporum)* was found at high abundance in all treatment plots, and the inoculated plot (55.7%) was well within the range of other treatments, which ranged from 33.2 to 89.9%. VTX00067 (corresponding to *Funneliformis mosseae)* was found in the control and cover crop and AD treatment plots, but absent from the inoculated treatment. Neither VTX00193 (corresponding to *Claroideoglomus claroideum*), nor VTX00104 (*corresponding to Glomus microaggregatum)* were found in the trial.

At the Allpress H48 site growing leek, VTX00065 (*Glomus caledonium)* was the dominant taxon, comprising 67.6% of reads in the cover crop and AD treatment. This figure dropped to 47.1% in the AD treatment plot, 27.7%

in the cover crop only plot, 15.7% in the control and only 6.9% in the AM fungal inoculated plot. VTX00113 (*Glomus MO-G3)* comprised 39.2% of reads in the AM fungal inoculated cover crop plots, 17.9% in the control plots, dropping to 10.5% in the AD treatment, 8.2% in the cover crop treatment, and 0.9% in the cover crop and AD treatment. VTX00114 (*Glomus MO-G17*) comprised 33% of reads in the control, dropping to 5.1% in the cover crop and AMF plot, 1.2% in the cover crop and AD plot, and absent in the cover crop only and AD only plots. The third most abundant taxon, VTX00281 (*Glomus laccatum)* was most abundant in the cover crop plot (23.5%), dropping to 11.1% in the cover crop and AMF treatment, 7.4% in the AD only treatment plot, 4.9% in the cover crop and AD plot, and 4.2% in the control. Once again, inoculation by AM fungi had no clear impact on the abundance of the component taxa. VTX00113 (corresponding to *Rhizophagus irregularis)* was considerably more abundant in the inoculated plots (39.2%) when compared to the other treatments (range 0.9-17.9%). Both VTX00114 (corresponding to *Rhizophagus irregularis)* and VTX00115 (corresponding to *Rhizophagus irregularis)* were found in inoculated plots, but were not found in higher abundance than in the control and cover crop plots, respectively. VTX00067 (corresponding to *Funneliformis geosporum)* was in lowest relative abundance (6.9%) in the inoculated plots, ranging from 15.7% in the control to 67.6% in the cover crop and AD treatment plot. VTX00067 (corresponding to *Funneliformis mosseae)* in the inoculated plot was in the second highest relative abundance (5.4%) after the cover crop only treatment (7.8%), although all plots had some representation of this taxon.

At the Euston site, the most common taxon, VTX00281 (*Glomus laccatum)* was found in high abundance at each of the three sequenced treatments. VTX00281 represented 26.5%, 49.5%, and 18.8% of reads in the control, cover cropped, and cover crop and AD treatment plots, respectively. The second most abundant taxon, VTX00065 (*Glomus caledonium)* represented 39.8% of reads in the control plot, decreasing to 15.5% in the cover cropped plot, and 9.2% in the cover crop and AD plot. The third most abundant taxon, VTX00113 (*Glomus MO-G3)* was only found in low abundance in the control and cover crop only treatment plots, representing 4.1% and 0.9% respectively. VTX00113 was the most dominant taxon in the cover crop and AD treatment, representing 54.6% of total reads. VTX00306 (*Diversispora sp.)* comprised 4.4% of reads in the plot receiving cover cropping without application of AD. This taxon was considerably less abundant in the control (1.3%) and cover crop and AD (0.2%) plots.

At Holkham, the most abundant taxon, VTX00065 (*Glomus caledonium)* made up 62.3%, 76.3% and 46.8% of reads for the cover crop only, cover crop and AD, and AD only treatment plots, respectively. Reduced abundance of this taxon in the AD only treatment plots was seemingly to the benefit of VTX00067 (*Glomus mosseae),* which increased from 2.2% and 1% in the cover crop and cover crop and AD treatments, to 15.6% in the AD only treatment. There were more subtle increases in VTX00342 (*Glomus VeGlo18*), which increased from 3.1% and 0.1% to 8.8% of treatment reads, as well as VTX00153 (*Glomus MO-G12* - increased from 8.8% and 6.9% to

14.8%) and VTX00283 (*Ambispora fennica* - increased from 0.2% and 1.7% to 5.6%).

Finally, at the North Moor farm site, the most abundant taxon, VTX00065 (*Glomus caledonium)* ranged from 76.3% of reads in the AD only treatment, to 69.7% and 68.6% in the cover crop and AD and control plots, respectively, and 45.8% in the cover crop plot. This reduction in proportional reads resulted in increased colonisation of VTX00052 (*Scutellospora MO-S2)* which represented 8.7% of reads, up from 1.1% in the cover crop and AD treatment and absent from the control and AD only treatments. Furthermore, 4.1% of reads from the cover crop only plot was attributed to VTX00067 (*Glomus mosseae)* which was not found in any other plot. VTX00064 (*Glomus MO-G18),* the third most abundant taxon was not found in this plot, but represented 16.6% of reads in the cover crop and AD treatment. The control, and AD only plots had 1.4% and 5.1% of these sequence reads, respectively. The second most abundant taxon VTX00281 (*Glomus laccatum)* was the second most abundant taxon in the control (25.6%), cover crop (30.5%), and AD only (14.9%) treatments, but only represented 4.9% of reads in the cover crop and AD plot. Breakdown of AM fungal community composition is visualised in Figure 5.31.

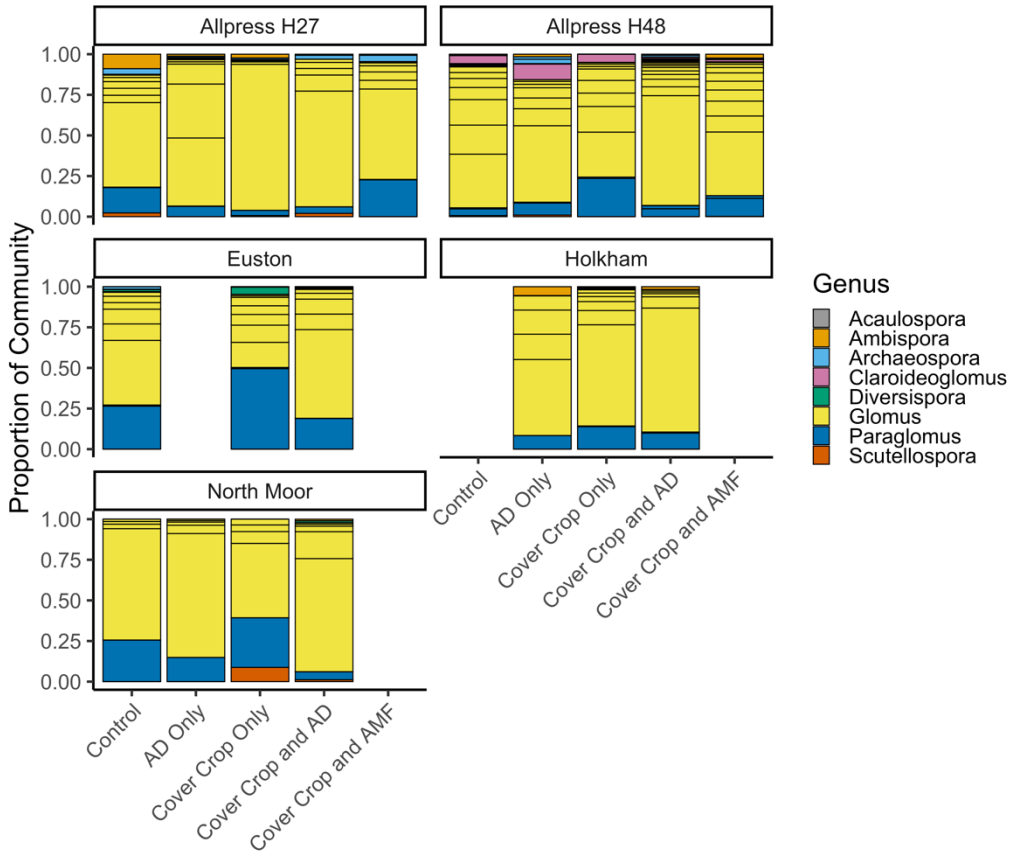


Figure 5.31 AM Fungal community composition in the IF trial.
Mean community composition of AM fungal VT for each experimental treatment, expressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT.

5.4 Discussion

In this chapter data is presented from the Innovative Farmers' Field Lab: Increasing Nutrient Efficiency From Anaerobic Digestate. All of the sites presented had good crop growth, both in terms of cover and cash crop, although this was not the case for all sites within the Field Lab. The cover crop at the Boxworth site failed to establish, due to application of AD scorching the crop. For this reason, as well as simplicity of analysis, the four farm sites growing maize were selected for analysis in this thesis. It was fortunate that the Allpress site was in discussion with PlantWorks, who had supplied the same AM fungal inoculum as was used in Chapter Three, to run a supplementary treatment in the IF trial. Analysis at the Allpress site therefore could compare the impact of the previously used AM fungal inoculum as well as the core IF experimental treatments, on two crop species in field conditions.

Maize growth was lower than expected, due to the hot and dry summer which also impacted the NFS trial in Chapter Four. For this reason, coupled with the difficulty of gaining true yield data for maize in a busy harvest period, all four farmers opted not to take field scale yield measurements for the trial. Although frustrating, plant growth responses have been shown as plant shoot biomass, and this experience has provided a valuable lesson on working with farmers on farm-based trials. Eventually, none of the experimental treatments had a positive impact on crop biomass across sites, in some cases having a negative effect compared to the control. This effect was

negated in both of the Allpress AM fungal inoculated plots, with the treatment having a more beneficial impact in the highly mycorrhizal leek crop. In leek, the cover crop and AD, and cover crop and AM fungal inoculated plot also appeared to result in increased shoot biomass, although again this was not statistically significant, and standard errors were large.

Quantification of RLC by trypan blue was considerably easier in maize and leek than for barley, as fungal structures in these crops are significantly clearer and crisper, an observation also made in in vitro barley plants grown by the Paszkowski lab. There were no resounding relationships to be drawn between experimental treatments and RLC, and at many sites standard errors were large. At the Euston site, application of AD to cover crops ahead of maize seemed to reduce maize RLC, but the same treatment had a small positive effect at the Holkham site. Addition of the AM fungal inoculum to the cover crop at the Allpress maize site also did not increase maize RLC. This is a similar finding to Berruti et al. (2017), who also found that a microbial inoculum containing eight AM taxa, as well as three fungal saprotrophs and one PGPR were unable to increase RLC of maize in Italy. In the Allpress Farm leek crop, the control achieved a RLC of 99.5%, which made evaluation for improvement in RLC difficult. However, the two cover crop treatments, both with and without addition of AD, decreased leek RLC, an observation found in contrast to Sorensen et al. (2005). When the cover crop was inoculated with an AM fungal inoculum, RLC was restored to the

levels achieved following the fallow plot (Figure 5.32). However, and as outlined below, this increase in RLC was not directly attributable to the components of the inoculum.

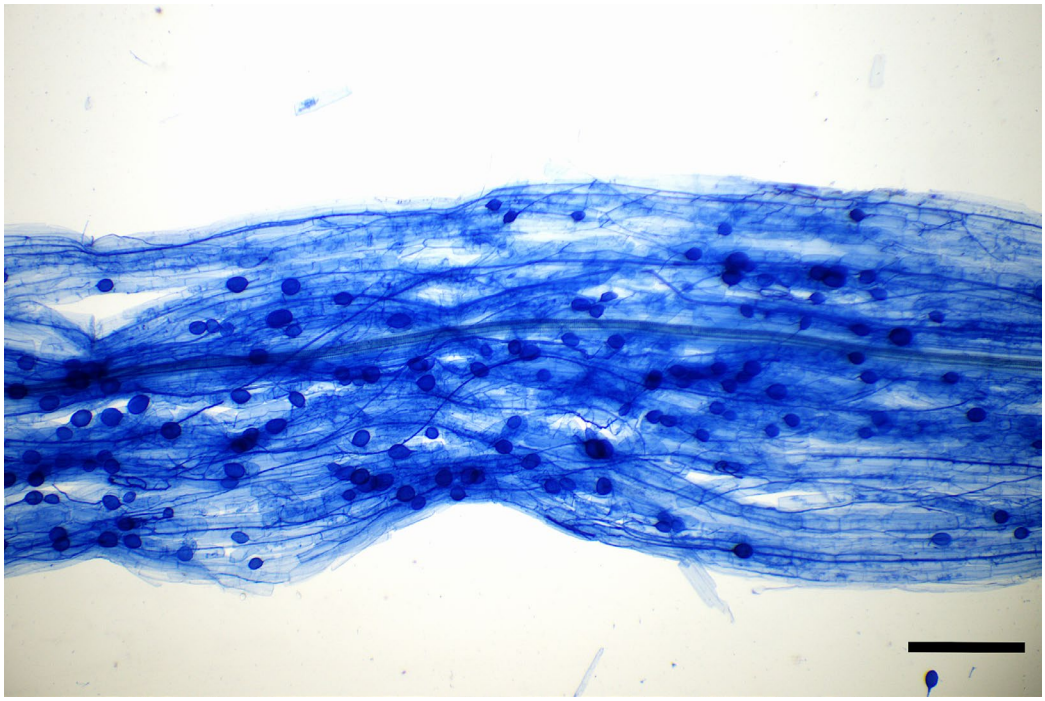


Figure 5.32 AM Fungal community colonisation of leek by large numbers of vesicles at the Allpress Farm site.

Unfortunately, despite considerable experimentation, the AM fungal specific 18S rDNA region was not able to be amplified from the AD treatment from Euston, or the control plot from Holkham. Once again, this was a disappointing outcome, least not as the reason behind the lack of amplification was never discovered. It is speculated that despite the relatively high (25-50%) RLC in these samples, actual fungal biomass in these samples may have been too low to amplify. Nevertheless, the remaining 20 samples were

successfully amplified, revealing differences in AM fungal community composition due to site, but not the preceding cover crop or AD application, in maize. This is an unsurprising outcome given the large plot areas and low numbers of replicates, and furthermore, geographic location has been shown to influence AM fungal communities to at least as greater an extent as biotic or abiotic treatments at a site (Kivlin et al. 2011; Yang et al. 2021). Additionally, results presented are in agreement with Coelho et al. (2020) who found that multiple additions of AD in a temperate grassland were unable to influence soil microbial communities.

Within each site, experimental treatments had variable impact on the observed numbers of AM fungal taxa. The hypothesis that addition of AD may reduce the diversity of experimental plots was not withstanding, having a positive, negative, and neutral effect on AM diversity at different sites. Likewise, addition of cover crops had impacts ranging from negative to positive. At the two Allpress farms sites, cash crops following the cover crop had reduced diversity in both instances. Addition of the AM fungal inoculum negated some of the negative impacts but did not restore levels back to that of the fallow plot. There was some evidence that the introduced inoculum taxa had established in these plots, with the most profound impacts being the increase in *Rhizophagus irregularis* taxa in both maize in leek, although to varying levels. This is in line with findings by Berruti et al. (2017), who found that inoculated AM fungal taxa were unable to colonise maize roots, and lacked persistence in arable soils. Like Berruti et al., this study found that community composition of the inoculated plots was more even than

some of the other treatment plots, but inconsistently, and in both cases, less than the fallow plots.

Analysis of differential abundance revealed that across the IF trial, VTX00153 (*Glomus MO-G12*) and VTX00114 (*Glomus MO-G17*) was significantly higher in abundance following the cover crop and AD treatment, when compared to the control and AD only treatments, respectively. Interestingly, Vályi et al. (2015) found that VTX00153 (*Glomus MO-G12*) and VTX00114 (*Glomus MO-G17*) were indicative of low and high land use intensity (LUI), respectively, where LUI was indexed by intensity of N fertilisation, frequency of mowing, and intensity of livestock grazing of the grassland sites. Despite the addition of N in the form of AD, VTX00153 (*Glomus MO-G12*) was more abundant than in the control, across trial sites, suggesting a role for cover crops in reducing the impact of the LUI resulting from N addition. However, the opposite is true of VTX00114 (*Glomus MO-G17*), which if this hypothesis were correct, would be lower in abundance following the cover crop plots receiving AD application. In addition to this, VTX00114 (*Glomus MO-G17*) was found to respond positively to drought conditions, suggesting that as well as being suited to high levels of LUI, this taxon is also tolerant of abiotic stress (Lumini et al. 2014). Finally, a study by Van Geel et al. (2017) investigating the drivers of AM fungal diversity in European seminatural grasslands found that both VTX00153 (*Glomus MO-G12*) and VTX00114 (*Glomus MO-G17*) were shown to have preference for alkaline pH sites, being completely absent at pH lower than 7.7. Chemical analysis of the AD, at least at the Allpress farm site, showed that the AD had

a pH of 8.1, slightly higher than some of the field sites. This may have increased pH sufficiently to improve soil conditions for these two taxa, which in some cases were more abundant in the higher pH sites, or low pH plots which had received addition of AD. Clearly, the interaction between geography, farming system, management, as well as other biotic and abiotic factors have complex and interdependent effects on AM fungal diversity, at the farm scale.

One of the main interests of the farmer group, was whether cover crops would be effective at taking up the available N from the applied AD, reducing any potentially detrimental impacts resulting from volatilisation or leaching. Cover crops grown alone, and with application of AD, reduced the available N in the soil profile, and especially at deeper (60-90cm) soil horizons. At the Allpress maize site, the additional AM inoculated cover crop treatment further reduced soil available N, and as cover crop shoot biomass was not increased in this treatment, it is speculated that root biomass may have been increased by AM fungal inoculation, although this was not reliably measured. This reduction in available N in the inoculated plot was not observed in the Allpress leek crop, despite considerably higher growth of the radish cover crop in this treatment. It is plausible that the slight increase in vetch biomass, coupled with increased levels of plant-released flavonoids resulted in an increased interaction with nitrogen fixing bacteria in vetch root nodules, which was then released to the soil. However, a more parsimonious conjecture would be that in-field variation, such as the aspect of the field, may have influenced the N availability. Regardless of this individual result,

it is clear that cover crops may have an important role in the reduction of volatilisation and leaching in NVZs.

Another area of interest for the farmer group was improvement of SOM, via cover cropping and addition of AD. Both the Allpress and North Moor maize fields benefitted from experimental treatments, with SOM increasing most prominently in the AD, and cover crop treatments at the North Moor site. Interestingly, the benefit resulting from the combination of cover crop and AD was not additive, although it was still improved compared to the control. The two sites on sandy soils showed neutral or negative responses to SOM resulting from cover crops or AD application. This has also been shown by Mouhamad et al. (2015), who found that soils with low clay content accumulated additional SOM at a slower rate than high clay soils. This observation has also been made by soil scientist Dr Lizzie Sagoo (ADAS, *Pers. comm.)*, and may be explained by if these sites lack the toolkit to effectively break down and store SOM. In the AM fungal inoculated plots at the Allpress Farm site, addition of the inoculum to the cover crop resulted in a negligible impact on SOM in the maize field. However, in the leek field H48, despite a reduction in SOM in the cover crop, AD, and cover crop and AD plots, the inoculated cover crop plot experienced a marginal increase in SOM compared to the control. This is in line with findings from an Annual Review by Frey (2019), who showed a strong link between AM fungi and accumulation of SOM, potentially via direct competition with free-living saprotrophs. It is important to highlight that as this trial is not spatially repli-

cated within sites, these results might simply be reflective of natural variation in SOM across the field. Nevertheless, experimentation at the field scale is invaluable for validating hypotheses in real world scenarios, and experimental error is an unavoidable outcome of these kinds of trials.

One final area of interest for the farmer group, was the ability of cover crops to improve soil structure. In the IF trial, soil structure was quantified by the VESS method. Despite being a relatively fast, and easily accessible method of measuring soil structure, results have been shown to correlate strongly with the use of a soil penetrometer (used in Chapter Three), which costs thousands of pounds, and will not be accessible to the majority of farmers and growers (Guimarães et al. 2013). Once more, there were inconsistent effects of experimental treatments, which may simply reflect in-field variation. Regardless, data from the North Moor site suggests that cover crops, grown with or without the addition of AD had a detrimental impact on soil structure. This same effect was seen to a lesser extent at the Allpress site, and to a very minor extent at the Holkham site with no differences in VESS at Euston. This result, although not entirely robust, is in contradiction to the commonly held understanding that over crops can alleviate compaction and improve soil structure (Stobart and Gosling 2015). At the Allpress leek field, there were very little differences between experimental treatments, with the cover crop only treatment resulting in slightly worse soil structure, but slightly improving soil structure when grown with AD. It should be noted that the soil quality and structure at both of the Allpress sites is a fantastic peaty soil, with very good structure, so the potential for treatments to provide benefit was more limited than at some of the other

sites. Nevertheless, addition of the AM fungal inoculum to the cover crop improved soil structure at both Allpress field sites, when compared to the experimental treatments and to a lesser extent, the control. This is one of the known benefits of increasing AM fungal abundance in soil, associated with the production of the stable glycoprotein glomalin. Glomalin is produced in abundance by mycorrhizal hyphae, and has been linked to remediation of polluted soils, soil structural improvement, and climate mitigation (González-Chávez et al. 2004; Wilson et al. 2009; Singh et al. 2013).

5.5 Conclusion

In this chapter, data is presented from five field sites at four farms from the Innovative Farmers Field Lab: Increasing Nutrient Efficiency From Anaerobic Digestate. The Innovative Farmers Programme is part of the Duchy Future Farming Programme, and funded by the Prince of Wales' Charitable Fund. The IF programme is a fantastic means of aligning the research conducted by universities and research institutions, to the needs of real farmers in the field. Furthermore, discussion between farmers, on a shared interest was stimulating, engaging, and truly innovative in nature. Continuation of the IF programme, or other similar programmes will help to bridge the knowledge gap between academia and farmers, and surely accelerate innovation in agriculture in the UK, and worldwide.

6 Chapter Six – General Discussion and Concluding Remarks

The present study aimed to determine the impact of cover cropping and soil amendments on crop growth and yield, as well as soil health characteristics focussing on AM fungal populations. With an approach spanning a range of scales, from glasshouse experiments to UK-wide trials, the impacts of cover crops and soil amendments have been rigorously investigated. In Chapter Two a UK-wide analysis of AM fungal diversity, exploring factors underpinning community composition was presented. Next, the impact of cover cropping, as well as a commercial AM fungal inoculum was tested at field scale, with results being compared to a controlled glasshouse trial. Three successional crops were assessed, to explore the longevity of the AM fungal inoculum, and assess the benefits from various cover crop mixtures. The New Farming Systems Fertility Building Rotations trial was the testbed for Chapter Four, focussing on the impact of cover crops, and different N fertilisation regimes on AM fungal diversity and abundance. These two replicated trials provided background for the field scale trials in Chapter Five, which were conducted as part of farmer-interactive Innovative Farmers Field Lab. The field lab utilised the cover crop mixture and AM fungal inoculum from Chapter Three, and further explored the impact of N fertilisation (Chapter Four) in this case in the form of AD, the biproduct of anaerobic digestion. Evaluating results across a range of spatial scales, culminating in field scale trials has allowed for project hypotheses to be rigorously evaluated.

It is anticipated that the results from this thesis will contribute to guidance for farmers and growers on the use of cover crops within a crop rotation, and the potential for cover crops and other farming practices to impact soil health, including the diversity and abundance of AM fungi.

6.1 Factors Underpinning the AM Fungal Symbiosis in UK Agriculture

The UK-wide analysis of AM fungal diversity in Chapter Two was conducted to establish a baseline for analysis of experimental trials, and to conduct high-level analyses on some of the factors underpinning AM fungal diversity in UK agriculture.

The project was established to investigate unusual findings from the FERA Big Soil Community project, which sequenced 16S and ITS amplicons for assessment of diversity of bacteria and fungi in UK agriculture. At the dissemination of results by the FERA group, it was concluded that only one species of AM fungi was found in the ~250 assessed samples, and at very low abundance. Following discussion with John Elphinstone, the project lead at FERA, a subset of samples was shared for ruderal RFLP analysis, which suggested that multiple AM fungal taxa were indeed present in the samples. A sequencing exercise using AM fungal specific primers on the Illumina Miseq identified 84 AM VT from 148 samples. It is possible that these taxa were initially missed for three reasons. Firstly: the ratio of AM fungal to

other DNA was too low for amplification. Even with AM specific primers, DNA must be diluted 100 fold for successful amplification, so it is likely that the DNA extraction method, chosen by FERA for high DNA yield, may have in fact hampered detecting the AM fungi that were only present at low abundance, as well as other rare species (Tedersoo et al. 2014). Two, commonly used primers for the ITS region are known to exhibit high levels of variation within species, and even spores of a single species for AM fungi (Sanders et al. 1995; Lloyd-Macgilp et al. 1996). Further to this, ITS primers are able to amplify fewer families than 18S, and are known to exhibit poor amplification of the AM fungi (Tedersoo et al. 2015; Lekberg et al. 2018). Finally, the SSU region is commonly used in identification of AM fungal taxa from field samples, therefore the MaarjAM database is more complete for this region. Taken in combination, the methodology of species detection in the FERA Big Soil Community was not suitable for detection of AM fungi.

Automatic forward and backward stepwise model selection of db-RDA showed that the most parsimonious model included cropping system as the sole covariate, that is, whether a site is arable or pasture. This finding was in agreement with others in the literature (Menéndez et al. 2001; Öpik et al. 2006), and the observed numbers of taxa in this study were similar to others reported in the literature, proving some assurance that the threshold values chosen in the bioinformatic pipeline were suitable. Cropping system, as a covariate encompasses a large number of other biotic and abiotic variables,

which will influence AM fungal diversity. Subsequently, after removing cropping system from the model, it was shown that soil texture, cultivation type, and fungicide application shaped AM fungal communities, which have all been shown to influence AM fungal diversity in the literature.

Frustratingly, yield data was not available for many of the arable samples and establishing a proxy for yield from permanent pasture was outside the scope of this PhD. There is much to learn from accurate description of the distribution and diversity of soil microbiota, but given the importance of yield to farmers and growers, yield should be an important consideration for similar trials in the future.

6.2 Role of Cover Crops and Other Amendments on AM Fungal Diversity and Abundance

Impact of Cover Crops on AM Fungal Diversity and Abundance

It is known that cover crops can provide a green bridge for actively growing AM fungi between cash crops, and can increase RLC of subsequent cash crops compared to fallow (Kabir et al. 1999; Lehman et al. 2012; Bowles et al. 2017). In terms of AM fungal diversity, cover cropping has provided inconsistent results from different studies reported in the literature. Authors find that cover crops can have a positive impact on AM fungal diversity (Ramos-Zapata et al. 2012; Higo et al. 2013, 2017) or community composition (Higo et al. 2018), but many authors have also seen no impact on either

diversity or community composition (Njeru et al. 2014b, a; Higo et al. 2014, 2015; Heberle et al. 2015; Turrini et al. 2016).

The impact of cover crops on AM fungal diversity was tested in two replicated trials, and one field scale trial in this thesis. Neither their use in the Bawburgh (Chapter Three), New Farming Systems (Chapter Four), nor Innovative Farmers (Chapter Five) field trials suggested that cover cropping was able to increase diversity of AM fungi under field conditions. However, in the NFS trial, cover cropping treatments led to a significant increase in RLC of the following spring barley crop, an impact more pronounced following the leguminous cover crop mixture. This may have been as a result of the release of rhizobial nod-factors into the soil, which are known to behave in a similar way to mycorrhizal myc-factors, shown to influence the AM symbiosis. The NFS trial was the only one of the three field trials to have featured more than one iteration of cover crop, and it could be that this factor has resulted in the increase in AM fungal spore or hyphal abundance in the soil, which is able to colonise following cash crops to a greater extent.

In the Bawburgh trial (Chapter Three) there was zero differentially abundant taxa due to cover cropping, or AM fungal inoculation. However, there were significant differences in community composition between cash crops/ years, with spring barley hosting 39 taxa, and winter oat hosting 27 taxa, with 13 and 1 taxa being novel to the barley and oat crop, respectively (Figure 6.1). This suggests that either oat is able to host fewer AM taxa than barley, or that the abiotic conditions in the second year of the trial

were not conducive to symbiosis. The 13 taxa associated with barley only included six *Glomus*, three *Diversispora*, two *Claroideoglomus*, and two *Paraglomus*, whereas the oat hosted only one additional *Claroideoglomus* taxon. In addition, two *Glomus*, one *Diversispora* and one *Ambispora* were significantly more abundant in the barley, whereas two *Archaeospora* were significantly more abundant in oat, as assessed by differential abundance analysis in DESeq2 (Love et al. 2014). As discussed in Chapter Three, this effect is likely to have been mainly influenced by abiotic conditions, as there is currently no literature suggesting that oat is disposed to hosting fewer AM taxa than barley, or incompatibility between oat and these absent AM taxa.

Figure 6.1 Winter oat var. Mascani at the Bawburgh trial.

Maize grown in the IF trial, reported to interact and benefit to a greater extent from AM fungi than cereal crops, showed negligible response to a cover crop mixture of fodder radish, oat, vetch, and buckwheat. This result was documented after the particularly hot and dry cropping season, which also impacted maize biomass, and may have influenced results in this cropping year. Cover crops have been shown to increase RLC of subsequent cash crops despite ploughing of soils, but there is also considerable evidence that many AM fungal taxa are strongly impacted by cultivations of all sorts, but especially ploughing (Kabir 2005). AM fungal community composition varied by site, however there was limited evidence that cover cropping, in combination with AD amendments could produce consistent impacts on AM diversity or composition. Impacts on AM fungal diversity ranged from negative to positive, with similar impacts when grown alongside AD application. There was some evidence that cover cropping with AD fertilisation can increase the abundance of two *Glomus* species, potentially due to their preference for alkaline soils, which had been influenced by the higher pH AD. Although this result makes no real contribution to the understanding of how AM fungi respond to farming systems and influence crop growth, it does reinforce the view advanced by many authors that these effects are controlled by complex, and interactive factors, spanning biotic and abiotic elements.

From the data presented, it seems plausible that the short duration of cover crop growth did not lead to a measurable increase in populations of AM

fungi after only one season. However, cover cropping may have minor impacts on the abundance of spores or hyphae in the soil, which are subsequently able to colonise following cash crops. These incremental increases in AM fungal abundance may be additive, and after the five iterations observed in the NFS trial, may have facilitated a considerable and statistically validated increase in fungal RLC. A similar trial by Higo et al. (2014) also had five cover crop iterations, alternating between soybean and cover crop. The authors found no effect of cover cropping on AM fungal diversity, but did not quantify AM RLC, or any other metric for AM fungal abundance. Results from Chapter Five provide some putative evidence for some level of plant – AM fungal host preference, but this may be confounded by other climatic or seasonal effects.

Impact of Nitrogen Amendments on AM Fungal Diversity and Abundance

The impact of N fertilisation is often overlooked in the AM fungal symbiosis, due to the more severe impact of insoluble P on plant RLC. Nevertheless, there is increasing evidence for the role of AM fungi in N uptake and cycling, and a new role for N availability in P mediated RLC suppression.

The impact of nitrogen fertiliser application was tested experimentally in two trials, via ammonium nitrate in the NFS trial (Chapter Four), and by nitrogen rich AD in the IF trial (Chapter Five), at the field scale. There is

some evidence that nutrients applied in an organic form can have less prominent negative impacts on the AM fungi, which should be considered when comparing these two trials.

Perhaps one of the most important observations of the study was found in the NFS trial (Chapter Four). Nitrogen application, especially the full N regime, significantly altered AM fungal community composition, hosting a subset of taxa of the 0% and 50% N regime plots. Most strongly impacted were the taxa within the genus *Glomus* including VTX00108 (*Glomus Whitfield type 7*) which was significantly less abundant in high N plots. In the UK-wide analysis of AM fungal diversity (Chapter Two), VTX00108 (*Glomus Whitfield type 7*) was present at around one quarter of analysed sites, but often at low abundance. This taxon was more abundant in light soil textures, which had received reduced levels of cultivation, further suggesting that this taxon is sensitive to disturbance.

There was no impact of N fertilisation on the observed number of taxa colonising barley roots, although this result did approach the $p < 0.05$ threshold for significance. This effect was not observed in result to application of N rich AD in the IF trial, which showed inconsistent and often conflicting impacts on both the diversity and community composition of AM fungal taxa across the five sites. This may be due to the organic source of N addition (as described above), or the single treatment of AD in these plots. The NFS trial had been running for 11 years at the time of sampling and would

have therefore had 11 years of variable N levels and non-cash crop species interactions to shape AM fungal communities.

Interestingly, despite altering AM fungal community composition at the NFS trial (Figure 6.2), there was no impact of N fertilisation on barley RLC, suggesting some role for N adaptation of AM fungal taxa, which is not controlled by the plant partner. Once again, there was no impact of AD application on maize RLC, but it is not certain whether the single application of AD in this experiment allows for robust conclusions to be made on the impact of AD on AM fungal RLC.

Figure 6.2 Cover crop plots under heavy snow at the NFS trial, January 2018.

These results suggest an effect of fertilisation by N on AM fungal communities, which may be negatively impacting taxa with a largely unknown role in plant N transfer. Further experimentation to understand the molecular mechanisms and control of N transfer in these species will increase our understanding around harnessing AM fungi for broad field N fertilisation of mycorrhizal cash crops. This knowledge may allow for the reduction of inorganic N fertilisation by farmers, whilst maintaining yields, therefore improving profitability, sustainability, and environmental goals.

Impact of AM Fungal Inoculation on AM Fungal Diversity and Abundance

Inoculation of spring barley with a five species AM fungal inoculum was conducted in semi-controlled glasshouse conditions, before assessing the impact of the same inoculum in the replicated Bawburgh trial (Chapter Three). The same AM fungal inoculum was then tested at the field scale, on maize and leek at the Allpress Farm site (Chapter Five).

Assessment of spring barley RLC from the glasshouse trial revealed that the model organism *R. irregularis* was the most prolific coloniser of barley, producing high numbers of vesicles, the lipid storage organ. Analysis of sequence reads revealed that in the mixed sample, 70% of Glomeromycotinian reads were of *R. irregularis*, reflecting the similar but subdued phenotype of the mixed inoculum samples. Similar dominance by a single taxon has been observed by other authors such as Gosling et al. (2016), who speculate that

the lower numbers of plant stressors in controlled conditions facilitate this effect. This increase in RLC resulted in significantly lower biomass, suggesting that *R. irregularis* can maintain high colonisation, even at the detriment of the plant partner.

The same five species AM fungal inoculum was drilled alongside nine cover crop treatments in the Bawburgh trial (Chapter Three). Overall, addition of the inoculum had no impact on barley RLC, growth or yield, and components of the inoculum were not found in higher abundance in inoculated samples. Similarly, yield models were not significantly improved by the addition of barley RLC or AM fungal richness. There were similar results for the winter oat crop in year two, but here, AM fungal inoculation appeared to increase AM fungal richness, though not directly as none of the inoculated AM taxa were found in higher abundance in the inoculated samples. Furthermore, the addition of oat RLC to yield models significantly improved model fit, suggesting some benefit of increased interaction with AM fungi in oat, but not barley. Despite this, oat hosted fewer AM fungal taxa overall, which may reflect some level of niche differentiation, or that there were different environmental factors influencing AM communities in this year, a result more commonly observed in other reported studies (Njeru et al. 2014a; Higo et al. 2014, 2015).

In the split-field scale plots of the IF trial, there was no impact of AM fungal inoculation on maize RLC, and only very minor, if any increase in the abundance of one component of the inoculum, *R. irregularis.* Once again, AM

fungal inoculation appeared to have indirect impact on the evenness of AM fungal communities, potentially resulting from the reduction in dominance of a single taxon, although this hypothesis is not without issues (Berruti et al. 2017). There was no clear link between inoculation, or increased AM diversity or abundance on maize RLC.

There were more significant positive results for the highly mycorrhizal leek, which displayed increased RLC compared to some other treatments. At this site, AM inoculation was associated with an increase in AM fungal diversity and evenness, but once again there were only small increases in the abundance of *R. irregularis,* which could not explain the appearance of other taxa in these samples. It is important to emphasise that extrapolating results from two, two-hectare plots in Cambridgeshire is not sufficiently robust; and further replicated trials would be needed to verify this result. It is also quite likely that the reported differences are simply in field variation, due to plot size and absence of replication. Regardless of this, the use of AM fungal inoculation has been demonstrated at the field scale, with limited return on investment, at least in terms of yield increases. Based on the experimental evidence, it appears likely that the success of AM fungal inoculation on AM diversity and abundance, and resulting crop benefit, is likely to be controlled by a combination of biotic and abiotic factors. Understanding the molecular mechanisms to why certain crop species interact more readily with, and receive greater benefit from the AM symbiosis will enable crop breeding platforms to target these genes or pathways. Similarly, a greater understanding of which AM fungal taxa, or which pairings of crop and AM fungal taxa

produce the greatest crop benefit, will increase the likelihood of successful application of AM fungal inoculums. In the short term, it should not be forgotten that AM fungi have been shown to improve resistance and sustainability of agricultural systems, so the lack of a negative impact on yield both at Bawburgh and Allpress Farms, is a positive result for the ongoing potential of these fungi and their use in agriculture (Hart and Trevors 2005; Sosa-Hernández et al. 2019).

6.3 Impact of AM Fungal Inoculation, Diversity and Abundance on Crop Growth and Yield

Impact of Crop RLC on Biomass and Yield

In the glasshouse trial (Chapter Three), spring barley shoot biomass decreased following the addition of AM fungal inoculum; with the greatest reduction associated with those AM taxa that developed higher RLC. This negative impact on barley biomass was not observed in the Bawburgh field trial, nor was there any impact, due to AM fungal inoculation, on spring barley yield. In the same trial, inoculation had no impact on a winter oat biomass or yield, however addition of oat RLC as a model covariate significantly improved model fit (Figure 6.3). This suggests that, unlike other cereal crops, oat may benefit from increased interaction with AM fungi. Similar findings were shown in petroleum contaminated soils growing oat in China (Xun et al. 2015), and may highlight an important consideration for

addition of amendments, or crop rotations to maximise the benefit of AM fungi in oat.

Figure 6.3 Harvesting of winter oat var. Mascani in the Bawburgh trial.

In the NFS trial (Chapter Four), the impact of AM fungal inoculation was not tested, however, as in the Bawburgh experiment described in Chapter Three, there was no impact of increased barley RLC on shoot biomass or yield. In Chapter Five, despite the hypothesis that C4 grasses receive more benefit from the AM fungal symbiosis, addition of maize RLC as a model covariate also did not improve crop biomass. Furthermore, AM fungal inoculation of maize neither impacted RLC nor crop biomass. Conversely, after inoculation of the cover crop, the following leek crop at the Allpress site, another highly mycorrhizal crop, did not show an increased RLC response when compared to the control but did prevent the decrease observed in other treatments. It may be speculated that leek RLC was associated with greater crop shoot biomass, with the inoculated plot yielding some of the largest biomass crops. In the literature, leek has been shown to associate readily with many AM fungal species, and studies document increases in crop growth and yield resulting from the interaction (Plenchette et al. 1983; Kahiluoto and Vestberg 1998; Tawaraya et al. 2012). As for maize, understanding the genetic underpinnings of mycorrhizal interaction and response has the potential to drastically impact how crops are nourished, with implications on food security, sustainability, and climate change. This research question is currently being addressed by the Paszkowski and Oldroyd Labs, and others, at the Cambridge Crop Science Centre.

Impact of AM Fungal Diversity on Biomass and Yield

Increasing diversity of AM fungi has been shown to improve crop growth and yield of a number of crop species (Jansa et al. 2008; Hoeksema et al. 2010; Verbruggen and Kiers 2010; Argüello et al. 2016). In this study, there was no evidence for this benefit, in spring barley (Chapters Three and Four) or winter oat (Chapter Three), with a mild, positive association in maize and leek (Chapter Five). However, it was not possible to determine whether the benefit observed in leek was attributable to the increase in RLC or diversity, as these factors themselves were correlated. It seems as though the findings are in line with the view of documented by Ryan & Graham (2018), who suggest that there is little evidence for farmers to consider the abundance (or diversity) of AM fungi when managing crops. However, as discussed in Chapter Three, this study focusses on crops that are known to respond poorly to AM fungi, and completely ignores the benefit of AM fungi to whole system performance and sustainability. The present study provides evidence for managing expectations of the benefit of AM fungi, at least in terms of short-term crop productivity, although the wider benefits demonstrated in the literature should not be ignored.

6.4 Impact of Cover Crops on Soil Physicochemical Factors

Cover crops are grown for the protection or improvement of soils between periods of regular crop production, encompassing soil fertility and structure, reduction of weeds and pests, and environmental management (Stobart and Gosling 2015).

During the present study a detailed and extensive analyses on a number of soil physicochemical parameters was undertaken, that are known to be influenced by cover crops. These measurements were made on the trial scale at Bawburgh (Chapter Three), and at five farm sites on the split field scale (Chapter Five). Soil improvement and sustainable use of nutrient resources was the prime focus of the Innovative Farmers Field Lab group, and management of soils will become an important task for all farmers in the short to medium timescale.

Impact of Cover Crops on Soil Structure

Soil structure was quantified by using a soil penetrometer in the Bawburgh trial (Chapter Three), and by the VESS method in the IF trial (Chapter Five). Both methods were found to have pros and cons, but both methods provided consistent and robust results. The penetrometer can measure to greater depths, is faster, intrinsically more repeatable and quantifiable without recourse to visual assessments that are necessarily based on a personal

opinion. It is, however, an expensive device, it performs poorly in stony ground, and the data capture and transfer is unreliable particularly in stony or very wet conditions. In contrast, the VESS method only measures down to a spade's depth, is considerably slower, and comparison of measurements between different researchers are often flawed, due to the subjectivity and opinion mentioned above. Nevertheless, the VESS method does have the benefit of being very low cost and requiring just a spade and technician time. It is therefore accessible to all farmers, and not limited by stony ground. It can also, provide the opportunity to make additional analyses, such as earthworm counts (Guimarães et al. 2013).

At the Bawburgh trial (Chapter Three), there was significant impact of the preceding cover crop on soil penetration resistance. Some of the best (lowest) measurements of penetration resistance were recorded in plots following a radish, or oat, vetch and fodder radish cover crop, although these were not significantly better than control plots. This result is in agreement with other research in the literature, suggesting that fodder radish, with a large diameter tap root, was most effective at penetrating compacted soil, and improving soil structure (Chen and Weil 2010; Stobart and Gosling 2015). However, the high seed rate fodder radish and oat, and fodder radish and vetch cover crops resulted in a significantly higher (worse) penetration resistance than the control plots, demonstrating the potentially variable nature of biological practices in farming.

In the IF trial (Chapter Five), there were inconsistent effects of the cover crop treatment on the resultant recorded VESS score. There was some evidence that cover crops, either grown alone or with the addition of AD had a detrimental impact on soil structure, which is largely in contrast to the commonly held understanding that over crops can alleviate compaction and improve soil structure (Stobart and Gosling 2015). Addition of the AM fungal inoculum to the cover crop at the two Allpress field sites, improved soil structure when compared to the experimental treatments and to a lesser extent, the fallow control. This is a known benefit of AM fungi, and may be attributable to the release of glomalin, which is discussed in Chapter One and Five.

Impact of Cover Crops on Levels of Soil Organic Matter

Soil organic matter underpins many other attributes of soil, and contributes to soil structure, water holding capacity, and nutrient cycling. Reductions in SOM at the field level are of increasing concern to farmers, and the long-term sustainability of the land as a growing medium comes into question. In addition, increasing SOM at the macro scale represents a significant C sink, which may contribute to UK climate change emission goals.

At the Bawburgh trial (Chapter Three), there was no impact of the cover crop on SOM, although there were some significant interactions between the cover crop and AM fungal inoculation. This suggests a level of interplay between crop residues and the soil microbiome, which has also been shown

by Martínez-García et al. (2018), who showed that cover crops can influence the abundance of bacteria and fungi, which in turn influence the concentration and aromaticity of SOM. In the IF trial (Chapter Five), there were inconsistent impacts of cover cropping and AD application on increased SOM. Generally, positive impacts were observed at sites with pre-existing higher levels of SOM, an observation made by other soil scientists and discussed in Chapter Five. It is speculated that sandy sites, or sites with inherently lower SOM lack the toolkit for the sequestration and accumulation of higher levels of SOM. One such tool would be a suitable range and balance of microorganisms for the breakdown and long-term storage of organic matter. In the AM fungal inoculated plot at the Allpress leek field, SOM was increased compared to the control, where other treatments had decreased. As discussed in Chapter Five, Frey (2019) shows a strong link between AM fungi and accumulation of SOM, potentially via direct competition with free-living saprotrophs. This further highlights the importance of soil microbial diversity, including that of AM fungi, for SOM accumulation.

Impact of Cover Crops on Excess Nutrient Scavenging

The nutrient scavenging capacity of cover crops was considered in Chapter Five, as part of the Innovative Farmers programme. As documented in this chapter, the ability of cover crops to take up N from the applied AD was of particular interest to the farmer group, as many were concerned about N leachate and impact on local water sources through diffuse pollution. Cover crops reduced the available N through the soil profile, especially at deeper

horizons. This suggests that the N that would have otherwise leached to the 60-90cm soil horizon or deeper, had been relocated into the cover crop biomass, which was then made available to the cash crop throughout the growing season. The rate at which this occurs is not known, and two means are suggested. Firstly, the cover crop is slowly broken down throughout the entire growing season, drip feeding the cash crop with nutrients which would otherwise have been lost. Secondly, the increased activity of saprotrophs throughout the soil profile as the soil warms in the late spring triggers rapid decomposition of cover crop biomass and flushes the system with the nitrogen which would otherwise have been lost out of the soil into water draining into local watercourses. The latter mechanism may increase the volatilisation and leaching of N, just later in the growing season, but depending on timing, may provide the plant with a climate-coordinated boost in nutrition. The rate at which cover crops degrade due to soil microbial saprotrophs, to release nutrients will be essential knowledge for farmers and agronomists, and requires further experimentation.

6.5 Impact of Cover Crops on Crop Growth and Yield

As well as benefitting soil physicochemical parameters, cover crops have been associated with increases in following cash crop growth and yield, either directly, or indirectly through long term soil improvement (Kabir and Koide 2002). Crop growth and yield was an important consideration for all trials in the present study, as it is the most easily quantifiable and recognisable form of economic return from investment in cover crops.

Impact of Cover Crops on Shoot Biomass

Crop shoot biomass was measured in the Bawburgh, NFS, and Innovative Farmers' trials (Chapters Three, Four, and Five), and yield was measured in the Bawburgh and NFS trials (Chapters Three and Four), following difficulty measuring the forage maize yield. In the Bawburgh trial, there was no impact of cover crop on spring barley shoot biomass, but there was a significant effect the following year in oat. Oat following vetch, and the high seed rate fodder radish and oat had significantly lower shoot biomass. This decline was not attributable to cover crop biomass, GAI, or oat RLC, and it is speculated that the impact of these cover crops impacted N availability in these plots more significantly than other cover crops, although the mechanism is unknown.

Despite no overall difference in shoot biomass in the NFS trial (Chapter Four) resulting from the preceding cover crop, there were some interesting qualitative differences. At the 0% N regime, both the fodder radish and oat, and legume mix cover crop increased the dry shoot weights of the following spring barley crop by around 15%. In the 50% N regime, this benefit was no longer present. However, at the 100% N regime the increased shoot biomass observed in the 0% regime had returned. The mechanism for crop benefit at the low and high, but not at intermediate levels of N fertilisation is not known, but may be attributable to the ratio of C:N in the soil, resulting in N lockup (Pinck et al. 1948). As discussed in Chapter Four, this is an important consideration for farmers applying organic nitrogen amendments, as

well as those who are thinking of integrating a cover crop or long-term ley into their rotation. Consideration of the C:N ratio of cover crop species to include in cover crop mixtures is an ongoing area of research, and may become more important as cover cropping becomes more widely adopted.

In the IF trial (Chapter Five), there were no clear benefits of either a single preceding cover crop, or surprisingly, addition of N-rich AD on crop biomass (Figure 6.4). It is possible that there was a weak negative effect of the cover crop treatment on maize biomass at the North Moor site, and a slight positive effect of the cover crop drilled with AD or the AM fungal inoculum at the Allpress maize site. This indicates that there is a complex relationship between the speed and extent of degradation, mineralisation, and availability of nutrients following a cover crop, and this must be considered alongside the amount and formulation of the organic fertiliser application. As cited above, this is not a new phenomenon, and may become more important to understand as farmers and growers increase the amount of organic amendments used on farm.

Figure 6.4 Maize crop at Holkham Farm, in July 2018.

Impact of Cover Crops on Crop Yield

Cover crop yield was measured for barley and oat in the Bawburgh trial (Chapter Three), and for barley in the NFS trial in Chapter Four. In Chapter Three, the impact of the preceding cover crop on spring barley yield was discussed, although this positive impact was not statistically validated at

the $p < 0.05$ level. Lower yields were observed in barley following the vetch, and high seed rate fodder radish and oat cover crop, providing further evidence of the detrimental nature of these two treatments on barley growth. Conversely, single species cover crops of fodder radish and oat produced some of the highest yields and around 0.5 t/ha higher than the fallow. This effect was not seen in the following oat crop, where the high rate radish and oat cover crop produced some of the highest oat yields across the trial. This observation provides further evidence that a negative impact on following crop yield due to these cover crops may be attributed to nutrient lockup due to C:N imbalance.

In the NFS trial, spring barley yields benefitted from a leguminous cover crop in the 0% N fertilisation regime, with the radish and oat producing a small yield decline. This benefit was not present in the 50% or 100% N fertilisation regimes, suggesting that the benefit of cover cropping is not as pronounced as N fertilisation, and that cover crops are using or locking up some of the N applied in the 50% or 100% N plots. Assessment of crop yields from other years of the NFS trial suggests that the particularly hot and dry weather conditions in the experimental year produced atypically negative results, as cash crops (including spring barley) in other years observed yield increases due to the preceding cover crop (Figure 6.5). It is interesting to note that in the full N fertilisation regime, both cover crop mixtures increased barley shoot biomass, but this increase was inversely proportional to increasing yield. This agrees with observations by Anindya Kundu (*Pers.*

Com.), that AM fungal inoculation was able to increase vegetative, but not reproductive growth in strawberry.

Figure 6.5 Soil cracking in response to drought at Northampton UK, in July 2018.

From the data presented, it is possible that introduction of a cover crops into an arable rotation may foster soil conditions, which promote crop

growth and yield, in the medium to long term. However, the timing of desiccation, establishment of the cash crop, and subsequent nutrient management clearly also have important, and potentially severe impacts on crop yield.

6.6 Evaluation of Experimental Design and Methodology

Evaluation of Trial Design Across Spatial Scales

One strength of the present study can be attributed to experimentation over a range of spatial scales, from glasshouse to field trials, and national scale analyses. The limitations of the observational nature of Chapter Two methodology has already been discussed, and a slightly more targeted approach, balancing the numbers of observations of each factor of interest, may have provided more robust results. The Bawburgh Trial (Chapter Three) suffered from low statistical power for dissection of cover crop effects. This resulted partly from inexperience, and partly as cover crop seed was supplied by the seed companies in exchange for inclusion in the trial. In future, similar trials, especially those investigating moderate impacts, should ask simple questions, to receive simple (but statistically more robust) answers.

There were no such issues at the New Farming Systems Fertility Building Rotations Trial (Chapter Four). This long running and large plot scale trial is an invaluable and well managed test bed for a number of questions around cover and companion cropping, and their interplay with N fertilisation. One

difficulty arising from this trial is finding and applying the most suitable type of statistical analysis. Unlike other split-plot trials, the N dose is not randomised within cover crop plots in this trial, making selection of a random effects model difficult. After consultation with Ian Mackay, a prominent statistician in the field of trial design and analysis, the decision was made to evaluate the trial in the manner described in Chapter Four, acknowledging limitations in the ability of this model to resolve in-field variation along a north south gradient. Similar trials have been established to explore a gradient in N application, such as the ADAS Opti-Plots trials, and suffer from similar limitations in analysis (ADAS and VSNi 2011). Like other areas of field-based research, the solution may be restricted to acknowledging the limitations of the trial, as there are currently no statistical models that are able to unpick the impact of a treatment gradient, and random effects gradient that may be correlated with the factor of interest.

Finally, the limitations of the split-field trials (Chapter Five) have been discussed at length. One method to overcome this in field variation is by increasing the overall number of sites, although this was outside the budget and feasibility of the IF project, and this PhD programme. Nevertheless, experimentation and validation at the farm scale, and especially farmer led, is an integral part of agricultural research, and should play a large part in research going forward.

Evaluation of Methodologies for Quantifying AM Fungal RLC

Many of the field-based evaluations were conducted with or after expert guidance from the NIAB trials delivery team at Morley, Norfolk. The trials team, and NIAB more generally, are expert in drilling, evaluating, and harvesting trials of a number of crop species, and the trials included in this PhD are no exception.

Methods around the staining, visualisation, and quantification of AM fungal structures in roots were adapted from well-established methods used daily in the Paszkowski Lab. Generally, field roots are stronger, and more lignified, and required more time and attention to achieve the same clarity as roots grown in controlled conditions. A large proportion of the work for this PhD was collecting, washing, staining, mounting, and quantifying RLC in crop roots. Since the beginning of this PhD study, two notable methodologies have been developed to increase the speed of quantification. The first involves the clearing and staining of whole root systems by conventional methods, digitally scanning root systems, and quantifying abundance of fungal structures by artificial intelligence (AI) (Evangelisti et al. 2021). At first glance, the utility of AI seems promising, but there are several drawbacks. Firstly, this method still requires the timely root clearing step, going as far as including an additional step to ensure clarity of images. Secondly, it is the time-consuming step of manually creating a reference database for which to build the supervised model, which may require thousands to hundreds of thousands of images, depending on the crop species and environment from

which the plant has been taken. The model has been demonstrated on roots of seedlings of model organisms, but achieving a suitable accuracy on field samples, even after training a moderate hand curated dataset, due to the difficulty in clearing and staining roots, and presence of other fungi, is highly unlikely. Subsequently, this method does not seem suitable for application to field based AM fungal research, at least in the short to medium term.

A more promising technology is the discovery of a correlation between increasing AM RLC, and accumulation of hydroxy- and carboxyblumenol C-glucosides in plant leaf tissue (Wang et al. 2018). These blumenols can then be quantified by mass spectrometry and correlated to a value from representative samples for whole root RLC. The method has been shown to be robust on samples grown in controlled and field conditions and could easily be extended to a wider range of crops. The method is significantly faster to conduct, requires less hands-on time, is relatively easy to conduct, and importantly, is non-destructive, allowing for measurement of the same plant multiple times over a growing season. This method will provide means for conducting experimentation on the temporal dynamics of RLC in crop plants across the growing season.

Evaluation of Methodologies for AM Fungal Bioinformatics

Optimal bioinformatic methodologies and thresholds were chosen after considerable exploration of the published literature, as well as through discus-

sion with eminent mycorrhizal researchers including Maarja Öpik, John Davison, Alex Dumbrell, and Martti Vasar. Pipelines were chosen to be robust, statistically valid, and comparable to other published literature in the field.

As previously discussed, bioinformatic methodology in AM fungal research is lagging behind the analysis of other fungi and bacteria, which utilise more complex algorithms to denoise sequence reads, and assign species IDs. This is partly due to the sequence variation within single species of AM fungi, and the current definition of what constitutes a species/VT of AM fungi. Clearly the biology of these types of fungal organisms are unlikely to change, but more complex analyses, such as the analysis of the OTUs/ ASVs which constitute VT may warrant some exploration. Indeed, studies such as Chen et al. (2018) have already conducted these analyses, showing that isolates of the species *R. irregularis* collected from the same field, display high intra-specific functional diversity.

One advancement which will make some significant improvement in the field is a high throughput sequencing technology which can span the ~1.5 kb 'Krüger' fragment, shown to be most efficient at amplifying and differentiating AM fungal VT (Krüger et al. 2009; Van Geel et al. 2014). Many of the current studies in AM fungal research utilise the 18S SSU, with primers such as NS31/ WANDA in the forward direction and AM1/ AML2 in the reverse, on the Illumina Miseq with 2 x 300bp read chemistry. Aside from being the sub-optimal primer choice, the amplicon at 550bp, relies on very high-quality

sequencing to ensure forward and reverse contigs can overlap. PacBio sequencing can reliably sequence longer amplicons, including the 1.5 kb Krüger fragment, but at considerably lower throughput that the Illumina system, making it economically unviable for many studies. Development of a high throughput system capable of sequencing these longer fragments would significantly improve research in the field, not to mention other areas such as genome assembly which rely on longer sequences for repetitive regions of the genome. This need has been identified by Illumina, who made a bid in 2018 to acquire Pacific Biosciences. This was eventually blocked by the Competition and Markets Authority (CMA) in 2020, but there is no doubt that Illumina are independently developing long read technology for the commercial market.

In addition to the results described, a considerable amount of additional work was conducted on the dataset produced in Chapter Two. Following the methodology of Davison et al. (2015), phylogenetic analysis was undertaken on those DNA sequence reads not matching to Glomeromycotina, in order to identify putative AM fungal taxa. In total, three putative taxa were discovered from the 148 samples from UK agricultural sites. Unfortunately, these were not robust across different thresholds for amplicon quality and length, so analysis, and inclusion of these taxa, was omitted for this thesis.

6.7 Evaluation of Realised Pros and Cons of Cover Cropping and Consideration of AM Fungi in UK Agriculture

In this thesis, a UK-wide analysis of AM fungal diversity is presented, before exploring the impact of cover crops, and other amendments, on soil health, AM fungal diversity and abundance, and crop growth and yield, over several spatial scales.

The data shows that UK agricultural sites host over 20% of the known global AM fungal diversity. This diversity is influenced by the cropping system, with long term pasture sites hosting significantly more and more diverse taxa than arable sites. This is likely to be underpinned by three factors: soil texture, type of cultivation, and whether fungicides had been applied to the crop. All these factors have previously been shown to influence AM fungal diversity and abundance, so there is reason to believe that this data, although observational in nature, is robust.

Throughout the thesis, cover cropping has had minor impacts on crop growth and yield, and a measurable impact is even less pronounced after a single season cover crop (Chapter Three and Five). However, cover crops were shown to influence some soil physicochemical parameters, such as N availability in the soil profile. Furthermore, multiple iterations of cover crops (Chapter Four) were shown to increase RLC of spring barley with the natural inoculum. This suggests that despite neutral to small negative impacts

on crop yield in the short term, cover crops are likely to be improving soil and system factors to ensure the sustainability of these yields in the medium to long term.

In the trials presented, several other experimental amendments were explored. Nitrogen fertilisation, as expected, had positive impacts on yield, which at low levels of fertilisation may have been supplemented by rhizobia associating with leguminous cover crops. N application did not influence spring barley RLC, but did influence AM fungal community composition, with full N fertilised barley hosting a simplified community of the 0% and 50% N regimes. In contrast to the literature, Chapter Four shows that *Glomus* species*,* including *Glomus Whitfield type 7,* and a Diversisporales, *Acaulospora Acau2* are particularly impacted by high N fertilisation. *Paraglomus* and *Archaeospora* species increased in their abundance to fill the niche abandoned by these N sensitive taxa. This provides further evidence for the potential benefit of increasing the diversity of AM fungi, either by natural or introduced inoculum, to occupy distinct niches and provide additive benefit.

A single N application, in the form of anaerobic digestion AD had variable impacts on AM fungal community composition, and no impact on cash crop RLC. The lack of replication in this trial makes robust conclusions hard to form, nevertheless, experimentation at the field scale has yielded valuable data on the feasibility and impact of cover cropping large acreages of arable cropland.

A commercially available AM fungal inoculum was tested over several scales, to test the impact on cash crop RLC, AM fungal diversity, and crop growth and yield. In controlled conditions, the inoculum decreased shoot biomass compared to the control, which was attributable to colonisation by the model organism *Rhizophagus irregularis,* which constituted 70% of reads in the mixed inoculum, used in field trials in Chapters Three and Five. In the Bawburgh trial (Chapter Three), the inoculum failed to influence RLC of either barley or oat, and DNA sequence reads of the inoculum taxa were not detected at higher abundance. Strangely, inoculation facilitated more biologically even AM fungal communities, an effect that has been observed by others in the literature.

For maize grown at the field-scale (Chapter Five), AM fungal inoculation did not increase RLC or diversity, with very minor, if any detection of the inoculum by molecular methods. Once again, however, AM fungal inoculation appeared to have indirect impact on the evenness of AM fungal communities. At the other Allpress Farms field site, the inoculated, highly mycorrhizal leek crop displayed increased RLC and diversity compared to some other treatments. In both of these plots, inoculation with AM fungi appeared to indirectly increase community evenness, although once again there was no parsimonious hypothesis for this effect.

Finally, there was only limited evidence that AM fungi could increase the growth and yield of cash crops. Increased RLC was associated with increased

yield/ biomass in oat and leek, but not in barley, where colonisation by *Rhizophagus irregularis* reduced biomass in controlled conditions. Increased AM fungal diversity had no impact on crop growth or yield, except in leek, where RLC, diversity, and biomass were all correlated, and subsequently the factor influencing growth and yield could not be established for this crop.

6.8 Summary of Project Hypotheses

In this study, it was shown that certain AM fungal taxa were impacted by high disturbance agriculture, whether this was cultivation, nitrogen fertilisation, or fungicide application, with undisturbed pasture hosting higher numbers and more diverse AM fungal taxa than arable sites. Cover crops did not influence the diversity of AM fungi that associate with the following cash crop, but were able to increase the RLC of spring barley in one trial. Observations indicate that this impact is only realised after a number of cover crops drilled over several years in a crop rotation. This requirement for multiple iterations of cover crops to achieve ecosystem or crop benefits has also been speculated by others in the literature (Chu et al. 2017; Pakarinen et al. 2021). Here, five iterations of cover crops were required to produce a 20-30% increase in barley RLC. Single iterations of cover crops did not significantly influence barley, oat, or maize RLC.

Cover crops were effective at improving some other elements of soil health, even after one iteration. Residual N was taken up by the cover crop in Chapter Five, preventing this N from leaching to deeper soil horizons, and

potentially into adjacent watercourses. There was some evidence that cover crops were able to increase SOM, especially on sites with higher levels of pre-existing SOM. In both the Bawburgh (Chapter Three) and IF (Chapter Five) trials, there was no evidence that cover crops measurably improved soil structure after only one year of treatment.

There was limited evidence that increasing AM fungal diversity or abundance could increase crop biomass and yield. In the Bawburgh trial (Chapter Three), increasing RLC was correlated with increased yield in oat, but this was not the case for the previous barley crop, and higher values of RLC were associated with reduced barley biomass in controlled conditions. Similarly, there was no impact of RLC on barley yield in the NFS trial (Chapter Four). In the IF trial (Chapter Five) there was no correlation between maize RLC and biomass, but there was a positive correlation between leek RLC, AM diversity, and yield, suggesting that either, or both of these factors may be positively impacting leek yield.

6.9 Conclusions

The present study aimed to determine the impact of cover cropping, and other soil amendments on crop growth and yield, as well soil health characteristics focussing on AM fungal populations. From the data presented, it seems likely that the soil is relatively resilient to the impact of any single amendment, but effects are additive if conducted over several years. This suggests that farmers should not be looking for 'quick fixes' from cover crops,

or any of the other treatments tested in this thesis, but should select and apply combinations of amendments or regimes for a number of years to realise the potential benefits. This highlights the importance of long-term experimental field sites, which facilitate the testing of hypotheses on soil conditions and health, which is not achievable during the length of a standard three to five year funded project. Finally, integration of farmers into participatory research, such as in the Innovative Farmers programme, is an invaluable way to accelerate and streamline academic research for agriculture, both in the UK and overseas.

7 Supplementary Figures

Table S5.1 Summary of soil physicochemical parameters for treatments within the four sites.

Site	Treatment	Available N 0-30cm	Available N 30-60cm	Available N 60-90cm	Available N Total	Nitrate 0-30cm (mg/kg)	Nitrate 30-60cm (mg/kg)	Nitrate 60-90cm (mg/kg)	Nitrate Total (mg/kg)	Ammonium 0-30cm (mg/kg)	Ammonium 30-60cm (mg/kg)	Ammonium 60-90cm (mg/kg)	Ammonium Total (mg/kg)	Available P (mg/l)	Available K (mg/l)	Available Mg (mg/l)	pH	SOM (%)
Allpress E27	Control	19.7	27.8	11.3	58.8	4.45	6.59	2.67	13.71	0.80	0.82	0.35	1.97	24.6	168	94	7.1	10.5
Allpress E27	Cover Crop and AD	19.4	5.3	3.3	28.0	4.19	0.94	0.83	5.96	0.97	0.47	0.06	1.50	28.4	174	122	7.2	11.7
Allpress E27	AD Only	31.1	39.6	25.4	96.1	7.50	10.07	6.38	23.95	0.78	0.48	0.41	1.67	27.6	165	106	7.1	10.9
Allpress E27	Cover Crop Only	14.8	5.3	3.7	23.8	2.63	0.90	0.64	4.17	1.32	0.52	0.35	2.19	30.2	268	118	7.3	10.7
Allpress E27	Cover Crop and AMF	7.2	1.8	2.6	11.6	1.38	0.06	0.40	1.84	0.54	0.41	0.28	1.23	23.0	174	99	6.9	10.7
Allpress H48	Control	244.9	219.7	102.6	567.2	64.56	53.6	26.14	144.3	0.73	4.98	1.21	6.92	26	237	140	6.7	43.4
Allpress H48	Cover Crop and AD	201.3	257.6	118.6	577.5	52.62	66.23	30.66	149.51	1.06	2.46	0.96	4.48	24.6	299	148	6.5	40.3
Allpress H48	AD Only	295.1	89.8	81	465.9	77.91	23.34	21.1	122.35	0.78	0.61	0.51	1.9	31.8	376	136	6.8	36.6
Allpress H48	Cover Crop Only	103.1	114	153.4	370.5	26.66	29.55	40	96.21	0.83	0.85	0.9	2.58	32.2	249	115	7.5	25.1
Allpress H48	Cover Crop and AMF	160.5	340.4	72.2	573.1	40.73	76.62	8.04	125.39	2.08	14.16	11.21	27.45	18.4	173	142	6.7	45.5
Euston	Control	12.1	15.0	11.1	38.2	2.43	3.07	2.43	7.93	0.79	0.93	0.52	2.24	29.6	110	41	7.3	2.4
Euston	Cover Crop and AD	13.7	10.8	14.9	39.4	2.99	2.03	3.34	8.36	0.67	0.85	0.63	2.15	32.8	110	21	6.8	2.4
Euston	AD Only	9.8	8.7	43.9	62.4	2.01	1.53	11.07	14.61	0.60	0.80	0.62	2.02	42.2	199	25	7.4	2.3
Euston	Cover Crop Only	9.5	5.4	2.6	17.5	1.66	0.69	0.06	2.41	0.86	0.75	0.62	2.23	34.2	89	34	6.4	2.4
Holkham	Control	11.9	7.2	NA	19.1	2.32	1.43	NA	3.75	0.86	0.49	NA	1.35	9.8	178	40	8.4	2.7
Holkham	Cover Crop and AD	12.4	6.4	NA	18.8	2.57	1.13	NA	3.70	0.73	0.59	NA	1.32	12.0	147	40	8.4	2.5
Holkham	AD Only	11.3	16.5	NA	27.8	2.31	2.29	NA	4.60	0.72	2.10	NA	2.82	10.8	169	37	8.5	2.7
Holkham	Cover Crop Only	10.0	7.8	NA	17.8	1.84	1.05	NA	2.89	0.82	1.05	NA	1.87	11.0	150	39	8.4	2.4
North Moor	Control	28.7	58.9	344.4	432.0	7.04	14.59	44.95	66.58	0.62	1.12	46.88	48.62	10.4	141	71	7.9	6.3
North Moor	Cover Crop and AD	32.8	75.9	189.3	298.0	8.00	17.61	10.91	36.52	0.74	2.63	39.56	42.93	11.2	133	73	8.1	7.5
North Moor	AD Only	26.4	43.1	292.3	361.8	5.87	8.14	4.52	18.53	1.16	3.35	73.42	77.93	10.4	122	82	7.9	8.8
North Moor	Cover Crop Only	58.1	164.7	375.5	598.3	14.85	39.85	20.41	75.11	0.65	4.08	79.72	84.45	14.2	171	95	7.5	10.8

Figure S5.1 SRUC Visual Evaluation of Soil Structure score chart

Visual Evaluation of Soil Structure

Soil structure affects root penetration, water availability to plants and soil aeration. This simple, quick test assesses soil structure based on the appearance and feel of a block of soil dug out with a spade.
The scale of the test ranges from Sq1, good structure, to Sq5, poor structure.

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Equipment:
Garden spade approx. 20 cm wide, 22-25 cm long.
Optional: light-coloured plastic sheet, sack or tray ~50 x 80 cm, small knife, digital camera.

When to sample:
Any time of year, but preferably when the soil is moist. If the soil is too dry or too wet it is difficult to obtain a representative sample.
Roots are best seen in an established crop or for some months after harvest.

Where to sample:
Select an area of uniform crop or soil colour or an area where you suspect there may be a problem. Within this area, plan a grid to look at the soil at 10, preferably more, spots. On small experimental plots, it may be necessary to restrict the number to 3 or 5 per plot.

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Method of assessment:

Step	Option	Procedure
Block extraction and examination		
1. Extract soil block	Loose soil	Remove a block of soil ~15 cm thick directly to the full depth of the spade and place spade plus soil onto the sheet, tray or the ground
	Firm soil	Dig out a hole slightly wider and deeper than the spade leaving one side of the hole undisturbed. On the undisturbed side, cut down each side of the block with the spade and remove the block as above.
2. Examine soil block	Uniform structure	Remove any compacted soil or debris from around the block
	Two or more horizontal layers of differing structure	Estimate the depth of each layer and prepare to assign scores to each separately.
Block break-up		
3. Break up block (take a photograph - optional)		Measure block length and look for layers. Gently manipulate the block using both hands to reveal any cohesive layers or clumps of aggregates. If possible separate the soil into natural aggregates and man-made clods. Clods are large, hard, cohesive and rounded aggregates.
4. Break up of major aggregates to confirm score		Break larger pieces apart and fragment it until a piece of aggregate of 1.5 - 2.0 cm. Look to their shape, porosity, roots and easily of break up. Clods can be broken into non-porous aggregates with angular corners and are indicative of poor structure and higher score.
Soil scoring		
5. Assign score		Match the soil to the pictures category by category to determine which fits best.
6. Confirm score from:		Factors increasing score:
	Block extraction	Difficulty in extracting the soil block
	Aggregate shape and size	Larger, more angular, less porous, presence of large worm holes
	Roots	Clustering, thickening and deflections
	Anaerobism	Pockets or layers of grey soil, smelling of sulphur and presence of ferrous ions
	Aggregate fragmentaion	Break up larger aggregates ~ 1.5 – 2.0 cm of diameter fragments to reveal their type
7.Calculate block scores for two or more layers of differing structure		Multiply the score of each layer by its thickness and divide the product by the overall depth, e.g. for a 25 cm block with 10 cm depth of loose soil (Sq1) over a more compact (Sq3) layer at 10-25 cm depth, the block score is (1 x 10)/25 + (3 x 15)/25 = Sq 2.2.

Scoring: Scores may fit between Sq categories if they have the properties of both.
Scores of 1-3 are usually acceptable whereas scores of 4 or 5 require a change of management.

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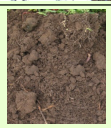
Structure quality	Size and appearance of aggregates	Visible porosity and Roots	Appearance after break-up: various soils	Appearance after break-up: same soil different tillage	Distinguishing feature	Appearance and description of natural or reduced fragment of ~ 1.5 cm diameter
Sq1 Friable Aggregates readily crumble with fingers	Mostly < 6 mm after crumbling	Highly porous Roots throughout the soil			 Fine aggregates	 The action of breaking the block is enough to reveal them. Large aggregates are composed of smaller ones, held by roots.
Sq2 Intact Aggregates easy to break with one hand	A mixture of porous, rounded aggregates from 2mm - 7 cm. No clods present	Most aggregates are porous Roots throughout the soil			 High aggregate porosity	 Aggregates when obtained are rounded, very fragile, crumble very easily and are highly porous.
Sq3 Firm Most aggregates break with one hand	A mixture of porous aggregates from 2mm -10 cm; less than 30% are <1 cm. Some angular, non-porous aggregates (clods) may be present	Macropores and cracks present. Porosity and roots both within aggregates.			 Low aggregate porosity	 Aggregate fragments are fairly easy to obtain. They have few visible pores and are rounded. Roots usually grow through the aggregates.
Sq4 Compact Requires considerable effort to break aggregates with one hand	Mostly large > 10 cm and sub-angular non-porous; horizontal/platy also possible; less than 30% are <7 cm	Few macropores and cracks All roots are clustered in macropores and around aggregates			 Distinct macropores	 Aggregate fragments are easy to obtain when soil is wet, in cube shapes which are very sharp-edged and show cracks internally.
Sq5 Very compact Difficult to break up	Mostly large > 10 cm, very few < 7 cm, angular and non-porous	Very low porosity. Macropores may be present. May contain anaerobic zones. Few roots, if any, and restricted to cracks			 Grey-blue colour	 Aggregate fragments are easy to obtain when soil is wet, although considerable force may be needed. No pores or cracks are visible usually.

8 References

Abdollahi L, Munkholm LJ (2014) Tillage System and Cover Crop Effects on Soil Quality:I. Chemical, Mechanical, and Biological Properties. Soil Sci Soc Am J 78:262. https://doi.org/10.2136/sssaj2013.07.0301

ADAS, VSNi (2011) Development of appropriate testing methodology for assessing nitrogen requirements of wheat and oilseed rape varieties

Aerts R (2003) The Role of Various Types of Mycorrhizal Fungi in Nutrient Cycling and Plant Competition. In: van der Heijden MGA, Sanders I. (eds) Mycorrhizal Ecology. Springer, Berlin, Heidelberg, pp 117–133

Agro Business Consultants (2021) The Agricultural Budgeting & Costing Book, 92nd Editi. ABC Books, Melton Mowbray

Akiyama K, Matsuzaki KI, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827. https://doi.org/10.1038/nature03608

Albiach R, Canet R, Pomares F, Ingelmo F (2001) Organic matter components and aggregate stability after the application of different amendments to a horticultural soil. Bioresour Technol 76:125–129. https://doi.org/10.1016/S0960-8524(00)00090-0

Alburquerque JA, de la Fuente C, Ferrer-Costa A, et al (2012) Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and Bioenergy 40:181–189. https://doi.org/10.1016/j.biombioe.2012.02.018

Alexandratos N, Bruinsma J (2012) World agriculture towards 2030/2050

Alguacil MM, Lumini E, Roldán A, et al (2008) The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. Ecol Appl 18:527–536. https://doi.org/10.1890/07-0521.1

Allan SN (2017) Disturbance and the Community Composition of Arbuscular Mycorrhizal Fungi in Ontario Tallgrass Prairies. University of Western Ontario

Altschul SF, Gish W, Miller W, et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Amir A, McDonald D, Navas-Molina JA, et al (2017) Deblur Rapidly Resolves Single-

Nucleotide Community Sequence Patterns. mSystems 2:. https://doi.org/10.1128/msystems.00191-16

Argüello A, O'Brien MJ, van der Heijden MGA, et al (2016) Options of partners improve carbon for phosphorus trade in the arbuscular mycorrhizal mutualism. Ecol Lett 19:648–656. https://doi.org/10.1111/ele.12601

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42. https://doi.org/10.1007/s005720100097

Bago B, Pfeffer PE, Shachar-Hill Y (2000) Carbon metabolism and transport in arbuscular mycorrhizas. Plant Physiol 124:949–958. https://doi.org/10.1104/pp.124.3.949

Bahram M, Peay KG, Tedersoo L (2015) Local-scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. New Phytol 205:1454–1463. https://doi.org/10.1111/nph.13206

Balu L. Bumb & Carlos A.Baanante (1996) Role of fertilizers in food security and protecting the environment to 2020. IFPRI Discuss. Pap. 54

Balzergue C, Puech-Pags V, Bécard G, Rochange SF (2011) The regulation of arbuscular mycorrhizal symbiosis by phosphate in pea involves early and systemic signalling events. J Exp Bot 62:1049–1060. https://doi.org/10.1093/jxb/erq335

Bankevich A, Nurk S, Antipov D, et al (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021

Bates D, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Batjes NH (2014) Total carbon and nitrogen in the soils of the world. Eur J Soil Sci 65:10–21. https://doi.org/10.1111/ejss.12114_2

Bennett AE, Bever JD (2007) Mycorrhizal species differentially alter plant growth and response to herbivory. Ecology 88:210–218. https://doi.org/10.1890/0012-9658(2007)88[210:MSDAPG]2.0.CO;2

Bennett AE, Bever JD (2009) Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in Plantago lanceolata. Oecologia 160:807–816. https://doi.org/10.1007/s00442-009-1345-6

Bennett EM, Carpenter SR, Caraco NF (2001) Human Impact on Erodable Phosphorus and Eutrophication: A Global Perspective. Bioscience 51:227–234. https://doi.org/10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2

Bennett JA, Maherali H, Reinhart KO, et al (2017) Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. Science (80-) 355:181–184. https://doi.org/10.1126/science.aai8212

Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes. Front Microbiol 6:1559. https://doi.org/10.3389/fmicb.2015.01559

Berruti A, Lumini E, Bianciotto V (2017) AMF components from a microbial inoculum fail to colonize roots and lack soil persistence in an arable maize field. Symbiosis 72:73–80. https://doi.org/10.1007/s13199-016-0442-7

Bever JD, Richardson SC, Lawrence BM, et al (2009) Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecol Lett 12:13–21. https://doi.org/10.1111/j.1461-0248.2008.01254.x

Biermann B, Linderman RG (1983) Use Of Vesicular-Arbuscular Mycorrhizal Roots, Intraradical Vesicles And Extraradical Vesicles As Inoculum. New Phytol 95:97–105. https://doi.org/10.1111/j.1469-8137.1983.tb03472.x

Bitterlich M, Krügel U, Boldt-Burisch K, et al (2014) The sucrose transporter SlSUT2 from tomato interacts with brassinosteroid functioning and affects arbuscular mycorrhiza formation. Plant J 78:877–889. https://doi.org/10.1111/tpj.12515

Bolyen E, Rideout JR, Dillon M, et al (2018) QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. PeerJ 37:852–857. https://doi.org/10.7287/peerj.preprints.27295

Boswell EP, Koide RT, Shumway DL, Addy HD (1998) Winter wheat cover cropping, VA mycorrhizal fungi and maize growth and yield. Agric. Ecosyst. Environ. 67:55–65

Bowles TM, Jackson LE, Loeher M, Cavagnaro TR (2017) Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. J Appl Ecol 54:1785–1793. https://doi.org/10.1111/1365-2664.12815

Breuillin-Sessoms F, Floss DS, Karen Gomez S, et al (2015) Suppression of arbuscule

degeneration in Medicago truncatula phosphate transporter4 mutants is dependent on the ammonium transporter 2 family protein AMT2;3. Plant Cell 27:1352–1366. https://doi.org/10.1105/tpc.114.131144

Bronick CJ, Lal R (2005) Soil structure and management: A review. Geoderma

Bruce A, Smith SE, Tester M (1994) The development of mycorrhizal infection in cucumber: effects of P supply on root growth, formation of entry points and growth of infection units. New Phytol 127:507–514. https://doi.org/10.1111/j.1469-8137.1994.tb03968.x

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275–304

Brundrett MC, Tedersoo L (2018) Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220:1108–1115

Bruns TD, Corradi N, Redecker D, et al (2018) Glomeromycotina: what is a species and why should we care? New Phytol 220:963–967. https://doi.org/10.1111/nph.14913

Buysens C, Dupré de Boulois H, Declerck S (2015) Do fungicides used to control Rhizoctonia solani impact the non-target arbuscular mycorrhizal fungus Rhizophagus irregularis? Mycorrhiza 25:277–288. https://doi.org/10.1007/s00572-014-0610-7

Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

Calonego JC, Rosolem CA (2010) Soybean root growth and yield in rotation with cover crops under chiseling and no-till. Eur J Agron 33:242–249. https://doi.org/10.1016/j.eja.2010.06.002

Camenzind T, Hempel S, Homeier J, et al (2014) Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest. Glob Chang Biol 20:3646–3659. https://doi.org/10.1111/gcb.12618

Caruso C, Maucieri C, Barco A, et al (2018) Effects of mycorrhizal inoculation and digestate fertilisation on triticale biomass production using fungicide-coated seeds. Irish J Agric Food Res 57:42–51. https://doi.org/10.1515/ijafr-2018-0005

Ceballos I, Ruiz M, Fernández C, et al (2013) The In Vitro Mass-Produced Model Mycorrhizal Fungus, Rhizophagus irregularis, Significantly Increases Yields of the

Globally Important Food Security Crop Cassava. PLoS One 8:e70633. https://doi.org/10.1371/journal.pone.0070633

Chen ECH, Morin E, Beaudet D, et al (2018) High intraspecific genome diversity in the model arbuscular mycorrhizal symbiont Rhizophagus irregularis. New Phytol 220:1161–1171. https://doi.org/10.1111/nph.14989

Chen G, Weil RR (2010) Penetration of cover crop roots through compacted soils. Plant Soil 331:31–43. https://doi.org/10.1007/s11104-009-0223-7

Choi J, Summers W, Paszkowski U (2018) Mechanisms Underlying Establishment of Arbuscular Mycorrhizal Symbioses. Annu Rev Phytopathol 56:135–160. https://doi.org/10.1146/annurev-phyto-080516-035521

Chu M, Jagadamma S, Walker FR, et al (2017) Effect of Multispecies Cover Crop Mixture on Soil Properties and Crop Yield. Agric Environ Lett 2:1–5. https://doi.org/10.2134/ael2017.09.0030

Cloutier ML, Murrell E, Barbercheck M, et al (2020) Fungal community shifts in soils with varied cover crop treatments and edaphic properties. Sci Rep 10:6198. https://doi.org/10.1038/s41598-020-63173-7

Coe S, Finlay J (2020) The Agriculture Bill (2019-2020). House Commons Libr

Coelho JJ, Hennessy A, Casey I, et al (2020) Biofertilisation with anaerobic digestates: A field study of effects on soil microbial abundance and diversity. Appl Soil Ecol 147:103403. https://doi.org/10.1016/j.apsoil.2019.103403

Cossio MLT, Giesen LF, Araya G, et al (2007) Managing Cover Crops Profitably. Sare 3:1–248

Crossay T, Majorel C, Redecker D, et al (2019) Is a mixture of arbuscular mycorrhizal fungi better for plant growth than single-species inoculants? Mycorrhiza 29:325–339. https://doi.org/10.1007/s00572-019-00898-y

Daniell TJ, Husband R, Fitter AH, Young JPW (2001) Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. FEMS Microbiol Ecol 36:203–209. https://doi.org/10.1016/S0168-6496(01)00134-9

Davidson-Lowe E, Ray S, Murrell E, et al (2021) Cover Crop Soil Legacies Alter Phytochemistry and Resistance to Fall Armyworm (Lepidoptera: Noctuidae) in Maize.

Environ Entomol 50:958–967. https://doi.org/10.1093/ee/nvab047

Davison J, Moora M, Öpik M, et al (2015) Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science (80-) 349:970–973. https://doi.org/10.5061/dryad.2m15n

Davison J, Öpik M, Daniell TJ, et al (2011) Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. FEMS Microbiol Ecol 78:103–115. https://doi.org/10.1111/j.1574-6941.2011.01103.x

Dawson CJ, Hilton J (2011) Fertiliser availability in a resource-limited world: Production and recycling of nitrogen and phosphorus. Food Policy 36:14–22. https://doi.org/10.1016/j.foodpol.2010.11.012

De La Providencia IE, De Souza FA, Fernández F, et al (2005) Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. New Phytol 165:261–271. https://doi.org/10.1111/j.1469-8137.2004.01236.x

De La Providencia IE, Fernández F, Declerck S (2007) Hyphal healing mechanism in the arbuscular mycorrhizal fungi Scutellospora reticulata and Glomus clarum differs in response to severe physical stress. FEMS Microbiol Lett 268:120–125. https://doi.org/10.1111/j.1574-6968.2006.00572.x

De Souza FA, Declerck S, Smit E, Kowalchuk GA (2005) Morphological, ontogenetic and molecular characterization of Scutellospora reticulata (Glomeromycota). Mycol Res 109:697–706. https://doi.org/10.1017/S0953756205002546

DeBary HA (1879) Die Erscheinung der Symbiose. Trübner, Strassburg

deCárcer DA, Denman SE, McSweeney C, Morrison M (2011) Evaluation of subsampling-based normalization strategies for tagged high-throughput sequencing data sets from gut microbiomes. Appl Environ Microbiol 77:8795–8798. https://doi.org/10.1128/AEM.05491-11

DEFRA (2018) Health and Harmony: the future for food, farming and the environment in a Green Brexit

DEFRA (2020) Countryside Stewardship Mid Tier and Wildlife Offers Manual

DEFRA (2017) Agriculture in the United Kingdom 2016. Natl Stat

DEFRA (2021) Sustainable Farming Incentive: Defra's plans for piloting and launching the scheme. In: gov.uk. https://www.gov.uk/government/publications/sustainable-farming-incentive-scheme-pilot-launch-overview/sustainable-farming-incentive-defras-plans-for-piloting-and-launching-the-scheme. Accessed 4 Dec 2021

DEFRA, Environment Agency (2018) Using Nitrogen Fertilisers in Nitrate Vulnerable Zones. In: Environ. Manag. L. Manag. https://www.gov.uk/guidance/using-nitrogen-fertilisers-in-nitrate-vulnerable-zones. Accessed 8 Apr 2020

Detheridge AP, Brand G, Fychan R, et al (2016) The legacy effect of cover crops on soil fungal populations in a cereal rotation. Agric Ecosyst Environ 228:49–61. https://doi.org/10.1016/j.agee.2016.04.022

Doran JW (2002) Soil health and global sustainability: translating science into practice. Agric Ecosyst Environ 88:119–127. https://doi.org/Doi 10.1016/S0167-8809(01)00246-8

Doran JW, Sarrantonio M, Liebig MA (1996) Soil Health and Sustainability. In: Advances in Agronomy. Academic Press, San Diego, CA.

Drew EA, Murray RS, Smith SE (2006) Functional diversity of external hyphae of AM fungi: Ability to colonise new hosts is influenced by fungal species, distance and soil conditions. Appl Soil Ecol 32:350–365. https://doi.org/10.1016/j.apsoil.2005.07.005

Egerton-Warburton LM, Johnson NC, Allen EB (2007) Mycorrhizal community dynamics following nitrogen fertilization: A cross-site test in five grasslands. Ecol Monogr 77:527–544. https://doi.org/10.1890/06-1772.1

Erisman JW, Galloway JN, Seitzinger S, et al (2013) Consequences of human modification of the global nitrogen cycle. Philos Trans R Soc B Biol Sci 368:. https://doi.org/10.1098/rstb.2013.0116

Erisman JW, Sutton MA, Galloway J, et al (2008) How a century of ammonia synthesis changed the world. Nat Geosci 1:636–639. https://doi.org/10.1038/ngeo325

Evangelisti E, Turner C, Mcdowell A, et al (2021) Artificial intelligence enables the identification and quantification of arbuscular mycorrhizal fungi in plant roots. bioRxiv

Faggioli VS, Cabello MN, Grilli G, et al (2019) Root colonizing and soil borne communities

of arbuscular mycorrhizal fungi differ among soybean fields with contrasting historical land use. Agric Ecosyst Environ 269:174–182. https://doi.org/10.1016/j.agee.2018.10.002

FAO (2018) FAOSTAT. In: FAOSTAT - Fertil. Arch. (Dataset). (Latest Updat. January 8, 2018). http://www.fao.org/faostat/en/#data/RA. Accessed 2 Aug 2018

FAO and ITPS (2015) Status of the World's Soil Resources (SWSR)

Faye A, Dalpé Y, Ndung'u-Magiroi K, et al (2013) Evaluation of commercial arbuscular mycorrhizal inoculants. Can J Plant Sci 93:1201–1208. https://doi.org/10.4141/cjps2013-326

Finney DM, White CM, Kaye JP (2016) Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. Agron J 108:39–52. https://doi.org/10.2134/agronj15.0182

Fischer T, Byerlee D, Edmeades G (2014) Crop yields and global food security

Frey SD (2019) Mycorrhizal Fungi as Mediators of Soil Organic Matter Dynamics. Annu Rev Ecol Evol Syst 50:237–259. https://doi.org/10.1146/annurev-ecolsys-110617-062331

Frink CR, Waggoner PE, Ausubel JH (1999) Nitrogen fertilizer: Retrospect and prospect. Proc Natl Acad Sci U S A 96:1175–1180. https://doi.org/10.1073/pnas.96.4.1175

Galloway JN, Townsend AR, Erisman JW, et al (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science (80-) 320:889–892. https://doi.org/10.1126/science.1136674

Galvez L, Douds DD, Drinkwater LE, Wagoner P (2001) Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. Plant Soil 228:299–308. https://doi.org/10.1023/A:1004810116854

Galvez L, Douds DD, Wagoner P, et al (1995) An overwintering cover crop increases inoculum of VAM fungi in agricultural soil. Am J Altern Agric 10:152–156. https://doi.org/10.1017/S0889189300006391

Gamper HA, Walker C, Schüßler A (2009) Diversispora celata sp. nov: Molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. New Phytol 182:495–506. https://doi.org/10.1111/j.1469-8137.2008.02750.x

Gange AC, Brown VK, Sinclair GS (1993) Vesicular-arbuscular mycorrhizal fungi: a determinant of plant community structure in early succession. Funct Ecol 7:616–622. https://doi.org/10.2307/2390139

Gehrig H, Schüßler A, Kluge M (1996) Geosiphon pyriforme, a fungus forming endocytobiosis with Nostoc (cyanobacteria), is an ancestral member of the glomales: Evidence by SSU rRNA analysis. J Mol Evol 43:71–81. https://doi.org/10.1007/BF02352301

Genre A, Chabaud M, Timmers T, et al (2005) Arbuscular Mycorrhizal Fungi Elicit a Novel Intracellular Apparatus in Medicago truncatula Root Epidermal Cells before Infection. Plant Cell 17:3489–3499

Giller KE, Ormesher J, Awah FM (1991) Nitrogen transfer from Phaseolus bean to intercropped maize measured using 15N-enrichment and 15N-isotope dilution methods. Soil Biol Biochem 23:339–346. https://doi.org/10.1016/0038-0717(91)90189-Q

Gobbato E, Wang E, Higgins G, et al (2013) RAM1 and RAM2 function and expression during Arbuscular Mycorrhizal Symbiosis and Aphanomyces euteiches colonization. Plant Signal Behav 8:e26049. https://doi.org/10.4161/psb.26049

Godfray HCJ, Beddington JR, Crute IR, et al (2010) Food security: the challenge of feeding 9 billion people. Science. https://doi.org/10.1126/science.1185383

Gollotte A, Van Tuinen D, Atkinson D (2004) Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species Agrostis capillaris and Lolium perenne in a field experiment. Mycorrhiza 14:111–117. https://doi.org/10.1007/s00572-003-0244-7

González-Chávez MC, Carrillo-González R, Wright SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323. https://doi.org/10.1016/j.envpol.2004.01.004

González-Guerrero M, Azcón-Aguilar C, Mooney M, et al (2005) Characterization of a Glomus intraradices gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet Biol 42:130–140. https://doi.org/10.1016/j.fgb.2004.10.007

Gosling P, Jones J, Bending GD (2016) Evidence for functional redundancy in arbuscular

mycorrhizal fungi and implications for agroecosystem management. Mycorrhiza 26:77–83. https://doi.org/10.1007/s00572-015-0651-6

Gosling P, Mead A, Proctor M, et al (2013) Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. New Phytol 198:546–556. https://doi.org/10.1111/nph.12169

Govindarajulu M, Pfeffer PE, Jin H, et al (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823. https://doi.org/10.1038/nature03610

Grafton RQ, Daugbjerg C, Qureshi ME (2015) Towards food security by 2050. Food Secur 7:179–183. https://doi.org/10.1007/s12571-015-0445-x

Grime JP, Mackey JML, Hillier SH, Read DJ (1988) Floristic diversity in a model system using experimental microcosms. Nature 328:420–422. https://doi.org/10.1038/328420a0

Grime JP, Pierce S (2012) The Evolutionary Strategies that Shape Ecosystems

Gryndler M (1992) The ecological role of mycorrhizal symbiosis and the origin of the land plants. Ces Mykol 46:93–98

Guimarães RML, Ball BC, Tormena CA, et al (2013) Relating visual evaluation of soil structure to other physical properties in soils of contrasting texture and management. Soil Tillage Res 127:92–99. https://doi.org/10.1016/j.still.2012.01.020

Gutjahr C, Banba M, Croset V, et al (2008) Arbuscular Mycorrhiza-Specific Signaling in Rice Transcends the Common Symbiosis Signaling Pathway. Plant Cell 20:2989–3005. https://doi.org/10.1105/tpc.108.062414

Gutjahr C, Radovanovic D, Geoffroy J, et al (2012) The half-size ABC transporters STR1 and STR2 are indispensable for mycorrhizal arbuscule formation in rice. Plant J 69:906–920. https://doi.org/10.1111/j.1365-313X.2011.04842.x

Gutser R, Ebertseder T, Weber A, et al (2005) Short-term and residual availability of nitrogen after long-term application of organic fertilizers on arable land. J Plant Nutr Soil Sci 168:439–446. https://doi.org/10.1002/jpln.200520510

Hamza MA, Anderson WK (2005) Soil compaction in cropping systems: A review of the nature, causes and possible solutions. Soil Tillage Res 82:121–145.

https://doi.org/10.1016/j.still.2004.08.009

Haramoto ER, Gallandt ER (2004) Brassica cover cropping for weed management: A review. Renew Agric Food Syst 19:187–198. https://doi.org/10.1079/RAFS200490

Hart MM, Antunes PM, Chaudhary VB, Abbott LK (2018) Fungal inoculants in the field: Is the reward greater than the risk? Funct Ecol 31:126–135. https://doi.org/10.1111/1365-2435.12976

Hart MM, Reader RJ, Klironomos JN (2003) Plant coexistence mediated by arbuscular mycorrhizal fungi. Trends Ecol Evol 18:418–423. https://doi.org/10.1016/S0169-5347(03)00127-7

Hart MM, Reader RJ, Klironomos JN (2001) Life-History Strategies of Arbuscular Mycorrhizal Fungi in Relation to Their Successional Dynamics. Mycologia 93:1186–1194. https://doi.org/10.2307/3761678

Hart MM, Trevors JT (2005) Microbe Management: Application of Mycorrhyzal Fungi in Sustainable Agriculture. Front Ecol Environ 3:533–539. https://doi.org/10.2307/3868609

Hartnett DC, Wilson GWT (1999) Mycorrhizae Influence Plant Community Structure and Diversity in Tallgrass Prairie. Ecology 80:1187–1195. https://doi.org/10.2307/177066

Hausmann NT, Hawkes C V. (2009) Plant neighborhood control of arbuscular mycorrhizal community composition. New Phytol 183:1188–1200. https://doi.org/10.1111/j.1469-8137.2009.02882.x

Hayashi T, Banba M, Shimoda Y, et al (2010) A dominant function of CCaMK in intracellular accommodation of bacterial and fungal endosymbionts. Plant J 63:141–154. https://doi.org/10.1111/j.1365-313X.2010.04228.x

He XH, Critchley C, Bledsoe C (2003) Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). CRC Crit Rev Plant Sci 22:531–567. https://doi.org/10.1080/713608315

Heberle E dos S, Armas RD de, Heberle DA, et al (2015) Occurrence and Structure of Arbuscular Mycorrhizal Fungal Communities in Cassava after Cultivation of Cover Crops as Observed by the “PCR-DGGE” Technique. Rev Bras Ciência do Solo 39:. https://doi.org/10.1590/01000683rbcs20140216

Helgason T, Daniell TJ, Husband R, et al (1998) Ploughing up the wood-wide web? Nature 394:431. https://doi.org/10.1038/28764

Higo M, Isobe K, Drijber RA, et al (2014) Impact of a 5-year winter cover crop rotational system on the molecular diversity of arbuscular mycorrhizal fungi colonizing roots of subsequent soybean. Biol Fertil Soils 50:913–926. https://doi.org/10.1007/s00374-014-0912-0

Higo M, Isobe K, Kondo T, et al (2015) Temporal variation of the molecular diversity of arbuscular mycorrhizal communities in three different winter cover crop rotational systems. Biol Fertil Soils 51:21–32. https://doi.org/10.1007/s00374-014-0945-4

Higo M, Isobe K, Yamaguchi M, et al (2013) Diversity and vertical distribution of indigenous arbuscular mycorrhizal fungi under two soybean rotational systems. Biol Fertil Soils 49:1085–1096. https://doi.org/10.1007/s00374-013-0807-5

Higo M, Sato R, Serizawa A, et al (2018) Can phosphorus application and cover cropping alter arbuscular mycorrhizal fungal communities and soybean performance after a five-year phosphorus-unfertilized crop rotational system? PeerJ 18:e4606. https://doi.org/10.7717/peerj.4606

Higo M, Takahashi Y, Gunji K, Isobe K (2017) How are arbuscular mycorrhizal associations related to maize growth performance during short-term cover crop rotation? J Sci Food Agric 98:1388–1396. https://doi.org/10.1002/jsfa.8606

Higo M, Tatewaki Y, Iida K, et al (2020) Amplicon sequencing analysis of arbuscular mycorrhizal fungal communities colonizing maize roots in different cover cropping and tillage systems. Sci Rep 10:. https://doi.org/10.1038/s41598-020-58942-3

Hiiesalu I, Pärtel M, Davison J, et al (2014) Species richness of arbuscular mycorrhizal fungi: Associations with grassland plant richness and biomass. New Phytol 203:233–244. https://doi.org/10.1111/nph.12765

Hijmans RJ (2020) raster: Geographic Data Analysis and Modeling

Hijri M, Sanders IR (2005) Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. Nature 433:160–163. https://doi.org/10.1038/nature03069

Hill J (2006) Inhibition of Vesicular–Arbuscular Mycorrhizae on Soybean Roots following

Brassica Cover Crop. J Nat Resour Life Sci Educ 35:158–160. https://doi.org/10.2134/jnrlse2006.0158

Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299. https://doi.org/10.1038/35095041

Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Natl Acad Sci U S A 107:13754–13759. https://doi.org/10.1073/pnas.1005874107

Hodge A, Storer K (2014) Arbuscular mycorrhiza and nitrogen: Implications for individual plants through to ecosystems. Plant Soil 386:1–19. https://doi.org/10.1007/s11104-014-2162-1

Hoeksema JD, Chaudhary VB, Gehring CA, et al (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol. Lett. 13:394–407

Hontoria C, García-González I, Quemada M, et al (2019) The cover crop determines the AMF community composition in soil and in roots of maize after a ten-year continuous crop rotation. Sci Total Environ 660:913–922. https://doi.org/10.1016/J.SCITOTENV.2019.01.095

House GJ, Parmelee RW (1985) Comparison of soil arthropods and earthworms from conventional and no-tillage agroecosystems. Soil Tillage Res 5:351–360. https://doi.org/10.1016/S0167-1987(85)80003-9

Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115:77–83. https://doi.org/10.1111/j.1469-8137.1990.tb00924.x

James TY, Kauff F, Schoch CL, et al (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822. https://doi.org/10.1038/nature05110

Janos DP (1980) Mycorrhizae Influence Tropical Succession. Biotropica 12:56–64. https://doi.org/10.2307/2388157

Jansa J, Erb A, Oberholzer HR, et al (2014) Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management

practices in Swiss agricultural soils. Mol Ecol 23:2118–2135. https://doi.org/10.1111/mec.12706

Jansa J, Mozafar A, Anken T, et al (2002) Diversity and structure of AMF communities as affected by tillage in a temperate soil. Mycorrhiza 12:225–234. https://doi.org/10.1007/s00572-002-0163-z

Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytol 177:779–789. https://doi.org/10.1111/j.1469-8137.2007.02294.x

Janse JM (1897) Les endophytes radicaux de quelques plantes Javanese. Ann du Jard Bot Buitenzorg 14:53–201

Javot H, Penmetsa RV, Terzaghi N, et al (2007) A Medicago truncatula phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci U S A 104:1720–1725. https://doi.org/10.1073/pnas.0608136104

Jin H, Germida JJ, Walley FL (2013) Suppressive effects of seed-applied fungicides on arbuscular mycorrhizal fungi (AMF) differ with fungicide mode of action and AMF species. Appl Soil Ecol 72:22–30. https://doi.org/10.1016/j.apsoil.2013.05.013

Johnson D, Leake JR, Read DJ (2002) Transfer of recent photosynthate into mycorrhizal mycelium of an upland grassland: Short-term respiratory losses and accumulation of 14C. Soil Biol Biochem 34:1521–1524. https://doi.org/10.1016/S0038-0717(02)00126-8

Johnson D, Vandenkoornhuyse PJ, Leake JR, et al (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytol 161:503–515. https://doi.org/10.1046/j.1469-8137.2003.00938.x

Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae? Ecol Appl 3:749–757. https://doi.org/10.2307/1942106

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol 135:575–585. https://doi.org/10.1046/j.1469-8137.1997.00729.x

Johnson NC, Wilson GWT, Bowker MA, et al (2010) Resource limitation is a driver of

local adaptation in mycorrhizal symbioses. Proc Natl Acad Sci U S A 107:2093–2098. https://doi.org/10.1073/pnas.0906710107

Joshi N, Fass J (2011) Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (Version 1.33) [Software]. Available at https://github.com/najoshi/sickle

Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ (2012) Mycorrhiza-Induced Resistance and Priming of Plant Defenses. J Chem Ecol 38:651–664. https://doi.org/10.1007/s10886-012-0134-6

Kabir Z (2005) Tillage or no-tillage: Impact on mycorrhizae. Can J Plant Sci 85:23–29. https://doi.org/10.4141/P03-160

Kabir Z, Koide RT (2002) Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake and yield of sweet corn in Pennsylvania, USA. Plant Soil 238:205–215. https://doi.org/10.1023/A:1014408723664

Kabir Z, Koide RT (2000) The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize. Agric Ecosyst Environ 78:167–174. https://doi.org/10.1016/S0167-8809(99)00121-8

Kabir Z, O'Halloran IP, Hamel C (1999) Combined effects of soil disturbance and fallowing on plant and fungal components of mycorrhizal corn (Zea mays L.). Soil Biol Biochem 31:307–314. https://doi.org/10.1016/S0038-0717(98)00124-2

Kahiluoto H, Vestberg M (1998) The Effect of Arbuscular Mycorrhiza on Biomass Production and Phosphorus Uptake from Sparingly Soluble Sources By Leek (Allium porrum L.) in Finnish Field Soils. Biol Agric Hortic 16:65–85. https://doi.org/10.1080/01448765.1998.9755219

Karasawa T, Takebe M (2012) Temporal or spatial arrangements of cover crops to promote arbuscular mycorrhizal colonization and P uptake of upland crops grown after nonmycorrhizal crops. Plant Soil 353:1–2. https://doi.org/10.1007/s11104-011-1036-z

Keating BA, Herrero M, Carberry PS, et al (2014) Food wedges: Framing the global food demand and supply challenge towards 2050. Glob Food Sec 3:125–132. https://doi.org/10.1016/j.gfs.2014.08.004

Kessavalou A, Doran JW, Mosier AR, Drijber RA (1998) Greenhouse Gas Fluxes following Tillage and Wetting in a Wheat-Fallow Cropping System. J Environ Qual 27:1105–

1116. https://doi.org/10.2134/jeq1998.00472425002700050016x

Kiers ET, Duhamel M, Beesetty Y, et al (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science (80-) 333:880–882. https://doi.org/10.1126/science.1208473

Kivlin SN, Hawkes C V., Treseder KK (2011) Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biol Biochem 43:2294–2303. https://doi.org/10.1016/j.soilbio.2011.07.012

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301. https://doi.org/10.1890/02-0413

Klironomos JN, McCune J, Hart M, Neville J (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecol Lett 3:137–141. https://doi.org/10.1046/j.1461-0248.2000.00131.x

Knight S, Knightley S, Bingham I, et al (2012) Desk study to evaluate contributory causes of the current 'yield plateau' in wheat and oilseed rape. Agric Hortic Dev Board - Proj Rep No 502

Kobae Y, Tamura Y, Takai S, et al (2010) Localized expression of arbuscular mycorrhiza-inducible ammonium transporters in soybean. Plant Cell Physiol 51:1411–1415. https://doi.org/10.1093/pcp/pcq099

Koch AM, Kuhn G, Fontanillas P, et al (2004) High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. Proc Natl Acad Sci U S A 101:2369–2374. https://doi.org/10.1073/pnas.0306441101

Köhl L, Lukasiewicz CE, Van der Heijden MGA (2016) Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. Plant Cell Environ 39:136–146. https://doi.org/10.1111/pce.12600

Krüger M, Krüger C, Walker C, et al (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol 193:970–984. https://doi.org/10.1111/j.1469-8137.2011.03962.x

Krüger M, Stockinger H, Krüger C, Schüßler A (2009) DNA-based species level detection of Glomeromycota: One PCR primer set for all arbuscular mycorrhizal fungi. New Phytol 183:212–223. https://doi.org/10.1111/j.1469-8137.2009.02835.x

Lal R (2004) Soil carbon sequestration impacts on global climate change and food security. Science (80-) 304:1623–1627. https://doi.org/10.1126/science.1097396

Lee J, Lee S, Young JPW (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65:339–349. https://doi.org/10.1111/j.1574-6941.2008.00531.x

Lee J, Young JPW (2009) The mitochondrial genome sequence of the arbuscular mycorrhizal fungus Glomus intraradices isolate 494 and implications for the phylogenetic placement of Glomus. New Phytol 183:200–211. https://doi.org/10.1111/j.1469-8137.2009.02834.x

Lehman RM, Taheri WI, Osborne SL, et al (2012) Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. Appl Soil Ecol 61:300–304. https://doi.org/10.1016/j.apsoil.2011.11.008

Leigh J, Hodge A, Fitter AH (2009) Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. New Phytol 181:199–207. https://doi.org/10.1111/j.1469-8137.2008.02630.x

Lekberg Y, Koide RT (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytol 168:189–204. https://doi.org/10.1111/j.1469-8137.2005.01490.x

Lekberg Y, Koide RT, Rohr JR, et al (2007) Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J Ecol 95:95–105. https://doi.org/10.1111/j.1365-2745.2006.01193.x

Lekberg Y, Vasar M, Bullington LS, et al (2018) More bang for the buck? Can arbuscular mycorrhizal fungal communities be characterized adequately alongside other fungi using general fungal primers? New Phytol 220:971–976. https://doi.org/10.1111/nph.15035

Lenth R, Singmann H, Love J, et al (2020) emmeans : Estimated Marginal Means, aka Least-Squares Means. R Packag. version 1.15-15

Li M, Jordan NR, Koide RT, et al (2019) Interspecific variation in crop and weed responses to arbuscular mycorrhizal fungal community highlights opportunities for weed biocontrol. Appl Soil Ecol 142:34–42. https://doi.org/10.1016/j.apsoil.2019.05.016

Lindahl BD, Nilsson RH, Tedersoo L, et al (2013) Fungal community analysis by high-throughput sequencing of amplified markers - a user's guide. New Phytol 199:288–299. https://doi.org/10.1111/nph.12243

Liu X, Gao X, Wang W, et al (2012) Pilot-scale anaerobic co-digestion of municipal biomass waste: Focusing on biogas production and GHG reduction. Renew Energy 32:2056–2060. https://doi.org/10.1016/j.renene.2012.01.092

Liu Y, Johnson NC, Mao L, et al (2015) Phylogenetic structure of arbuscular mycorrhizal community shifts in response to increasing soil fertility. Soil Biol Biochem 89:196–205. https://doi.org/10.1016/j.soilbio.2015.07.007

Lloyd-Macgilp SA, Chambers SM, Dodd JC, et al (1996) Diversity of the ribosomal internal transcribed spacers within and among isolates of Glomus mosseae and related mycorrhizal fungi. New Phytol 133:103–111. https://doi.org/10.1111/j.1469-8137.1996.tb04346.x

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:. https://doi.org/10.1186/s13059-014-0550-8

Lumini E, Voyron S, Berruti A, et al (2014) Do arbuscular mycorrhizal fungi exhibit phylogenetic conservatism in their response to drought? In: First Global Soil Biodiversity Conference--Assessing soil biodiversity and its role for ecosystem services. Dijon, France

Maclean AM, Bravo A, Harrison MJ (2017) Plant signaling and metabolic pathways enabling arbuscular mycorrhizal symbiosis. Plant Cell 29:2319–2335. https://doi.org/10.1105/tpc.17.00555

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science (80-) 316:1746–1748. https://doi.org/10.1126/science.1143082

Maherali H, Oberle B, Stevens PF, et al (2016) Mutualism Persistence and Abandonment during the Evolution of the Mycorrhizal Symbiosis. Am Nat 188:113–125. https://doi.org/10.1086/688675

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–

102. https://doi.org/10.1007/BF00000098

Martin F, Gianinazzi-Pearson V, Hijri M, et al (2008) The long hard road to a completed Glomus intraradices genome. New Phytol 180:747–750. https://doi.org/10.1111/j.1469-8137.2008.02671.x

Martínez-García LB, Korthals G, Brussaard L, et al (2018) Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties. Agric Ecosyst Environ 263:7–17. https://doi.org/10.1016/j.agee.2018.04.018

Martínez-García LB, Pugnaire FI (2011) Arbuscular mycorrhizal fungi host preference and site effects in two plant species in a semiarid environment. Appl Soil Ecol 48:313–317. https://doi.org/10.1016/j.apsoil.2011.04.003

Masella AP, Bartram AK, Truszkowski JM, et al (2012) PANDAseq: Paired-end assembler for illumina sequences. BMC Bioinformatics 13:. https://doi.org/10.1186/1471-2105-13-31

McGonigle TP, Fitter AH (1990) Ecological specificity of vesicular-arbuscular mycorrhizal associations. Mycol Res 94:120–122. https://doi.org/10.1016/S0953-7562(09)81272-0

McMurdie PJ, Holmes S (2014) Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLoS Comput Biol 10:e1003531. https://doi.org/10.1371/journal.pcbi.1003531

McMurdie PJ, Holmes S (2013) Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One 8:e61217. https://doi.org/10.1371/journal.pone.0061217

Menéndez AB, Scervino JM, Godeas AM (2001) Arbuscular mycorrhizal populations associated with natural and cultivated vegetation on a site of Buenos Aires province, Argentina. Biol Fertil Soils 33:373–381. https://doi.org/10.1007/s003740000336

Middleton EL, Richardson S, Koziol L, et al (2015) Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere 6:1–16. https://doi.org/10.1890/ES15-00152.1

Miller JB, Pratap A, Miyahara A, et al (2013) Calcium/calmodulin-dependent protein kinase is negatively and positively regulated by calcium, providing a mechanism for

decoding calcium responses during symbiosis signaling. Plant Cell 12:5053–5066. https://doi.org/10.1105/tpc.113.116921

Moora M, Davison J, Öpik M, et al (2014) Anthropogenic land use shapes the composition and phylogenetic structure of soil arbuscular mycorrhizal fungal communities. FEMS Microbiol Ecol 90:609–621. https://doi.org/10.1111/1574-6941.12420

Morgan BST, Egerton-Warburton LM (2017) Barcoded NS31/AML2 Primers for Sequencing of Arbuscular Mycorrhizal Communities in Environmental Samples. Appl Plant Sci 5:1700017. https://doi.org/10.3732/apps.1700017

Morton JB, Benny GL (1990) Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. Mycotaxon 37:471–491

Morton JB, Msiska Z (2010) Phylogenies from genetic and morphological characters do not support a revision of Gigasporaceae (Glomeromycota) into four families and five genera. Mycorrhiza 20:483–496. https://doi.org/10.1007/s00572-010-0303-9

Mosse B (1953) Fructifications associated with mycorrhizal strawberry roots. Nature 171:974. https://doi.org/10.1038/171974a0

Motisi N, Montfort F, Faloya V, et al (2009) Growing Brassica juncea as a cover crop, then incorporating its residues provide complementary control of Rhizoctonia root rot of sugar beet. F Crop Res 113:238–245. https://doi.org/10.1016/j.fcr.2009.05.011

Mouhamad R, Atiyah A, Mohammad R, Iqbal M (2015) Decomposition of organic matter under different soil textures. Curr Sci Perspect 1:22–25

Munkholm LJ, Heck RJ, Deen B (2013) Long-term rotation and tillage effects on soil structure and crop yield. Soil Tillage Res 127:85–91. https://doi.org/10.1016/j.still.2012.02.007

Nikolenko SI, Korobeynikov AI, Alekseyev MA (2013) BayesHammer: Bayesian clustering for error correction in single-cell sequencing. BMC Genomics 14:. https://doi.org/10.1186/1471-2164-14-S1-S7

Njeru EM, Avio L, Bocci G, et al (2014a) Contrasting effects of cover crops on 'hot spot' arbuscular mycorrhizal fungal communities in organic tomato. Biol Fertil Soils

51:151–166. https://doi.org/10.1007/s00374-014-0958-z

Njeru EM, Avio L, Sbrana C, et al (2014b) First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize growth. Agron Sustain Dev 34:841–848. https://doi.org/10.1007/s13593-013-0197-y

Nurk S, Bankevich A, Antipov D, et al (2013) Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol 10:714–737. https://doi.org/10.1089/cmb.2013.0084

Oehl F, Da Silva GA, Goto BT, Sieverding E (2011) Glomeromycota: Three new genera and glomoid species reorganized. Mycotaxon 116:75–120. https://doi.org/10.5248/116.75

Oehl F, De Souza FA, Sieverding E (2008) Revision of Scutellospora and description of five new genera and three new families in the arbuscular mycorrhiza-forming Glomeromycetes. Mycotaxon 106:311–360

Oehl F, Laczko E, Oberholzer HR, et al (2017) Diversity and biogeography of arbuscular mycorrhizal fungi in agricultural soils. Biol Fertil Soils 53:777–797. https://doi.org/10.1007/s00374-017-1217-x

Oehl F, Sieverding E, Ineichen K, et al (2003) Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Appl Environ Microbiol 69:2816–2824. https://doi.org/10.1128/AEM.69.5.2816-2824.2003

Ofori F, Stern WR (1987) Cereal–Legume Intercropping Systems. Adv Agron 41:41–90. https://doi.org/10.1016/S0065-2113(08)60802-0

Oksanen J, Blanchet FG, Kindt R, et al (2013) Package vegan: Community Ecology Package

Oldroyd GED (2013) Speak, friend, and enter: Signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263. https://doi.org/10.1038/nrmicro2990

Öpik M, Davison J, Moora M, et al (2016) Response to Comment on "Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism." Science (80-) 351:826. https://doi.org/10.1126/science.aad5495

Öpik M, Davison J, Moora M, Zobel M (2014) Dna-based detection and identification of

glomeromycota:The virtual taxonomy of environmental sequences. Botany 92:135–147. https://doi.org/10.1139/cjb-2013-0110

Öpik M, Metsis M, Daniell TJ, et al (2009) Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytol 184:424–437. https://doi.org/10.1111/j.1469-8137.2009.02920.x

Öpik M, Moora M, Liira J, Zobel M (2006) Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. J Ecol 94:778–790. https://doi.org/10.1111/j.1365-2745.2006.01136.x

Öpik M, Vanatoa A, Vanatoa E, et al (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188:223–241. https://doi.org/10.1111/j.1469-8137.2010.03334.x

Öpik M, Zobel M, Cantero JJ, et al (2013) Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. Mycorrhiza 23:411–430. https://doi.org/10.1007/s00572-013-0482-2

Pakarinen A, Fritze H, Timonen S, et al (2021) Boreal soil microbial diversity and seed onion mycorrhizal colonization is unaffected by preceding one season crop cultivation. Eur J Soil Biol 105:103335. https://doi.org/10.1016/j.ejsobi.2021.103335

Paszkowski U, Kroken S, Roux C, Briggs SP (2002) Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci U S A 99:13324–13329. https://doi.org/10.1073/pnas.202474599

Paul Schreiner R, Koide RT (1993) Antifungal compounds from the roots of mycotrophic and non-mycotrophic plant species. New Phytol 123:99–105. https://doi.org/10.1111/j.1469-8137.1993.tb04535.x

Pawlowska TE, Taylor JW (2004) Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. Nature 427:733–737. https://doi.org/10.1038/nature02290

Pearson JN, Abbott LK, Jasper DA (1993) Mediation of competition between two colonizing VA mycorrhizal fungi by the host plant. New Phytol 123:93–98.

https://doi.org/10.1111/j.1469-8137.1993.tb04534.x

Pellegrino E, Turrini A, Gamper HA, et al (2012) Establishment, persistence and effectiveness of arbuscular mycorrhizal fungal inoculants in the field revealed using molecular genetic tracing and measurement of yield components. New Phytol 194:810–822. https://doi.org/10.1111/j.1469-8137.2012.04090.x

Pellerin S, Mollier A, Morel C, Plenchette C (2007) Effect of incorporation of Brassica napus L. residues in soils on mycorrhizal fungus colonisation of roots and phosphorus uptake by maize (Zea mays L.). Eur J Agron 26:113–120. https://doi.org/10.1016/j.eja.2006.07.007

Peoples MB, Herridge DF, Ladha JK (1995) Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? Plant Soil 174:3–28. https://doi.org/10.1007/BF00032239

Peterson RL, Piché Y, Plenchette C (1984) Mycorrhizae and their potential use in the agricultural and forestry industries. Biotechnol. Adv. 2:101–120

Pianka ER (1970) On r- and K-Selection. Am Nat 104:592–597. https://doi.org/10.1086/282697

Pierce D (2019) ncdf4: Interface to Unidata netCDF (Version 4 or Earlier) Format Data Files

Pinck LA, Allison FE, Gaddy VL (1948) The Effect of Green Manure Crops of Varying Carbon-Nitrogen Ratios upon Nitrogen Availability and Soil Organic Matter Content. Agron J 40:237–248. https://doi.org/10.2134/agronj1948.00021962004000030004x

Plenchette C, Fortin JA, Furlan V (1983) Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility - I. Mycorrhizal dependency under field conditions. Plant Soil 70:199–209. https://doi.org/10.1007/BF02374780

Pretty JN, Mason CF, Nedwell DB, et al (2003) Environmental costs of freshwater eutrophication in England and Wales. Environ Sci Technol 37:201–208. https://doi.org/10.1021/es020793k

R Core Team (2019) R: A language and environment for statistical computing.

Ramos-Zapata JA, Marrufo-Zapata D, Guadarrama P, et al (2012) Impact of weed control on arbuscular mycorrhizal fungi in a tropical agroecosystem: A long-term experiment.

Mycorrhiza 22:653–661. https://doi.org/10.1007/s00572-012-0443-1

Rapparini F, Peñuelas J (2014) Mycorrhizal fungi to alleviate drought stress on plant growth. In: Use of Microbes for the Alleviation of Soil Stresses. pp 21–42

Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science (80-) 289:1920–1921. https://doi.org/10.1126/science.289.5486.1920

Redecker D, Raab P (2006) Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): Recent developments and new gene markers. Mycologia 98:885–895. https://doi.org/10.3852/mycologia.98.6.885

Redecker D, Schüßler A, Stockinger H, et al (2013) An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza 23:515–531. https://doi.org/10.1007/s00572-013-0486-y

Reid DA, Gerdemann JW, Trappe JM (1975) The Endogonaceae in the Pacific Northwest. Kew Bull. https://doi.org/10.2307/4102919

Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc Natl Acad Sci U S A 91:11841–11843. https://doi.org/10.1073/pnas.91.25.11841

Reuter JA, Spacek D V., Snyder MP (2015) High-Throughput Sequencing Technologies. Mol Cell 58:586–597. https://doi.org/10.1016/j.molcel.2015.05.004

Rillig MC, Aguilar-Trigueros CA, Camenzind T, et al (2019) Why farmers should manage the arbuscular mycorrhizal symbiosis. New Phytol 222:1171–1175. https://doi.org/10.1111/nph.15602

Rodríguez-Echeverría S, Teixeira H, Correia M, et al (2017) Arbuscular mycorrhizal fungi communities from tropical Africa reveal strong ecological structure. New Phytol 213:380–390. https://doi.org/10.1111/nph.14122

Rodriguez A, Sanders IR (2014) The role of community and population ecology in applying mycorrhizal fungi for improved food security. ISME J 9:1053–1061. https://doi.org/10.1038/ismej.2014.207

Rognes T, Flouri T, Nichols B, et al (2016) VSEARCH: A versatile open source tool for metagenomics. PeerJ 4:e2584. https://doi.org/10.7717/peerj.2584

Rosendahl S, Matzen HB (2008) Genetic structure of arbuscular mycorrhizal populations

in fallow and cultivated soils. New Phytol 179:1154–1161. https://doi.org/10.1111/j.1469-8137.2008.02535.x

Rosewarne GM, Barker SJ, Smith SE (1997) Production of near-synchronous fungal colonization in tomato for developmental and molecular analyses of mycorrhiza. Mycol Res 101:966–970. https://doi.org/10.1017/S0953756297003626

Roth R, Paszkowski U (2017) Plant carbon nourishment of arbuscular mycorrhizal fungi. Curr Opin Plant Biol 39:50–56. https://doi.org/10.1016/j.pbi.2017.05.008

RStudio Team (2015) RStudio: Integrated Development for R. . RStudio, Inc

Ryan MH, Graham JH (2018) Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. New Phytol. 220:1092–1107

Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: A continuum of interactions with host plants. Annu Rev Ecol Syst 29:319–343. https://doi.org/10.1146/annurev.ecolsys.29.1.319

Säle V, Aguilera P, Laczko E, et al (2015) Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. Soil Biol Biochem 84:38–52. https://doi.org/10.1016/j.soilbio.2015.02.005

Sanders IR, Alt M, Groppe K, et al (1995) Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. New Phytol 130:419–427. https://doi.org/10.1111/j.1469-8137.1995.tb01836.x

Schalamuk S, Cabello M (2010) Arbuscular mycorrhizal fungal propagules from tillage and no-tillage systems: Possible effects on Glomeromycota diversity. Mycologia 102:261–268. https://doi.org/10.3852/08-118

Schloss PD, Westcott SL, Ryabin T, et al (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541. https://doi.org/10.1128/AEM.01541-09

Schnoor TK, Lekberg Y, Rosendahl S, Olsson PA (2011) Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland.

Mycorrhiza 21:211–220. https://doi.org/10.1007/s00572-010-0325-3

Schoch CL, Seifert KA, Huhndorf S, et al (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci U S A 109:6241–6246. https://doi.org/10.1073/pnas.1117018109

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: Phylogeny and evolution. Mycol Res 105:1413–1421. https://doi.org/10.1017/S0953756201005196

Schwarzott D, Walker C, Schüßler A (2001) Glomus, the largest genus of the arbuscular mycorrhizal fungi (Glomales), is nonmonophyletic. Mol Phylogenet Evol 21:190–197. https://doi.org/10.1006/mpev.2001.1007

Schwieger F, Tebbe CC (1998) A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. Appl Environ Microbiol 64:4870–4876. https://doi.org/10.1128/aem.64.12.4870-4876.1998

Selosse MA, Le Tacon F (1998) The land flora: A phototroph-fungus partnership? Trends Ecol Evol 13:15–20. https://doi.org/10.1016/S0169-5347(97)01230-5

Simon L, Lalonde M, Bruns TD (1992) Specific amplification of 18S fungal ribosomal genes from vesicular- arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58:291–295

Singh PK, Singh M, Tripathi BN (2013) Glomalin: An arbuscular mycorrhizal fungal soil protein. Protoplasma 250:663–669. https://doi.org/10.1007/s00709-012-0453-z

Smil V (1999) Nitrogen in crop production: An account of global flows. Global Biogeochem Cycles 13:647–662. https://doi.org/10.1029/1999GB900015

Smith SE, Read D (2008) 1-The symbionts forming arbuscular mycorrhizas. Mycorrhizal Symbiosis. https://doi.org/10.1016/B978-012370526-6.50003-9

Smith SE, Read DJ (2010) Mycorrhizal symbiosis, Third edition edn. Academic, New York

Smith SE, Smith FA (2011) Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. Annu Rev Plant Biol 62:227–250. https://doi.org/10.1146/annurev-arplant-042110-103846

Smith SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol 133:16–20.

https://doi.org/10.1104/pp.103.024380

Snapp SS, Swinton SM, Labarta R, et al (2005) Evaluating cover crops for benefits, costs and performance within cropping system niches. Agron. J. 97:322–332

Sokolski S, Dalpé Y, Séguin S, et al (2010) Conspecificity of DAOM 197198, the model arbuscular mycorrhizal fungus, with glomus irregulare: Molecular evidence with three proteinencoding genes. Botany 88:829–838. https://doi.org/10.1139/B10-050

Sorensen JN, Larsen J, Jakobsen I (2005) Mycorrhiza formation and nutrient concentration in leeks (Allium porrum) in relation to previous crop and cover crop management on high P soils. Plant Soil 273:101–114. https://doi.org/10.1007/s11104-004-6960-8

Sosa-Hernández MA, Leifheit EF, Ingraffia R, Rillig MC (2019) Subsoil arbuscular mycorrhizal fungi for sustainability and climate-smart agriculture: A solution right under our feet? Front Microbiol 10:744. https://doi.org/10.3389/fmicb.2019.00744

Spatafora JW, Chang Y, Benny GL, et al (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108:1028–1046. https://doi.org/10.3852/16-042

Stobart R, Gosling P (2015) Opportunities for cover crops in conventional arable rotations. Summer:1–4

Stobart R, Morris NL, Fielding H, et al (2015) Developing the use of cover crops on farm through the Kellogg 's Origins grower programme. Asp Appl Biol 129:27–34

Stobart RM, Morris NL (2014) The impact of cover crops on yield and soils in the New Farming Systems programme. Asp Appl Biol 127:223–232

Stockinger H, Walker C, Schüßler A (2009) "Glomus intraradices DAOM197198", a model fungus in arbuscular mycorrhiza research, is not Glomus intraradices. New Phytol 183:1176–1187. https://doi.org/10.1111/j.1469-8137.2009.02874.x

Sýkorová Z, Ineichen K, Wiemken A, Redecker D (2007a) The cultivation bias: Different communities of arbuscular mycorrhizal fungi detected in roots from the field, from bait plants transplanted to the field, and from a greenhouse trap experiment. Mycorrhiza 18:1–14. https://doi.org/10.1007/s00572-007-0147-0

Sýkorová Z, Wiemken A, Redecker D (2007b) Cooccurring Gentiana verna and Gentiana acaulis and their neighboring plants in two Swiss upper montane meadows harbor

distinct arbuscular mycorrhizal fungal communities. Appl Environ Microbiol 73:5426–5434. https://doi.org/10.1128/AEM.00987-07

Tawaraya K, Hirose R, Wagatsuma T (2012) Inoculation of arbuscular mycorrhizal fungi can substantially reduce phosphate fertilizer application to Allium fistulosum L. and achieve marketable yield under field condition. Biol Fertil Soils 48:839–843. https://doi.org/10.1007/s00374-012-0669-2

Tedersoo L, Bahram M, Põlme S, et al (2014) Global diversity and geography of soil fungi. Science (80-) 346:1256688. https://doi.org/10.1126/science.1256688

Tedersoo L, Bahram M, Põlme S, et al (2015) Response to Comment on "Global diversity and geography of soil fungi." Science (80-) 349:936. https://doi.org/10.1126/science.aaa5594

Thomson BD, Robson a D, Abbott LK (1986) Effects of Phosphorus on the Formation of Mycorrhizas by Gigaspora-Calospora and Glomus-Fasciculatum in Relation to Root Carbohydrates. New Phytol 103:751–765

Tilman D (1999) Global environmental impacts of agricultural expansion: The need for sustainable and efficient practices. Proc Natl Acad Sci 96:5995–6000. https://doi.org/10.1073/pnas.96.11.5995

Tilman D, Cassman KG, Matson PA, et al (2002) Agriculture Sustainability and Intensive Production Practices. Nature 418:671–677. https://doi.org/10.1007/s13398-014-0173-7.2

Tisserant E, Malbreil M, Kuo A, et al (2013) Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. Proc Natl Acad Sci U S A 110:20117–22. https://doi.org/10.1073/pnas.1313452110

Tobar R, Azcón R, Barea JM (1994) Improved nitrogen uptake and transport from 15N-labelled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. New Phytol 126:119–122. https://doi.org/10.1111/j.1469-8137.1994.tb07536.x

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. New Phytol 164:347–355. https://doi.org/10.1111/j.1469-8137.2004.01159.x

Tulasne L-R, Tulasne C (1844) Fungi nonnulli hypogaei, novi v. minus congnoti auct. G Bot Ital 2:55–63

Turrini A, Sbrana C, Avio L, et al (2016) Changes in the composition of native root arbuscular mycorrhizal fungal communities during a short-term cover crop-maize succession. Biol Fertil Soils. https://doi.org/10.1007/s00374-016-1106-8

Uibopuu A, Moora M, Saks Ü, et al (2009) Differential effect of arbuscular mycorrhizal fungal communities from ecosystems along management gradient on the growth of forest understorey plant species. Soil Biol Biochem 41:2141–2146. https://doi.org/10.1016/j.soilbio.2009.07.026

UNEP, WHRC (2007) Reactive Nitrogen in the Environment; Too Much or too Little of a Good Thing

United Nations Department of Economic and Social Affairs (2017) World Population Prospects: The 2017 Revision

Vályi K, Rillig MC, Hempel S (2015) Land-use intensity and host plant identity interactively shape communities of arbuscular mycorrhizal fungi in roots of grassland plants. New Phytol 205:1577–1586. https://doi.org/10.1111/nph.13236

Van Der Heijden MGA (2010) Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology 91:1163–1171. https://doi.org/10.1890/09-0336.1

Van Der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998a) Different Arbuscular Mycorrhizal Fungal Species are Potential Determinants of Plant Community Structure. Ecology 79:2082–2091. https://doi.org/10.1890/0012-9658(1998)079[2082:DAMFSA]2.0.CO;2

Van Der Heijden MGA, Klironomos JN, Ursic M, et al (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72. https://doi.org/10.1038/23932

Van Der Heijden MGA, Scheublin TR (2007) Functional traits in mycorrhizal ecology: Their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. New Phytol 174:244–250. https://doi.org/10.1111/j.1469-8137.2007.02041.x

Van Elsas JD, Chiurazzi M, Mallon CA, et al (2012) Microbial diversity determines the

invasion of soil by a bacterial pathogen. Proc Natl Acad Sci U S A 109:1159–1164. https://doi.org/10.1073/pnas.1109326109

Van Geel M, Busschaert P, Honnay O, Lievens B (2014) Evaluation of six primer pairs targeting the nuclear rRNA operon for characterization of arbuscular mycorrhizal fungal (AMF) communities using 454 pyrosequencing. J Microbiol Methods 106:93–100. https://doi.org/10.1016/j.mimet.2014.08.006

Van Geel M, De Beenhouwer M, Lievens B, Honnay O (2016) Crop-specific and single-species mycorrhizal inoculation is the best approach to improve crop growth in controlled environments. Agron Sustain Dev 36:37. https://doi.org/10.1007/s13593-016-0373-y

Van Geel M, Jacquemyn H, Plue J, et al (2018) Abiotic rather than biotic filtering shapes the arbuscular mycorrhizal fungal communities of European seminatural grasslands. New Phytol 220:1262–1272. https://doi.org/10.1111/nph.14947

Van Kauwenbergh SJ (2010) World Phosphate Rock Reserves and Resources

Vance CP (2001) Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiol 127:390–397. https://doi.org/10.1104/pp.010331

Varela-Cervero S, Vasar M, Davison J, et al (2015) The composition of arbuscular mycorrhizal fungal communities differs among the roots, spores and extraradical mycelia associated with five Mediterranean plant species. Environ Microbiol 17:2882–2895. https://doi.org/10.1111/1462-2920.12810

Vasar M, Andreson R, Davison J, et al (2017) Increased sequencing depth does not increase captured diversity of arbuscular mycorrhizal fungi. Mycorrhiza 27:761–773. https://doi.org/10.1007/s00572-017-0791-y

Verbruggen E, Kiers ET (2010) Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. Evol Appl 3:547–560. https://doi.org/10.1111/j.1752-4571.2010.00145.x

Verbruggen E, Kiers ET, Bakelaar PNC, et al (2012) Provision of contrasting ecosystem services by soil communities from different agricultural fields. Plant Soil 350:43–55. https://doi.org/10.1007/s11104-011-0828-5

Verbruggen E, Röling WFM, Gamper HA, et al (2010) Positive effects of organic farming on below-ground mutualists: Large-scale comparison of mycorrhizal fungal communities in agricultural soils. New Phytol 186:968–979. https://doi.org/10.1111/j.1469-8137.2010.03230.x

Veresoglou SD, Menexes G, Rillig MC (2012) Do arbuscular mycorrhizal fungi affect the allometric partition of host plant biomass to shoots and roots? A meta-analysis of studies from 1990 to 2010. Mycorrhiza 22:227–235. https://doi.org/10.1007/s00572-011-0398-7

Verma BC, Datta SP, Rattan RK, Singh AK (2010) Monitoring changes in soil organic carbon pools, nitrogen, phosphorus, and sulfur under different agricultural management practices in the tropics. Environ Monit Assess 171:579–593. https://doi.org/10.1007/s10661-009-1301-2

Vrindts E, Mouazen AM, Reyniers M, et al (2005) Management zones based on correlation between soil compaction, yield and crop data. Biosyst Eng 92:419–428. https://doi.org/10.1016/j.biosystemseng.2005.08.010

Walker C (1992) Systematics and taxonomy of the arbuscular endomycorrhizal fungi (Glomales)- a possible way forward. Agronomie 12:887–897

Walker C, Błaszkowski J, Schwarzott D, Schüßler A (2004) Gerdemannia gen. nov., a genus separated from Glomus, and Gerdemanniaceae fam. nov., a new family in the Glomeromycota. Mycol Res 108:707–718. https://doi.org/10.1017/S0953756204000346

Walker C, Schler A (2004) Nomenclatural Clarifications and New Taxa in the Glomeromycota. Mycol Res 108:979–982. https://doi.org/10.1017/S0953756204231173

Walsh JJ, Jones DL, Edwards-Jones G, Williams AP (2012) Replacing inorganic fertilizer with anaerobic digestate may maintain agricultural productivity at less environmental cost. J Plant Nutr Soil Sci 175:840–845. https://doi.org/10.1002/jpln.201200214

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Wang B, Yeun LH, Xue JY, et al (2010) Presence of three mycorrhizal genes in the common

ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. New Phytol 186:514–525. https://doi.org/10.1111/j.1469-8137.2009.03137.x

Wang M, Schäfer M, Li D, et al (2018) Blumenols as shoot markers for root symbiosis with arbuscular mycorrhizal fungi. Elife 7:e37093. https://doi.org/10.7554/eLife.37093

Wang S, Chen A, Xie K, et al (2020) Functional analysis of the OsNPF4.5 nitrate transporter reveals a conserved mycorrhizal pathway of nitrogen acquisition in plants. Proc Natl Acad Sci U S A 117:16649–16659. https://doi.org/10.1073/pnas.2000926117

White CA, Holmes HF, Morris NL, Stobart RM (2016) A review of the benefits , optimal crop management practices and knowledge gaps associated with different cover crop species. Agric Hortic Dev Board - Cereal Oilseeds Res Rev 90:

White CM, Weil RR (2010) Forage radish and cereal rye cover crop effects on mycorrhizal fungus colonization of maize roots. Plant Soil. https://doi.org/10.1007/s11104-009-0131-x

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Williams SM, Weil RR (2004) Crop Cover Root Channels May Alleviate Soil Compaction Effects on Soybean Crop. Soil Sci Soc Am J 68:1403–1409. https://doi.org/10.2136/sssaj2004.1403

Wilson GWT, Rice CW, Rillig MC, et al (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments. Ecol Lett 12:452–461. https://doi.org/10.1111/j.1461-0248.2009.01303.x

Withers PJA, Neal C, Jarvie HP, Doody DG (2014) Agriculture and eutrophication: Where do we go from here? Sustain 6:5853–5875. https://doi.org/10.3390/su6095853

Wortman SE, Francis CA, Bernards ML, et al (2012) Optimizing cover crop benefits with diverse mixtures and an alternative termination method. Agron J 104:1425–1435. https://doi.org/10.2134/agronj2012.0185

Wubet T, Weiß M, Kottke I, et al (2004) Molecular diversity of arbuscular mycorrhizal fungi in Prunus africana, an endangered medicinal tree species in dry Afromontane forests of Ethiopia. New Phytol 161:517–528. https://doi.org/10.1046/j.1469-

8137.2003.00924.x

Xiang D, Verbruggen E, Hu Y, et al (2014) Land use influences arbuscular mycorrhizal fungal communities in the farming-pastoral ecotone of northern China. New Phytol 204:968–978. https://doi.org/10.1111/nph.12961

Xun F, Xie B, Liu S, Guo C (2015) Effect of plant growth-promoting bacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) inoculation on oats in saline-alkali soil contaminated by petroleum to enhance phytoremediation. Environ Sci Pollut Res 22:598–608. https://doi.org/10.1007/s11356-014-3396-4

Yang H, Zhang Q, Koide RT, et al (2017) Taxonomic resolution is a determinant of biodiversity effects in arbuscular mycorrhizal fungal communities. J Ecol 105:219–228. https://doi.org/10.1111/1365-2745.12655

Yang W, Li S, Wang X, et al (2021) Soil properties and geography shape arbuscular mycorrhizal fungal communities in black land of China. Appl Soil Ecol 167:104–109. https://doi.org/10.1016/j.apsoil.2021.104109

Yoneyama K, Xie X, Kusumoto D, et al (2007a) Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. Planta 227:125–132. https://doi.org/10.1007/s00425-007-0600-5

Yoneyama K, Yoneyama K, Takeuchi Y, Sekimoto H (2007b) Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. Planta 225:1031–1038. https://doi.org/10.1007/s00425-006-0410-1

Young JPW (2012) A molecular guide to the taxonomy of arbuscular mycorrhizal fungi. New Phytol 193:823–826. https://doi.org/10.1111/j.1469-8137.2011.04029.x

Zhang J, Kobert K, Flouri T, Stamatakis A (2014) PEAR: A fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30:614–620. https://doi.org/10.1093/bioinformatics/btt593

Zhang Q, Blaylock LA, Harrison MJ (2010) Two Medicago truncatula half-ABC transporters are essential for arbuscule development in arbuscular mycorrhizal symbiosis. Plant Cell 22:1483–1497. https://doi.org/10.1105/tpc.110.074955

Zhu YG, Miller RM (2003) Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. Trends Plant Sci 8:407–409. https://doi.org/10.1016/S1360-1385(03)00184-5