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Cover Cropping for Arbuscular Mycorrhizal Fungi

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1. Industry Summary

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 could foster these fungal populations to increase crop and soil benefits, including yield. Cover cropping, (cultivation of crops or crop mixtures, usually during winter months) is used to protect and improve soil, and potentially to contribute to ecosystem services; such as both the diversity and abundance of AM fungi.

This study aimed to determine the impact of cover cropping and other amendments including mycorrhizal inoculum, and nitrogen in various forms, on the diversity and abundance of AM fungi, and critically, the resulting impact on crop yield and quality.

A large-scale analysis of AM fungal diversity in UK agriculture, in collaboration with FERA, provided a framework for further analysis of how cover crops, and soil amendments influence AM fungal communities. In total, 84 AM fungal Virtual Taxa were identified from across the 67 independent sites in the trial.

Building on this baseline understanding, results from replicated field trials provided evidence that multiple iterations of cover crops can increase the extent to which plants are colonised by AM fungi, but single iterations had no measurable impact. In the New Farming Systems: Fertility Building Rotations trial, it was shown that repeated applications of nitrogen fertiliser over several years influenced AM fungal community composition, but this observation was not made in a shorter validation experiment conducted at the field scale using AD (digestate from anaerobic digestion) as the nitrogen source. In this trial, there was no link between increased diversity/ abundance of AM fungi, and increased crop growth or yield.

In another trial based at Bawburgh, Norfolk, 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 other soil benefits.

Finally, results from experimentation at the field scale provided some evidence that cover crops or AM fungal inoculum could positively influence AM fungal abundance or diversity, or improve crop yield. However, cover crops were found to assimilate soil nitrogen, following autumn applications, especially from deeper soil horizons, which may reduce environmental impact, such as diffuse pollution resulting from application of nitrogen based fertiliser.

2. Introduction

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).

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 some practices that have 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 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. 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, high inputs of N and P fertiliser can be detrimental to terrestrial and aquatic ecosystems through diffuse pollution, and contribute to greenhouse gas emissions through the release of nitrous oxide (Smil 1999; Bennett et al. 2001; Tilman et al. 2002).

The UK Department for Environment, Food & Rural Affairs (DEFRA) acknowledges the unsustainable nature of some aspects 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 have been considered throughout this PhD project, not only in terms of agricultural sustainability, but also food production, economics, and ease of adoption.

2.1. 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. This research focused on the biological aspect; in particular the diversity and abundance of arbuscular mycorrhizal fungi. It also considered the physical and chemical aspects in the wider context of cover cropping and sustainable farming.

2.2. Biology and Diversity of Arbuscular Mycorrhizal Fungi

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 many benefits including nutrient uptake, increased pest and pathogen resistance, and drought tolerance (Figure 1), in exchange for plant derived carbon (Jakobsen and Rosendahl, 1990; Marschner and Dell, 1994; Augé, 2001; Jung *et al.*, 2012; Rapparini and Peñuelas, 2014). 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).

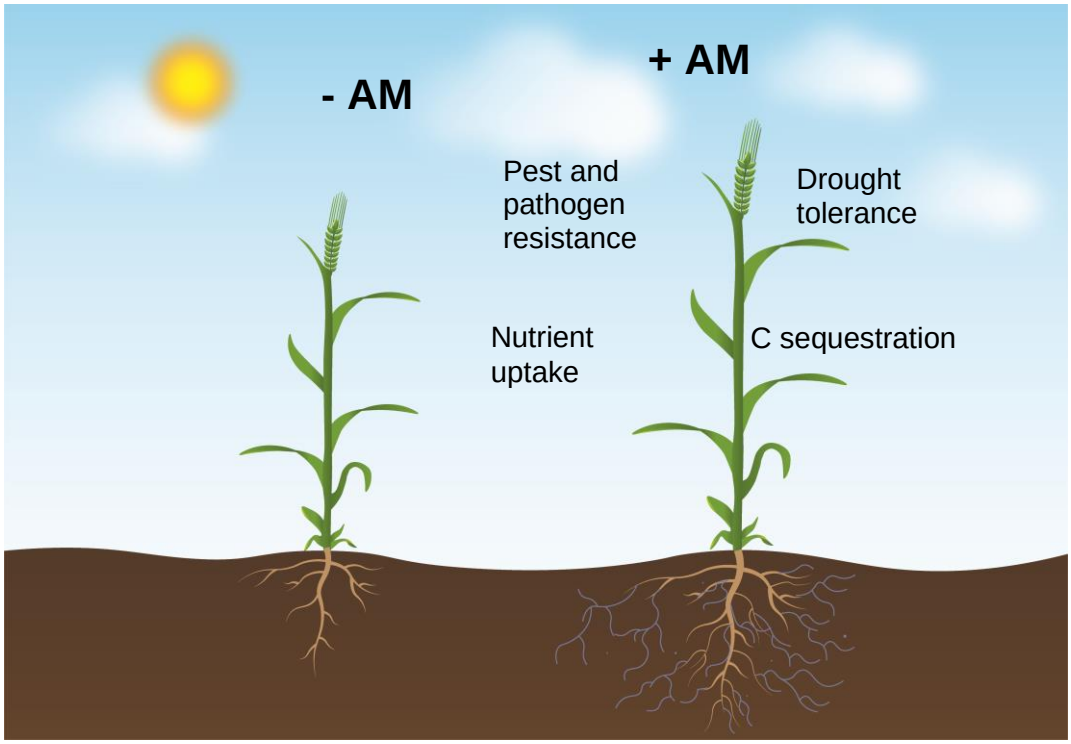

Figure 1 – Diagram representing the plant benefit from increasing interaction with AM fungi.

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).

2.2.1. Impact of AM Fungi on Plant Growth in Agriculture

Studies have shown a positive relationship between AM fungal diversity and colonisation; leading to increases in plant productivity, or biomass in natural systems (Van Der Heijden et al. 1998; Klironomos et al. 2000). Veresoglou et al. (2012) reported differences following inoculation with different fungal isolates, and that AM fungal mixtures were generally more effective at conferring benefit to the plant host 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 biomass of a range of plant species was on average 34.9% higher in inoculated vs non-inoculated crops (Van Geel et al. 2016). Similarly, a meta-analysis undertaken by Lekberg and Koide (2005) indicated that increased AM colonisation resulted in a 23% increase in yield for a range of crops.

2.2.2. 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 by AM fungi (Akiyama et al. 2005). Application of fertiliser to increase crop yield can elevate soil phosphate to many times the levels found at some mature, undisturbed sites, and caused 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 structure 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. Absence of living plant hosts prevents active mycelial growth fed via plant roots. These obligate biotrophic symbionts and can only persist in the form of resting spores, and hyphal networks break down.

AM fungi that are adapted to highly disrupted soils are likely to be characterised by fast spore germination, and a larger investment in reproductive spores (Figure 2). It has been suggested that this ruderal strategy, leads to those species commonly found in agricultural sites being less beneficial to their plant hosts (Johnson 1993; Menéndez et al. 2001; Verbruggen and Kiers 2010; Van Geel et al. 2016). 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).

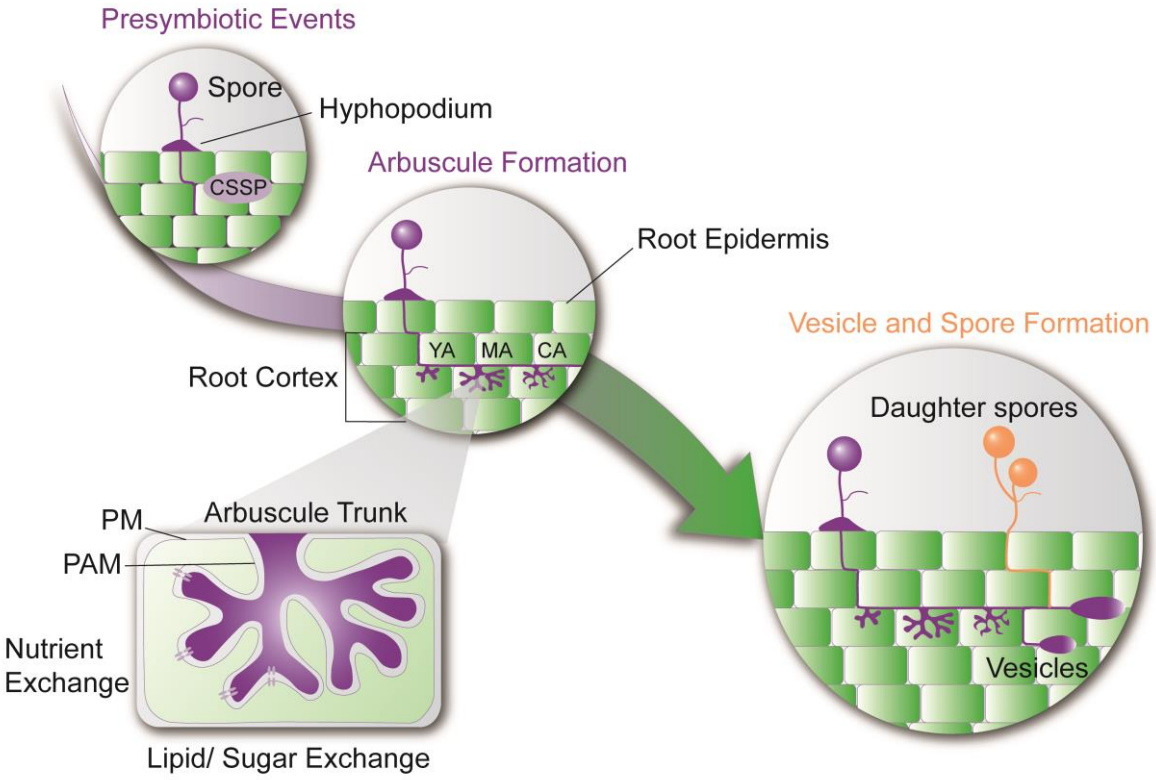

Figure 2 – Diagram representing the stages of AM colonisation in plant roots. Figure represents the three stages of AM fungal symbioses, including early symbiosis, formation of Young (YA), Mature (MA), and Collapsing (CA) Arbuscules, and finally formation of vesicles and spores. CSSP is the Common Symbiosis Signalling Pathway, a set of genetic controls for the symbiosis. Magnified diagram (bottom left) shows a mature arbuscule surrounded by the Peri-Arbuscular Membrane (PAM) and the Plant Membrane (PM).

2.2.3. Role of AM Fungi in Plant Nitrogen Uptake and Cycling

The AM fungi are often associated with their role in plant inorganic phosphate (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).

2.3. Cover Cropping as a Method for Soil Improvement

Cover crops (Figure 3) are grown for the main purpose of 'protecting or improving' soils between periods of regular crop production (Stobart and Gosling 2015). They can be effective at improving soil fertility and structure, reducing weeds and pests, and helping prevent erosion and diffuse pollution. 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 3 – A cover crop of radish, oat, and vetch, at Euston Farm in January 2018

2.3.1. 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, 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 borne 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).

2.3.2. 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. A farmer may, for example, be interested in enabling more N-fixation via the symbiotic route, and deterring weeds, so may opt to mix a legume species with a second cover crop species that has allelopathic properties. However, other studies have shown that the benefit of increased cover crop diversity does not always sufficiently improve soil conditions or crop yields to justify the additional price (Wortman et al. 2012; Finney et al. 2016).

2.4. Cover Crops to Improve Populations of Mycorrhizal Fungi

It is widely accepted that cover crops can 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 in the absence of a living 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.

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). Many studies have found positive effects of cover cropping on mycorrhizal abundance in the following crop (above), but literature concerned with the importance of AM species diversity is less common. Advances in high-throughput DNA sequencing have led to increased accuracy, and reduced price of microbiome studies, which have consequently become a popular methodolgical strategy.

2.4.1. 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 maize/ soybean 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).

2.5. 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 root length colonisation (RLC, Figure 4), 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.*

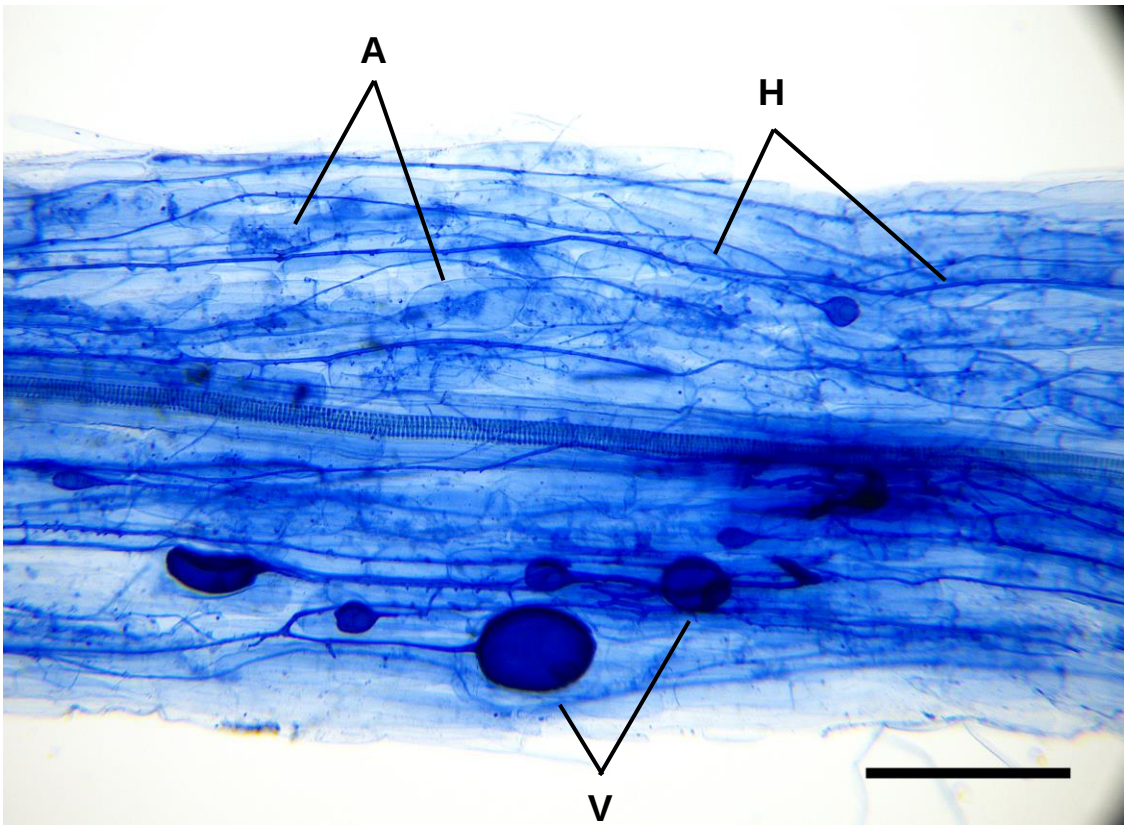

Figure 4 – Mycorrhizal hyphae (H), arbuscules (A) and vesicles (V) stained with trypan blue, present within a large lateral root of leek (*Allium ampeloprasum*). Root material was sourced from Allpress Farm, UK during January 2019. Scale bar represents 200 µm.

2.5.1. AM Fungal Inocula 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. 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 inocula increased shoot biomass compared to single species, but there was no overall increase in colonisation, nutrition, or yield.

There is surprisingly little direct evidence for successful establishment of AM fungal interactions following use of inoculum (Hart et al. 2018). It seems likely that increasing AM fungal abundance

per se will provide less benefit than promoting species that 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).

2.6. Project Overview

This PhD programme explored how cover crops, and other amendments including mycorrhizal inoculum, and nitrogen in various forms influenced the diversity and abundance of AM fungi, whether these populations were maintained in subsequent cash crops, and critically, the resulting effect on crop yield. Experiments included 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.

2.7. Hypotheses

The key hypotheses the project addressed include:

1. Certain AM fungal taxa 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 were 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, there have not been any comprehensive UK studies that 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. This project evaluated 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 were considered. Most importantly, the effect of cover crops, and AM fungal diversity and abundance were quantified in terms of cash crop benefit, including yield. The project aimed 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.

3. Materials and methods

3.1. Large Scale Analysis of Arbuscular Mycorrhizal Fungi in UK Agricultural Systems, and Implications for Sustainable Agriculture

3.1.1. Study Sites and Sampling

To assess diversity of AM fungi in UK agricultural systems, soil samples were collected and sent from farm sites as part of the FERA Big Soils project, representing 67 individual field locations (Figure 5).

Figure 5 – Geographic distribution of the 67 independent sites that were sampled for AM fungal microbiome analysis

3.1.2. UK Climatological Data for Study Sites

Weather data was extracted from the Met Office HadUK gridded climate observations dataset, at 1km resolution and included in statistical models.

3.1.3. DNA Extraction from Big Soil Community Soil Samples

To assess the microbiome of soil or root samples, purified DNA was extracted from the sample of interest. In brief, 10g of soil was taken from soil samples at random for DNA extraction, using the PowerSoil DNA kit following the manufacturers protocol (QAIGEN, UK). DNA samples were diluted 100-fold to improve amplification of AM fungal DNA.

3.1.4. DNA Amplification of Soil Samples using AM fungal Specific Primers

To increase abundance of specific regions of DNA, samples were amplified using AM fungal specific primers NS31 (Simon et al. 1992) and AML2 (Lee et al. 2008). 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).

3.1.5. Indexing and Library Preparation of Study Samples

To prepare samples for DNA sequencing, Illumina Nextera XT indexes were attached to PCR amplicons following the manufacturers recommendations. The resulting PCR product was purified with AMpure beads. 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. The pooled library was run on a BluePippin using a 1.5% agarose cassette, to remove a small ~180bp amplicon. 40% PhiX was added to the denatured library to increase heterogeneity of base calling, to improve read quality (Dumbrell, A. *pers. com.*), and denatured following the manufacturers protocols.

3.1.6. 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.

3.1.7. Bioinformatical Analysis of Study Sequence Data

To prepare DNA reads for downstream analysis, raw FASTQ files were quality trimmed using SICKLE version 1.33 (Joshi and Fass 2011). 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), to reduce the likelihood of false observations. Forward and reverse reads were then aligned using the PEAR algorithm (Zhang et al. 2014) in PANDAseq version 2.11 (Masella et al. 2012). 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 (equivalent of species)

using BLAST+ (Altschul et al. 1990) following the open reference OTU picking approach of Davison et al. (2015).

3.1.8. 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. This prevents bias in diversity from samples with small or large numbers of DNA reads.

3.2. Cover Cropping and AM Fungal Inoculation to Improve Soil Health and Increase Crop Yield

3.2.1. 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 AM 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 (see below) 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 live propagules between treatments. This reflects the manufacturer’s preparation and description of innocula which are made up of combined 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.

3.2.2. Soil Conditions in the Glasshouse Trial

Autoclaved John Innes Number 1 was mixed in equal volume with autoclaved yellow Garside silica sand. 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/ha available N, 6.8 mg/l P, 34 mg/l K, and 56 mg/l Mg.

3.2.3. 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 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 5 days,then transferred to pre-prepared 400ml/ 9cm Lily of the Valley pots, prepared with the sand-soil mixture, and inoculated with crude inoculum if appropriate. In total, there were 10 replicates, with one plant in each 400ml pot.

3.2.4. Glasshouse Conditions and Plant Care

Barley plants were watered using reverse osmosis (RO) water only for two weeks, to allow for germination and establishment of the AM symbiosis which is sensitive to even low levels of P. After two weeks, plants were watered twice a week with low P Hoaglands solution, containing 10 µM P.

3.2.5. Plant Harvest and Sample Storage

Plants were harvested at seven weeks post inoculation. Representative plants of each experimental treatment are shown in Figure 6, recorded immediately before harvest.

Figure 6 – 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, then inoculated with *F. mosseae, F. geosporum, C. claroideum, R. irregularis, G. microaggregatum,* 5 species commercial mix

3.2.6. Staining and Quantification of Root Length Colonisation by Trypan Blue

Root samples were rinsed with double distilled (dd) H_2O, transferred to 10% (w/v) potassium hydroxide (KOH), 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. Ten 1cm root fragments were stained and mounted per plant.

Root length colonisation was calculated using an adapted version of the gridline intersect method, following staining with trypan blue (Gutjahr et al. 2008).

3.2.7. DNA Extraction with an Adapted CTAB Protocol

For plant root samples across the glasshouse and replicated trials, DNA was extracted using an adapted cetrimonium bromide (CTAB) method, tailored for extraction of fungal DNA from plant tissue.

3.2.8. 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.

3.2.9. Site Selection and Preparation for the Bawburgh Cover Crop Inoculation Trial

The Bawburgh trial site was located with a combination of yield maps and NDVI images.The chosen trial site was located at 52°37'37.9"N 1°11'03.5"E, approximately 7.5km west of Norwich, UK. 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.

3.2.10. 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. Three species, one oat, one vetch, and one radish were chosen as to represent a cereal, legume, and brassica cover crop. 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). Each experimental treatment was replicated three times.

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.

3.2.11. Cover Crop Sampling and Storage

Cover crops were sampled on 3rd January 2019, following the method of Higo et al. (2014), pooling a total of 15 whole, individual plants into three technical replicates of five plants per plot. Representative plots of each cover crop are shown in Figure 7. Whole plants were stored in polythene bags at 4°C for a maximum of 48 hours before being processed for microscopy and DNA extraction.


a
b
c
d
e
f

Figure 7 – 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.

3.2.12. 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. Samples were sent via chilled courier to NRM labs (Bracknell, UK) for analysis of soil nutrients.

3.2.13. 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.

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

Whole spring barley plants were sampled for biomass, as well as microscopic and DNA-based analysis of AM fungal abundance and diversity. Plant sampling was conducted on 29th May 2019, again following the method of Higo et al. (2014). This date corresponded to growth stage (GS) 38-40 on Zadoks growth scale, at the end of stem elongation but prior to booting. Spring barley was harvested for each plot on 15th August 2019 and standardised to 15% moisture content for further analysis.

3.2.15. 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.

3.2.16. 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.

3.2.17. 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. Winter oat was harvested for each plot on 24th August 2020 (Figure 8). and standardised to 15% moisture content for further analysis.

3.2.18. Measurement of Soil Physicochemical Properties in Glasshouse and Field Trials

Soil physicochemical properties were assessed by NRM Laboratories (Bracknell, UK), following standard protocols.

3.2.19. 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. At least 5 and up to 10 measurements (due to difficulty measuring penetration resistance in stony ground), were taken per plot using a soil penetrometer, 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 8 – Harvesting of winter oat var. Mascani at Bawburgh on 24th August 2020.

3.2.20. Statistical Analysis of Plant and Fungal Traits in Field Conditions

Two measures of AM fungal diversity, alpha and beta diversity were calculated in the Phyloseq package (McMurdie and Holmes 2013) in R (R Core Team 2019).

Maximum likelihood (ML) 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). 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. Post-hoc multiple comparisons were conducted using the contrast function in the package emmeans (Lenth et al. 2020).

3.3. Influence of Nitrogen Application on AM fungal communities, and benefit of symbiosis

3.3.1. Experimental Site and Sampling

The New Farming Systems (NFS) Fertility Building Rotation experiment is an incomplete factorial design with four replicates of four cover crop systems, three rotation systems, and three N treatments (Annex 1). The trial is located at Morley, Norfolk, on a sandy clay loam with the

physicochemical parameters outlined in Table 1. The trial was established in 2008 and at the time of sampling, with five iterations of cover crop within the rotation .

Table 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

3.3.2. Selection of Rotations in the NFS Trial

The three crop rotation approaches are based around winter wheat, with different break crop approaches. 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 report will consider only the spring break and mixed cropping approaches.

3.3.3. 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, with 8 replicates per treatment. Cropping systems include:

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

3.3.4. Nitrogen Dose Application for Laureate Spring Barley

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

1. 100% of standard dose for the crop being grown
2. 50% of standard dose for the crop being grown
3. No nitrogen application

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

3.3.5. Plant Sampling and Yield Measurement in Spring Barley

Spring barley was sampled on 24th June 2018, following the method of Higo et al. (2014). This date corresponded to growth stage 38-40 on Zadok's growth scale, at the end of stem elongation but prior to booting. Spring barley was subsequently harvested for each plot on 3rd August 2018.

3.3.6. 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 above (Section 3.2.6). As above, ten 1cm root fragments were quantified.

3.3.7. DNA Extraction, PCR Conditions, Sequencing, and Bioinformatics

Methods for DNA extraction, amplification by PCR, and sequencing were conducted as above (Sections 3.1.4 to 3.1.7 and 3.2.7).

3.3.8. Statistical Analyses in the NFS Fertility Building Rotations Trial

Restricted maximum likelihood (REML) estimates of the predictors of linear mixed effects models were determined with the lmer function in the package lmerTest. Cover crop treatment plot encompassing the three levels of N dose, was nested within experimental block and used as a random effect to account for the split-plot trial design. Post-hoc multiple comparisons were conducted in the function emmeans in the package emmeans (Lenth et al. 2020).

3.4. Use of Cover Crops, and Soil Amendments at the Farm Scale

3.4.1. Sites and Crop Establishment

The study made use of the ongoing Innovative Farmers project: 'Increasing Nutrient Efficiency from Anaerobic Digestate' aiming 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 9). 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. At one site growing maize, cover crops failed to establish due to late drilling, and was subsequently omitted from further analyses.

Figure 9 – Arrangement of experimental treatments at the Holkham field site, representative of others in the Innovative Farmers trial

The four field sites with maize as the cash crop were chosen for this report, and are shown in Figure 10. 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. Drilling, sampling, and desiccation dates for these sites are shown in Table 2.

Figure 10 – Geographical arrangement of Innovative Farmers trial sites in the East of England.

3.4.2. Mycorrhizal Inoculant for Allpress Farm Cover Crop and AMF Treatment

Mycorrhizal inoculum for the IF trial was sourced from PlantWorks Ltd (Sittingbourne, UK). The components of this inoculum are described above (Section 3.2.1).

Table 2 – Drill, sampling, and desiccation dates for cover crops and maize at each site in the Innovative Farmers trial.

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

3.4.3. Soil Sampling for Physicochemical Analysis

Soil samples were collected between December 2017 and February 2018. Seven repeats per plot were pooled by three depths (0-30cm, 30-60cm, 60-90cm) and were thoroughly mixed before saving a subsample of approximately 300g for laboratory analysis.

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 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. 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 (Guimarães et al. 2013).

3.4.4. Plant Sampling for Biomass and Colonisation Assessment

Ten plants of each cover crop species, and later maize as the cash crop were randomly sampled per treatment (Table 2). Approximately 200mg of root material was saved for molecular and microscopic analysis.

3.4.5. Soil Physicochemical Properties

Soil physicochemical properties were assessed by NRM Laboratories, Bracknell, UK.

3.4.6. 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 above. A representative root fragment from a maize plant, sampled at the Allpress Farm site is shown in Figure 11.

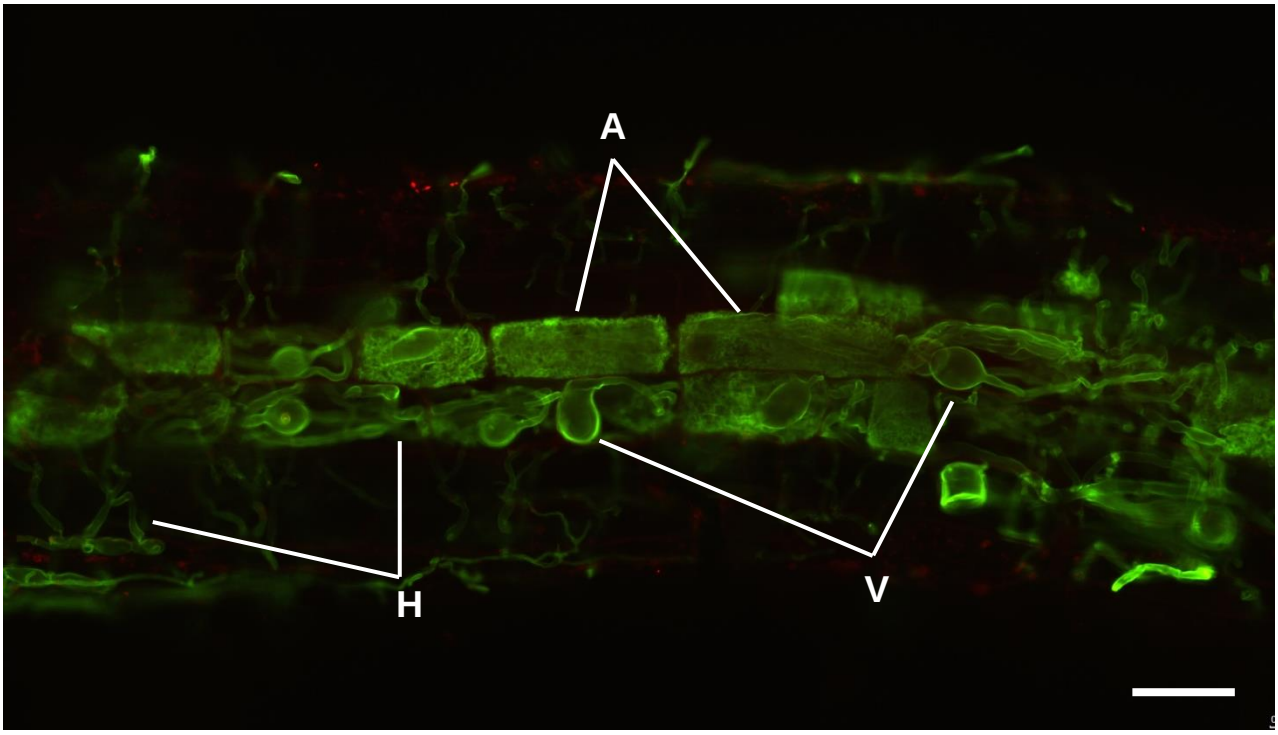

Figure 11 – Wheat Germ Agglutin (WGA) 488 stained micrograph of oat root from the Allpress site, showing AM fungal structures (green)
Image shows mycorrhizal hyphae (H), arbuscules (A) and vesicles (V). Scale bar represents 25µm.

3.4.7. DNA Extraction, Amplification and Sequencing of Maize and Leek

Protocols for DNA extraction, PCR amplification, DNA sequencing, and bioinformatics were conducted as above (Sections 3.1.4 to 3.1.7 and 3.2.7).

3.4.8. 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). 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 is an invaluable validation step to confirm whether findings from experimental setups are robust in real world situations.

4. Results

4.1. Large Scale Analysis of Arbuscular Mycorrhizal Fungi in UK Agricultural Systems, and Implications for Sustainable Agriculture

4.1.1. 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 12.

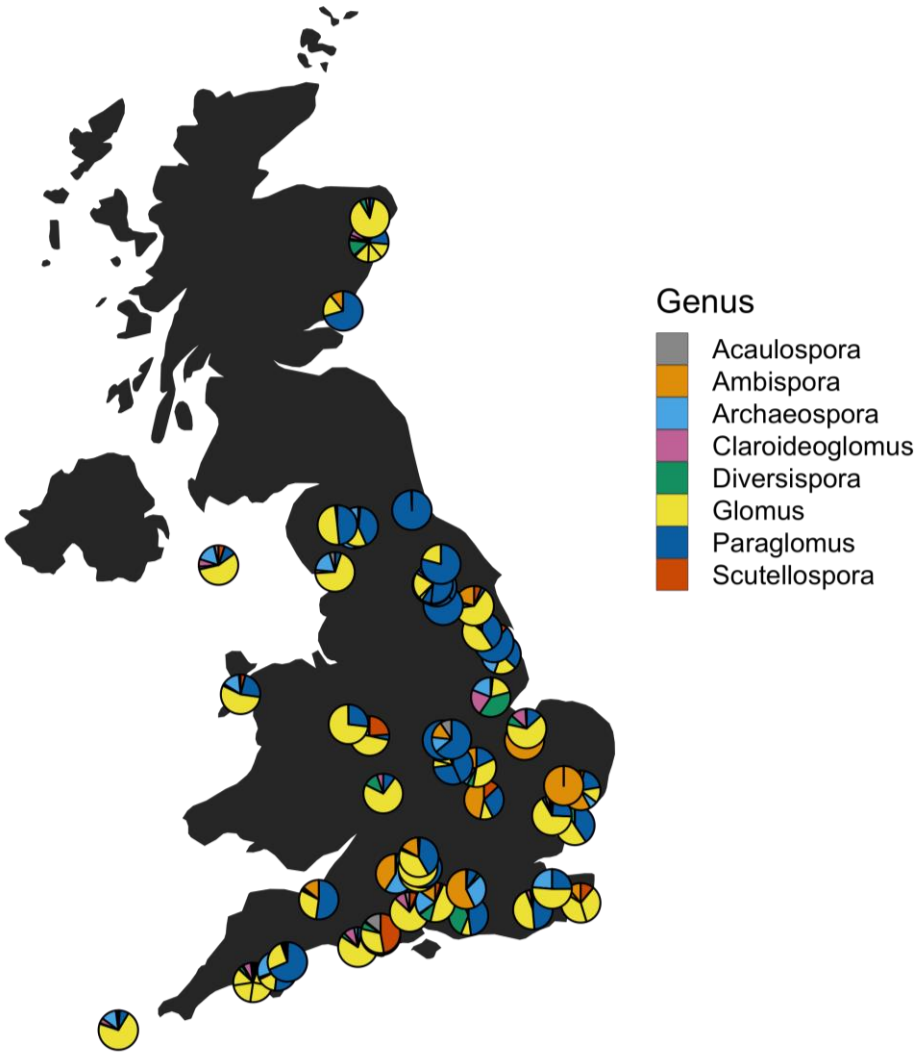

Figure 12 – Proportion of AM Fungal Genera at the 67 farm sites from the FERA Big Soil Community trial.
Pie charts represent proportion of AM fungal composition, at the genus level.

4.1.2. AM Fungal Abundance by Prevalence at a Site in the FERA trial

The top 10 most abundant taxa across the FERA 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 13.

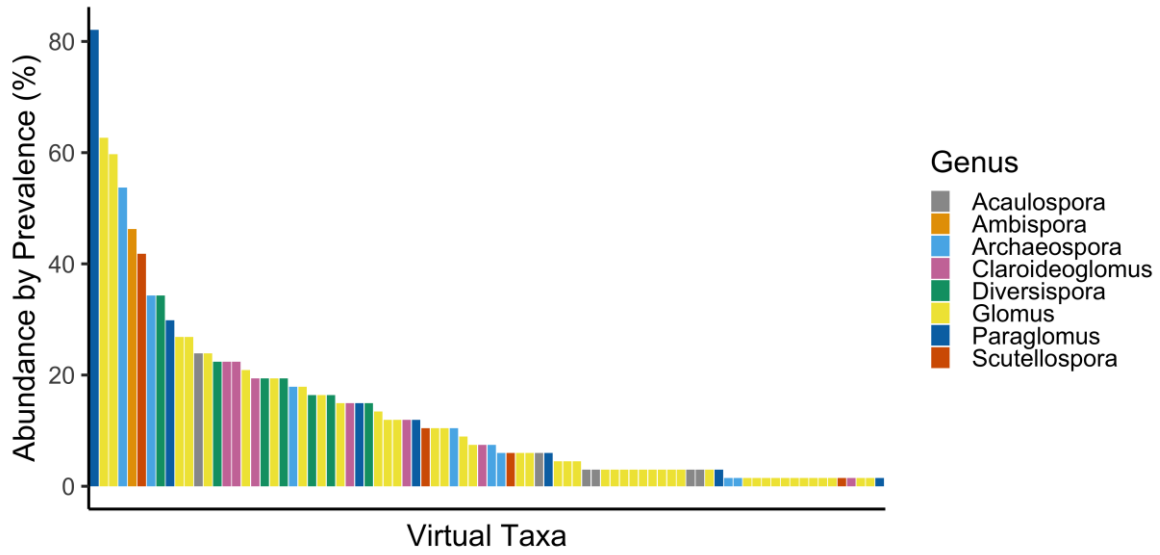

Figure 13 – Most abundant AM fungal virtual taxa by prevalence in the FERA trial showing distribution of all 84 VT identified
Abundance of the top AM fungal VT, expressed as a percentage of the number of times a VT appears at a site. Colours represent the genus of taxa.

4.1.3. 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 be 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, having not been supplied by the grower to the Big Soil Community project. 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.

4.1.4. 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 in place of 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 are visualised in Figure 14.

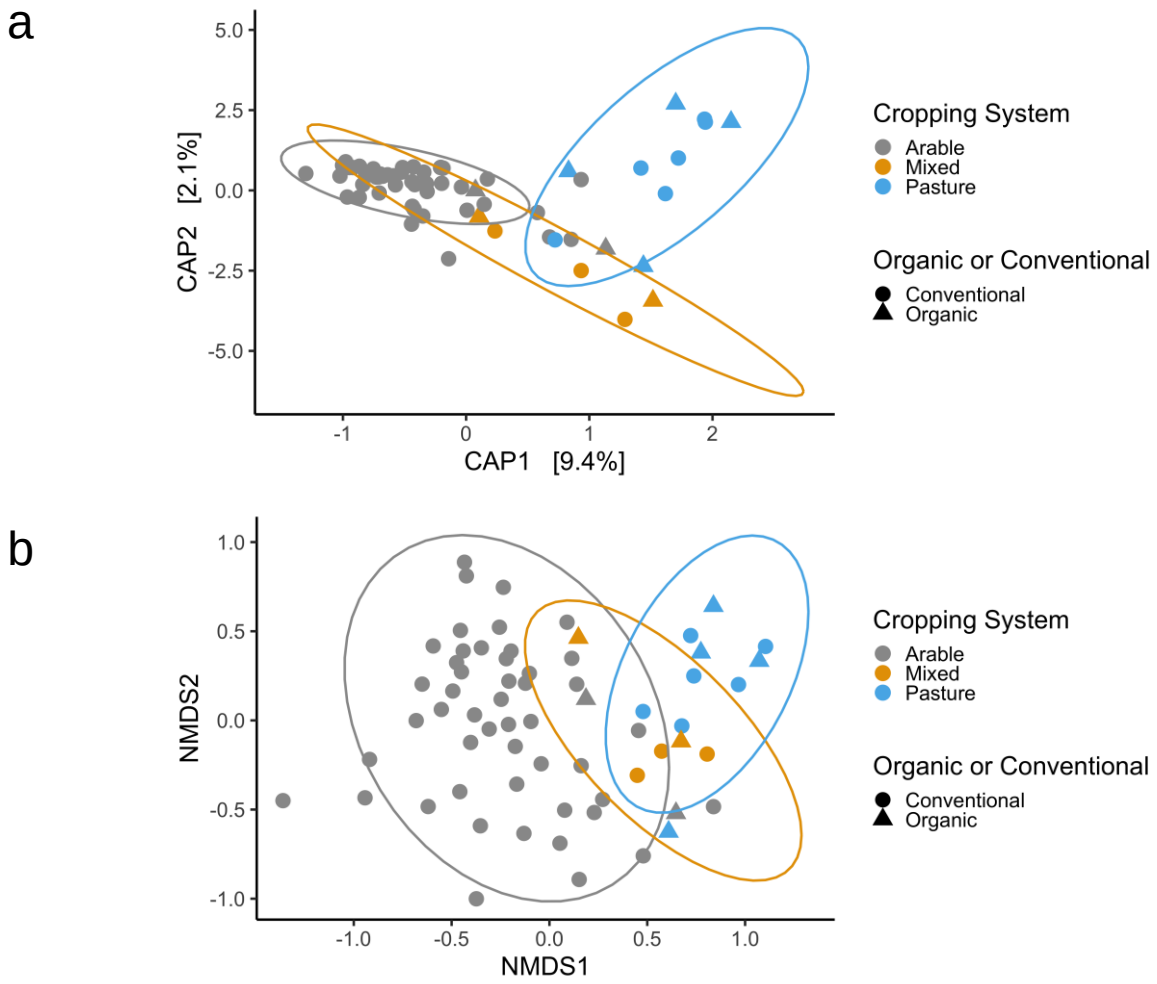

Figure 14 – a) db-RDA ordination of Bray-Curtis distances b) NMDS ordination of Bray-Curtis distances split by the cropping system, from 67 UK agricultural sites in the FERA trial
Both distanced based RDA ($F_{2,63} = 3.60$, $p = 0.005$), and ANOSIM ($R = 0.256$, $p = 0.004$) suggested that the cropping system had a significant impact on AM fungal community composition.

4.2. Cover Cropping and AM Fungal Inoculation to Improve Soil Health and Increase Crop Yield

4.2.1. 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 15), 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 5 species mix (0.756g) were not significantly different to the control, or *R. irregularis* at the $p < 0.05$ level.

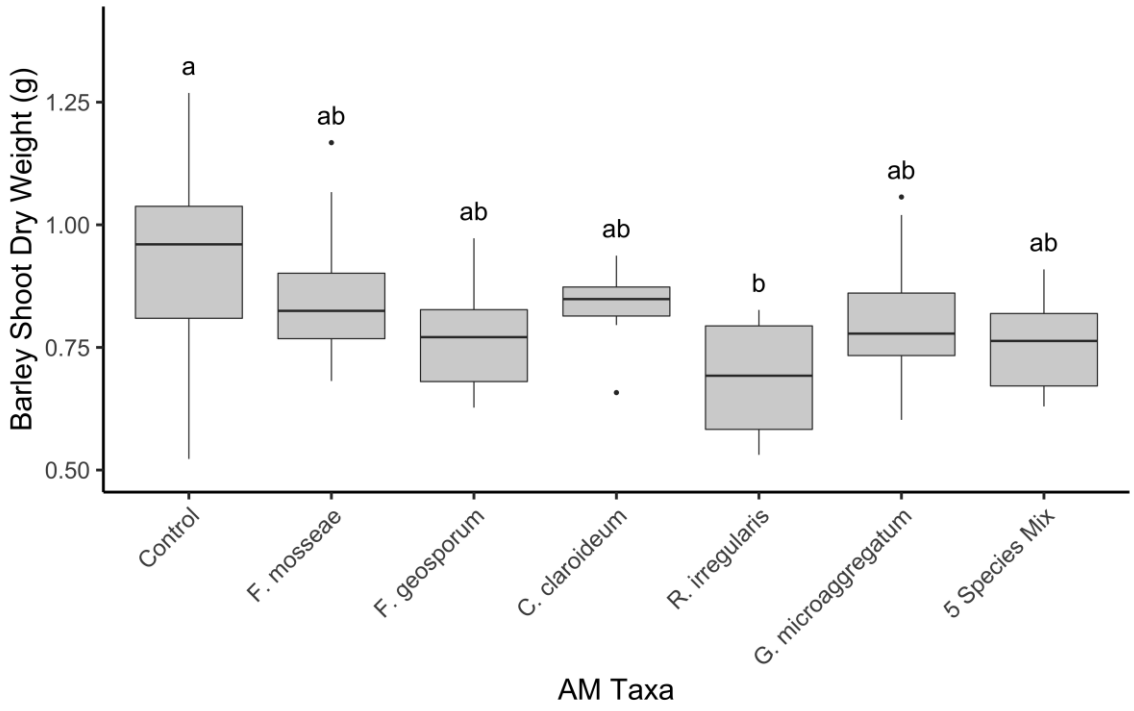

Figure 15 – Dry weight of spring barley (var. Laureate), grown in glasshouse conditions following inoculation of each of the five AM taxa, and the five species mix, compared to the control. Means followed by the same letter did not differ significantly (Tukey test, p<0.05).

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

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 $p < 0.001$, Figure 16). 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, confirming the absense of colonisation by naturally occuring AM fungi. There was a significant effect of inoculum on barley root length colonisation (ANOVA: $F_{6,63}$ = 72.14, p<0.001).

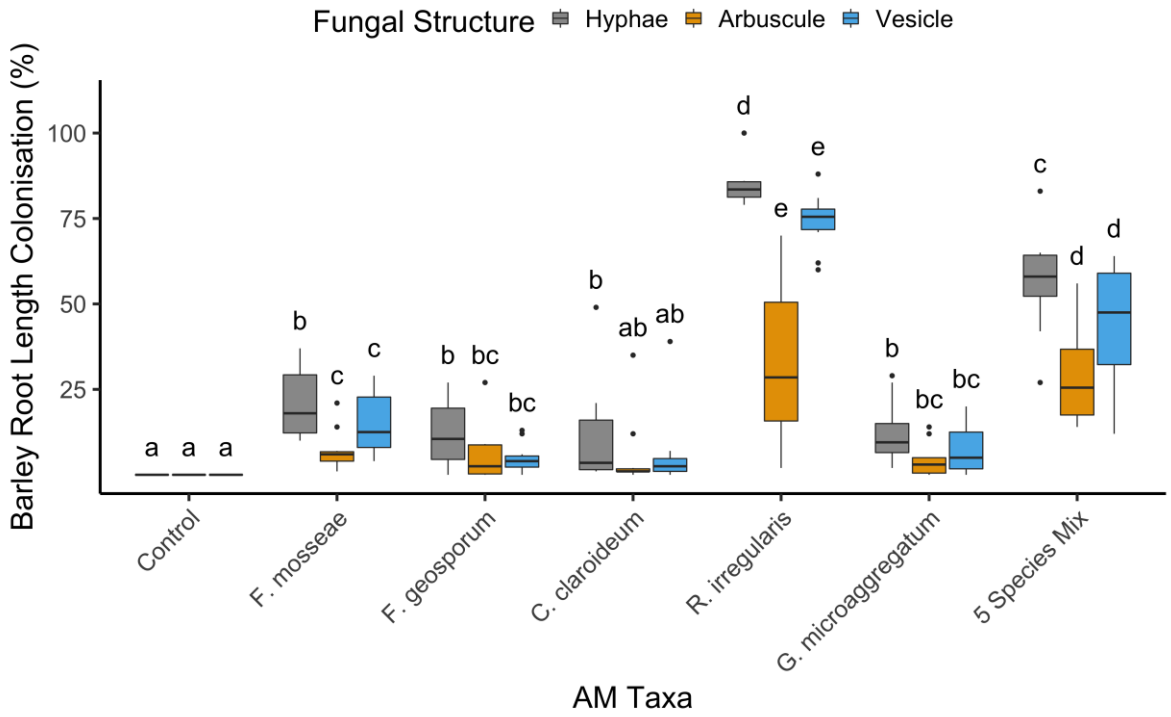

Figure 16 – Root length colonisation (RLC) of spring barley (var. Laureate) grown in glasshouse conditions, following inoculation of each of the five AM taxa, and the five species mix, compared to the control treatment. Multiple comparisons were conducted across treatments only. Means followed by the same letter did not differ significantly (Tukey test, p<0.05). Dots represent outliers which are greater than 1.5 times the interquartile range from the lower or upper quartile.

4.2.3. Relative Colonisation of AM Taxa in the 5 Species Mixed Inoculum

To determine the relative proportion of AM fungal taxa colonising spring barley roots, a DNA based approach was taken. All 10 glasshouse DNA samples were successfully amplified by PCR using AM fungal specific primers . 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.

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 17.

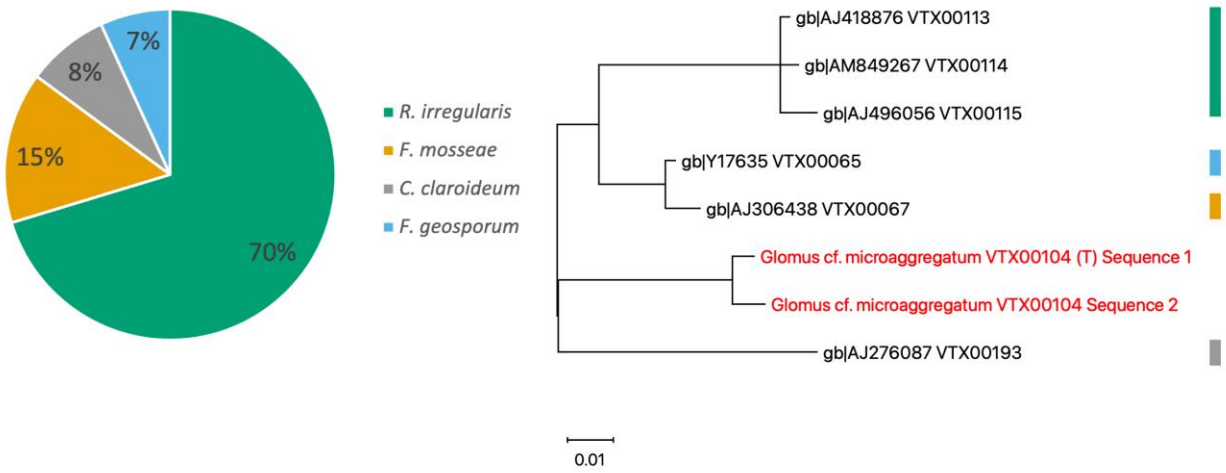

Figure 17 – Relative colonisation of inoculated AM fungal taxa colonising spring barley grown in glasshouse conditions.
Proportion of the five AM species colonising spring barley (var. Laureate) in the glasshouse trial.

4.2.4. Spring Barley Shoot Biomass at the Bawburgh Trial

Whole plant samples were removed from cold storage, washed to remove soil, and separated into root and shoot. Barley shoots were blotted dry and five shoots constituting one biological replicate 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 in the inoculated low rate radish and oats treatment (Figure 18). The non-inoculated fallow control had the third highest shoot biomass, at 18.70 ± 0.15g, which fell to 17.53 ± 0.29g in the inoculated fallow treatment. This marked reduction in shoot weight, resulting from AM fungal inoculation was also observed in 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.

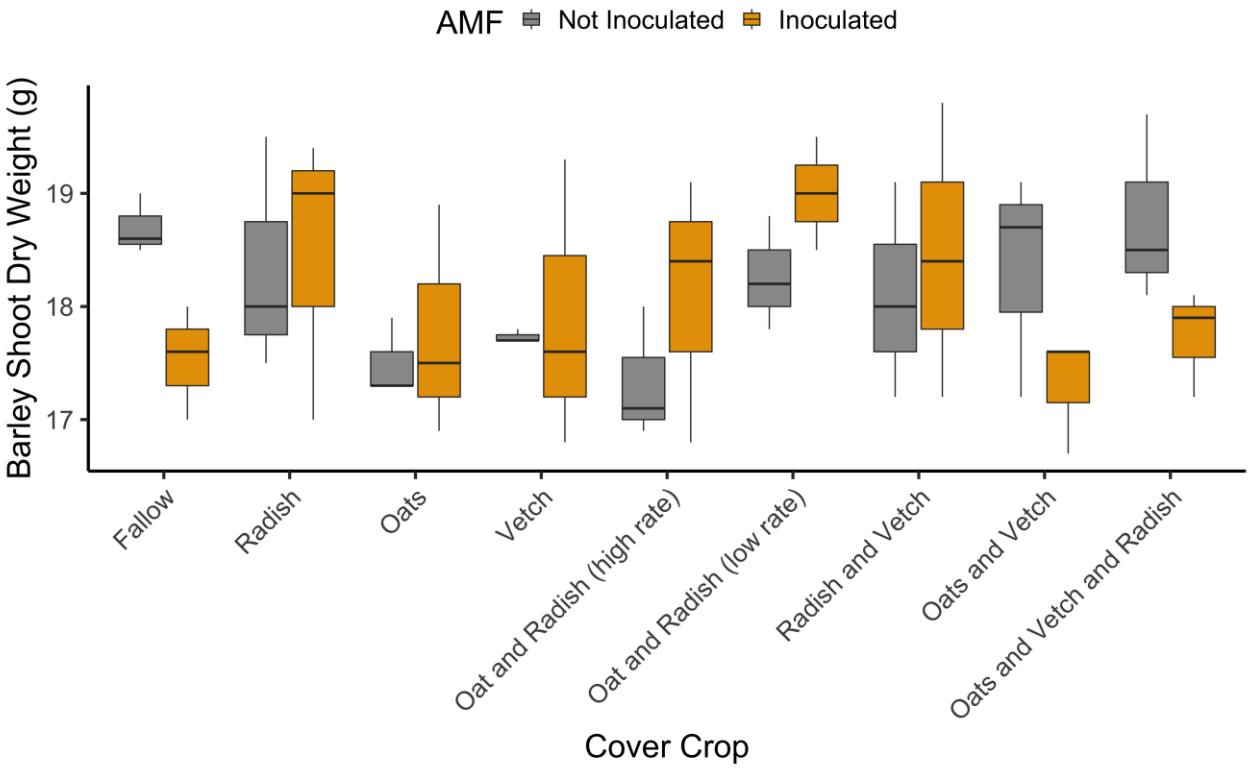

Figure 18 – Spring barley dry shoot weight from year one of the Bawburgh trial
Dry shoot weights represent the green shoot of five spring barley plants, classified as one biological replicate.

4.2.5. 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 19). The non-inoculated fallow control had a barley 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 (χ^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).

Biomass, RLC, and yield measurements were also made for winter oat in year two (data not shown). There were no significant differences resulting from the preceding cover crop, or from AM fungal inoculation.

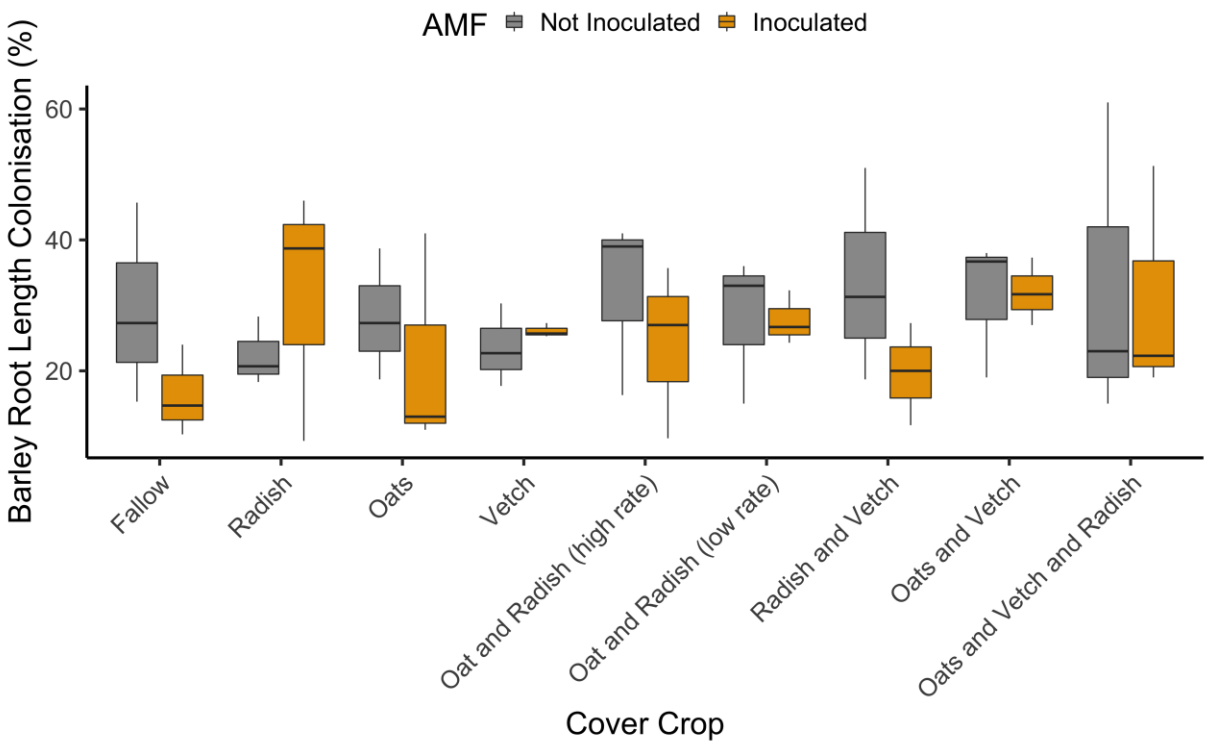

Figure 19 – Spring barley root length colonisation (RLC) by AM fungi, from year one of the Bawburgh trial.
Fungal structures were visualised using trypan blue. RLC expressed as a percentage of the total root length colonised, following measurement of an adapted version of the grid intersect method.

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

Analysis of differential abundance of virtual taxa, 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 20). 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.

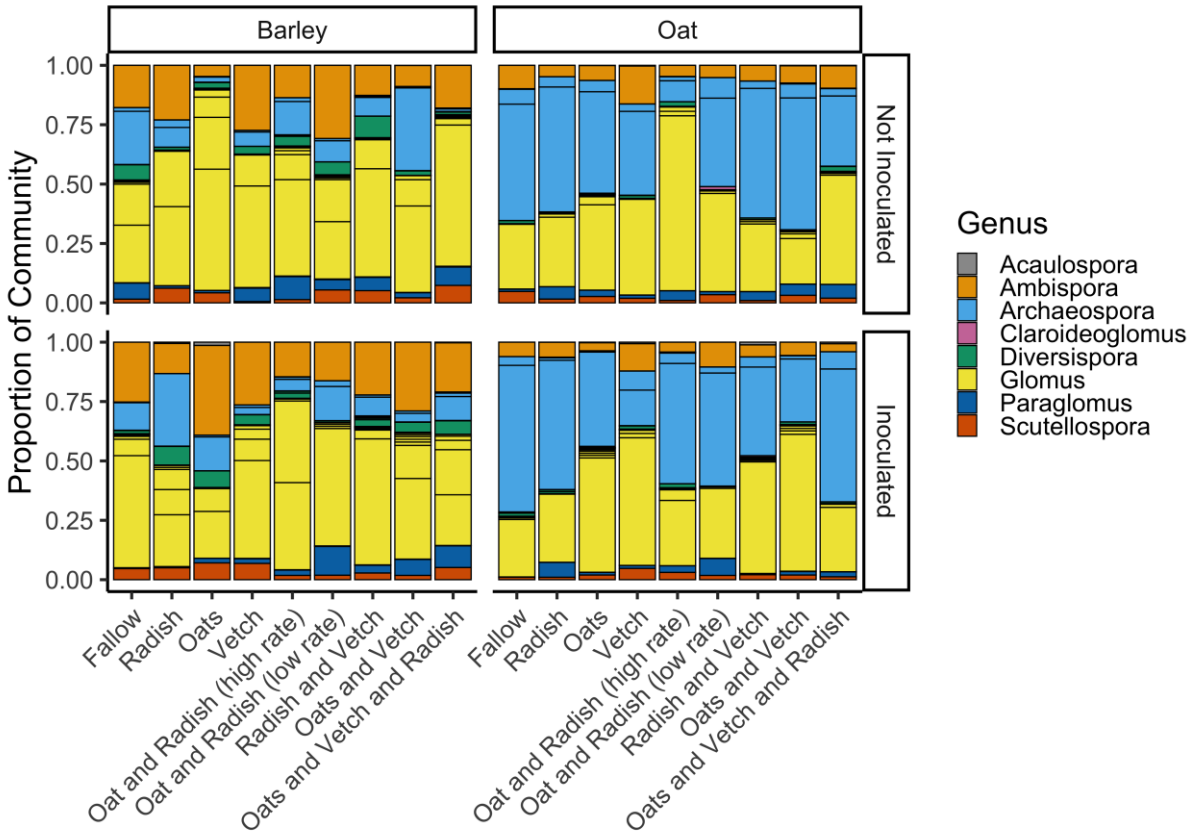

Figure 20 – AM Fungal community composition associated with spring barley and oats, following cover cropping at the Bawburgh trial.
Mean community composition of AM fungal VT for each experimental treatment, expressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT.

4.3. Influence of Nitrogen Application on AM fungal communities, and benefit of symbiosis

4.3.1. Barley Root Length Colonisation in the New Farming Systems trial

Total RLC varied from 18.7% in the 100% N fallow treatment, to 72.3% observed in the 50% N legume mix treatment (Figure 21). The lowest mean RLC of 32.2% was observed in 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, the fallow treatment had a RLC of 33.4%. This was significantly increased to 45.4% (Tukey $p = 0.033$) in the radish and oat cover crop treatment, and 51.8% (Tukey $p < 0.001$) in the legume mix, which was the highest mean RLC in the trial. This treatment also had the highest individual measurement of barley RLC, at 72.3%. In the 100% plots, the fallow mean RLC was 34.1%. Only the legume mix cover crop was significantly higher than the fallow treatment, exhibiting a mean RLC of 45.5% (Tukey $p = 0.045$). The 100% N radish and oat

treatment had a 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.

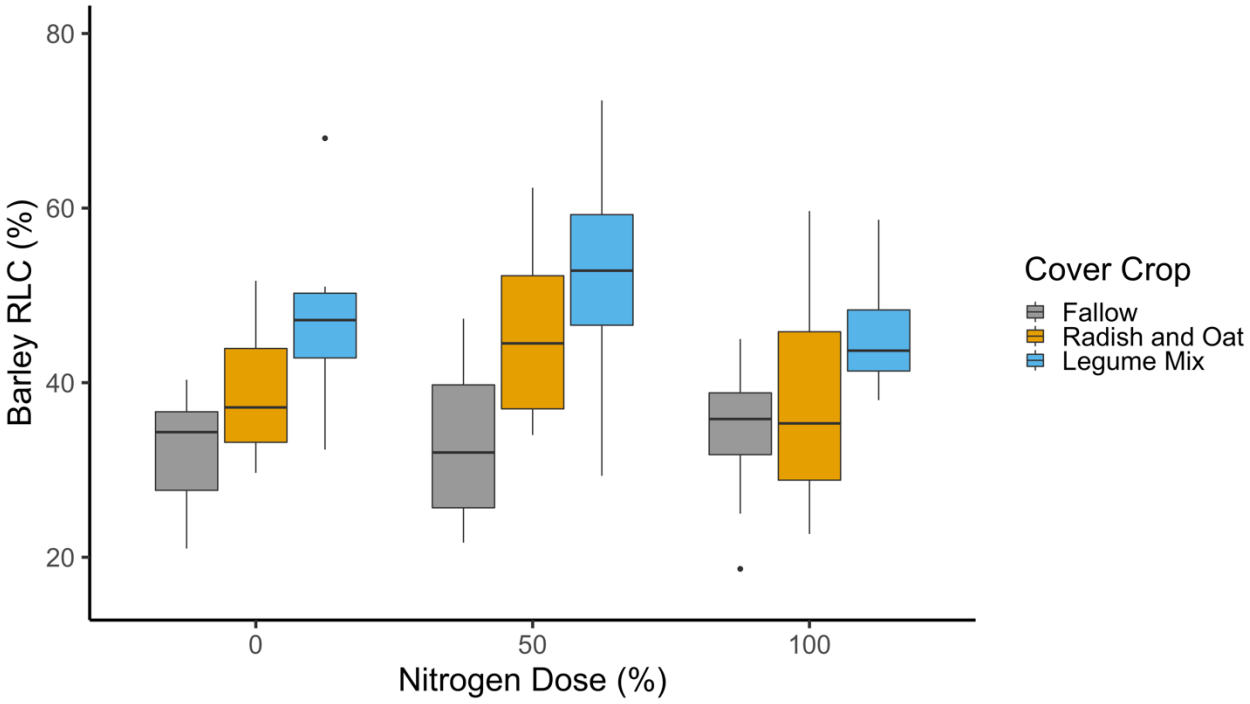

Figure 21 – Spring barley root length colonisation (RLC) at different N doses, in the New Farming Systems trial, stained using trypan blue.
RLC expressed as a percentage of the total root length colonised, following measurement by an adapted version of the grid intersect method. Dots represent outliers which are greater than 1.5 times the interquartile range from the lower or upper quartile.

4.3.2. Spring Barley Yield in the NFS Trial

Spring barley yield ranged from 2.92 t/ha in the 0% N radish and oat treatment, to 6.96 t/ha in the 100% N fallow treatment (Figure 22). 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 in 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 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 the radish and oat (Tukey $p < 0.001$) and legume mix (Tukey $p < 0.001$) cover crops, which themselves did not differ in yield across 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, $p = 0.996$).

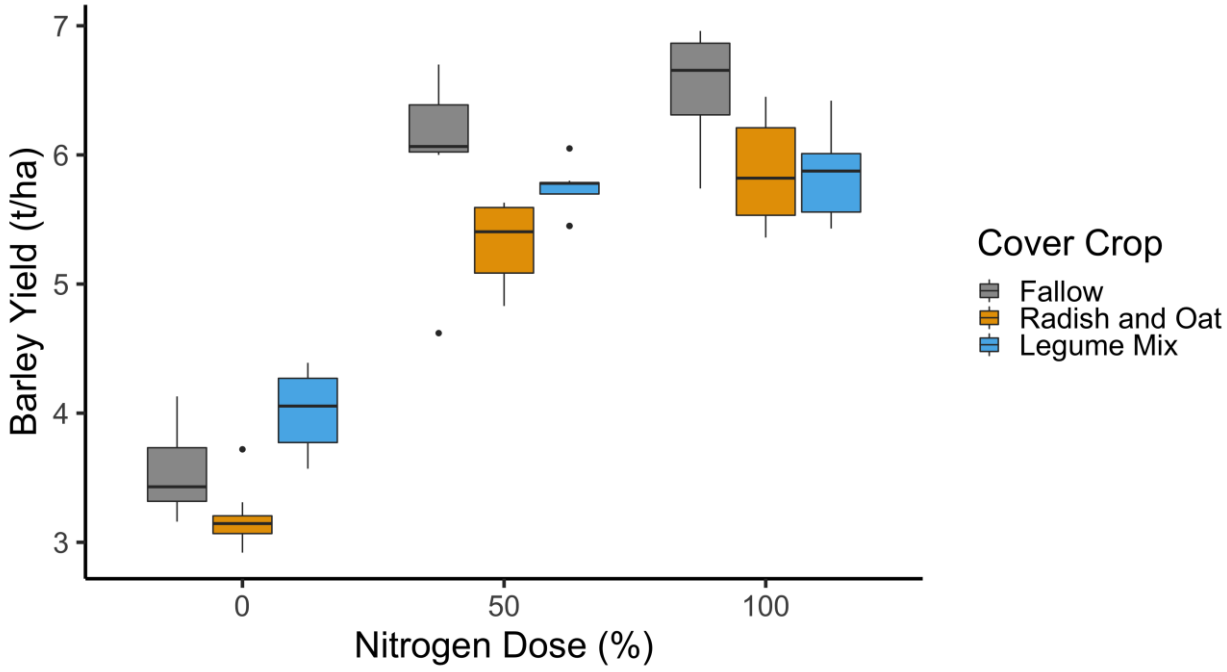

Figure 22 – Spring barley yield at varying N doses, following each cover crop regime in the New Farming Systems trial.
Barley yield expressed in tonnes per hectare, following standardisation to 15% moisture content. Dots represent outliers which are greater than 1.5 times the interquartile range from the lower or upper quartile.

4.3.3. AM Fungal Community Composition and Analysis of Differential Abundance in the NFS trial

Analysis of differential abundance of virtual taxa 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 23). VTX00108 (*Glomus Whitfield type 7)* was significantly less abundant in the 100% treatment plots ($log_2FC = 9.18$, $p = 0.006$), as was VTX00030 (*Acaulospora Acau2,* $log_2FC = 4.79$, $p = 0.007$). One AM VT, VTX00245 (*Archaeospora trappei*) was significantly more abundant in the 100% N plots, when compared to the 0% N treatment ($log_2FC = 1.55$, $p = 0.041$)

Two virtual taxa were differentially expressed when comparing the 50% and 100% N treatments. Two taxa were significantly less abundant in the 100% N plots, including VTX00108 (*Glomus Whitfield type 7,* $log_2FC = 8.20$, $p = 0.026$), and VTX00030 (*Acaulospora Acau2,* $log_2FC = 4.13$, $p = 0.036$).

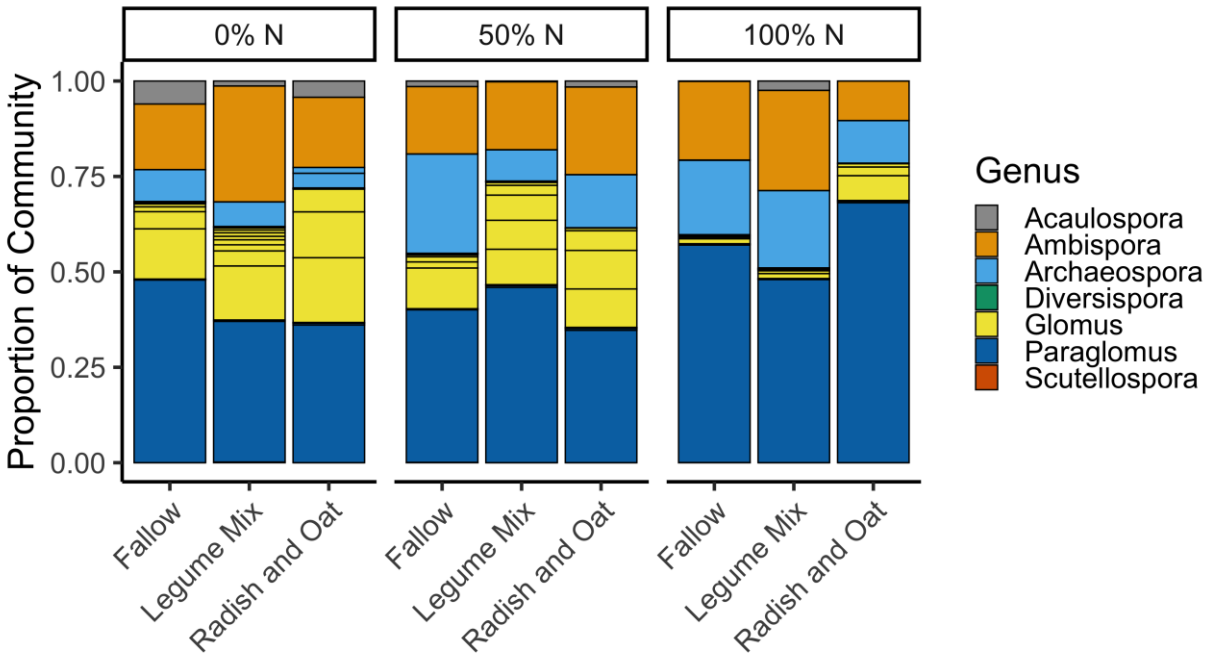

Figure 23 – AM Fungal community composition in the New Farming Systems trial.
Mean community composition of AM fungal VT for each experimental treatment, expressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT.

4.4. Use of Cover Crops, and Soil Amendments at the Farm Scale

4.4.1. Available Nitrogen in the Soil Profile in the IF trial

Soil nutrient testing was conducted to assess how AD impacted soil available N, and whether cover crops were effective at preventing N leaching. Application of AD was effective at increasing soil N at three of the four sites (excluding the North Moor Farm site), but large amounts of this nutrient were leached to the 30-60cm, or 60-90cm depths, with little effect at the 0-30 level (Figure 24). When applied with cover crops, leaching to lower horizons was reduced. This effect of cover crops to take up available N from the soil was also observed at the Allpress and Euston sites in cover crop plots which had not received an AD application. The North Moor site was hypothesised to exhibit this effect due to severe waterlogging at the site.

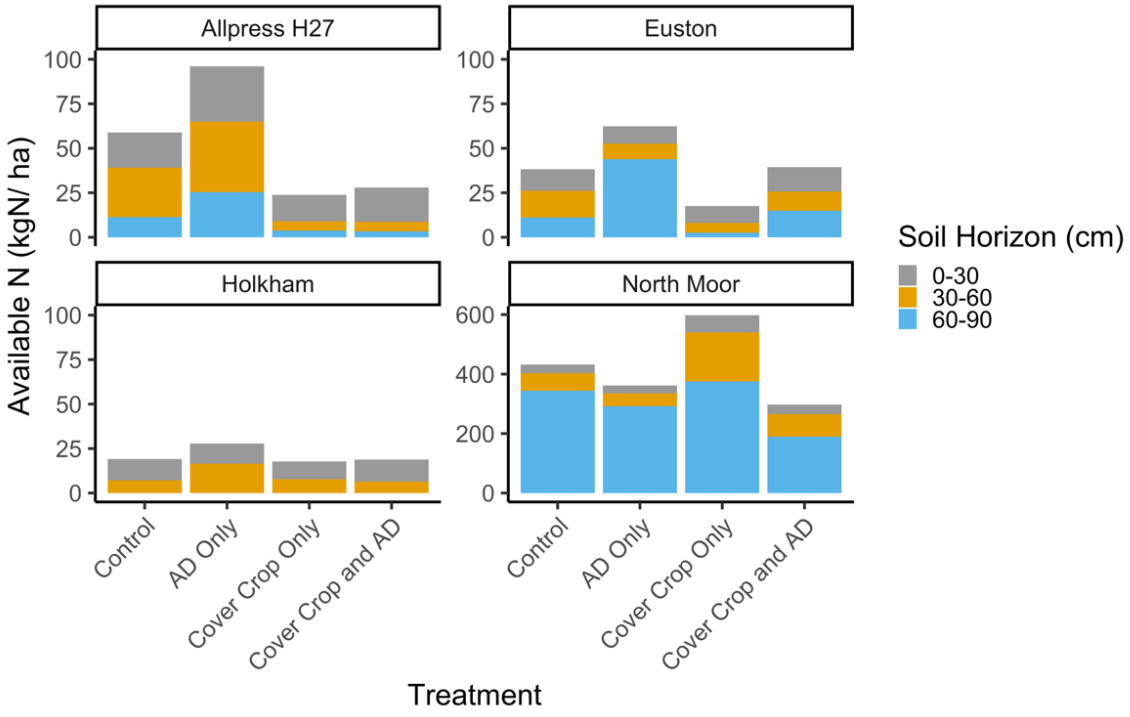

Figure 24 – Crop available nitrogen in the soil profile from four sites in the Innovative Farmers field trial.
Accumulation of available N in each treatment, at depths of 0-30cm, 30-60cm, and 60-90cm. The Holkham sites omits the 60-90cm depth, as the soil profile was not this deep. The four sites are plotted on variable axis, due to the large differences in available N between sites.

4.4.2. Maize RLC by AM Fungi in the Innovative Farmers trial

Maize colonisation by AM fungi ranged from 2% in the Euston Control to 99% in the Allpress cover crop and AD treatment (Figure 25). 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.

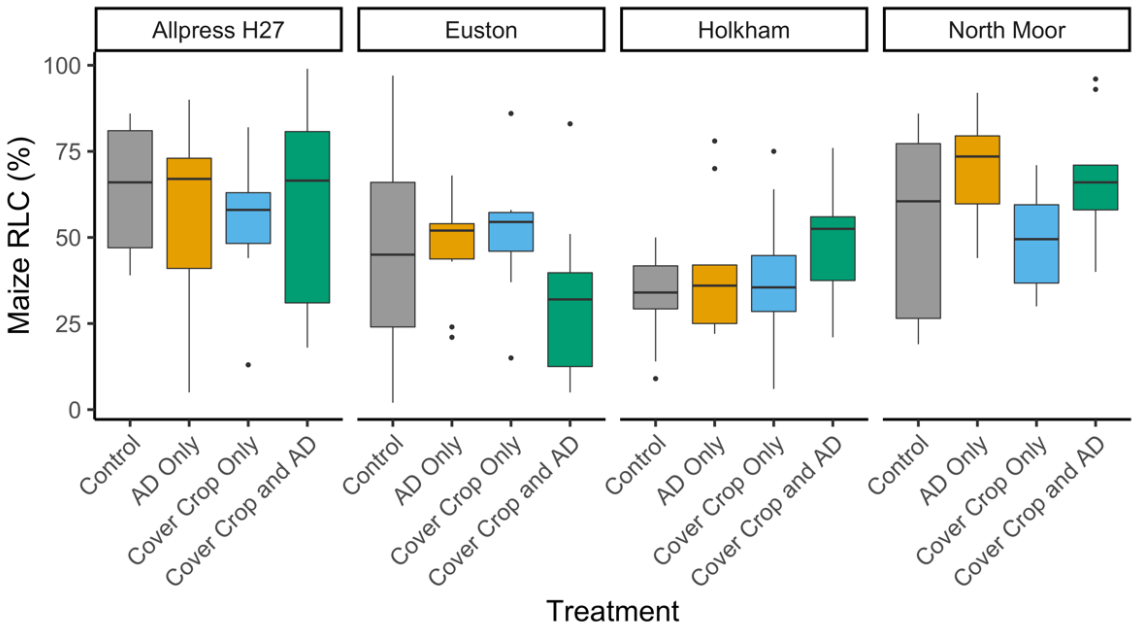

Figure 25 – Colonisation of maize by AM fungi, from four sites in the Innovative Farmers trial. Boxes display mean, interquartile range, minimum, and maximum colonisation of each plot. Dots represent outliers which are greater than 1.5 times the interquartile range from the lower or upper quartile.

4.4.3. Maize Biomass in the Innovative Farmers trial

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 26). The North Moor site also exhibited the greatest biomass across treatments, with a mean biomass of 78.3 ± 4.6g. The next largest 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: $F_{9,144} = 1.11$, $p = 0.357$), 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, $p = 0.192$).

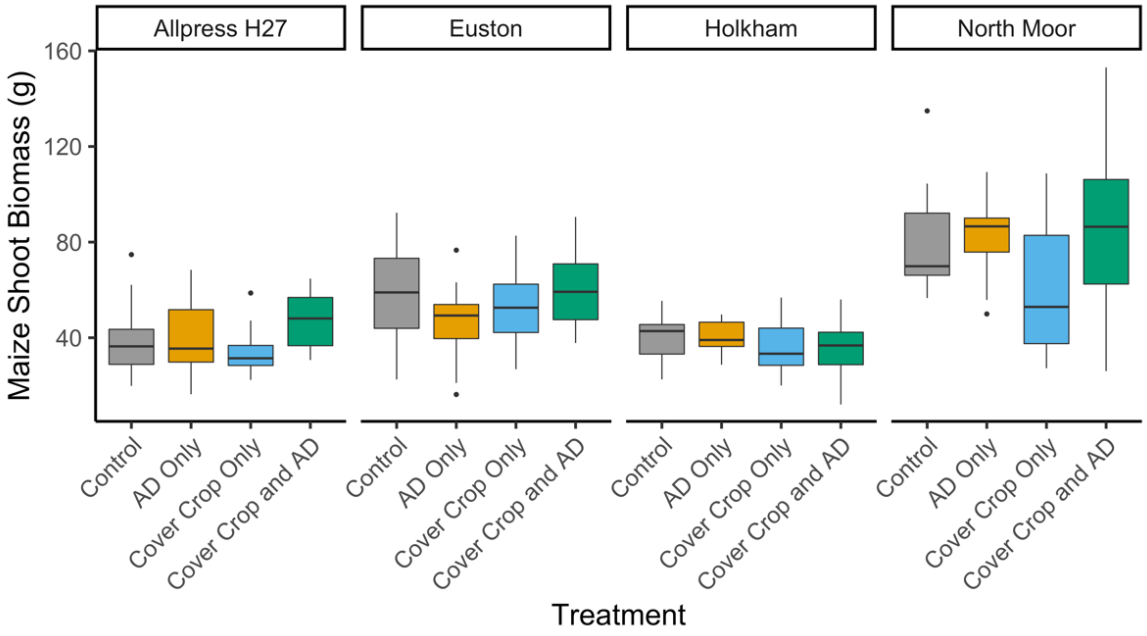

Figure 26 – Maize dry shoot weight at four sites from the Innovative Farmers trial.
Dry shoot weight of maize at each site following the four experimental treatments

4.4.4. Observed Number of AM Fungal Taxa in the IF trial

As a measure of diversity, the total unique number of AM taxa per site per treatment was used. For comparison, the Allpress H48 site growing leek is also included. Mean observed taxa across the IF trial was 11.35 ± 1.01, ranging from 5 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 27). 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 observed 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.

When considering the effect of treatment across sites, the cover crop and AMF treatment (tested at the Allpress site only) 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 in 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).

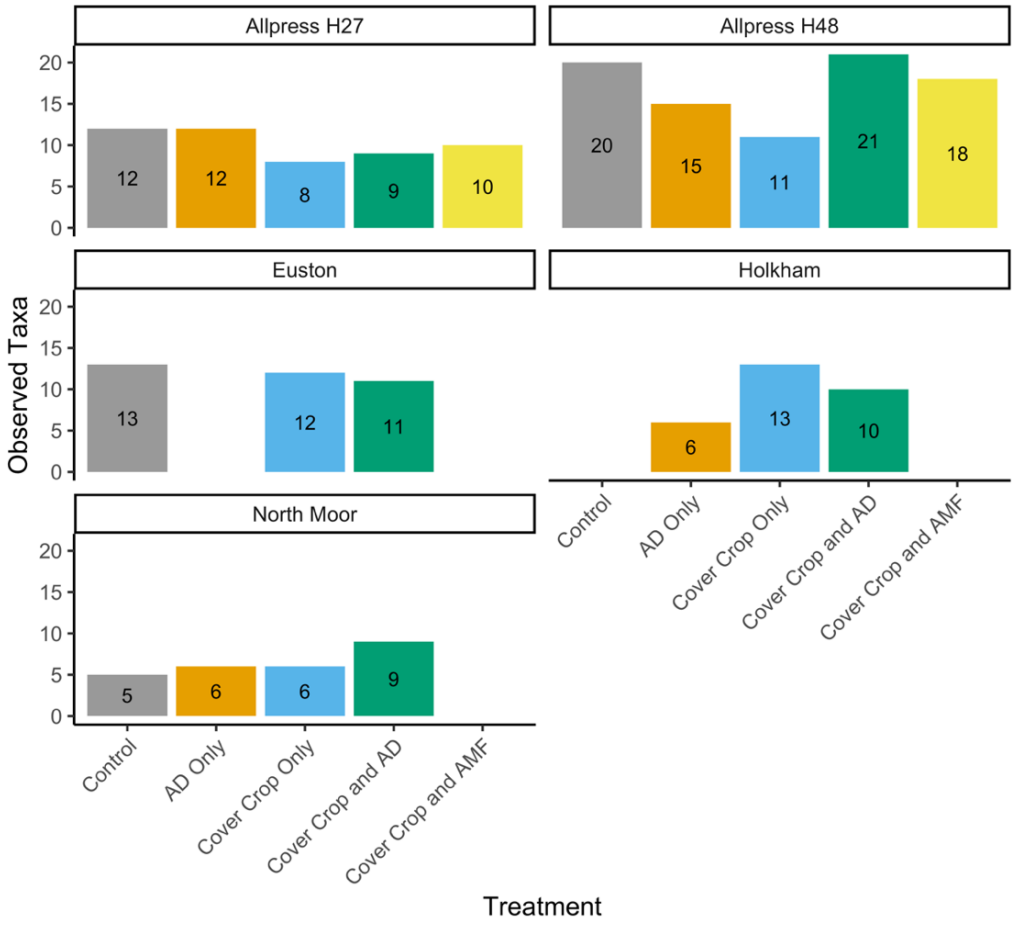

Figure 27 – Observed AM fungal virtual taxa at five sites from the Innovative Farmers trial. AM fungal VT observed in each experimental treatment per site. There was no abundance threshold for classification of present vs absent to account for lowly abundant VT. Allpress H27 and H48 were the only sites with an additional treatment – cover crop with AM fungal inoculum. The AD only sample at Euston and the Control sample from Holkham failed to amplify using AM fungal specific DNA primers.

4.4.5. Differential Abundance of AM fungal taxa in the IF trial

Analysis of differential abundance of virtual taxa was conducted to further confirm how individual taxa had responded to experimental treatments. For comparison, the Allpress H48 site growing leek is also included. The relative proportion of AM fungal genera in each treatment and site is shown in Figure 28. One taxon, VTX00153 (*Glomus MO-G12)* was significantly higher abundance in the treatment following the cover crop and AD, when compared to the control ($log_2FC = 24.6$, $p < 0.001$). 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 ($log_2FC = 23.9$, $p < 0.001$). 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.

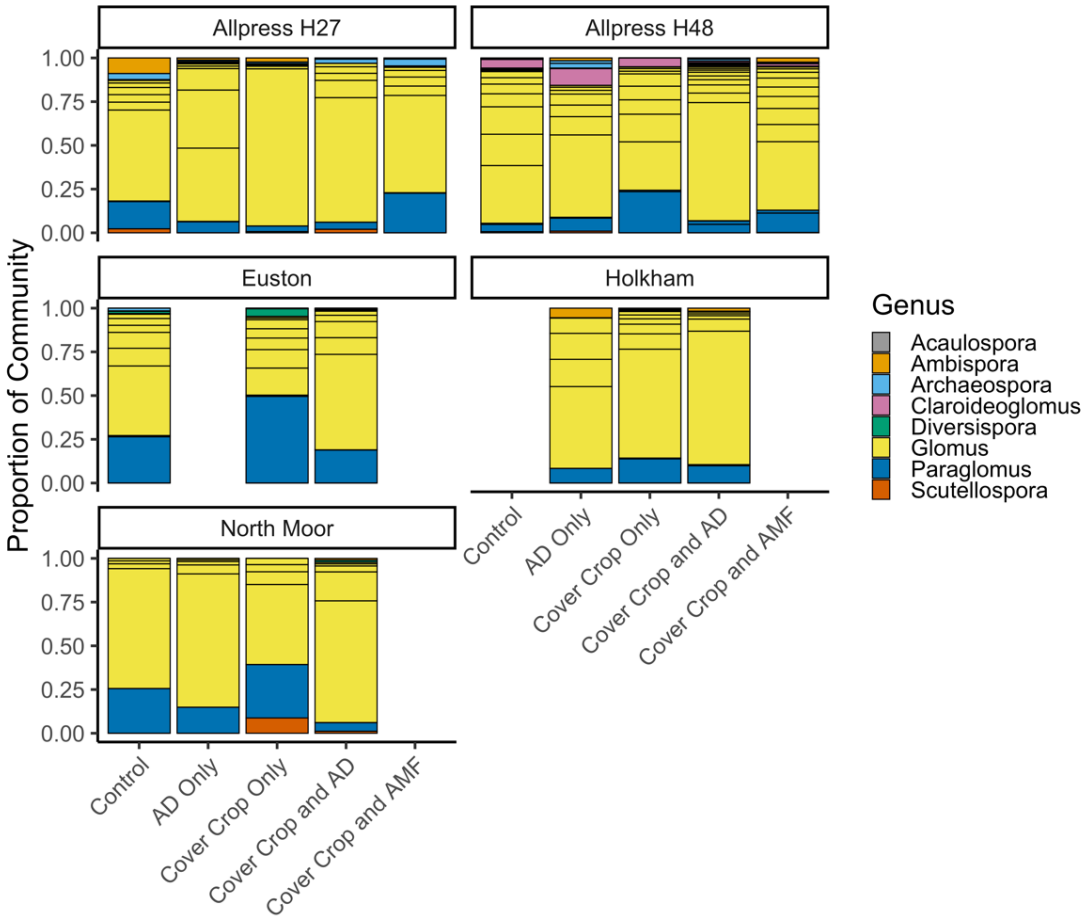

Figure 28 – AM Fungal community composition at five sites from the Innovative Farmers trial. Mean community composition of AM fungal VT for each experimental treatment, expressed at a proportion of total number of DNA reads matching to Glomeromycotina. Each stacked bar represents one AM fungal VT, while the colour of the bar represents the genus of that VT. Allpress H27 and H48 were the only sites with an additional treatment – cover crop with AM fungal inoculum. The AD only sample at Euston and the Control sample from Holkham failed to amplify using AM fungal specific DNA primers.

5. Discussion

In this report, a UK-wide analysis of AM fungal diversity is presented, before exploring the impact of cover crops, and other amendments, on 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 diversity can be explained by three factors: the soil type as defined by texture, type of cultivation, and whether fungicides had been applied to the crop (data not shown). 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 project, 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. 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 in the NFS trial 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 NFS trial, the leguminous cover crop mixture increased barley RLC to a greater extent than the radish and oat. This is potentially due to the non-mycorrhizal nature of the radish cover crop, or that legumes may promote the symbiosis to a greater extent due to increased release of hormonal signals. Conversely, there was no measurable impact on RLC or AM fungal diversity in spring barley following a radish cover crop (single or in a mixture) in the Bawburgh trial. More, long term studies will be required to validate this outcome.

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% regimes. In contrast to the literature, *Glomus* species*,* including *Glomus Whitfield type 7,* and a Diversisporales, *Acaulospora Acau2* were particularly impacted by high N fertilisation. *Paraglomus* and *Archaeospora* taxa increased in their abundance to fill the niche abandoned by these taxa that were potentially sensitive to N. This provides further evidence for the potential benefit of strategies that result in an increase to the diversity of AM fungi, to occupy distinct niches and provide additive benefit.

A single N application, in the form of anaerobic digestate 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 *Rhizophagus irregularis,* which constituted 70% of DNA reads in the mixed inoculum, also used in two field trials. In the Bawburgh trial, the inoculum failed to influence AM fungal RLC of either barley or oat, and DNA sequence reads of the inoculum taxa were not detected at higher abundance. Anecdotally, inoculation did appear to reduce the dominance of common AM fungal taxa in spring barley (Section 4.2.6), an effect that has been observed by others in the literature. However, this effect was short lived and not observed in the following winter oat crop.

At the field-scale, 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 following 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.

5.1. Summary of Project Hypotheses

In this study, it was shown that 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 from the NFS trial 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 a radish and oat, or legume mix cover crop 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 did result in other quantifiable benefits, even after one iteration. Residual N was taken up by the cover crop in Section 4.4.1, preventing this N from leaching to deeper soil horizons, and potentially into adjacent watercourses.

It was shown that certain AM fungal taxa exhibited differing levels of RLC, and subsequent impact on spring barley growth in controlled conditions. However higher values of RLC were associated with reduced spring barley biomass, which is in contrast to the understanding that increasing AM fungal colonisation results in increased plant benefit. Furthermore, there was limited evidence that increasing AM fungal diversity or abundance could increase crop biomass and yield in field conditions. In the Bawburgh trial, increasing RLC was correlated with increased yield in oat, but this was not the case for the previous barley crop. Similarly, there was no impact of RLC on barley yield in the NFS trial. In the IF trial there was no correlation between maize RLC and yield, 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.

5.2. Conclusions

The main aims of this study were to determine the impact of cover cropping and other amendments (including mycorrhizal inoculum, and nitrogen in various forms) on the diversity and abundance of AM fungi. A further aim was to determine the resulting effect on crop yield. From the data presented, it seems likely that the soil microbiome is resilient to the impact of an amendment applied only once, but positive effects are additive if repeated over several years. This suggests that farmers should not be looking for 'quick fixes' from cover crops, or any of the other amendments tested in this project, 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.

REP 3

	Spring Break Clover	Winter Break Clover	Spring Break Fallow	Spring Break Legume Mix	Mixed Cropping Clover	Mixed Cropping Legume Mix	Mixed Cropping Radish & Oat	Winter Break Fallow	Spring Break Radish & Oat	Mixed Cropping Fallow
100% N										
50% N										
0% N										

REP 4

	Mixed Cropping Legume Mix	Mixed Cropping Clover	Spring Break Clover	Spring Break Radish & Oat	Winter Break Clover	Winter Break Fallow	Spring Break Fallow	Mixed Cropping Fallow	Spring Break Legume Mix	Mixed Cropping Radish & Oat
100% N										
50% N										
0% N										

REP 1

	Winter Break Clover	Winter Break Fallow	Spring Break Radish & Oat	Mixed Cropping Fallow	Spring Break Fallow	Mixed Cropping Clover	Mixed Cropping Legume Mix	Mixed Cropping Radish & Oat	Spring Break Legume Mix	Spring Break Clover
100% N										
50% N										
0% N										

REP 2

	Mixed Cropping Fallow	Mixed Cropping Legume Mix	Spring Break Clover	Winter Break Clover	Mixed Cropping Radish & Oat	Spring Break Legume Mix	Spring Break Radish & Oat	Spring Break Fallow	Mixed Cropping Clover	Winter Break Fallow
100% N										
50% N										
0% N										

= 12 x 12 m²

Figure A1 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.

7. References

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