The improvement of plant performance in reduced peat growing media using arbuscular mycorrhizal fungi

A thesis submitted to the University of London for the degree of Doctor of Philosophy

By

Lauren Carys Edwards

School of Biological Sciences

Royal Holloway, University of London

2017

Declaration of Authorship

I, Lauren Carys Edwards, hereby declare that this thesis and the work presented in it is entirely my own. Where I have consulted the work of others, this is always clearly stated.

Signed……

Date……..

Acknowledgements

Firstly, I would like to thank Alan Gange, Tony Stead, Paul Alexander and Paul Devlin for all their support, help and advice throughout my study. Thank you to Gracie Barrett, Martin Ward, Neil Bragg and Simon Budge for their valuable insight, advice and supply of materials which enabled me to carry out this research. I would also like to thank the RHS, Symbio and the BBSRC for funding this project. Thank you also to the RHS staff who helped with all my experiments but also for supporting my development as a researcher and science educator.

Thanks to Royal Holloway technicians Pauline Bake and Neil Morley for always having a solution and being around when I needed them. Thank you to Tracey Jeffries, Nicola Moss and Mel Kiukkenen for chats, printing help, and all things admin related! And finally, thanks must go to Alice Milner, Jon Beauchamp, Dave Morritt, Mark Brown and Enrique Lopez for their unwavering support with my research, teaching and personal development.

A huge thank you to all my project students who helped me gather data for this research and made lab/field work more enjoyable: Kirsty, Sadie, Mera, Holly, Rebecca, Victoria and Clive.

Thank you to my fellow PhD students Kate Orman, Roseina Woods, Matthew Casey and Bianca Lear for all your support, tea and gin-based therapy sessions, kindness, laughter and generally making this experience immeasurable better. Thank you also to the Gange lab group for your help and support, but especially Sue Broughton for her proofreading skills!

A special thank you to my Mum, Dad, Sister and all my family for always being understanding and ready to help and support including with field work, even in the rain! A big thank you must also go to my partner Jamie Hampshire for all his love, support, washing up skills, and putting up with me during the hardest of times.

Abstract

The unsustainable and destructive nature of peat extraction and its extensive use in the horticultural industry requires a suitable alternative to be found. Alternative sources are being considered, but they all have properties that make them unfavourable to both commercial and amateur growers, because of their inconsistent quality and performance. For any of them to succeed in replacing peat, a solution must be found to increase their ability to produce a similar standard of plant performance currently achieved, whilst also providing other benefits which will make these media favourable to growers.

The aim of this PhD research was to further test the effects of adding commercial AMF inoculum to reduced peat growing media with commercially relevant plant species in both outdoor and commercial glasshouse style experiments. In both environments, plant performance in each combination of AMF treatment and peat amendment has been directly compared to commercial peat standards. DNA extraction has been used to identify the AMF species responsible and improve understanding on the function of commercial AMF inocula.

Results showed that bedding plants grown in bark chip and wood fibre-based growing media were not significantly smaller than those grown in peat, and that the addition of live AMF inoculum significantly reduced the inconsistency in size between plants in both of these treatments. The number of marigold plants showing signs of nutrient stress with purple colouring to the leaves was also reduced with addition of AMF inoculum. In the wood fibre growing media, colonisation by AMF was also found to directly increase the water holding capacity of pots. Molecular work indicated that colonised AMF species varied between different commercial inocula and growing media treatments.

Table of Contents

List of Tables

List of Figures

Clarifications

Subject	Description
AHDB	Agriculture and Horticulture Development Board
AM or AMF	Arbuscular Mycorrhizal Fungi
AO	Antioxidant
Biomass	Total dry weight of shoot tissue (not including roots)
DEFRA	The Department for Environment, Food, and Rural Affairs
PCR	Polymerase chain reaction
PGPR	Plant growth promoting rhizobacteria
SGMTF	The Sustainable Growing Media Task Force

Chapter 1 - General Introduction

1.1 Rationale for Peat Reduction

Peat is formed in boggy areas where carbon rich plant material decays in anaerobic conditions caused by waterlogging. In the UK *Sphagnum* moss species form the majority of peat. The UK is one of the top twenty nations in the world in terms of total peatland area, as it makes up to 15% of the total for Europe (Bain *et al.*, 2011).

Peatlands are nationally and internationally important as they provide unique habitats for typical peatland flora and fauna, act as a site of carbon storage (with an estimated 3.2 billion tonnes sequestered in remaining UK sites), produce cleaner water which requires less treatment for drinking, and have preserved some of the most important and intriguing archaeological finds in the history of the UK (Bain et al. 2011).

Use of peat as a growing media for container plants in the in the UK has been popular since the 1970s (Bragg 1998). In addition to peat extraction for growing media and fuel, draining and destruction of peatlands for agriculture and forestry practices has also damaged the remaining peatlands so much that it is estimated that less than 20% of the UKs peatlands are undamaged. In 2008, only 9,000 ha out of 70,000 ha of lowland raised bogs were classified as near natural or primary degraded bog which is thought to be capable of natural regeneration (Great Britain), and up to 4000 ha still had consent for extraction. The main source of peat for the UK is in the Republic of Ireland, where 308,742 ha of original peatlands have been reduced to just 25,189 ha considered viable for conservation (Alexander et al. 2008). Even those now protected under EU wildlife and water legislation have suffered, with less than 50% remaining in a potentially reversible condition (Bain et al. 2011).

As peat accumulates very slowly it cannot be replaced to its original thickness in our lifetime, and in England the horticultural use of peat is five times greater than the volume of peat extracted, which means the majority is now imported from the Republic of Ireland, Scotland, Northern Ireland, and continental Europe (Waste and Resource Action Programme 2012). Not only is continued peat extraction unsustainable, it is contributing to increased greenhouse gas emissions, population decline of peatland species, and, through drainage and erosion ditches, damaged peatlands also exacerbate flood events which cause significant damage to downstream areas (Bain et al. 2011).

Attempts at large scale restoration of protected peatlands in the UK are now underway, with programmes such as the International Union for the Conservation of Nature (IUCN) Peatland Programme setting a target of one third (one million ha) of the UKs peatlands to be in good condition or under restoration management by 2020 (The Wildlife Trusts 2013).

1.2 Government Targets and the Effect on the Horticultural Industry

The UK garden market has an annual turnover of £5 billion, and the edible horticultural sector also contributes an annual turnover of £3 billion (DEFRA 2013), so the horticultural sector is therefore very important to the UK economy. In June 2011 the government Department for Environment, Food, and Rural Affairs (DEFRA) published their Natural Environment White Paper which set targets to phase out peat usage by 2030 for professional growers, and by 2020 in the amateur garden market, as it represents the majority (69%) of the three million cubic meters of peat used per year for horticulture.

The unique properties of peat have resulted in consistent plant growth for decades. It is such a highly sought-after material because it is the perfect substrate for growing plants; it contains large amounts of air space, whilst also having a high water holding capacity; it has low pH and nutrient content which allows fertilisers and additives to adjust levels to specific plant requirements; and, due to its formation, it is relatively pest and pathogen free, so it does not require additional sterilisation (Schmilewski 2008). The issues facing the supply and demand of peat alternatives are caused by not only their expense and comparative unavailability, but because they cannot match the consistent quality and performance of peat. As the result of a consultation aimed at reducing the horticultural use of peat in England, DEFRA appointed the Sustainable Growing Media Taskforce (SGMT) to work to address these issues.

With this government focus, more pressure has been put on professional growers and retailers that sell peat products to make their supply chains more sustainable. Information guidelines (Waste and Resource Action Programme 2012) and support are available to help them achieve this, but the government have acknowledged that this transition is going to be difficult, and suggest that technological developments and advances may be needed to facilitate the changeover (DEFRA 2013) to alternative growing media. Guidelines (Waste and Resource Action Programme 2014b) state that any new media must be trialled by each grower in order to allow nurseries to understand how to adapt management practices to suit the new material. This need to adapt or change production set-ups is another part of the reason growers have been resistant to the change from peat.

The same reasons outlined above have been attributed to reluctance of amateur gardeners to buy or use peat free products. In 2014 less than half (45%) of the volume of materials sold in growing media in the UK was made up of peat alternatives. There is evidence that attitudes are changing, as the 45% of peat alternatives in growing media sold sees an increase from 2011 (39%) when the taskforce was set up (Denny & Waller 2016). Which? magazine runs extensive compost trials each year, and are a trusted source for gardeners, with trial results also being published in

the press (Gray 2010) and they have gone from producing negative reviews of peat free compost trials in 2013: "*Peat-free composts have never done brilliantly in our trials…none was good enough from 2013 trials to recommend*." to having peat-free products rated the best in 2015: *"We were very pleased that among the Best Buys were two peat-free composts."* (National Farmers Union 2014; Nicholsons 2016). This suggests that the quality and performance of peat-free composts has improved. However, this apparent change in attitudes has not resulted in a reduction of the sale of peat based products. In fact, there has been an increase in peat use in the retail market from 50 to 53% between 2011 and 2015 (Denny & Waller 2016). This could be because, despite regulations such as those for green compost (British Standards Institute 2011) put in place to improve consistency, plant performance is still not considered as good as with peat. This could also be due to poor understanding of the differences in alternatives to peat, which results in amateur gardeners not being aware of how to change their watering and management systems to get the best out of the alternative based mixes.

It appears that in order to improve the adoption of alternative peat materials in the horticultural sector, a solution needs to be found which not only increases the ability of the peat free media to produce a similar standard of plant performance achieved using peat, but one which also reduces the variability of plant performance, perhaps by reducing the change in requirements of water and nutrients.

1.3 Peat Alternatives

There are four main alternative materials that have been considered suitable replacements and have been incorporated, sometimes in combination, in both professional and retail mixes in recent years. Unlike the retail market, the amount of peat in professional growing media has decreased since 2011 from 72% to 63.9% in 2015 (Denny & Waller 2016). This has been replaced with mixtures of the peat alternatives outlined here. Their contribution to improving sustainability in horticulture is increased further by all of them being waste products produced by other processes. They have different properties which, as previously stated, currently cannot match the ideal combination of those found in peat. This is often why they are used as amendments to reduce peat content in growing media, or used in combination with each other. The following sections outline the physical, chemical, biological, and economic properties of each of the main peat replacement materials that have been investigated and analysed in depth in recent years. They will also all feature in the experimental work of this thesis.

1.3.1 Coir

Coir is formed from the ground husk (mesocarp) which surrounds coconuts and is a waste product of the coconut (*Cocos nucifera*) industry, with the majority being produced in India, Indonesia, and Sri Lanka (Drewe 2012). The transportation costs are partly responsible for the high cost of this material, although it can be dried to reduce weight because it has excellent re-wetting capabilities (Schmilewski 2008). It is the most similar of the alternative materials to peat because it has similar structure and texture which give it similar water and air holding properties (Schmilewski 2008). Previous issues with high salt content as a result of washing the material in sea water during processing, or as a result of coconuts which were grown by the sea, has been reduced in recent years with the introduction of a buffering step. Treatment with calcium nitrate solution to remove excess sodium and potassium with fresh water can reduce the phytotoxic levels which can make coir unsuitable for horticultural use (Schmilewski 2008; Nichols 2013). As coir is a waste product which is not manufactured specifically for horticultural use as a growing medium its processing is not regulated, and this step is not always carried out as it increases the cost of producing, and therefore the overall cost of coir. The physical and chemical variability of the material as a result of different storage times and length of drying and buffering processes (Schmilewski 2008) can also make it unreliable to work with. Therefore, despite there being sufficient amounts of coir to supply the demand for the horticultural sector in the UK, supply would be restricted to countries which have suitable quality control and infrastructure to handle the demand for consistent, good quality material. (Drewe 2012; Waste and Resource Action Programme 2012; Nichols 2013) Holman et al. (2005) found that plant growth in different brands of coir from different origins was consistently poor, and not able to match the standard of plant growth in peat.

Despite its initial problems, as the market for coir has grown the production of it has become more consistent, and it is now a very popular peat amendment in growing media produced for professional use in the horticultural sector due to its similarity in properties to peat (Schmilewski 2008). The physical texture of coir also makes it ideal for integrating into mechanised potting systems (Waste and Resource Action Programme 2012). In the Which? magazine compost trial mentioned earlier, a peat free, coir based multipurpose compost was awarded best buy in 2015. Indeed, studies which have compared peat alternative-based multipurpose composts found that there were no significant differences in growth, flower number and plant quality of bedding plants in peat and coir based composts, including when they were maintained under the same watering regimes (Alexander et al. 2013; Alexander et al. 2014). These favourable properties are likely to see coir use in horticulture increase in future, despite its high cost.

1.3.2 Green Waste Compost

Composted green waste (CGW) is another product produced from waste, and often involves recycling dead or decaying household plant waste from municipal waste streams. It often includes woody materials, leaves, branches, grass clippings and sometimes spent growing media, but this will vary throughout the year depending on garden management during different seasons. Also, as there is no real control over what can get taken to council collection sites or put in garden waste bins, other materials such as rubble, brick, gravel, plastic, glass and even wire or cable can sometimes be included. There is also the risk that herbicides (Blewett et al. 2005), plant pests and pathogens (Noble & Roberts 2004) could find their way into CGW growing media if sufficient composting procedures are not in place. After significant poor quality and inconsistencies were found with CGW, regulations were tightened in 2011 to reduce allowances for physical contaminants such as those mentioned (British Standards Institute 2011), but the SGMT found that confidence in GWC still remained low from previous bad experiences and in 2013 the government agreed (DEFRA 2013). This resulted in clarification and improved best practice guidelines for producing composts for growing media (Waste and Resource Action Programme 2014a; Waste and Resource Action Programme 2014b).

The positive properties of green waste compost are that it has good water holding capacity and it is cheaper than peat and the other alternatives. It has also been shown to suppress a range of plant diseases including root rot diseases due to its microbial populations (Hoitink et al. 1997; Reuveni et al. 2002; Noble & Coventry 2005; Sabet et al. 2013). It also has high nutrient content, but this is due to its high organic content which also results in high pH, so CGW must always be blended with other materials, typically only up to 30% to reduce negative effects on plants. Its high bulk density also makes it costly to transport due to increased weight (Schmilewski 2008; Waste and Resource Action Programme 2012).

Research has suggested different ways of improving the quality and properties of GWC, either with shredding material and the addition of wood shavings which were shown to improve organic matter concentration and pH (Tognetti et al. 2007), extending the composting time as immature composts were found to contain herbicide compounds which had not successfully broken down (Blewett et al. 2005), and increasing temperatures, along with ensuring even temperature treatment to result in the significant reduction of pests and pathogens (Noble & Roberts 2004).

Experiments which have used green waste multipurpose composts showed significantly poor results when compared to peat, with it producing small plants with uneven stem elongation and limited flower production, regardless of watering regime (Alexander et al. 2013; Alexander et al. 2014). When GWC was added as an amendment to peat or other materials in low concentrations

positive plant growth results were seen due to improved physical or chemical properties (Benito et al. 2005; Perner et al. 2006; Perner et al. 2007; Matysiak & Falkowski 2010).

1.3.3 Soft-wood Bark

Bark chips are produced as a by-product of the forestry industry because the bark of trees is not required in timber production. Soft woods such as pine, spruce and larch are used because hard wood barks contain tannins and terpenes which can affect plant growth (Waste and Resource Action Programme 2012). They are often composted or matured in piles outdoors in order to improve stability (Barrett et al. 2016). This process often involves the addition of nitrogen to prevent the material from immobilising nitrogen and reducing plants access to it (Schmilewski 2008). Pine bark is one of most common peat alternatives, and has been used to reduce the peat content in professional growing media for some time, but it is not widely used in retail growing media because it is expensive (Waste and Resource Action Programme 2012). In parts of the USA, New Zealand and Australia pine bark-based growing media dominate in the container plant industry (Barrett et al. 2016). Due to its larger particles and open structure it has high air holding capacity and can often be added to other materials to improve drainage (Schmilewski 2008). This open structure also means that it cannot support good plant growth alone (with the exception of orchids), so it has to be mixed with other materials, often GWC (Else 2013). Wood materials are also acidic, so must be mixed with lime (calcium carbonate) in order to increase the pH to make it suitable for plant growth (Barrett et al. 2016). Due to the composting or ageing process, bark is biologically active and can suppress plant pathogens (Calvet et al. 1992; Hoitink et al. 1997). Pine bark can also contain species of the fungus *Trichoderma* which are commonly found on decaying wood. These species have been directly shown to interact and support other microbes such as arbuscular mycorrhizal fungi (Calvet et al. 1992).

One of the disadvantages of bark is that, in various places, the price is being driven higher due to competition with other land uses (such as agriculture), and raw bark being used for other purposes such as burning for energy or bio-ethanol production (Schmilewski 2008; Barrett et al. 2016). If the demand for wood increases for the production of green energy then it may outcompete the use of wood for horticulture.

1.3.4 Wood Fibre

Wood fibre production uses machinery, high pressure, and steam to force a change in the structure of wood chips so they become fluffy and fibrous. The resulting material is very uniform (Barrett et al. 2016) and, unlike bark, it is sterile (Schmilewski, 2008). In a similar way to the processing of bark chips, wood chips are often impregnated with nitrogen before the material is extruded to prevent nitrogen immobilisation (Gruda et al. 2000). The use of wood fibre in horticulture is very popular in Europe, and the technology and popularity is now spreading within the UK (Waste and Resource Action Programme 2012). The wood fibre material produces a lightweight substrate which has high air capacity but poor water holding capacity. For this reason, it is normally added with other materials. Professional standard growing media can contain up to 30% wood fibre alongside peat (Schmilewski 2008). The majority (51%) of alternative materials supplied in retail growing media was made up of wood based materials (wood fibre and bark) in 2014. This shows an increase from 45% in 2011, and in professional growing media the amount of wood based materials (including wood fibre and bark) has increased from 6.7-11.9% from 2011 to 2015. This is in line with the decrease in the volume of peat used (Denny & Waller 2016).

No extensive research has been conducted using wood fibre as a majority soilless substrate, but Gruda & Schnitzler (2004a; 2004b) have shown that although the water holding capacity was reduced in wood fibre substrates, the volume weight and pore space was similar to peat, and this translated to comparable shoot and even enhanced root growth of tomatoes as long as the wood fibre was compressed into pots to help retain more water through the summer months. This is in contrast to Alexander et al. (2013; 2014) who compared retail compost containing wood fibre to peat and found that plants in wood fibre were consistently poorer in growth, flower number and quality compared to peat, and this was significantly exacerbated when they received reduced watering, which did not have the same effect on peat. Wood Fibre plants required more water to achieve the same quality as peat and this appeared to be due to poor water distribution through the media in pots.

There are disadvantages of using large amounts of wood fibre as a growing media substrate. Although it can prevent slumping in peat (Schmilewski 2008) it can compress and lose volume when watered during plant growth (Gruda & Schnitzler 2004b). The process of machine extrusion requires a lot of energy and water, and is a costly process that, combined with the increased cost of wood chip due to competition for biofuels, makes this material expensive.

1.3.5 Implications for this Research

It will not be enough to be able to show that these alternative media can produce plants that are of comparable size to those grown in peat. To find an alternative that commercial growers are happy to adopt, it must be able to produce plants that live up to the quality expectations set by the growers, distributors, and consumers. This will include consistency in the plant performance, and this will require the supply and quality of the media to also be consistent. If possible, a growing media which requires fewer changes to watering regimes already in place would lend itself to current industrial set ups, and would be more favourable to home gardeners.

1.4 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are soil living organisms in the phylum *Glomeromycota*. They are obligate symbionts that associate with the roots of plants, around 80% of *all* vascular plants will have been colonised by at least one species of AMF (Smith & Read 2002b). They create an extensive network of hyphae which forage for nutrients that are limiting to plants, through their symbiotic relationship with plant hosts they exchange these nutrients (particularly soluble phosphorus as it is often only found in very low concentrations in soil) for photosynthetic carbon from the plant (Smith & Read 2002a). They achieve this through special structures formed from hyphal branches within individual root cells called arbuscules. These are highly branched tree-like structures which are the sites of nutrient exchange and vesicles which could act as storage structures or have a propagule function supporting hyphal regrowth (their specific function is still debated today) (Biermann & Linderman 1983; Jin et al. 2017). This fundamental relationship greatly affects both plant growth and fitness (van der Heijden et al. 2015) and the effect of AMF colonisation on different plant species in both field and controlled environments has been widely studied. The following sections highlight the main effects of AMF symbiosis that make them of specific interest to the horticultural industry.

1.4.1 Increasing Biomass

Due to their ability to provide plants with more nutrients than they could obtain alone, mycorrhizae can increase the size plants can grow to. Studies have shown that the addition of mycorrhizal fungi can be used to improve both the yield and nutrient quality of important commercially produced plants such as basil (Rasouli-sadaghiani et al. 2010), chive (Ustuner et al. 2009), and oranges (Ortas & Ustuner 2014b; Ortas & Ustuner 2014a). Even field crops such as

wheat (Pan et al. 2003), cassava (Ceballos et al. 2013), tomato (Poulton et al. 2002; Ziane et al. 2017) and pea (Jin et al. 2013) have shown increases in biomass when AMF are present, as have ornamentals (Mcgraw & Schenck 1980; Linderman & Davis 2003; Meir et al. 2010; Puschel et al. 2014). However, their ability to do this can often be limited by the growing conditions. There are many studies that attempt to study the relationship between mycorrhizas and the concentration of soil nutrients, especially phosphorous and nitrogen. In high nutrient environments, the mycorrhiza can become unnecessary, or even a carbon drain on the plant, and thus the symbiosis may become parasitic. In such circumstances, it is likely that the symbiosis will end. However, it has been shown that only if both N *and* P are readily available to the plant will the carbon exchange with the fungus be reduced (Baath & Spokes 1989; Blake et al. 2011). Growth enhancement has been seen as a result of colonisation at low, intermediate (Baath and Spokes, 1989), and high fertiliser concentrations (Peng et al. 1993). The ability of AMF to boost plant growth in low nutrient environments makes them ideal for use as biofertilisers in horticultural and agricultural systems, and especially in organic or sustainable systems as they would allow the reduction in the use of chemical fertilisers.

1.4.2 Other Benefits of AMF

As plants grow larger and more nutrients are available flower number and size has been shown to increase in plants colonised with AMF (Gange & Smith 2005), along with nutritional weight and oil content of seeds such as sunflowers (Gholamhoseini et al. 2013). This is obviously a very important feature to growers of ornamental plants.

The ability of AMF to act as biocontrol agents has been widely demonstrated with their ability to protect plants against root diseases (Nemec et al. 1996) and plant pathogens such as *Pythium* (Calvet et al. 1993), *Cryptocline* (Dubsky et al. 2002) and *Fusarium* (McAllister et al. 1994; Datnoff et al. 1995; St-Arnaud et al. 1997; Martínez-Medina et al. 2009; Hage-Ahmed et al. 2013). Colonisation by certain AMF species such as *Glomus intraradices* have also been shown to cause physiological and biochemical changes within the plant, which can reduce the performance (damage and survival rates) of general chewing insects (Koricheva et al. 2009). This has formed part of the interest of using AMF in horticultural and agricultural systems as an organic method of biocontrol to reduce the use of chemical pesticides and fungicides.

The presence of AMF has also been shown to confer tolerance to various abiotic stress conditions, including improving growth and nutrient uptake in drought stressed plants (Nelsen & Safir 1982; Allen & Boosalis 1983; Ruiz Lozano et al. 1995; Bryla & Duniway 1997; Asrar & Elhindi 2011;

Gromberg et al. 2015; Ruiz-Lozano et al. 2016) and increased growth, water content and antioxidant production to reduce effects of salt stress (Giri et al. 2003; Porras-Soriano et al. 2009; Evelin & Kapoor 2014). This is especially important to field grown crops, but also to outdoor nursery grown plants which can also suffer abiotic stress (Davies et al. 2000). The mechanisms which allow AMF to induce drought tolerance could result in the reduction of water usage to grow plants in professional and home gardens.

In medicinal plants, AMF have been shown to improve the amount (Pedone-Bonfim et al. 2015), consistency and quality of important secondary metabolites such as terpenes, phenols, and alkaloids (Zeng et al. 2013). In potted herbs sold commercially to supermarkets such as basil (Copetta et al. 2006; Toussaint et al. 2007; Rasouli-sadaghiani et al. 2010; Taie et al. 2010; Srivastava et al. 2016) and chives (Mnayer et al. 2014), plants that were colonised with AMF species have been shown to have increased antioxidants and essential oil content. For growers of medicinal and food crops such as herbs, where levels of these substances can improve the smell, taste and nutritional or therapeutic benefit of plants, this effect has great importance.

Colonisation and presence of AMF hyphae has also been shown to alter the structure, and therefore properties, of soil by causing an increase in soil aggregates (Rillig & Mummey 2006). This ability is most likely due to the production of the glycoprotein glomalin found to be produced by the extraradical mycelium (Wright & Upadhyaya 1996). Using immunofluorescence this insoluble glycoprotein coating has been found on the outside of hyphae (Driver et al. 2005) and spores, as well as in soil aggregates found around colonised plants, and its concentration was positively correlated with the number and hydrophobic nature of those aggregates (Rillig et al. 1998; Purin & Rillig 2008). This has led to the observation that the production of these insoluble proteins helps to form water stable aggregates that aid water infiltration of soil, as well as oxygenation of root tissue. The, as yet undescribed, gene products of AMF and fractions of them in soil are collectively grouped and known as glomalin related soil proteins (GRSPs) as extraction methods will always include a mixture, but the relationship between them and glomalin is still not fully clear. This phenomenon has been shown to occur in container experiments that used soil where aggregate size and stability were directly related to presence of AMF hyphae (Wu et al. 2008; Samaei et al. 2015). This has implications for improving dry, contaminated, or waterlogged soils for growing field crops (Medina et al. 2004), and although the effect has not been demonstrated in soilless media, it shows the potential AMF could have to improve the physical structure of growing media.

1.4.3 Commercial AMF Inocula

Commercial mycorrhizal inocula for home garden use have been produced as a natural alternative to fertilisers, and are currently marketed to amateur gardeners to use as natural growth stimulants. They all contain a mixture of arbuscular, and sometimes other mycorrhizal species in order to increase the chances of their compatibility with as many plant species as possible. They can also vary in their production methods (*in vitro* or *in vivo*), propagule type (spores, hyphae, or colonised root material) and carrier material. In some cases, other microorganisms, such as plant growth promoting rhizobacteria (PGPR) species and other soil dwelling fungal species that may be beneficial to plants such as *Trichoderma,* may also be added to the mix. For mycorrhizas to work as a commercial product they must also be 'compatible' with the different composts gardeners like to use. It is unlikely that this 'one size fits all' approach will have consistent results across all plant species and substrates likely to be used in home gardens. Indeed, research using commercial AMF products has seen great variability in their colonisation power and effect on plant growth. Colonisation with commercial AMF has been shown to be successful and produce plant growth enhancement, but the range in colonisation was often variable: 5-20% (Carpio et al. 2003), 17-39% (Matysiak & Falkowski 2010), 20-30% (Perner et al. 2006), and 17-68% (Puschel et al. 2014). This variation was often attributed to the use of different plant species and substrates. There have been negative reports of commercial inocula resulting in plant growth depression (Cerruti et al. 2013), and when multiple products have been used the success rate of colonisation was not consistent; Gaur et al. (1998) found that only one out of three inocula resulted in colonisation, Tarbell & Koske (2007) found that five out of eight products failed to colonise the roots of plants, Faye et al. (2013) also reported very low colonisation in three out of 12 commercial inoculants, and in another trial only six out of 10 products resulted in colonisation (Corkidi et al. 2004). This inconsistency could explain why commercial inocula have only been used in 15% of all studies using AMF from 2001 to 2015 (Berruti et al. 2016).

1.4.4 Use in the Horticultural Industry

Arbuscular mycorrhizal fungi are currently used in the UK in some commercial sectors, namely in forestry and orchid cultivation. but successful growth of many tropical plant species often requires specialist AMF as well. The addition of mycorrhizal inoculum is not standard practice in the UK for large scale crop or commercial plant production systems (Smith & Read 2002c). However, a recent review highlighted how the use of AMF in horticulture has significantly increased in the past two decades, and it has been suggested that they will play an important role

in the future move towards sustainable and organic horticulture (Rouphael et al. 2015) but there are still many challenges to overcome.

It has been suggested that, much like alternative growing media, farmers and growers have been resistant to invest in microbial biofertiliser products due to their poor quality and inconsistency (Herrmann & Lesueur 2013). Poor inoculum production techniques, including quality control and ability to mass produce, are one of the main barriers (Azcón-Aguilar & Barea 1997). *In vitro* production is possible, and could result in successful mass production, but these inocula are normally spore based (Mohammad & Khan 2002), and it has been shown that they are not the most effective propagule for colonisation for some AMF species (Biermann & Linderman 1983; Gaur et al. 1998; Klironomos & Hart 2002).

Most studies still use AMF in controlled environments, but significant benefits of AMF have been achieved in field crop species (Ceballos et al. 2013). Outdoor effects on AMF in container grown plants are less frequent. Issues raised with global transport and inoculation with AMF in the field on indigenous species suggests that containers and indoor planting should be the focus in future (Schwartz et al. 2006).

One of the main barriers to directly introducing AMF and other microbes into managed horticultural systems is the need to carefully match up host plant, AMF species and substrate with adjusted nutrient and watering levels to obtain positive results. Without prior knowledge or examples, it cannot be known how a specific symbiotic relationship will perform in certain substrates and environments (Azcón-Aguilar & Barea 1997). Thus, studies comparing the use of AMF in constant conditions are not representative; other factors must also be varied if the maximum benefits of AMF as commercial biofertilisers are to be observed. Indeed, it has been shown that plant benefit from AMF can differ between nutrient levels (Nouri et al. 2014), substrate (Linderman & Davis 2003), host plant species (Linderman & Davis 2003), and presence of other microbes (Linderman 2008; Nadeem et al. 2014). Recent work has highlighted functional diversity amongst AMF species which explains how the symbiosis is not always reciprocal and is highly context dependent, with nutrient availability, plant/fungal species combination and competition amongst AMF species being the main causes (Smith et al. 2009; Hoeksema et al. 2010; Mensah et al. 2015; Gosling et al. 2016). This has gone so far as to suggest that both plant and AMF species undergo partner selection in order to allocate resources to higher quality partners, which helps to stabilise relationships (Werner & Kiers 2015). As many previous studies have focused on trials with single species it may be that the use of commercial inocula containing mix of AMF species may be the best approach when working with a range of growing media to allow plants the best chance of forming a beneficial symbiotic relationship.

1.5 Previous Research

The role AMF could play in improving fertilisation in organic production, as well as reducing peat usage in horticulture, has been investigated, but not widely. The majority of research looking at the effects of AMF colonisation in different peat reduced substrates focuses on the addition of various plant-based, or green waste, composts in increasing concentrations. Across these studies, it was shown that AMF colonisation increased with increasing addition of composted plant material, but it was negatively affected by high fertilisation levels (Matysiak & Falkowski 2010). Positive effects on plant performance, including biomass, flower number and nutrient content, were seen but these were not consistent and did not always correlate with increased AMF colonisation (Perner et al. 2006; Matysiak & Falkowski 2010). These studies often showed that plant response to different AMF species varied with compost amendment (Ustuner et al. 2009; Ortas & Ustuner 2014a). In one study, two different combinations of AMF species and compost addition were shown to produce plants comparable to those grown with full fertiliser (Ustuner et al. 2009).

There are also a few examples of studies that have used AMF in combination with the other alternatives outlined in section 1.3. Linderman & Davis (2003) used different amounts of coir amended peat to grow different plant species inoculated with one species of AMF. Although colonisation was increased in the peat amended growing media in all plants, this did not translate into positive growth responses. Where pine bark-based media have been used, it has been mixed with other materials such as sand (Corkidi et al. 2004) and peat (Carpio et al. 2003). Again, different substrates and plant species resulted in different responses from the AMF inocula, but overall colonisation was increased in the pine bark-based media. In nursery conditions, AMF colonisation was shown to increase the growth of plants in pine-bark amended peat when half the recommended fertiliser rate was used (Carpio et al. 2003).

DNA extraction methods have been widely used for AMF community analysis in field soils (Osborn et al. 2000; Appoloni et al. 2008; Jin et al. 2013; Kohout et al. 2014) and more sparingly to extract DNA from known AMF species in controlled environment experiments (Alkan et al. 2006; Kiers et al. 2011). The method of identifying AMF species to confirm their presence in roots of plant species is as a result of their presence in the inoculum mix is not widely used. Two such studies using commercial inocula have been found where AMF species were identified from roots using spore morphology (Faye et al. 2013) and phylogenetic analysis using extracted DNA (Berruti et al. 2013). Despite the fact that only morphological techniques are currently used to characterise and check the presence of species which make up commercial inoculum mixes, it has now been recognised that molecular tracing of AMF species in both the inoculum and colonised root

material post-inoculation is vital in order to assess the success of the inoculum and identify which species in the mix are responsible for any effects (Vosátka et al. 2012; Berruti et al. 2013).

1.6 Focus, Aims and Objectives

One of the working groups of the SGMT which tracked peat usage in growing media from 2011 to 2015 found that the bedding, pot plant and nursery stock sector consistently accounted for the majority of peat based professional growing media use in the UK (Denny & Waller 2016). It has also been identified that AMF biotechnology would best suit the parts of the industry where plants have a transplant stage, including those produced in containers (Azcón-Aguilar & Barea 1997), as the benefits of AMF provide more robust plants in containers and once planted out (Davies et al. 2000). However, AMF research in outdoor (nursery style) experiments using containers is rare; only 7% of studies involving AMF were conducted outside in pots since 2001 (Berruti *et al.*, 2016). This is possibly due to the increase in variables and decreased ability to keep control plants free from AMF colonisation (Davies et al. 2000; Carpio et al. 2003; Corkidi et al. 2004).

The aim of this project was therefore to test combinations of peat reduced growing media with AMF in commercial style environments in order to provide growers with an increased understanding of how these media will perform in these settings, and how AMF could improve the performance of plants in these media and conditions. It is also hoped that by reducing the effects of abiotic stresses AMF treatment will produce repeatable effects in outdoor experiments over multiple seasons, something that has rarely been demonstrated. The combining of physiological data with molecular data will also allow the effect of different commercial AMF inocula on plant performance, and more specifically their interactions in different substrates and on different plant species to be investigated for the first time.

With this data, it is hoped that more informed recommendations could be made to growers on how to get the best plant performance in different commercial environments with peat reduced media, and how using AMF can help them achieve this.

The overall objectives of this study were:

- To test the effect on plant performance (biomass, plant height, flower number etc.) of combinations of peat reduced mixes made with different raw materials with different commercial AMF inoculum products.

- To conduct trials in pots with hardy annual bedding plants in outdoor experiments in order to replicate the use of peat alternative media and commercial AMF products in the largest peat-using sectors of the horticultural industry (bedding, nursery stock and amateur gardener).
- To include controlled environment experiments using the same peat reduced media and AMF combinations to observe the effect of a controlled environment on these treatments, and to replicate the production systems of the potted herbs industry, which significantly contributes to peat usage in potted plants in the UK.
- To use materials that are currently mass produced, which would be available to both professional and amateur growers, so that any results should be applicable and transferable to both areas of the horticultural market where reliance on peat needs to be reduced.
- To use DNA extraction to identify the number and species of AMF from each commercial inoculum product which has colonised the roots of different species of plants. This would allow physiological effects to be related to a particular AMF species or groups of species in each treatment, which would ultimately help improve the understanding of the interactions of commercial inocula with different plant species and substrates.

1.6.1 Introduction to Chapters

The majority of the experimental work for this thesis is described in Chapters 2, 3 and 4. Chapter 2 details the initial experiment using retail composts each containing a main peat alternative material, and describes how the experimental method was adapted to reduce the number of media and improve their reliability and consistency. Chapters 3 and 4 describe similar experiments designed to show how consistent the combinations of two wood-based reduced peat growing media and different commercial inoculum treatments would be across consecutive seasons.

A series of trials which investigated colonisation of potted basil and chives in a controlled glasshouse environment can be found in Chapter 5.

Different DNA extraction methods were trialled and eventually modified for successful extraction of DNA from both chive (*Allium schoenoprasum L.*) and marigold (*Tagetes erecta L.*) roots. The results of those extractions as well as data on the AMF sequences identified from roots in different treatments is described in Chapter 6.

Chapter 2 - Effect of commercial mycorrhizal inoculum on growth of bedding plants in reduced peat commercial composts.

2.1 Introduction

This chapter outlines the preliminary experiment which aimed to expand on a 2012 undergraduate project (Edwards, 2012), the outcomes of which underpin this research into the interactions between commercial mycorrhizal inocula and peat free growing media and their effect on plant performance. The aim of this experiment was to test whether biomass (and other measures of plant performance) could be increased in common commercial bedding plants with the addition of commercial AM inoculum and to investigate the interaction effect of different peat free composts.

A main objective was to test and identify peat free substrates which would be suitable to recommend to both commercial and amateur growers to combine with these commercial inocula.

2.2 Materials and Methods

2.2.1 Nutrient Analysis of Growing Media

Two different soil analysis techniques were used to identify the quantity of available levels of total nitrogen, phosphate, and potassium as well as chloride, and sodium in each multipurpose compost. The following methods used should not be considered definitive but more an estimation of the nutrients present as they were developed to be used to extract from nutrient rich soils or organic materials such as raw peat and not on multipurpose composts which contain mixtures of highly organic material as well as inorganic fertilisers.

Six replicate samples of each compost, composed of 400g homogenised material taken from different areas of each bag were stored in the fridge at 4˚C in sealed plastic bags before being used for analysis. In total, three replicates for each compost were subject to water and digestion extract methods, to obtain soluble and available nutrients respectively. By measuring the weight of the fresh compost before and after it had been dried, average water content of each compost was calculated.

2.2.1.1 Soluble nutrients

This method is designed to extract nutrients that are soluble in water, the most available to the plant as they are not trapped in the media itself. A sample of each compost was dried in an oven overnight at 40^{o}C. Exactly 10g of dry compost was placed in a conical flask and saturated with 100ml of deionized water before being placed on a shaker rack for 1 hour. The mixture was then filtered through 1mm Whatman paper using a Büchner funnel. The extracts were placed in a 50ml

falcon tube and centrifuged to remove large soil particles. The supernatant was decanted and micro filtered using 0.25mm Whatman paper into individual acid washed vials. These were then capped and stored at 4˚C in a fridge until needed.

Ion chromatography was used to analyse samples for soluble ions including nitrate, nitrite, and phosphate. A Dionex DX 500 chromatography system equipped with a GP40 gradient pump, CD20 conductivity detector, EG50 eluent generators and an Ion Pack AS19 4x250mm chromatography column was used attached to an AS autosampler which took 25 microlitres of each extract. Charged ions bonded to resin (stationary phase) in the column which measured their conductivity and displayed them in the form of peaks. Ions were then identified by their retention time using Chromeleon® Chromatography Management System software.

A standard sample which contained a mix of 6 anions (fluoride, chloride, bromide, nitrate, sulphate, and phosphate) was used for water extract samples and before they were run three blank (dH_2O) samples were put through, the results of the blank analyses were averaged for each ion and taken away from the average of the samples. Ammonium was measured in each sample separately using a set of ammonium chloride standards (0, 5, 10, 12, and 15). All results presented as mg per litre or kilo of dry compost.

2.2.1.2 Insoluble Nutrients

These methods involve special reagents that are designed to extract insoluble ions of nutrients such as potassium and sodium which cannot be measured with water extraction methods. This, combined with water extracts can give values of total available nitrogen and phosphorus. Unless otherwise stated, methods for digest extracts were as outlined in Okalebo et al. (1993).

2.2.1.2.1 NH_4^- and NO_3^- Extraction

A sample of each compost was dried in an oven overnight at 40^{o}C. Exactly 10g of dry compost was placed in a conical flask and saturated with 100ml of 1M KCl before being placed on a shaker rack for 1 hour. The addition of KCl causes the displacement of exchangeable NH_4 and soluble NO_3 is also extracted. Method was then continued as for soluble nutrients (2.2.9.1).

2.2.1.2.2 PO_4^{3-} Extraction

Olsen's reagent (sodium hydrogen carbonate adjusted to pH 8.5 with 50% NaOH) was prepared and left overnight to acidify. A sample of each compost was dried in an oven overnight at 40^{o}C. Exactly 2.5g of dry compost was placed in a conical flask and saturated with 50ml of Olsen's reagent and added to the sample in a 1:5 ratio (Olsens:sample). Method modified from Olsen *et al.*, (1954). Method was then continued as for soluble nutrients (2.2.9.1).

2.2.1.2.3 Cation Exchange (K^+ and Na^+) Extraction

A sample of each compost was dried in an oven overnight at 40°C. Exactly 2.5g of dry compost was placed in a conical flask and saturated with 63ml of 1M ammonium acetate (NH_4OAc, buffered to pH7) before being placed on a shaker rack for 1 hour. The addition of NH_4^+ causes rapid displacement of exchangeable alkaline cations from soil particles, these will be present in extracts which can then be quantified. Method was then continued as for soluble nutrients (2.2.9.1).

Flame and Graphite Furnace Atomic Absorption Spectrometry, (FAAS)/(GFAAS) was used to determine levels of potassium from digestion extracts as well as levels of sodium in water extracts. By vaporising samples, the specific absorption frequency of each element was measured relative to a known standard. Standards were analysed after three blank (deionized water) samples at the beginning of the run, standard concentrations used were as follows: potassium 0, 100, 200 and 400ppm and sodium 0, 25 and 50ppm. A Perkin Elmer AAnalyst 800 was used along with Winlab 32 – AA software to analyse samples, no auto sampler was used, capillary tubing was placed into each sample in turn by hand.

2.2.2 Growing Media

These composts all contain a mixture of materials to improve structure and nutrients and were all available at local garden centres, they will be referred to as the following:

- Peat (industry standard)

For this experiment peat was sourced from a commercial grower (Vitacress) who use it in all their potted herb production. It is imported and shipped in bales from Europe at low cost and consists of 100% peat.

- Low Peat

'Levington® Multipurpose Compost' produced in Scotland by Everris Limited (Figure 2.1a). It is not peat free but has a very low peat component (between 1-5%), with some added potting bark. It also contains a 'waterlock system' which claims to allow it to absorb 25% more water than ordinary multipurpose composts. This brand dates back to the 1950s and is trusted by amateur gardeners promising to provide 'professional quality'. The company have a growing media policy and say that they source peat only from responsibly managed sites.

- Coir

'Fertile Fibre multipurpose compost' produced by Fertile Fibre Ltd is a popular, organic, peat-free, multipurpose compost made from ground coir pith. This brand is a leader in the coir industry, its multipurpose compost contains raw coir shipped in bales from Asia, predominantly India and Sri Lanka. This is soaked and organic nutrients are added along with; Melcourt Growbark® to improve bulk density; Melcourt Potting Bark™ which increases air filled porosity to reduce waterlogging and encourage root growth; vermiculate for aeration; and, perlite for improving permeability and preventing compaction.

- Wood Fibre

'Professional Peat-Free Multipurpose Compost' is manufactured by Bulrush Ltd and has a base made of machine extruded wood fibre. The woodchips are from Forest Stewardship Council (FSC®) certified sources and are imbibed with nitrogen. The wood fibre increases the water holding capacity of the substrate; composted bark (Melcourt Growbark®) is added to improve the bulk density; and, clay is added to improve nutrient holding capabilities.

- Green Waste

'Verve' peat free multi-purpose compost was sourced from a local branch of a leading garden retailer (B&Q). It contains composted green waste (CGW) or recycled materials. As this CGW will most likely have been sourced from a local municipal waste facility it will vary greatly between batches but all bags were purchased on the same date from the same branch to try and minimise this variability. It is likely that other materials, potentially bark, will have been added to this mix as CGW is not normally used to make up any more than 50% of growing media. This is due to its high bulk density, salinity, and high pH; however, as this compost is not produced by an independent company few details about the mix were available. It should be noted that some sharps (glass shards) as well as stones and brick fragments were occasionally found in the substrate, a common problem with retail CGW composts.

Levington and Fertile Fibre composts were used in the 2012 but the other brands of compost were different due to lack of availability. Whilst the main ingredients remain significantly different there are some elements of the mixes that feature in more than one of the composts: potting or growing bark features in all four alternative composts and the low peat mix also contains "recycled nutrients" otherwise known as green waste compost.

Figure 2.1 Photograph of bags of the four peat free retail composts used in this experiment. a) Bulrush Professional peat free multi-purpose compost. b) B&Q Verve Peat free multi-purpose compost c) Levington multi-purpose compost. d) Fertile Fibre premium organic quality compost.

2.2.3 Commercial Arbuscular Mycorrhizal Inocula

Two brands of commercial endomycorrhizal inocula which are produced on a large scale were used for all experiments. Due to commercial sensitivity they will be referred to as arbuscular mycorrhiza one (AM1) and arbuscular mycorrhiza two (AM2). AM1 contains a mix of propagules (colonised root fragments and hyphae) and spores from five different species of arbuscular mycorrhizal fungi. These propagules are mixed with the inert granular clay carrier; the substrate they are cultured in (*in vivo*). The manufacturer recommended dose is 7mls per litre of pot size, this equates to approximately 7500 propagules. This inoculum was measured and added to pots using graduated 15ml falcon tubes. AM2 contains nine different species of arbuscular mycorrhizal fungi, two of which are also present in AM1. The *in vitro* cultured spores and hyphal fragments are dried and suspended in a fine pumice powder. The number of propagules per kilo/litre is two and a half times less than that for AM1 but the manufacturers recommended dose is also smaller. Only 0.2g of AM2 was added per L of pot size, this equates to approximately 100 spores. It was weighed and added using a 1.5ml Eppendorf tube. A well was made in each pot and each inoculum was added by sprinkling in and around the sides of the well to ensure as much contact with the plant roots as possible. Roots of plug plants were teased and exposed as much as possible to increase contact with the inoculum. Control plants had no inoculum added. No additional control treatments were used, such as microbial wash (Koide and Li, 1989) because the aim was to test the addition of the inoculum itself as a product; not the mycorrhizal species themselves.

2.2.3.1 Measuring AMF Root Colonisation

Root samples were taken from each pot and fixed in 70% ethanol prior to staining for mycorrhizal colonisation analysis (Figure 2.2a). The method of staining root material was modified from Vierheilig et al. (2005). Fixed roots were washed under running water in a 1mm sieve to remove ethanol and any remaining soil particles. Roots were placed in labelled tissue cassettes (Histosette® II, Simport) and cleared in 10% potassium hydroxide (10% w/v: 10g KOH in 100ml aqueous solution) that had been preheated to 75 ^{o}C in a water bath. Marigold roots were cleared in 15 minutes. When the KOH became visibly discoloured (yellow) cassettes were removed and put in fresh KOH for the remainder of the time. Roots were considered cleared after becoming pale yellow and translucent with a slight gelatinous consistency (Figure 2.2b). After clearing, roots were rinsed in their cassettes with running water to remove all KOH. Roots were stained in acidified ink (blue ink, Quink) solution (84.4:15:0.6, dH2O:1%HCL:Ink) also heated to 75 ^{o}C. Cassettes were placed in the beaker of stain in the water bath and left for 20 minutes. Root length colonised by AMF (%RLC) was calculated using five to ten ~3cm pieces of stained root from each pot. These were placed on a microscope slide (Figure 2.2c) and using the crosshair eyepiece method (McGonigle, et al., 1990) presence of hyphae was recorded for 100 views at 400x magnification. Arbuscules and vesicles were also noted and where possible, quantified for each view. The number of recorded hyphal presences out of the total was used to calculate percentage colonisation, and the mean percentage of ten replicates was used to observe differences between the different growing media and AMF inoculum combinations.

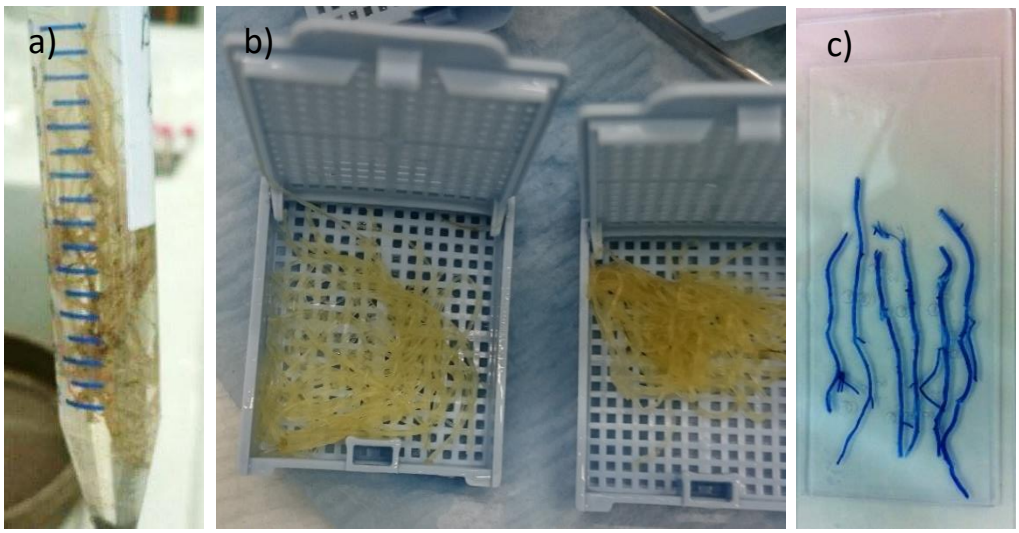


Figure 2.2 a) Marigold roots fixed in 70% ethanol in a 15ml falcon tube. b) Marigold roots in tissue cassettes which have been cleared with KOH. c) Stained marigold roots on a microscope slide.

2.2.4 Plant Species

French marigolds, (*Tagetes erecta L.)* were grown from seed under glass. *T. patula* 'Bonita mixed' seeds were sourced from Thompson & Morgan. They were grown under glass as small plugs for two weeks until ready to be transferred to their two litre pots at the start of the experiment.

2.2.5 Experimental Design

The five different growing media (Section 2.2.1) were each used to fill 30 two-litre (20cm diameter) pots. For each compost both AM1 and AM2 were added to 20 pots each and the remaining 10 were planted without inoculum. These 15 treatments were planted with ten replicate pots for each.

2.2.6 Site

Pots were randomly distributed in a semi-sheltered area between two glasshouses at RHUL on MyPex® (weed proof breathable matting). The pots were shuffled every two weeks as they were measured. Watering was conducted as and when plants were considered in need, for example they received more on hot dry days than on cool rainy days. This was in accordance to our effort to treat the plants in a similar way to an amateur or 'home' gardener who would be using the products being investigated.

2.2.7 Growth Recordings

Measurements were taken at regular two-week intervals throughout the growing season (June to September) to monitor growth of each plant: the number of leaves and flowers were counted and recorded every 3 weeks. At the end plant height was measured using a 30cm ruler from the base of the shoot to the tallest leaf.

2.2.8 Above Ground Biomass

Final harvest of above ground biomass was carried out after 12 weeks. Fresh weight was recorded. Plants were then placed in labelled envelopes and dried in an oven at approximately 40^{o}C until constant weight. Once dried, envelopes were weighed and the weight of the envelope removed to calculate the total shoot biomass for each pot. Mean dry biomass taken from ten replicates were used to observe differences between the different growing media and AMF inoculum combinations.

2.3 Statistical Analysis

For flower number, final leaf number, biomass, and root length colonisation a two-way ANOVA was used to analyses differences between and interactions within compost and inoculum type (live or control) for AM1 and AM2. For leaf number, a repeated measures ANOVA was used. For nutrient level and moisture means were separated with Tukey's HSD post hoc test. Where interactions between growing media and inoculum were found to be significant, independent sample t-tests were used to look at differences between the effect of live and sterile treatments on plants grown in each medium. Where data were not normally distributed values were transformed with squares or logarithms.

As root length colonisation was measured as a percentage for each root sample these data (hyphae, arbuscules and vesicles) were transformed using the arcsine transformation. It has been claimed that the arcsine transformation should not be used to transform proportional binomial data because it reduces power and does not take into account additional unexplained variation (Warton and Hui, 2011). Part of this claim is that only when the same samples size is used to estimate each proportion is the variance made equal by the transformation. In this case, as all microscope slides were measured with 100 fields of view each percentage had the same number of observations so this transformation was considered to be acceptable. To identify if plant biomass could be predicted by root length colonisation, these data for replicate plants were subjected to linear regression analysis. All analyses were conducted using IBM SPSS 21.

2.4 Results

2.4.1 Media Nutrients

The average amount of nitrogen, phosphorus and potassium in each compost is shown in Figure 2.3. The wood fibre compost contained significantly more nitrogen than the green waste compost (F_4=3.647). No significant differences were found between the amounts of total phosphorus and potassium between composts (Table 2.1). Total chloride levels were significantly higher in coir than in any other compost (F_4= 29.264, Table 2.1) and the levels of sodium were also the highest and were found to be significantly higher than those measured in wood fibre and peat (F_4=3.457, Table 2.1, Figure 2.4). This was even though the sodium solution extracts had to be diluted 1:10 to produce readings that fit within the range of the standards so it is likely the actual amount of sodium would be significantly higher than all nutrients.

Nutrient	d.f	F Value	Sig
Total N	4	3.647	**P<0.05**
Phosphate	4	1.647	p>0.05
Potassium	4	2.781	p>0.05
Sodium	4	3.457	**P<0.05**
Chloride	4	29.264	**P<0.001**
Sulphate	4	2.943	P>0.05

Table 2.1 Results of one-way ANOVAs on the nutrient content in each compost.

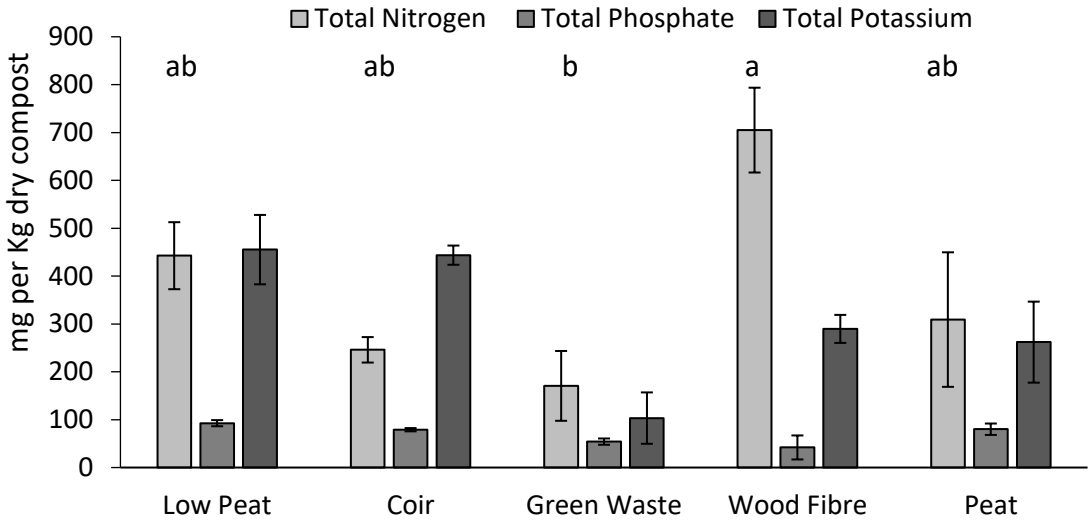


Figure 2.3 Average of total nutrients from combining soluble salts in water extracts and available nutrients from digestion extracts for nitrogen (ammonia + nitrate + nitrite), phosphate and potassium. Letters indicate significantly different means between composts (total nitrogen only). n=3, bars±S.E

Green waste had the highest water content and the water content of both the green waste and wood fibre compost was significantly higher than peat, with green waste also holding significantly more water than low peat and coir (Figure 2.5, F_4=24.739, p<0.001)

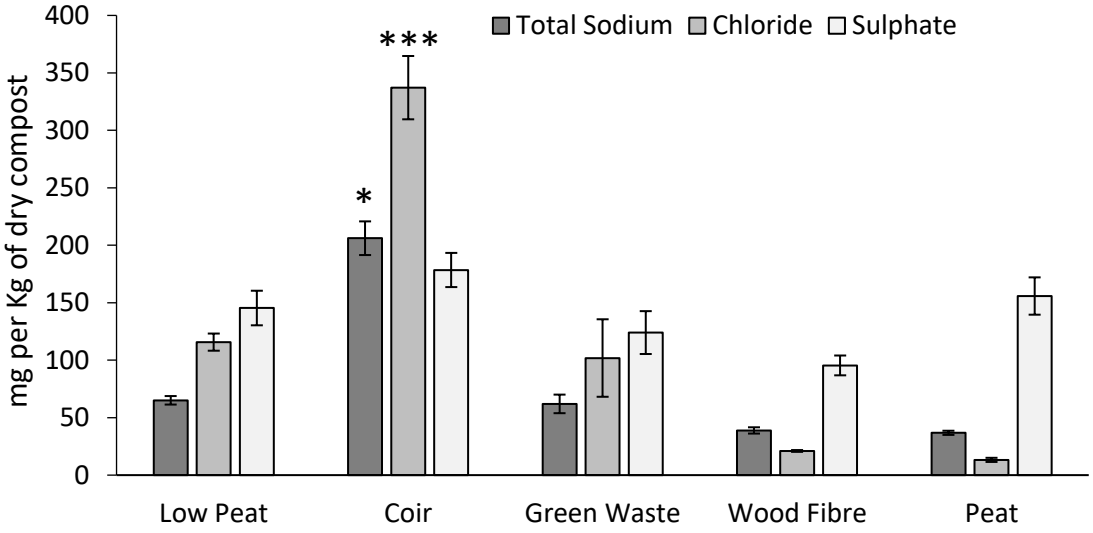


*Figure 2.4 Average amount of total sodium (water and digest extract combined) along with soluble chloride and sodium salts of each growth medium. n=3, bars = S.E. Asterisk indicate significantly different means between composts: *=p<0.05, ***=p<0.001.*

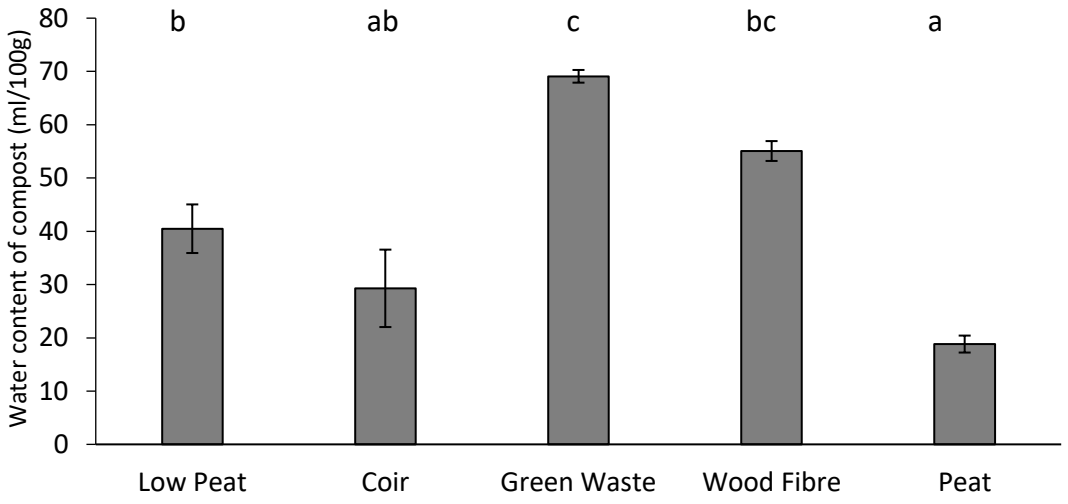


Figure 2.5 Average moisture content of compost samples used for nutrient analysis. Bars with different letters have significantly different means. n=3, bars±S.E.

2.4.1 AMF Root Colonisation

2.4.1.1 Identification of arbuscular mycorrhizal fungi

Root staining was successful and in many samples hyphae, vesicles and arbuscules were clearly distinguishable from root cells as they stained a darker blue (Figure 2.6). Different types of hyphae were sometimes seen, if they were particularly large and appeared outside or on top of the cells as in Figure 2.6g or very fine and net-like as in Figure 2.6h, they were not counted towards the root length colonisation.

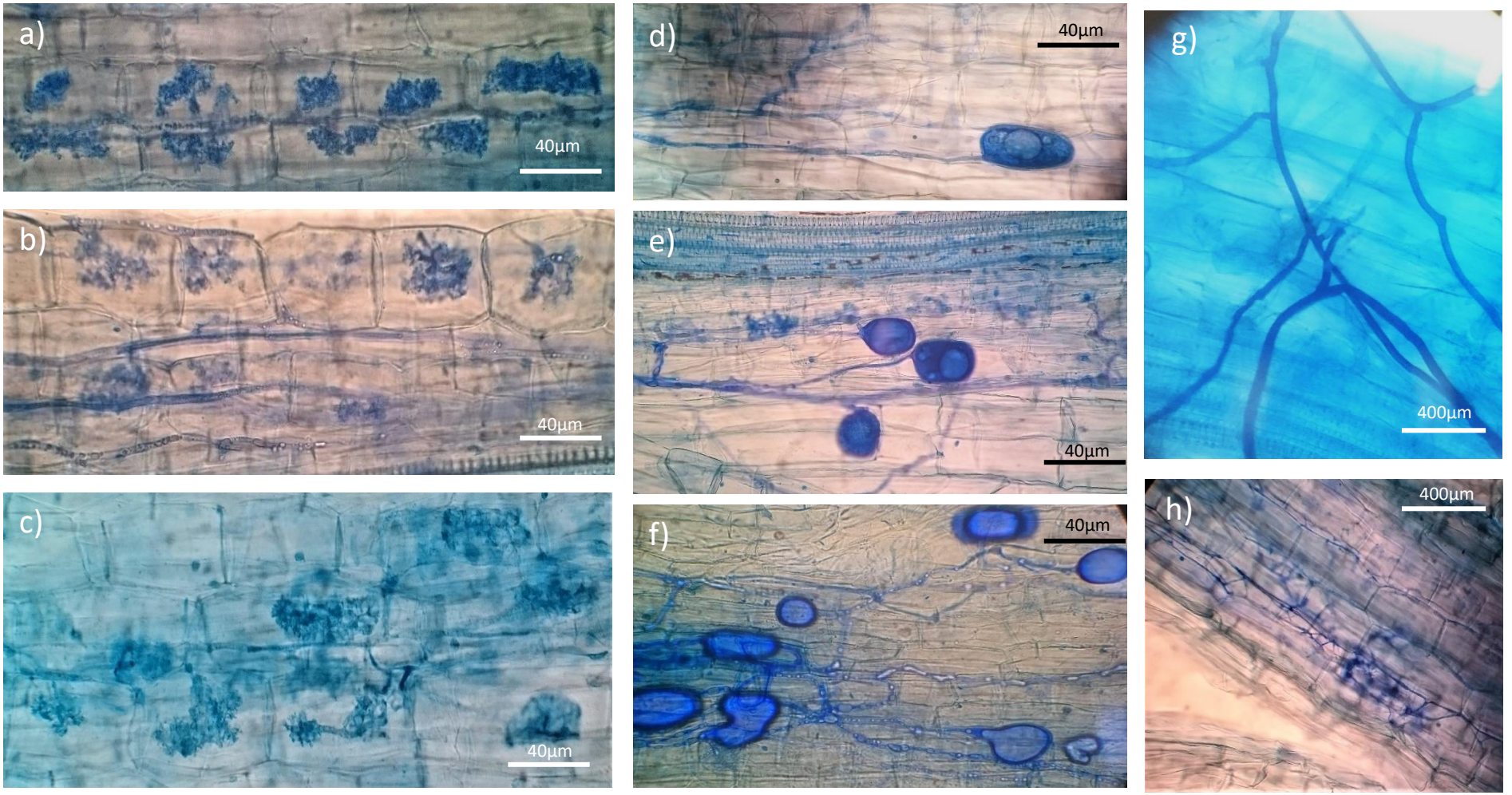


Figure 2.6 Microscope images of mycorrhizal structures identified in roots of Tagetes Erecta L. plants inoculated with AM1 or AM2. Arbuscules within root cells connected to hyphae (a-c), vesicles connected to hyphae (d-f) and two types of hyphae: large, thick hyphae seen outside of root cells (g) and very thin fine net-like hyphae (h). Magnification: a-f = 400x and g-h = 100x.

2.4.1.2 Effect of colonisation on biomass

The range of colonisation varied considerably between individual plants. Treatment with AM1 produced plants with root samples that were 100% colonised with hyphae, however both inocula produced a few plants that were found to contain no evidence of mycorrhizal hyphae (Figure 2.7). No significant relationship was found between percentage root length colonisation and biomass for either inoculum. AM1: R squared=0.041, F_1=2.997, p>0.05 (Figure 2.7a) and AM2: R squared=0.008, F_1=1.345, P>0.05 (Figure 2.7b). Control plants were found not to contain evidence of colonisation.

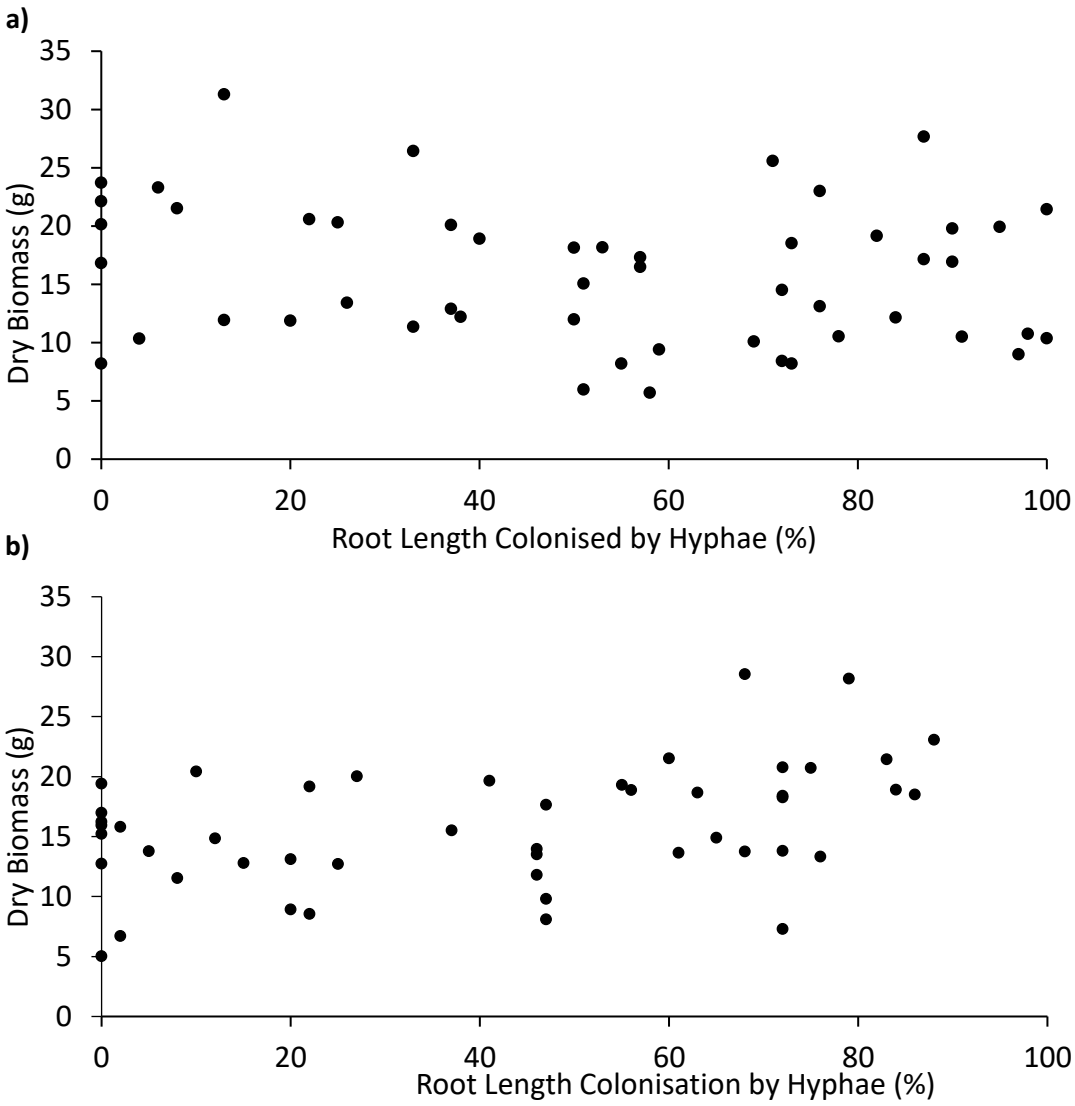


Figure 2.7 Relationship between biomass and colonisation of marigolds grown in peat free composts and peat with each commercial inoculum: a) AM1 and b) AM2.

2.4.1.3 Commercial AMF product performance

There was a highly significant difference between the colonisation ability of the two commercial inocula with roots from AM1 treated plants consistently containing a higher percentage of hyphae than AM2 roots (F_1=4.806, Figure 2.8).

	d.f	F Value	Sig
AM	1	4.806	**P<0.05**
Compost	4	3.291	**P<0.05**
*AM*Compost*	4	0.326	P>0.05

Table 2.2 Results of Two-way ANOVA on average percent root length colonisation. Error degrees of freedom = 90.

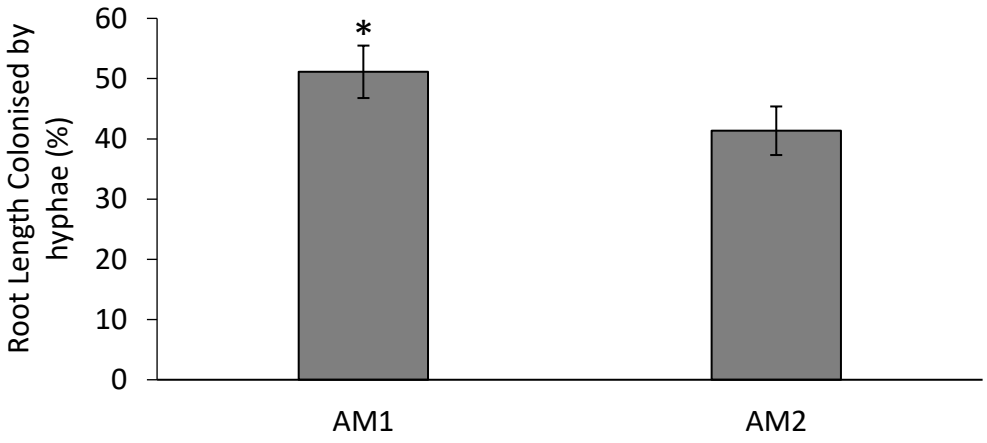


Figure 2.8 Difference between the average colonisation of marigold roots treated with AM1 and AM2 across composts. Asterisk denotes statistical difference between inoculum and control treatments. n=50, bars±S.E.

A significant effect of compost on AMF colonisation was seen (Table 2.2) but only in AM2 plants. Peat grown plants had the lowest level of colonisation, significantly lower than those grown in green compost which had the highest (Figure 2.9b). Similar levels of colonisation were seen in low peat, coir and wood fibre grown plants. Although colonisation seemed to vary a little between composts in AM1 treated plants, and peat also produced plants with roots that were colonised the least, there was no significant difference between the performance of each inoculum in each compost (Figure 2.9a).

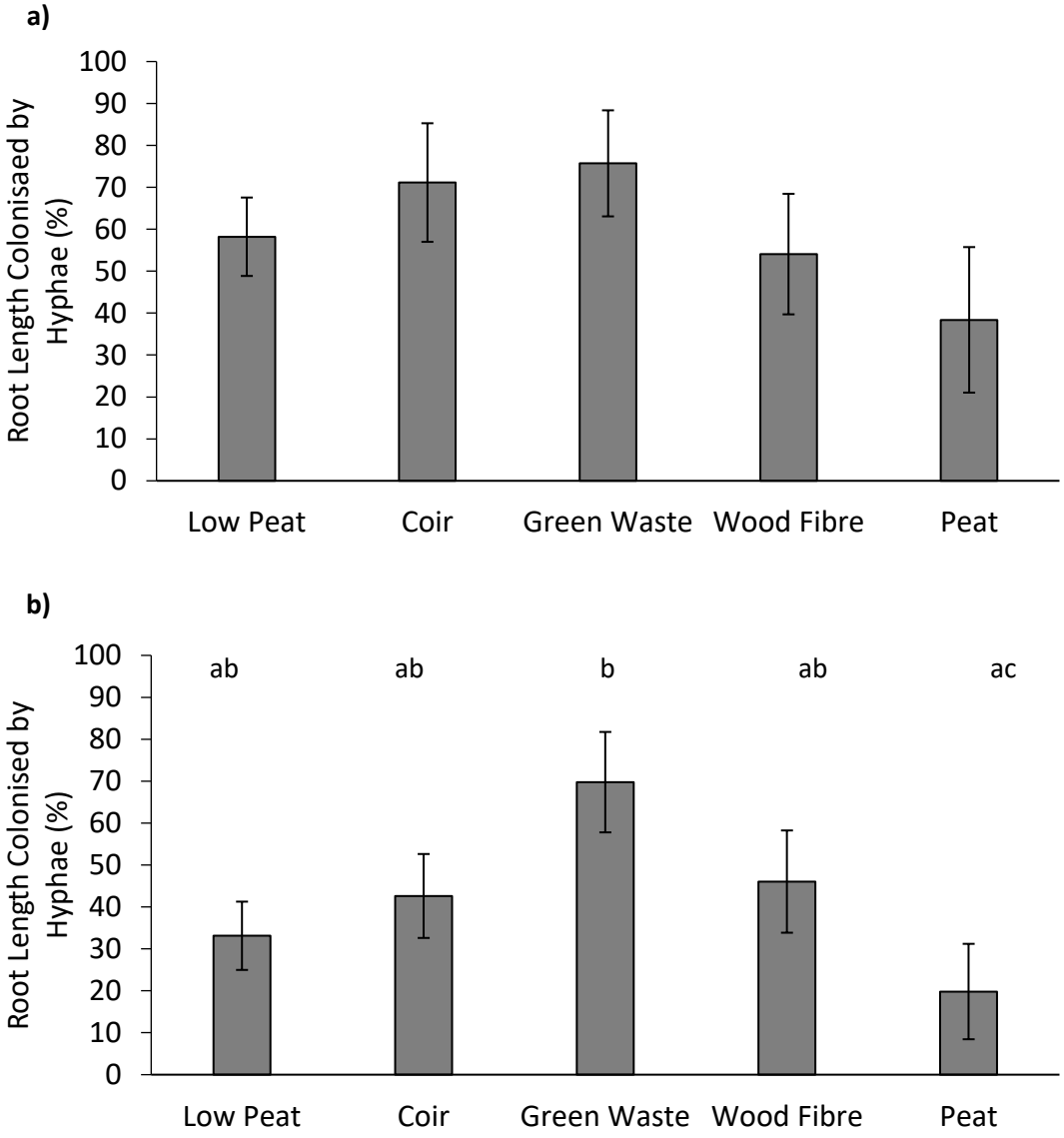


Figure 2.9 The average colonisation of marigold roots grown in each compost with each inoculum: a) AM1, b) AM2. Composts with different letters have statistically different means. n=10, bars± S.E

2.4.2 Plant Growth

2.4.2.1 Plant Height

The largest plants were produced by the wood fibre compost and these plants were found to be significantly taller than all but peat-grown plants when treated with AM1. (F_4=6.428, Figure 2.10a). No effect of compost was seen on the height of plants grown with AM2 (Table 2.3). Coir was the only compost treatment where the addition of both inocula appeared to have a negative effect on plant height, coir also produced the shortest plants overall. The addition of AM1 was only seen to have a positive effect on plant height in wood fibre and this plus a significant

negative effect of adding AM1 on the height of plants in green waste was responsible for a significant interaction effect of AM with compost (F_4=2.693, Figure 2.10a).

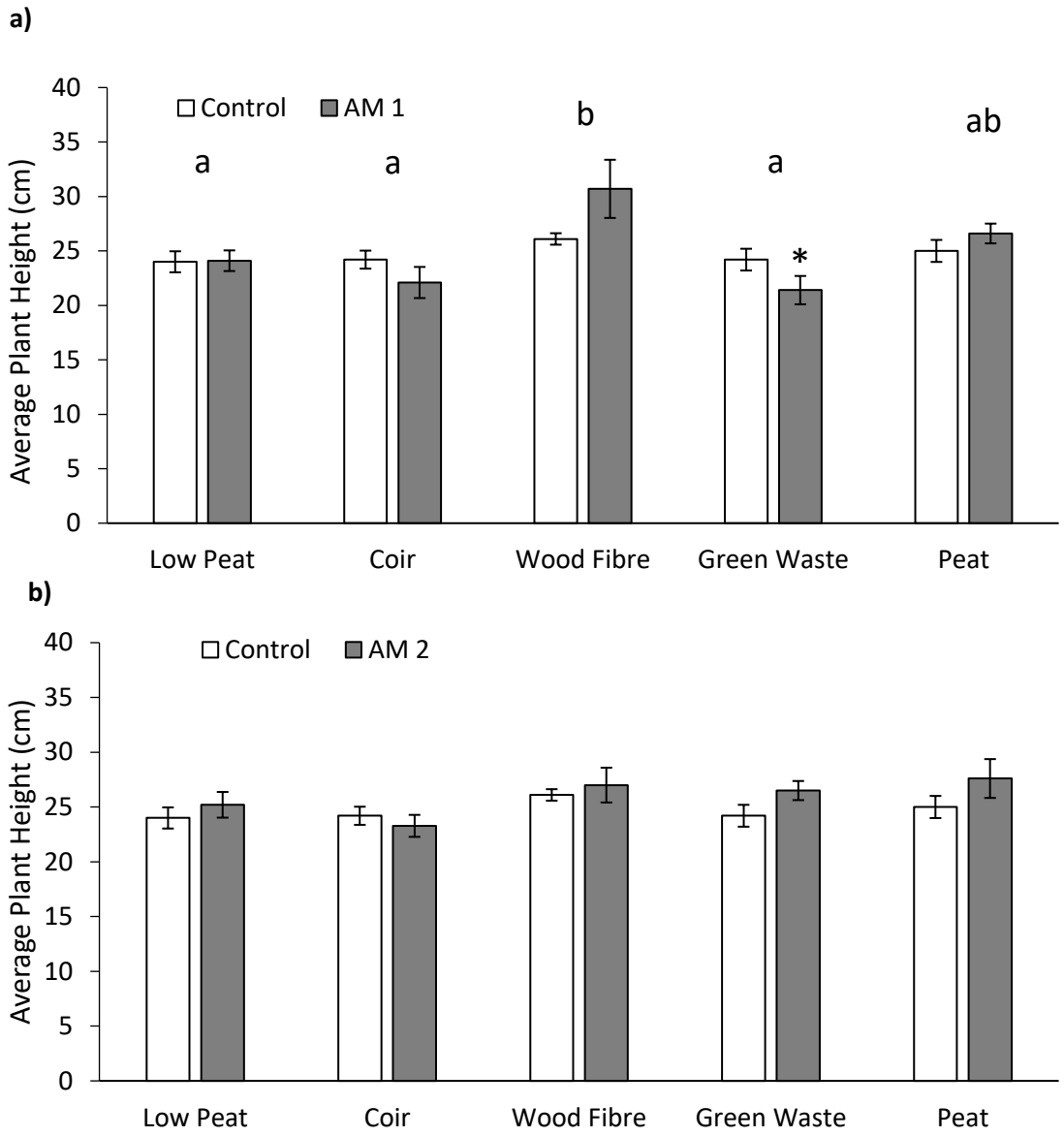


Figure 2.10 Difference in height (cm) between plants grown in each compost and inoculum treatment: a) AM1, b) AM2. Letters indicate statistically different means between composts. Asterisk denotes statistical difference between inoculum and control treatments within each compost. n=10 Bars±S.E

AM 1	d.f	F Value	Sig
AMF	1	0.119	P>0.05
Compost	4	6.428	**P<0.001**
AMF*Compost	4	2.693	**P<0.05**
AM 2			
AMF	1	2.941	P>0.05
Compost	4	2.149	P>0.05
AMF*Compost	4	0.764	P>0.05

Table 2.3 Results from two-way ANOVA on plant height. Error degrees of freedom: AM1=90, AM2=88.

2.4.2.2 Flower Number

Plants grown with both AM1 and 2 had fewer flowers than control plants but only plants treated with AM2 had significantly fewer flowers overall (F_1=5.303, Figure 2.11b). The addition of AM1 resulted in significantly reduced flower number compared to control plants only in wood fibre and green waste composts resulting in a significant interaction term (Table 2.4, Figure 2.11a). The number of flowers produced by plants treated with AM1 and grown in peat was found to be significantly higher than for plants grown in green waste and low peat composts (F_4=3.092) No significant difference was found between the number of flowers on plants grown in each compost with AM2 (Table 2.4).

AM 1	d.f	F Value	Sig
AMF	1	3.316	P<0.05
Compost	4	3.092	**P<0.01**
AMF*Compost	4	3.636	**P<0.01**
AM 2			
AMF	1	5.303	**P<0.05**
Compost	4	2.062	P>0.05
AMF*Compost	4	1.694	P>0.05

Table 2.4 Results from two-way ANOVA on flower number. Error degrees of freedom: AM1=90, AM2=90.

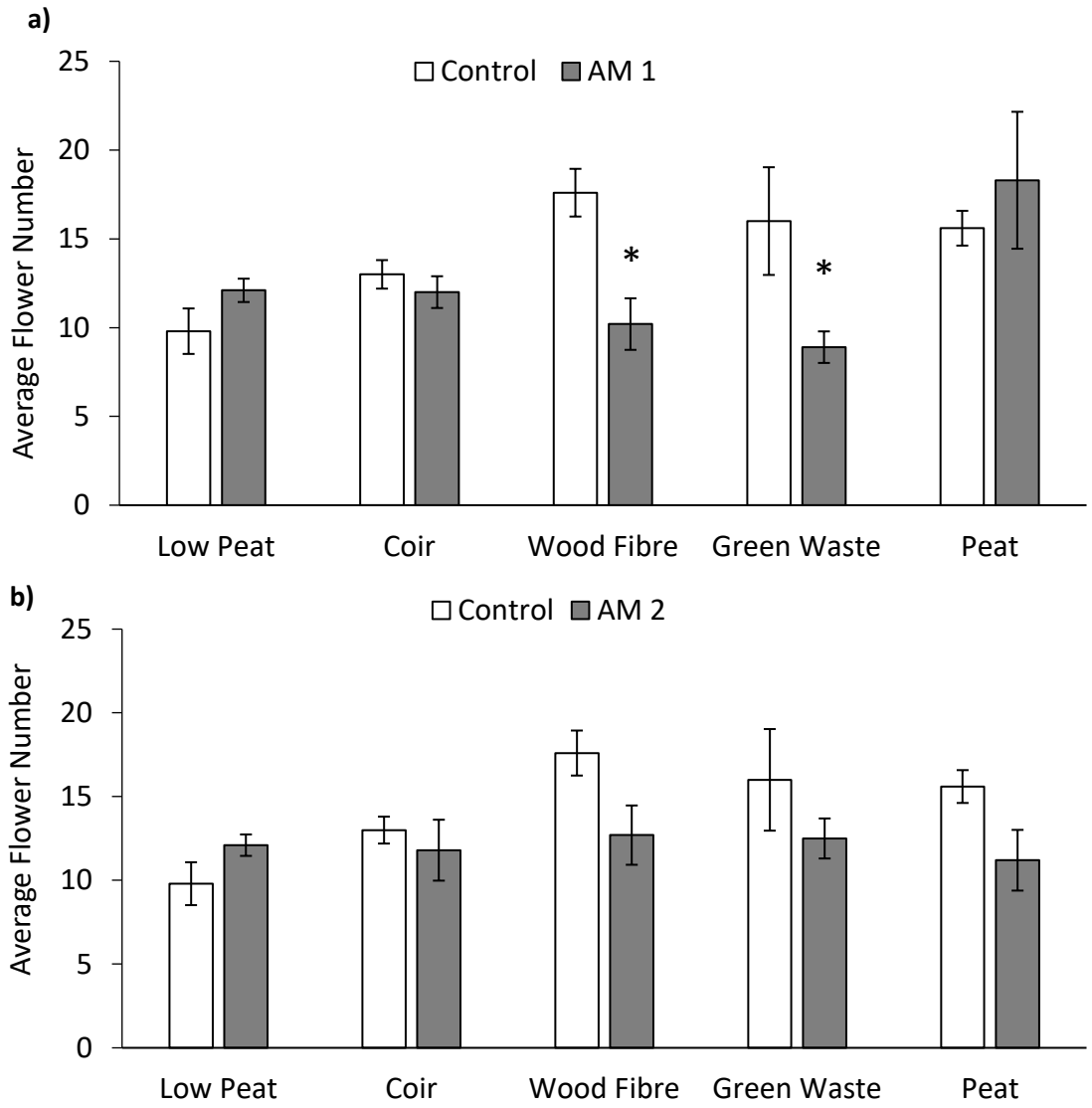


*Figure2.11 Difference in average flower number between plants grown in each compost and inoculum treatment: a) AM1, b) AM2. Asterisk denotes statistical difference between inoculum and control treatment for each compost *=p<0.05. n=10, bars±S.E*

2.4.2.3 Leaf Number

Patterns of compost effect on leaf number results were very similar to plant height and for plants treated with both AM1 and AM2. Overall plants grown in wood fibre were the only plants found not to have a significantly lower number of leaves than those grown in peat which produced the plants with the most leaves (Table 2.5, Figure 2.12). Coir compost also produced plants with the smallest leaf number and the final leaf number of coir grown plants was significantly lower than that of plants grown in peat, wood fibre and green compost (Figure 2.12).

		AM1		AM2	
Repeated Measures	**d.f**	**F Value**	**Sig**	**F Value**	**Sig**
Time	3	361.3	**P<0.001**	380.0	**P<0.001**
Time*AM	3	0.600	P>0.05	1.277	P>0.05
Time*Compost	12	2.261	**P<0.01**	4.165	**P<0.001**
Time*AM*Compost	12	0.836	P>0.05	1.331	P>0.05
Final Leaf Number					
AM	1	3.439	P>0.05	4.557	**P<0.05**
Compost	4	7.610	**P<0.001**	9.403	**P<0.001**
AM*Compost	4	1.25	P>0.05	2.170	P>0.05

Table 2.5 Results of two-way ANOVA with repeated measures on leaf number. Repeated measures error degrees of freedom: AM1=264, AM2=261. ANOVA error degrees of freedom: AM1=88, AM2=87.

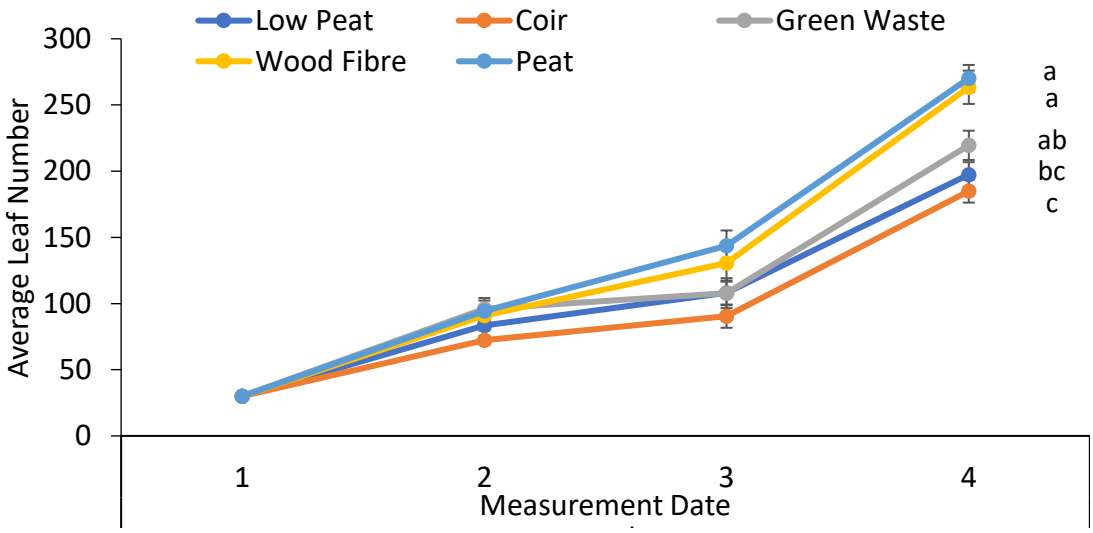


Figure 2.12 Overall effect of compost treatment on leaf number measured over four weeks. Letters indicate significant difference between treatments. n=30 and bars±S.E

The only significant effect of adding inoculum was seen in low peat in AM2 where plants treated with AM2 had significantly fewer leaves than control plants (F_1=4.557, Figure 2.13a). Although differences were not significant, both AMF inocula produced plants with fewer leaves than non-inoculated plants in peat and green waste composts (Figure 2.13a, c), but in wood fibre and peat the different inocula (AM1 and AM2 respectively) produced plants with the same amount or more leaves than the control plants (Figure 2.13d-e)

There was a significant interaction between time and compost for both inoculum treatments (Table 2.5) although the effect was more significant with AM2 (F_{12}=4.165). The graphs in Figure 2.13 show how the change in leaf number varied in the rate of production between weeks in different composts. Some showed consistent increases each week (Coir (a), Wood Fibre (d) and Peat (e)) and others showed reductions or little increase between weeks 2 and 3 (Low peat (a) and green waste (b)). Peat and wood fibre grown plants also showed a greater increase of leaf number between the penultimate and final time points, compared to plants in the other composts.

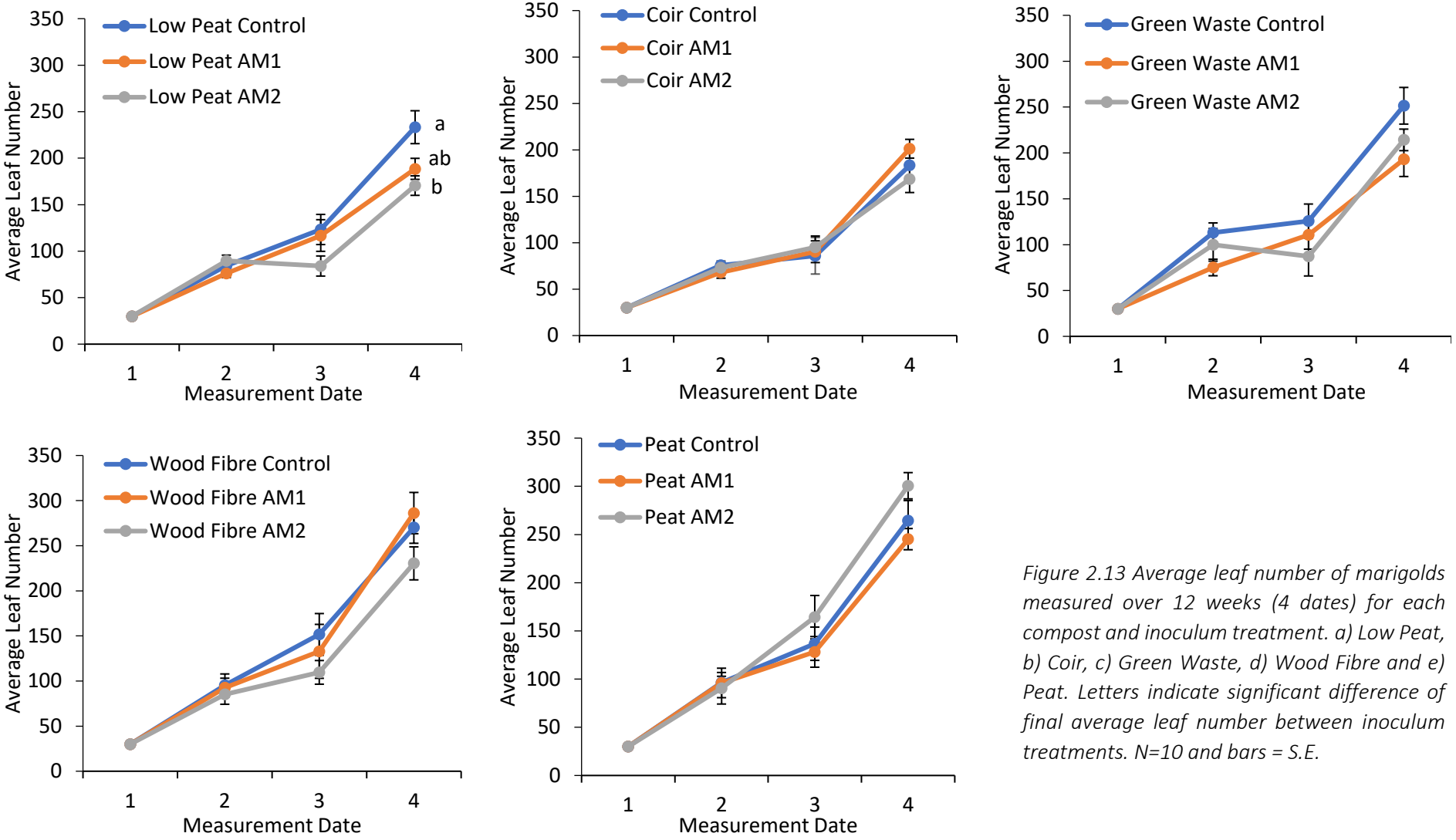


Figure 2.13 Average leaf number of marigolds measured over 12 weeks (4 dates) for each compost and inoculum treatment. a) Low Peat, b) Coir, c) Green Waste, d) Wood Fibre and e) Peat. Letters indicate significant difference of final average leaf number between inoculum treatments. N=10 and bars = S.E.

2.4.3 Biomass

Similar to plant height and leaf number, wood fibre produced the largest plants and the only plants that were not significantly smaller in terms of biomass than those grown in peat (F_4=21.420, Figure 2.14a). In AM1 green compost produced the smallest plants but this was most likely due to the significant negative effect adding AM1 had on biomass (t_{17}=3.964, p<0.01, Figure 2.14a). In AM2 treated plants, coir based compost produced plants that were significantly smaller than plants from all growing medias except those grown in the low peat mix and green waste plants were found to be similar in size to peat and wood fibre grown plants (F_4=12.174, Figure 2.14b).

In wood fibre grown plants AM1 continued to have a positive effect on biomass as seen with height and leaf number, but the opposite effect was seen with AM2, a decrease in coir plant biomass was also seen when AM2 was added.

AM 1	d.f	F	Sig
AMF	1	3.011	P>0.05
Compost	4	21.420	**P<0.001**
AMF*Compost	4	5.608	**P<0.001**
AM 2			
AM	1	0.645	P>0.05
Compost	4	12.174	**P<0.001**
AM*Compost	4	1.765	P>0.05

Table 2.6 Results from two-way ANOVA on plant biomass for each inoculum treatment. Error degrees of freedom: AM1=85, AM2=83.

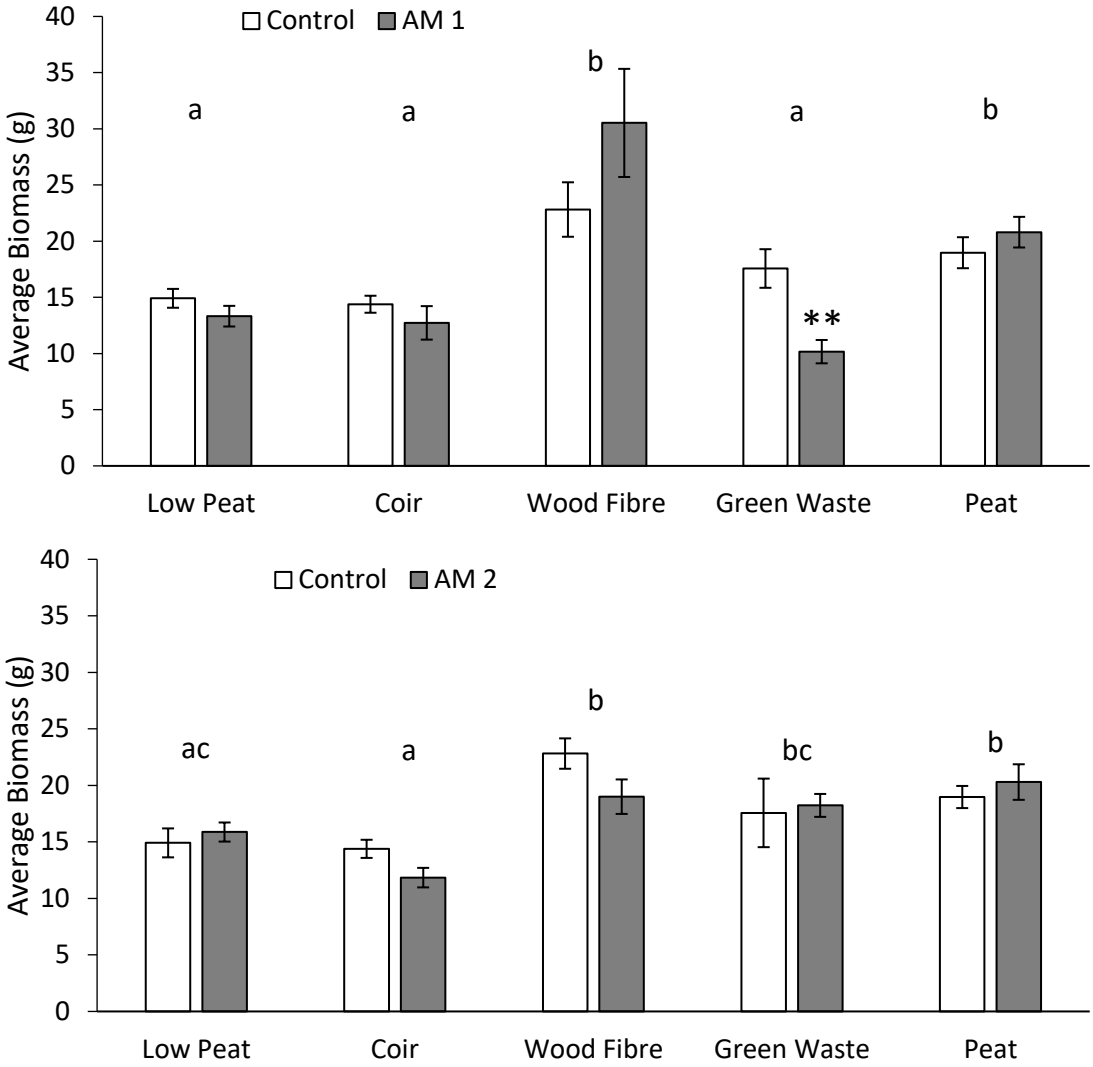


Figure 2.14 Difference in biomass between plants grown in each compost for each inoculum treatment. Letters indicate statistically different means between composts. Asterisk denotes statistical difference between inoculum and control treatments within each compost.. n=10, bars±S.E

2.5 Discussion

2.5.1 Media Nutrients

There is a lot of evidence to support the idea that the addition of AMF will increase plant biomass as well as nutritional value in crops (Klironomos and Hart, 2002; Baum, El-Tohamy and Gruda, 2015; Rouphael *et al.*, 2015) in field conditions, and with normal and reduced fertiliser levels (Ceballos *et al.*, 2013; Bona *et al.*, 2015).Their ability to do this however can often be limited by the growing conditions, as their symbiosis greatly depends upon nutrient levels in the surrounding substrate (Biermann & Linderman 1983; Koide 1985; Nouri et al. 2014). There are many studies that attempt to look at the relationship between mycorrhizas and the concentration of nutrients especially phosphorus and nitrogen, both with field and pot based trials. It has been shown that only if both nitrogen and phosphorus are readily available to the plant will the carbon exchange with mycorrhizas be reduced (Blake, et al., 2011). Severe lack of nitrogen can override the negative effects of high phosphorus levels so that plants will maintain AMF symbiosis as long as they are lacking one of these two main nutrients (Nouri *et al.*, 2014). One of the appeals of retail multipurpose composts to gardeners is that levels of additional fertiliser aim to provide enough nutrients to ensure optimum growth over a long period before extra feeding is required. Given the nature of the relationship between AMF and plants high levels of fertiliser in commercial compost could have a detrimental effect on the mycorrhizas' ability to germinate, colonise and function as an effective nutrient transfer system (Breuillin *et al.*, 2010; Balzergue *et al.*, 2013). Going forward with this research it is important to know what levels of essential nutrients mixed with growing media will allow successful mycorrhizal symbioses to occur to make sure plants receive the most benefit.

Nitrogen levels were high in all composts except the peat composts where levels of nitrogen and potassium were equal, this is not surprising as nitrogen is essential for photosynthesis as well as growth. Despite expected high levels of nutrients expected phosphorus levels were close to what is suggested as the optimal level of 50ppm for AMF to produce the most benefits to plants (Smith and Read, 2002a) as the range was between 93 and 41 mg/Kg or ppm. Studies have shown positive responses on plant growth with AMF with similar phosphorus input (Sylvia and Schenck, 1983; Gaur and Adholeya, 2000) and also no effect of colonisation (Biermann and Linderman, 1983a). Wood fibre had the lowest amount of phosphorus (41mg/Kg) which could therefore promote mycorrhizal colonisation however plants grown in wood fibre compost were not found to have higher percentages of colonisation in their roots than plants grown in any other peat free compost or the low peat.

Coir compost was shown to be rich in anions due to its high salinity and it was the only compost with significantly less nitrogen than potassium. The reason for the high salinity of coir compost can be traced back to the coir production process, where coconuts can be grown by the sea and coir processed by soaking the pith in sea water where fresh water is hard to come by in Sri Lanka, Indonesia and India (Drewe, 2012). A buffering process using calcium nitrate is supposed to reduce harmful levels of potassium and sodium but it clearly is not always enough (Schmilewski, 2008).

The lowest levels of nitrogen were seen in the green waste compost. This is unsurprising given its high organic content which is likely to be decomposed by microbes who also use up nitrogen and phosphorus (Handreck 1992a,1996), nutrients are broken down only to be used and locked up in microbial cells so it does not become available to plants. This microbial activity could depend on how the green waste was composted (Tognetti, Mazzarino and Laos, 2005). Municipal waste streams have guidelines for sterilising recycled waste to be used for growing media but these cannot guarantee to be 100% effective (Noble and Roberts, 2004), as such it cannot be sure that this media would be free from micro-organisms including pathogens. A previous project (Edwards, 2012) which featured a different brand of retail multipurpose GWC showed different species of fungal fruiting bodies emerging from pots including *Coprinus* species and *Peziza repanda*, along with various mildews on the compost surface. The unsightly nature of this is obviously an unwanted trait by gardeners but particularly for growers of live plants to be sold in containers such as potted herbs this is a major problem as it would directly affect sales.

2.5.2 AMF Root Colonisation

2.5.2.1 Commercial inocula effectiveness

No significant difference in colonisation was found between each inoculum treatment AM1 and AM2 in each compost or between composts but overall plants treated with AM2 exhibited significantly lower levels of root colonisation. This could be due to several factors because the two inocula are very different: AM2 contains a mix of nine species compared to AM1 and only two of those species are common to both mixes, AM2 is made predominantly of spores instead of spores and larger propagules such as in AM1, as outlined in section the recommended dose rate of AM2 meant that pots contained considerably less spores than pots inoculated with AM1.

Using combined mixes of inoculum have been shown to have an additive effect and provide more plant benefits than single species of inoculum. Mixes of both indigenous and non-

indigenous species of AMF produced taller citrus plants with a higher percentages of phosphorus than plants inoculated with only one species of AMF. (Ortas and Ustuner, 2014b). (Ustuner *et al.*, 2009) also showed that different combinations of AMF species produced plants with higher shoot and root biomass in different growing media. Despite there being fewer species in AM1 than AM2, levels of colonisation were higher and increases in biomass were more frequent in plants inoculated with AM1; this could be because there is a more beneficial mix with complimentary species that have an additive effect. AMF species have shown to demonstrate species specificity in terms of functional diversity, where similar amounts of colonisation by the same species of AMF can occur in different plant species but the benefit to plant growth can be the same (Klironomos, 2000).

There are two other marked differences between the two inoculum treatments which would affect their ability to successfully colonise the same plant in the same environment. Colonisation has been shown to be variable between inocula that have different propagule types (Klironomos and Hart, 2002; Faye *et al.*, 2013; Herrmann and Lesueur, 2013), as AM1 contains hyphae and colonised root material as well as spores this could be the reason for higher colonisation levels. The dosage level could also have caused a difference, this has often been linked to biomass (Clapperton & Reid 1992). As previously outlined (Section 2.2.2) the recommended dosage for AM2 resulted in a smaller number of spores being added to each pot compared to AM1, this could result in reduced colonisation but also reduced plant performance. It should be determined if this difference is consistent, including in other plant species and if so which factor allows for one inoculum to dominate.

2.5.2.2 Compost effects on colonisation

Although levels of colonisation between plants grown in peat free composts were similar there was a significant difference between root length colonisation in plants grown in green waste compost and peat. It is unsurprising that the highest levels of colonisation were recorded in green waste and the lowest recorded in peat and low peat given their origins. CGW for multipurpose compost is often sourced from municipal waste streams that include garden waste, this is likely to contain a lot of bacteria and fungi. It should be noted that this could have resulted in green waste grown plants being infected with multiple endophytic fungi and that some hyphae may have been misidentified during mycorrhizal colonisation scoring contributing to the higher average colonisation. It has been shown that mycorrhizal fungi often need a community of bacteria and other soil microbes to thrive and studies have shown that plant

growth promoting rhizobacteria (PGPR) can help to improve plant performance when combined with AMF (Lavakush *et al.*, 2014; Nadeem *et al.*, 2014). Another fungal group beneficial to plants *Trichoderma* spp. have also been shown to increase AMF spore germination when they are present together in soils (Calvet, Pera and Barea, 1990; Calvet, Barea and Pera, 1992). Mixed commercial inoculum containing AMF, *Trichoderma spp*. and PGPRs was shown to increase the frequency and amount of mycorrhizal colonisation compared to single species inoculum, single species inoculum was also shown to decrease shoot and leaf biomass compared to control plants. (Berruti *et al.*, 2013) As PGPRs and *Trichoderma spp*. could be found in plant material it is reasonable to assume they may also be present in green waste compost. Calvet et al. (1993) demonstrated this synergistic effect of *Trichoderma* and *Funneliformis mosseae* in marigolds grown in a peat based medium, where plants inoculated with both fungi had increased root colonisation by *F. mosseae* combined with an increase in plant biomass. Contrastingly peat is naturally relatively biologically inactive due to its high pH and formation in cold environments, given the evidence a relatively sterile medium like peat might not provide the ideal conditions for AMF to germinate and colonise successfully. It has also been shown that physical and chemical properties of some peats can negatively affect germination of AMF spores and colonisation (Ma et al. 2007, Linderman & Davis 2003). It is also likely that even given the slightly fewer nutrients available in peat the plants were able to successfully uptake nutrients and water without the help of mycorrhizae due to the properties of peat.

In coir combined levels of low nitrogen and phosphorus could be the reason why coir grown plants had the second highest percentage of roots colonised by mycorrhizas. Despite seeing high levels of colonisation coir grown plants in this study there was a negative rather than a positive effect (although not significant) on all plant growth measurements with the addition of both mycorrhizal inocula. Linderman & Davis (2003) found that addition of AMF consistently improved growth of *Tagetes patula* and colonisation by *Glomus intraradices* increased as the percentage of coir in a peat:coir mixed medium was increased. This was suggested by the authors to be connected to the increased microbiological properties of coir compared to peat. In this study, the high levels of salinity in the compost may have prevented the positive effects of the mycorrhizal symbiosis. Research has shown that increasing salinity reduces root colonisation by AMF (Manaf and Zayed, 2015) and this has been attributed to inhibiting germination, hyphal growth and hyphal spreading (McMillen, Juniper and Abbott, 1998).

Despite these slight differences, it is promising that there was no significant effect of the peat free compost on colonisation as this supports the distribution of these products for amateur garden use as their producers claim that it can be used universally on different substrates from

soil to specialised growing media. This could be due to the mix of species in each inoculum which help to make sure that there will be successful colonisation in different nutrient, pH, and moisture conditions. It would therefore be expected that different AMF species or combinations of species colonise plants grown in different composts. Ortas (2014) showed colonisation and effect differed between growing media, further detail on which AMF species have colonised plants in future experiments would be an interesting next step in examining these differences to further understand how AMF and growing media interact.

2.5.3 Plant Performance

Overall no significant effect of colonisation on biomass was found within or across treatments despite multiple studies showing that across plant species and environments addition of AMF does increase shoot biomass (Berruti *et al.*, 2016), including in marigolds (Linderman and E. a. Davis, 2003; Linderman and Davis, 2004). There was no pattern to suggest the addition of either inoculum would have a consistent increase in the biomass of plants as the results across composts and treatments were very variable. The high variability in biomass between control and treated plants along with inconsistent colonisation levels is most likely the reason for this. However, trends could be observed that do indicate mycorrhizal treatments did have some effect on plant performance.

Wood fibre grown plants did not have significantly reduced height, biomass or leaf number when compared to peat grown plants. Plants grown in wood fibre treated with AM1 also had the biggest increase in plant height, biomass and leaf number compared to controls of any treated plants which suggests that they were obtaining the most benefit from the AMF symbiosis. This difference was not found to be statistically significant but the effect could have been limited due to the high amount of nitrogen also present in this compost. Overall coir compost produced the smallest (biomass and leaf number) plants. This is surprising given a large body of research which shows AMF colonisation ameliorating the effects of salt stress in plants and increasing their biomass (Al-Karaki, 2000; Giri, Kapoor and Mukerji, 2003; Colla *et al.*, 2008). Manaf and Zayed (2015) however saw a reduction in dry biomass of mycorrhizal plants compared to uninoculated controls in both their saline treatments. Access to more phosphorus through mycorrhizas is suggested as the process by which colonised plants deal with higher levels of salts. In this instance, due to limiting phosphorus availability in small enclosed rhizome there may not have been enough and the presence of AMF could have caused the plant further stress by being a drain on carbon resources.

Other negative effects of inoculum addition on plant biomass were seen in low peat and green waste compost. These differences were found to be significantly different to control plants and those treated with AM2, as this effect was only seen with AM1 it could be a species-specific issue determined by the AMF species that colonised the roots of those plants. There could be many other factors that have previously been reported with CGW that could cause negative impact of mycorrhizas. Biological instability (Burger et al., 1997; Nichualain and Prasad,2009) or presence of various bacteria and fungi could cause competition with AMF for nutrients as well as reduced levels of available nitrogen through respiration. High bulk density of GWC has also been reported (Benito *et al.*, 2005) which can result in restricted root growth and waterlogging. It should be noted green waste pots were noticeably heavier on lifting compared to all other composts, but especially pots filled with wood fibre which were noticeably lighter. It was shown that green waste compost contained significantly more moisture than all other composts except wood fibre. Water stress in the form of waterlogging could be the result of anaerobic conditions but also in this case release of more phosphorus and other nutrients (Mendoza, Escudero and García, 2005) which could have caused AMF to have parasitic effects. Differing responses to water in peat free growing media are being investigated (Alexander, Williams and Nevison, 2013) as growers will need detail on how to change watering regimes from optimums set for peat in order to make sure plant performance is maintained. By using components such as composted bark as a main ingredient which is normally used to increase water holding capacity of other media there is a risk of waterlogging and *vice versa* with aerating materials. Mycorrhizal interaction with water management in plants and the effect of growing media will need to be considered in future experiments.

Plant height showed very similar patterns to biomass except differences in inoculum effect were more pronounced with biomass, this was shown by more significant differences in biomass between treatment types. Biomass was therefore considered a more reliable measurement to monitor the effects of mycorrhizal colonisation.

Flower number patterns did not match those of biomass suggesting that plants with high biomass were not the result of more flowers but larger vegetative growth, this makes sense as leaf number and plant height patterns matched biomass. Flower number was not affected by compost and only in peat based composts did inoculum treatments have a positive effect on flower number. Overall AM2 inoculated plants produced significantly fewer flowers than control plants, this negative effect was also seen with AM1 inoculation in wood fibre and green waste grown plants. Plants that had higher average biomass seemed to have fewer flowers suggesting extra nutrition may have been used for vegetative growth rather than producing flowers. It has

been demonstrated that AMF can significantly increase flower number in marigolds fertiliser levels are low (Bi *et al.*, 2010) but flower number in marigolds has also been shown to not be affected by AMF treatment (Linderman and Davis, 2004).

2.6 Conclusions

In summary, this experiment revealed the variability of peat free multipurpose composts from nutrient levels to their effect on plant performance and mycorrhizal colonisation. The data gathered highlighted the benefits and drawbacks of the different peat alternative source materials, most of these observations match what has previously been debated when considering these materials as replacements for peat in the horticultural industry (Barrett et al. 2016).

Whilst coir appeared to promote mycorrhizal colonisation the high salt content and its effect was not ameliorated by the addition of AMF and overall it produced the smallest plants. The risk of this negative effect on plant performance could be too high. To consider coir as a reliable substrate for future experiments. Similarly, the biological instability, waterlogging and nutrient levels of green waste compost creates too many variables which could negatively affect the growth of plants as well as induce negative effects of AMF colonisation on growth. The production of such GWC from municipal streams would also make a consistent, reliable source impossible which would not be productive to a comparative, multi-experiment study.

Wood fibre produced the largest plants, and was the only peat free compost to produce plants with biomasses not significantly smaller than peat. Inoculation with AM1 in wood fibre composts produced the largest increase in biomass compared to control plants. Increases in plant height and biomass of plants inoculated with AM2 were also seen in low peat and green compost, both contain bark fines. Soft-wood pine bark is used widely for container plant cultivation in southern Europe, south east USA and New Zealand because of its high availability and high air holding capacity (Barrett *et al.*, 2016). For this reason it is also used to amend other materials, potting and grow bark have been shown to reduce pH and electrical conductivity of CGW (Tognetti, Mazzarino and Laos, 2005) along with being added to peat to reduce it as a component like in our low peat mix.

With clear options for which media performed best and which were to be avoided, the peat alternative media treatments were reduced to wood based mixes for the future experiments in this thesis.

The variability of retail multipurpose composts was considered too great to continue using them for this research, similar results with another study showed that plant growth of multiple plants species, including marigolds, varied significantly between different brands of retail composts as well as within brands themselves i.e. between bags of the same compost (Wiberg, Koenig and Cerny-Koenig, 2006). As a result, the growing media mixes to be used in the rest of this thesis were specifically engineered as part of another project also looking at sustainable media. The raw materials have been kept consistent in their source and production and, based on results from this experiment, additional nutrients were controlled so as not to be too high to interfere with colonisation. This is also more representative of the level of consistency which would be required of growing media materials to be used in professional mixes.

It was highlighted in this study that knowing the physical properties of growing media, with special focus on water holding ability would be necessary to identify differences between the growing media which could explain plant responses and the effects of interactions between growing media and AMF on plant performance. The significant difference between the colonisation ability of the two inocula should also be explored further including identifying if this difference is a result of species mix, propagule type, or dosage. Given previous research it would also be expected that growing media could influence the performance of different AMF species.

Chapter 3 - The interactions between commercial mycorrhizas and reduced peat growing media on plant performance

3.1. Introduction

As part of this investigation into the effects of commercial mycorrhizal inoculum, biomass was a key area of study as these products are marketed to increase plant size by acting as bio-stimulants. By providing plants with more nutrients than they could obtain alone arbuscular mycorrhizas could increase the size plants can grow to. Plant size is important to amateur gardeners as well as commercial producers. Peat alternatives are often rejected by growers for producing plants of significantly smaller size.

Studies have shown that the addition of AMF can be used to improve yield and nutrient quality of crops. (Raviv 2010; Hart & Forsythe 2012) The same effect has also been demonstrated with commercially produced inoculum in the field. (Ceballos *et al.*, 2013) In these cases levels of phosphorus and nitrogen were monitored and controlled to ensure colonisation by AMF was beneficial. The previous experiment in this thesis used commercial composts with relatively unknown and uncontrollable levels of nutrients (section 2.2.2) however, these highly fertilised substrates did not appear to inhibit mycorrhizal colonisation (section 2.4.1).

The aim of this experiment was to test whether the positive effects of the commercial inocula used in Chapter two were repeatable. The objectives were to continue to test these inocula in nursery stock style conditions with reduced peat substrates and known nutrient levels to see if these treatments could be applied here in future.

3.2 Materials and Methods

3.2.1 Growing Media

One mix included pine bark chips and the other had fibrous steam treated wood and these were mixed with peat at 30% by volume (Table 3.2.1). A benchmark mix of 100% peat was used to replicate the current industry standard used in the majority. Each growing media contained enough to support an annual plant for at least three months.

Table 3.1 shows the mixture of nutrients used in each growing media. The N:P:K ratio was approximately 15:10:20. Table 3.2 shows the complete make-up of each growing media mix. All three contained the same amount of base nutrients, lime to control the pH and a wetting agent. Wetting agents are likely to be soap or alcohol based to assist with the re-wetting and drainage of the media. Both reduced peat composts contained an added dose of nitrogen in the form of calcium ammonium nitrate.

The peat in each media contained a mix of light, dark and sod peat in the following ratio: 0.6:0.3:0.1. The bark media contained matured pine bark chips (potting mix grade) sourced from responsibly managed forests by Melcourt. The wood fibre media contained the same machine extruded wood fibre material again sourced from pine. This product is produced by Bulrush Ltd and is known as Forest Gold Plus®. The machine extruding process involves high temperature and pressure which means that wood fibre was the only sterilised mix ingredient. This was the same ingredient in the Bulrush multipurpose compost used in the preliminary experiment in 2014 (section 2.2.2). All seeds were germinated and grown in seed trays before being transplanted into pots. They were grown in SylvaGrow®, a commercial sustainable multipurpose compost produced by Melcourt which contains fine bark and wood fibre (from sustainably managed British forests) and coir (from a single, known source). (Melcourt Industries, no date)

Nutrient	Soluble in Water	%	Trace Element	Soluble in Water	%
Nitrogen (N)	No	15.50	Boron (B)	Yes	0.03
Phosphorus (P_2O_5)	Yes	9.00	Copper (C)	Yes	0.15
Potassium (K_2O)	Yes	19.90	Manganese (Mn)	Yes	0.18
Magnesium (MgO)	Yes	3.30	Molybdenum (Mo)	Yes	0.20
Calcium (CaO)	No	4.80	Iron (Fe)	Yes	0.11
			Zinc (Zn)	Yes	0.04

Table 3.1 Data on percentage component of each nutrient in the base fertiliser (Control 50) mixed into each growing medium.

	GROWING MEDIA		
Component	**All Peat**	**Bark**	**Wood Fibre**
Base Fertiliser - Control 50	1kg	1kg	1kg
Lime	4kg	4kg	4kg
Wetting Agent	0.4L	0.4L	0.4L
Calcium Ammonium Nitrate (26% nitrogen)	X	0.3kg	0.3kg
Peat	1000L	700L	700L
Bark Fines	X	300L	X
Forest Gold Plus	X	X	300L

*T*able 3.2 Complete mix of ingredients in each growing media

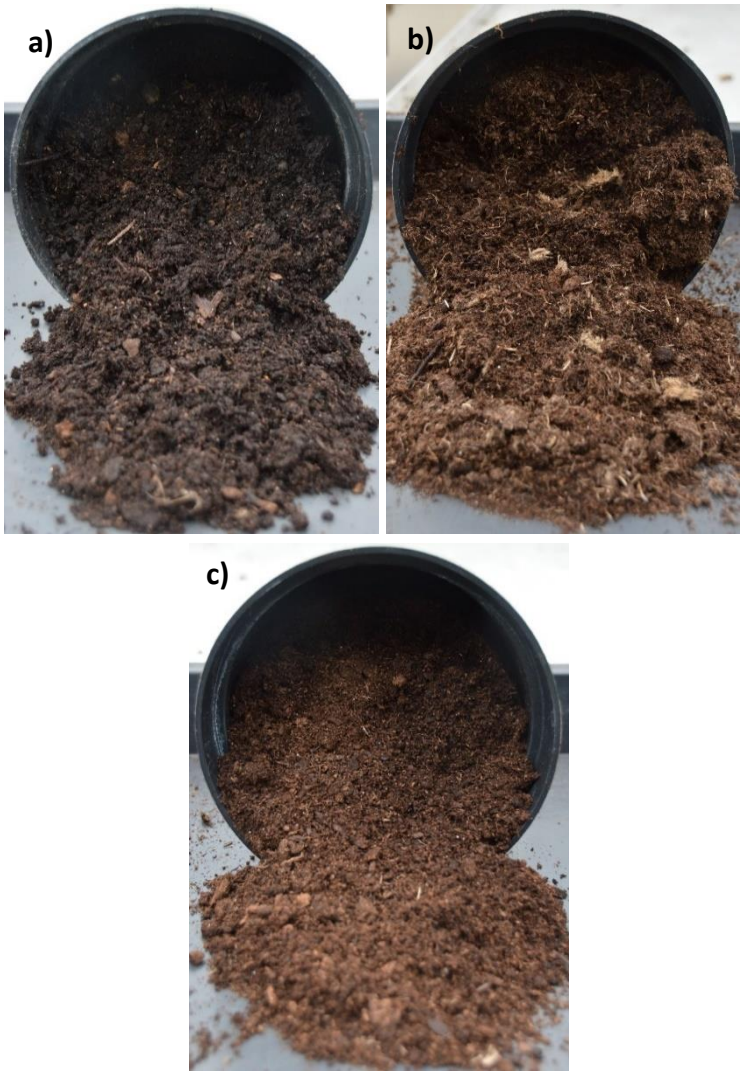


Figure 3.1 Growing media used to grow plants: a) bark media b) wood fibre media c) peat media

3.2.1 Commercial AMF

AM1 and AM2 were used as in section 2.2.3 but they were also added to control pots in a sterilised form. Following methods used by Hourston (2015) inoculum was baked in a muffle oven at 500 ^{o}C for an hour before being left to cool and then the process was repeated once more. Sterilised inoculum was kept in sealed containers for no more than a week before use.

3.2.1.1 Root Staining for AMF Colonisation Analysis

This was carried out as described by the methods in section 2.2.3.1.

3.2.2 Plant Species

French marigolds, (*Tagetes erecta L.)* were grown from seed under glass for three weeks until ready to be transferred into pots. *T. patula* 'Bonita mixed' seeds were sourced from Thompson & Morgan. A single marigold seedling was planted in each pot and they were grown for a total of three months.

3.2.3 Experimental Design

Three custom growing media were used: peat, bark chip and wood fibre (section 3.2.1) along with commercial inocula AM1 and AM2. In order to examine the effect of physically adding each mycorrhizal inoculum along with its carrier material each inoculum was added in a sterile form as a control treatment. There were six replicates of each of the 18 treatments. In total 108 pots were planted in the second week of June 2015, this experiment was then repeated with another 108 pots planted in the second week of July 2015. The plants were all grown for 12 weeks but this separation allowed for a staggered harvest, one in early and one in late September, to allow for such a large number of plants. Due to the fact that this method resulted in data from each repeat not being able to be combined for some measurements it was not used again. In this experiment, it was used to examine seasonal effects on plant growth parameters.

3.2.4 Site and Weather Data

The site used in 2014 (2.2.6) was not available so for this experiment an exposed field area at the RHS Field Research Facility at Deers Farm (Wisley, Guildford) was used. Pots were placed in a randomised block design in squares of 5x5 pots directly on MyPex® weed proof matting. Plastic circles were placed underneath pots when roots reached the MyPex® to prevent them growing through. Plants were watered with automatic overhead sprinklers morning and evening for one hour every day.

Weather data including rainfall, sunshine hours, maximum and minimum temperature was measured at a weather station on site during the entire period of growth, this data was then supplied by the RHS.

3.2.5 Plant Growth Measurements

3.2.5.1 Plant Height

To account for variation, height of seedlings was measured at the start of the experiment the day after they had been transplanted into two litre pots. A 30cm ruler was used to measure the height from the base of the stem at compost level to the tallest leaf. This process was repeated the day before the above ground biomass was harvested so that the increase in height over the growth period could be calculated for each plant. The average was then taken for replicates of each treatment.

3.2.5.2 Flower Number

Cumulative flower number was recording by totalling the number of open flowers, dead flowers and seed heads of each plant the day before the final above ground biomass was taken. This was then averaged across replicate plants for each treatment.

3.2.6 Leaf discolouration scoring

Towards the end of the experiment there were obvious visual differences between individuals, where some had very purple leaves. Plant groups grown with sterilised AMF appeared to show more replicates with purple leaves than those grown with live inoculum. To quantify this, each plant was given a score to indicate purpling, photos were used to assign scores. Plants were given a score of one if they had purple leaves (Figure 2.2b) and zero if their leaves were green (Figure 2.2a). The difference in total number of purple plants between inoculum treatments was then tested.

Figure 3.2 Plants that had green leaves compared to plants that exhibited purple leaves. a) two plant plants that scored 0 and b) two plant plants that scored 1

3.2.7 Porosity

To investigate the different physical properties of each growing media as well as the effect of the addition of the mycorrhizal inoculum carrier and colonisation water holding capacity was measured. Three replicate pots were taken from each treatment after the biomass was removed at soil height and harvested. Media volume was calculated using the diameter of the pot and media depth (measured with a ruler). Each pot had a silicone seal (Re-usable silicone stretch-cover lids, Amazon,) placed over the base (Figure 2.1a) and was weighed. Sealed pots were filled with water until no more water was absorbed by the media and a layer of water could be seen covering the surface of the substrate. (Figure 2.1c) Filled pots were then reweighed to determine how much water was added. Pots were then left to soak for an hour with water being topped up if necessary. Seals were then removed from pots and they were left at bench height to free drain overnight into trays. (Figure 2.1d) Pots were then reweighed along with the water that had drained into the tray.

Percentage porosity was calculated by dividing the amount of water added to each container by the container volume. Air-filled porosity (AFP) was calculated by dividing the amount of drained gravitational water by the container volume. Water Retention Porosity (WRP) could then be calculated by subtracting AFP from percent porosity.

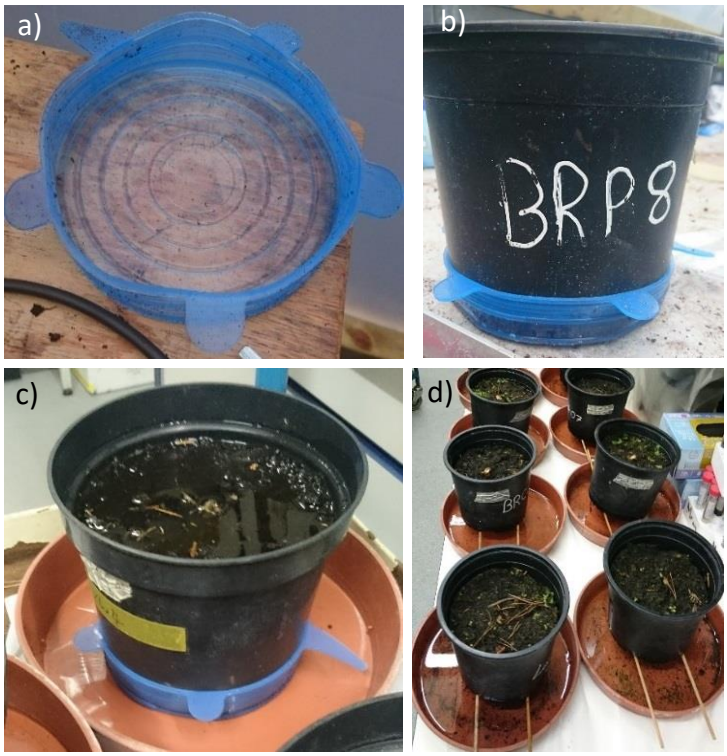


Figure 3.3 Process of measuring porosity and water holding capacity. a) A single silicone seal. b) A sealed 2L pot. c) A 2L pot that has been filled with water and left to soak. d) Pots with seals removed left to drain overnight with drained water collecting in trays.

3.2.8 Biomass

Final harvest of above ground biomass was carried out as described in section 2.2.8.

3.3 Statistical Analysis

To identify differences in abiotic factors affecting the plants at each harvest weather data (temperature and sunlight hours) collected for each week of growth was tested between the two harvests using independent sample t-tests. The factors of reduced sunshine hours and maximum temperature during harvest two resulted in a significant effect of harvest on various growth measurements when it was used as a co-variate in ANOVAs. Plants from different harvests were shown to have significantly different numbers of flowers, biomass and coefficient of variation of biomass so analyses for these data were conducted separately for each harvest. Plant height difference and porosity of growing media did not differ between different harvests so for these analyses harvest data was combined.

As root length colonisation was measured as a percentage for each root sample these data (hyphae, arbuscules and vesicles) were transformed using the arcsine transformation. To identify if plant biomass could be predicted by root length colonisation, these data for replicate plants were subjected to linear regression analysis.

For height increase, flower number, porosity, biomass, and root length colonisation a two-way ANOVA was used to analyses differences between and interactions within media and inoculum type (live or control) for AM1 and AM2. Tukey's HSD was used to perform post-hoc tests for growing media treatments. Where interactions between growing media and inoculum were found to be significant, independent sample t-tests were used to look at differences between the effect of live and sterile treatments on plants grown in each medium. Where data were not normally distributed values were transformed with square roots or logarithms.

A Man-Whitney U test was used to look at the difference between the number of purple leaved plants grown with each inoculum type (live or sterile) for each growing medium. All these analyses were conducted using IBM SPSS 21.

To measure size inequality the coefficient of variation and Gini coefficient were calculated for the biomass of replicate plants grown with live and sterile inoculum in each growing media. The coefficient of variation (COV) measured the variability in biomass of a group of replicates in relation to their population mean, the difference between the COV of biomass for live and sterile inoculum treated plants was analysed using a Z test for COV, originally described by Miller (1991). Gini coefficients are calculated from Lorenz curves: these are plotted using the ranked biomass of replicate plants from each treatment. The cumulative percentage of plants is plotted against the cumulative percentage of their total biomass, totally uniform groups would have straight, diagonal lines (these are plotted on Lorenz graphs as the line of equality). Comparing the area above and below the curves under the line of equality can be used to calculate the total amount of size inequality using the Gini coefficient. Gini coefficients measure the inequality of dispersion of each group of replicates on a scale from all plants having a uniform biomass (0) to complete biomass inequality (1). Bootstrapping was used to produce 95% confidence intervals for COV and Gini coefficients to allow them to be directly compared (Dixon *et al.*, 2016). As Lorenz curves were drawn for each set of replicates the Lorenz asymmetry coefficient could be calculated to help describe the causes of inequality in each treatment. The Lorenz coefficient is a measure of skewness with values below 1 representing a right skew and above one representing a left skew (Damgaard and Weiner, 2000). As values are ranked by size left skew

will be the result of smaller individuals and a skew to the right would be caused by larger individuals. All these analyses were conducted using R version 3.4.1 (Team, 2017).

3.4 Results

3.4.1 AMF Colonisation

Only data from harvest one is presented. Plants grown with AM1 were colonised successfully, hyphae were noted to be present in nearly all roots sampled, including some sterilised inoculum controls. Only five individuals (out of thirty-six) treated with AM1 were found to contain no presence of hyphae in their root samples. In contrast, only five plants treated with AM2 were found to contain evidence of hyphae in root samples taken from them, this resulted in no effects of AM or media being found.

Plants grown with AM1 in peat showed higher levels of colonisation in controls than with live inoculum in peat, this resulted in peat-grown plants having significantly higher levels of colonisation in their roots than those in wood fibre and bark (F_2=11.666, Figure 3.4a). There was a significant interaction between growing media and AM treatment (F_2=5.084) because unlike peat, the root colonisation by hyphae of control plants grown in wood fibre and bark were significantly lower than with the live inoculum treatment. (Bark: p<0.05, Wood Fibre: $t_{5.4}$=3.317, p<0.05, Figure 3.4a).

		Hyphae		Arbuscules		Vesicles	
AM 1	**d.f**	**F**	**Sig.**	**F**	**Sig.**	**F**	**Sig.**
Media	2	11.666	**P<0.001**	5.997	**p<0.01**	10.28	**p<0.001**
AM	1	3.304	P>0.05	1.490	p>0.05	0.005	p>0.05
Media*AM	2	5.084	**P<0.05**	1.699	p>0.05	4.017	**p<0.05**
AM 2							
Media	2	0.526	P>0.05	0.647	P>0.05	0.285	p>0.05
AM	1	0.168	P>0.05	2.062	P>0.05	1.100	p>0.05
Media*AM	2	2.428	P>0.05	0.907	P>0.05	0.799	p>0.05

Table 3.3 Results of two-way ANOVAs on plant root length colonisation by hyphae, arbuscules and vesicles in each inoculum. Error degrees of freedom: AM1=29, AM2=28.

In both bark and peat pots treated with AM2 evidence of hyphal colonisation wasn't recorded at all in control plants and in wood fibre plants the colonisation level was very low (<3%, Figure 3.4b).

The percentage of root length colonised by vesicles and arbuscules for plants treated with AM1 is shown in Figure 3.4a and the results of ANOVA on these data is shown in Table 3.3. The percentage of arbuscules found in roots grown in peat was significantly higher than that of bark plants (F_2=5.997, Table 3.3) and the percentage of vesicles in the roots of peat grown plants was also significantly higher than roots of plants grown in both reduced peat media (F_2=10.28, Table 3.3). As expected, the high levels of colonisation by hyphae in peat controls corresponded to higher percentage colonisation by arbuscules and vesicles and this resulted in a significant interaction effect of inoculum and media on the presence of vesicles in plant roots (F_2=4.017, Table 3.3).

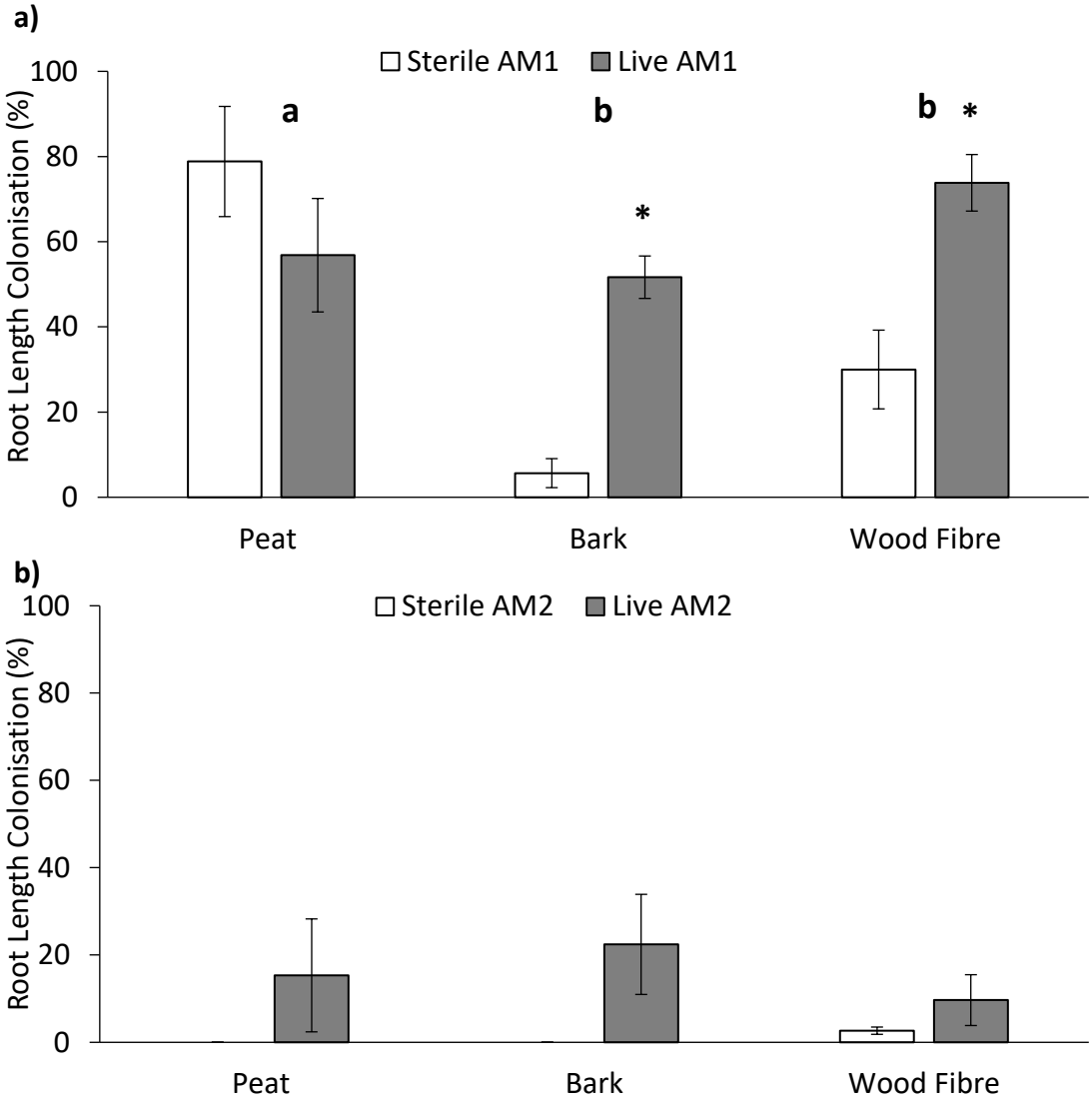


Figure 3.4 Root length colonised by hyphae in each growing medium for both inocula: a) AM1, b) AM2. Groups of bars with different letters have significantly different means and asterisks denote statistical difference between mean pairs, p<0.05. n=6, bars±SE

In contrast, the overall presence of nutrient exchange structures in roots of plants grown with AM2 was lower, with only one arbuscule being found on average in 100 counts in wood fibre roots (Figure 3.5b). Unsurprisingly with large numbers of zero values there was no significant difference between the amount of colonisation but mycorrhizal structures across roots of plants grown with AM2 (Table 3.3).

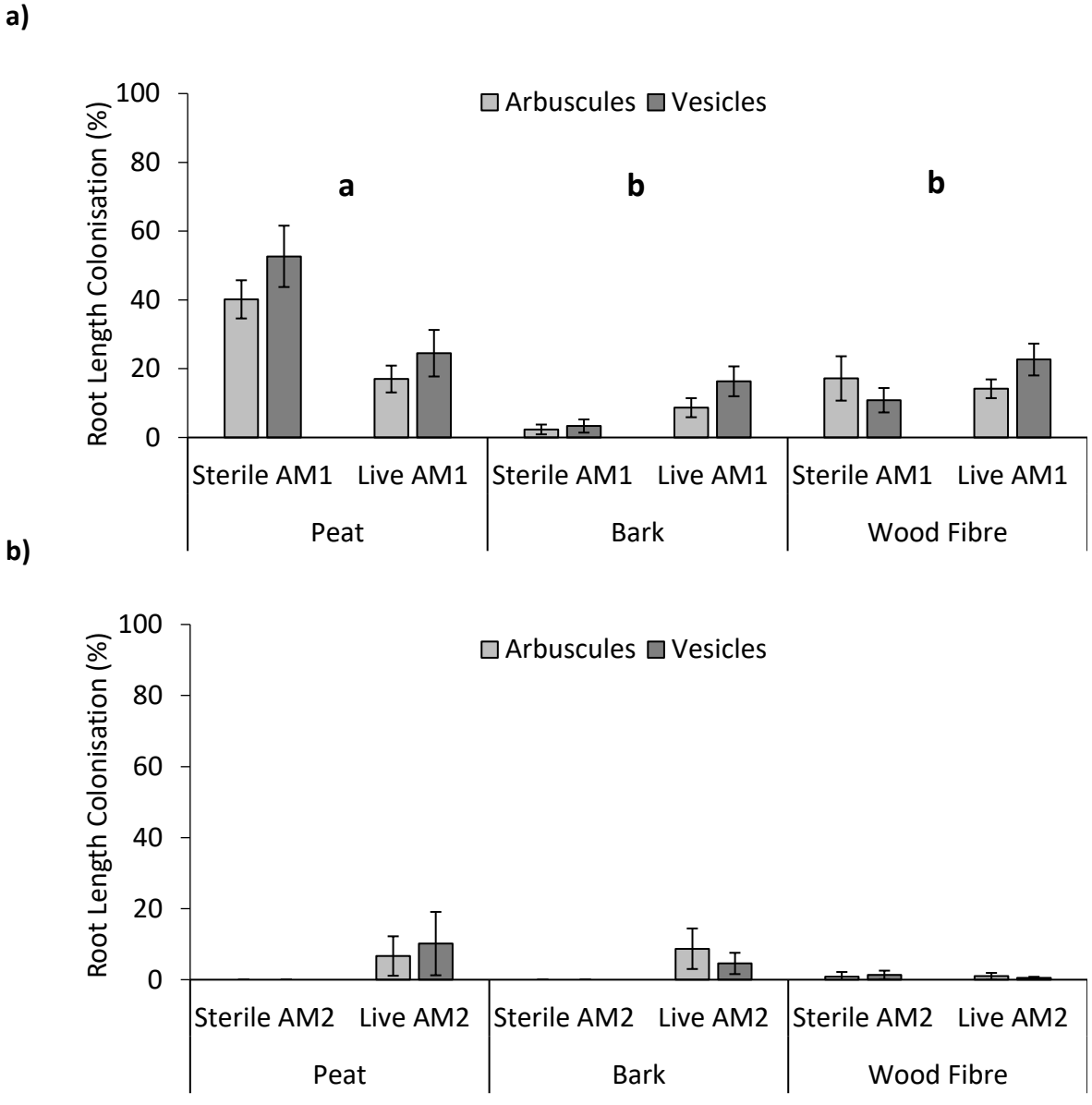


Figure 3.5 Root length colonised by arbuscules and vesicles in roots grown in each media with live inoculum: a) AM1, b) AM2. n=6, bars±SE

3.4.2 Weather Data

Seasonal changes resulted in plants in the second harvest experiencing less favourable conditions than in the first harvest: lower temperatures (Figure 3.7) and light levels (Figure 3.6). With a few exceptions (weeks one and four, Figure 3.6) plants in the second harvest experienced lower sunshine hours than in harvest one for the first nine weeks of growth, in weeks two and three the average number of sunshine hours was significantly lower for harvest two plants (t_{12}=2.503, p=0.028, and t_{12}=2.326, p=0.038 respectively).

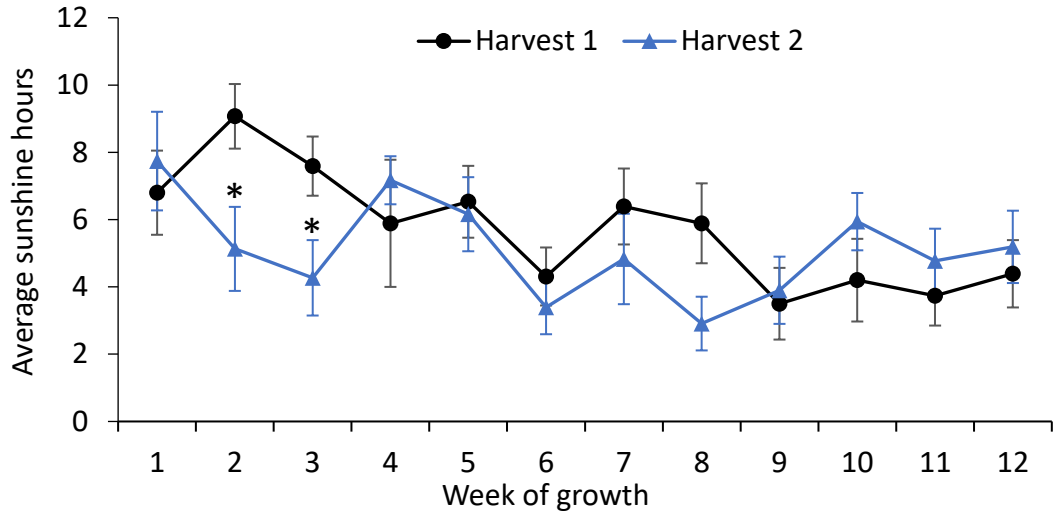


Figure 3.6 Average number of sunshine hours recorded for each week of plant growth for harvests 1 and 2 at Deer's Farm site. n=7, bars SE. Asterisk denotes statistical significance between harvests, p<0.05.

Maximum temperature data followed a similar pattern to sunshine hours, except for week six where harvest two plants experienced significantly higher maximum average temperature than in harvest one (t_{12}=-2.426, p=0.043, Figure 3.7). As expected, because harvest two plants were left to grow further into September, despite increased sunshine hours they experienced a reduction in maximum temperature compared to those grown at harvest one. This difference can be seen in the last four weeks of growth and was found to be significant at weeks eight and nine (t_{12}=4.027, p=0.002 and t_{12}=5.154, p=0.001 respectively, Figure 3.7). The average minimum temperature was also found to be significantly higher for the entire 12-week growth period for harvest one compared to harvest two (t_{166}=2.470, p=0.015, Figure 3.8).

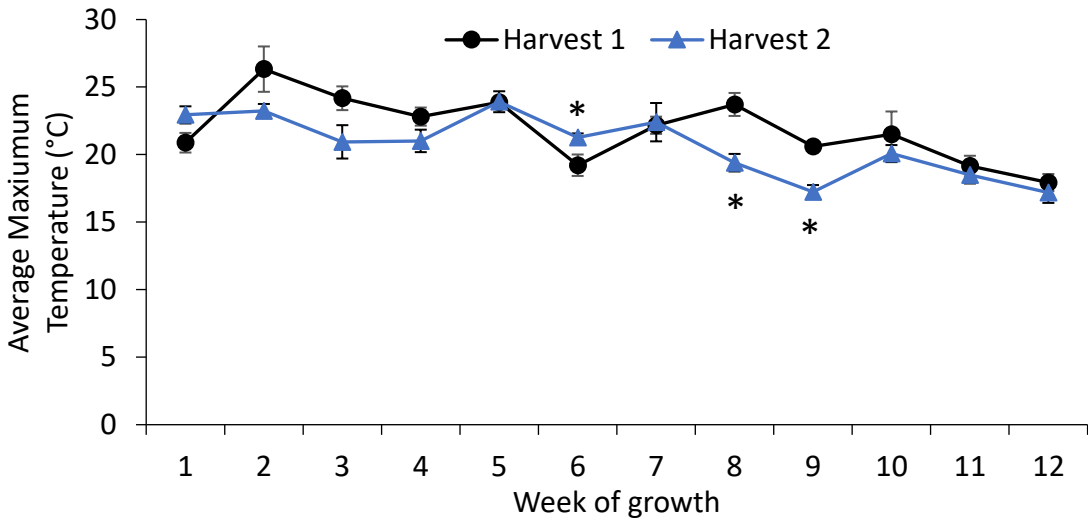


Figure 3.7 Average maximum temperature recorded for each week of plant growth for harvests 1 and 2 at Deer's Farm site. n=7, bars±SE. Asterisk denotes statistical significance between harvests, p<0.05.

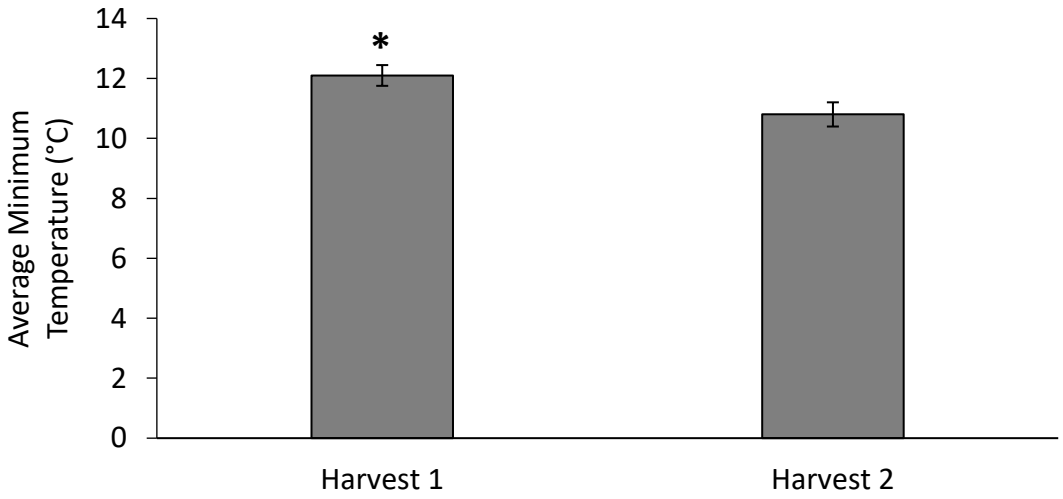


Figure 3.8 Average minimum temperature recorded over 12 weeks for each harvest at Deer's Farm site. Harvest 1: n=74, Harvest 2: n=65. Bars±SE. Asterisk denotes statistical significance between harvests, p<0.05.

3.4.3 Plant Growth Measurements

3.4.2.1 Plant Height

The factors of reduced sunshine hours and maximum temperature during harvest two did not result in a significant effect of harvest on plant height difference when it was used as a co-variate in the ANOVA, so for these analyses harvest data was combined.

The average height increase did not significantly differ between plants in different growing media, this was the same for both inoculum treatments. Plants treated with AM1 in bark were significantly shorter than those treated with sterile inoculum (t_{22}=-3.088, p=0.005) but this difference was not seen with AM2. Despite a significant interaction between AM and growing media in AM2, and wood fibre plants treated with live AM2 appearing to be a lot shorter than those with sterile AM2; no significant difference was found between the heights of control and live inoculum treatments in AM2 for any growing media (Figure 3.9).

AM1	d.f	F	Sig
Media	2	1.347	P>0.05
AM	1	0.02	P>0.05
Media*AM	2	2.414	P>0.05
AM 2			
Media	2	3.092	P>0.05
AM	1	1.092	P>0.05
Media*AM	2	4.097	**P<0.05**

Table 3.4 Results of two-way ANOVAs on height increase of plants grown with each commercial inoculum. Error degrees of freedom=66.

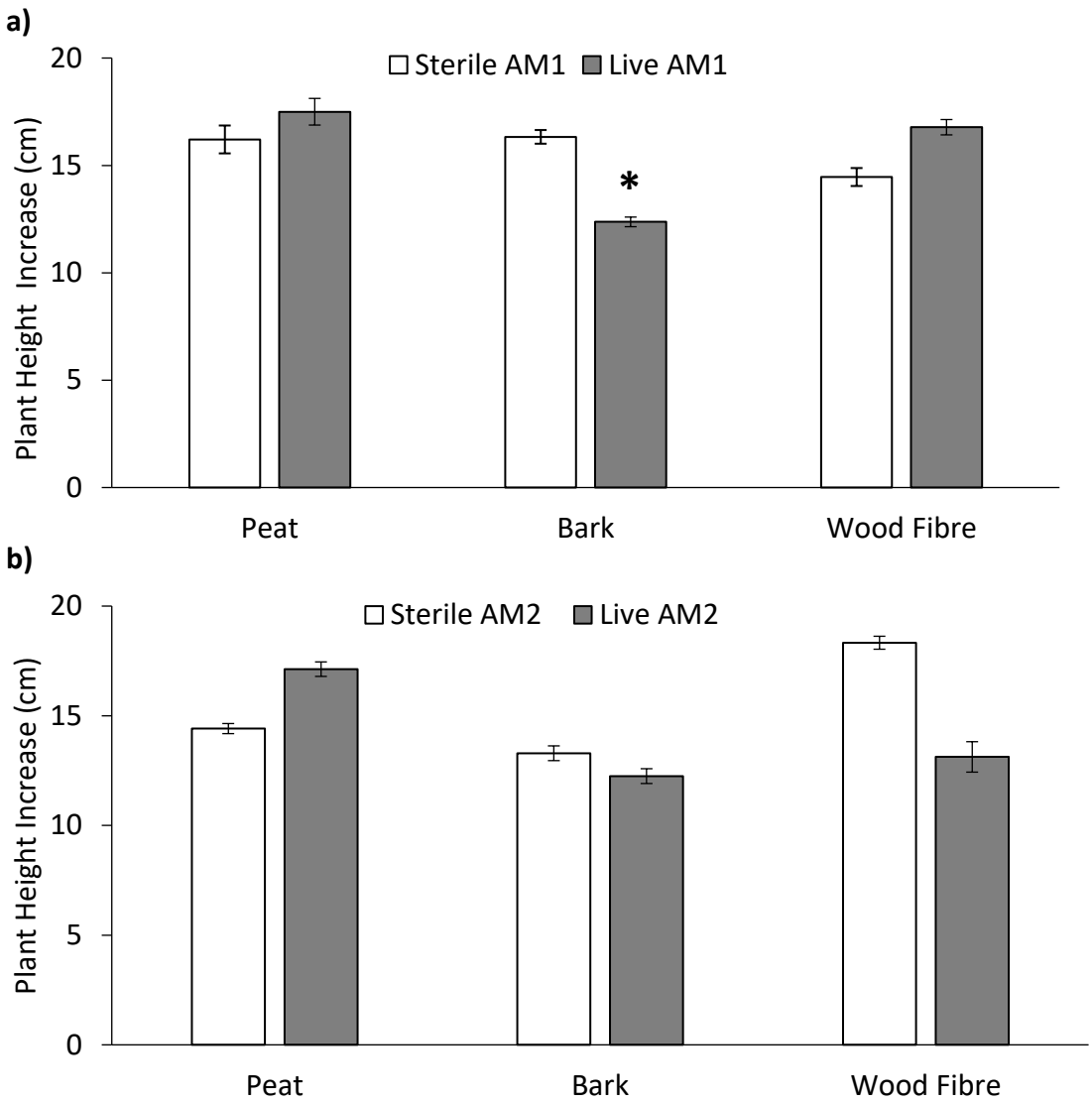


Figure 3.9 Average height of plants from both harvests grown in each media with each inoculum: a) AM1 Asterisk denote statistical difference between pairs of means, p<0.05. b) AM2. n=12, bars±SE

3.4.2.2 Flower Number

Treatment with either live inoculum did not have any effect on flower number in either harvest but growing media did (Table 3.5). In harvest one, plants grown in bark had significantly more flowers than plants grown in peat and wood fibre in AM1 treated plants (F_2=18.132, Figure 3.10a) and peat in AM2 treated plants (F_2=6.338, Figure 3.10b). In harvest two there was no difference in the number of flowers of plants grown in different compost and the addition of inoculum did not have any effect on flower number (Table 3.5, graphs in Appendix II).

		Harvest 1		Harvest 2	
AM1	**d.f**	**F**	**Sig**	**F**	**Sig**
Media	2	18.132	**P<0.001**	1.088	P>0.05
AM	1	2.585	P>0.05	0.869	P>0.05
Media*AM	2	0.250	P>0.05	1.008	P>0.05
AM 2					
Media	2	6.338	**P<0.01**	2.211	P>0.05
AM	1	2.422	P>0.05	0.042	P>0.05
Media*AM	2	1.392	P>0.05	0.052	P>0.05

Table 3.5 Results of two-way ANOVAs on flower number produced by plants grown with each commercial inoculum. Error degrees of freedom: Harvest 1 AM1=30, AM2=29. Harvest 2 AM1=29, AM2=30.

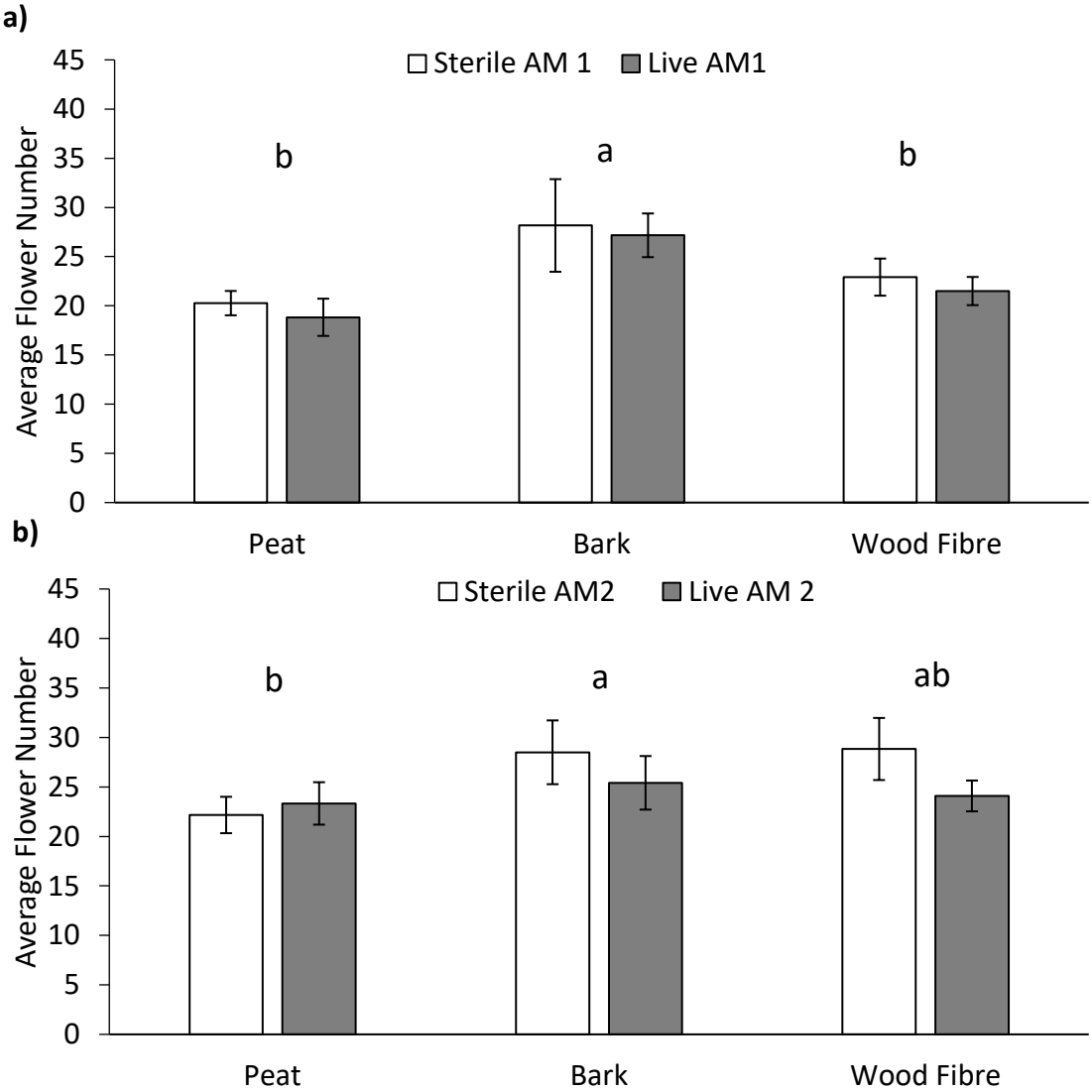


Figure 3.10 Average flower number of plants grown in each media with each commercial inoculum: a) AM1 and b) AM2. Bars with different letters have statistically different means, p<0.05. n=12, bars±SE.

3.4.4 Porosity

There was no difference found between total, air filled and water retention porosity in pots from each harvest which allowed the data to be combined. There was also no difference found between porosity measurements in pots treated with live inoculum compared to control plants for either AM1 or AM2 (Table 3.6). In plants treated with AM1 there were no differences found between the total, air filled or water retention porosity between pots of different growing media (Table 3.6, F_2=2.178, F_2=2.184 and F_2=1.533 respectively). However, there were differences in porosity between different growing media in pots treated with AM2: overall bark pots had the highest total porosity, this was only found to be significantly higher than peat in AM2 treated pots (Figure 3.11b). No differences were seen between the water retention in porosity in AM2 pots of different growing media (F_2=1.775, Table 3.6) but peat was found to have significantly lower air filled porosity than both bark and wood fibre (F_2=13.048, Figure 3.12b).

		Total Porosity		AFP		WRP	
AM1	**df**	**F**	**Sig**	**F**	**Sig**	**F**	**Sig**
Media	2	2.178	P>0.05	2.184	P>0.05	1.533	P>0.05
AM	1	0.002	P>0.05	0.143	P>0.05	0.015	P>0.05
*Media*AM*	2	1.581	P>0.05	2.437	P>0.05	1.034	P>0.05
AM 2							
Media	2	4.304	**P<0.05**	13.048	**P<0.001**	1.775	P>0.05
AM	1	3.206	P>0.05	0.017	P>0.05	3.409	P>0.05
*Media*AM*	2	2.797	P>0.05	2.210	P>0.05	1.987	P>0.05

Table 3.6 Results of two-way ANOVAs on total porosity, air-filled porosity (AFP) and water retention porosity (WRP) for each commercial inoculum treatment. Error degrees of freedom: AM1=30, AM2=29.

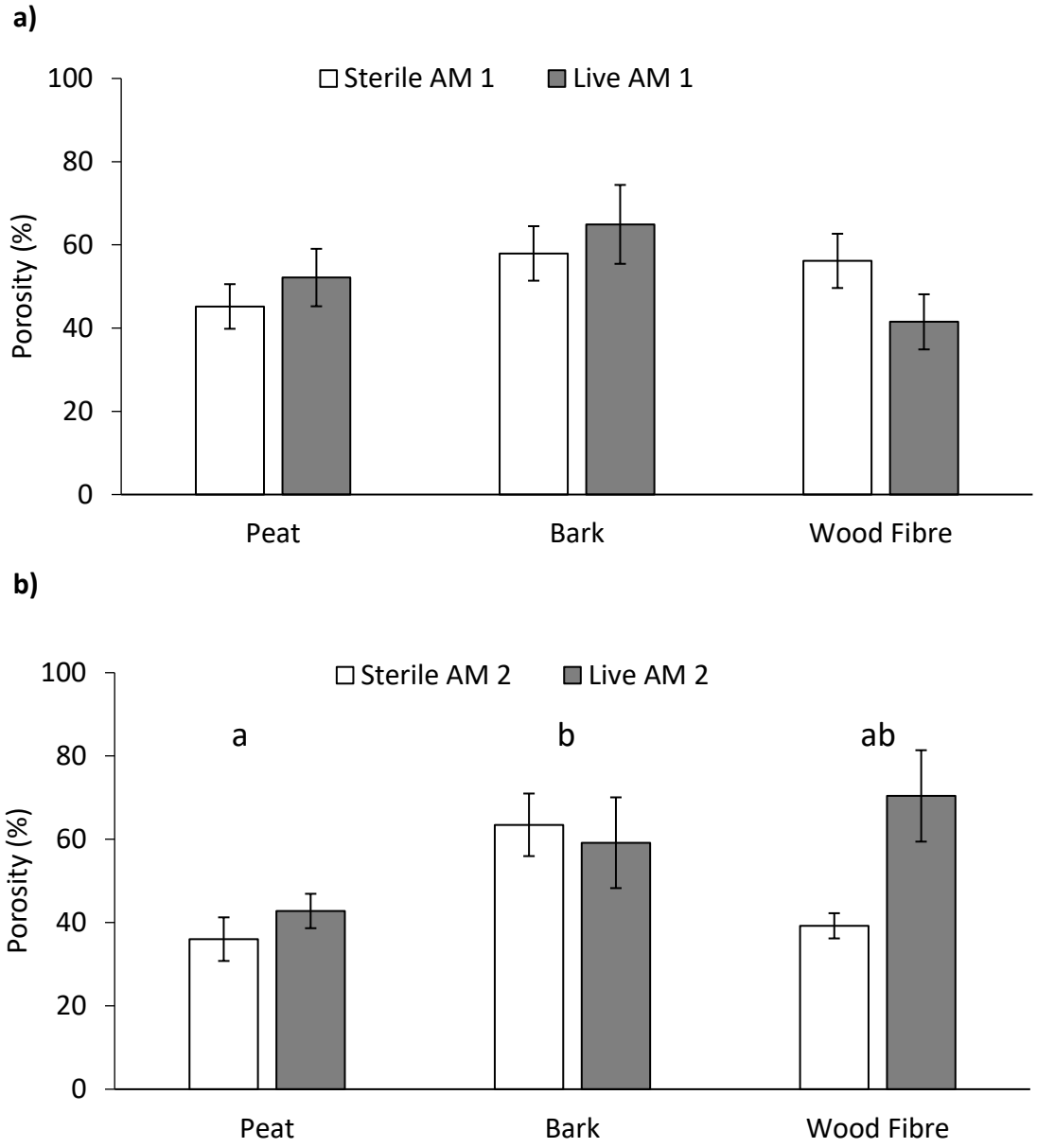
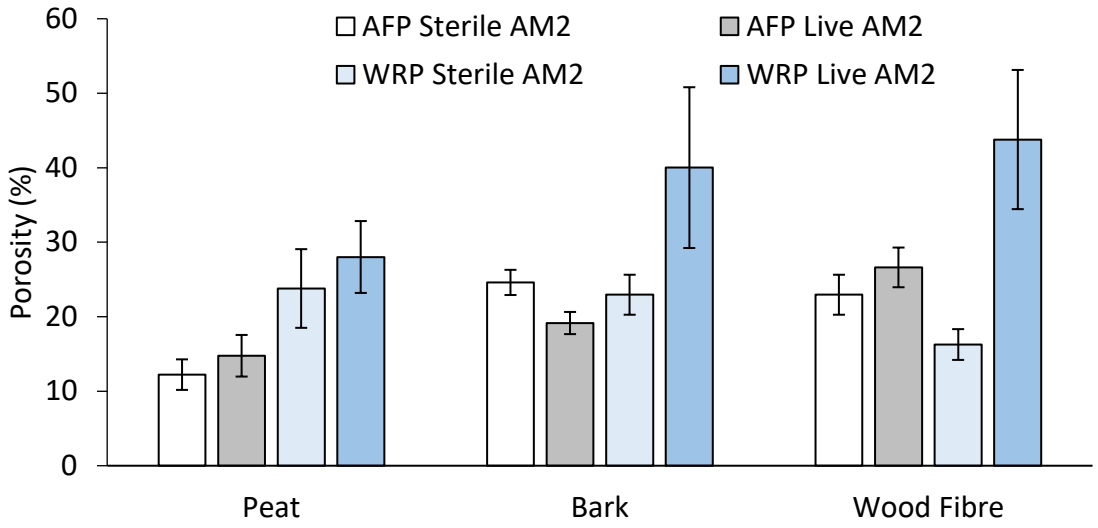


Figure 3.11 Average total porosity of pots containing each growing media treated with a) AM1 or b) AM2. Letters denote statistical differences between growing media means and asterisks denote statistical differences between live and sterile inoculum treatments. p<0.05. n=6, bars±S.E

a)



b)

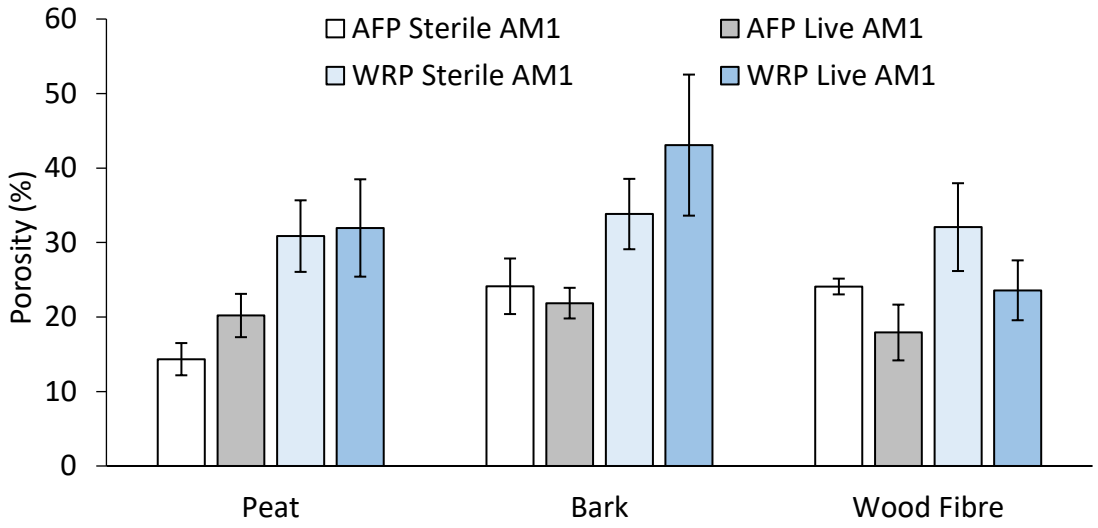


Figure 3.12 Average air-filled porosity (AFP) and water retention porosity (WRP) for each growing media for each commercial inoculum treatment: a) AM1, b) AM2. n=6, bars±SE.

3.4.5 Leaf discolouration scoring

The number of individual plants (out of ten replicates) with purple leaves in plants treated with AM1 was shown to be reduced in all plants that received live inoculum (Figure 3.13a) but this difference was only shown to be significant in plants grown in wood fibre ($t_{17.1}$= -2.419, $p<0.05$). The number of plants with purple leaves in AM2 treated plants did not follow the same consistent pattern because in wood fibre a higher proportion of plants grown with live inoculum had more purple leaves (Figure 3.13b). A reduction in the number of plants with purple leaves with the addition of live AM2 was seen in both peat and bark but only in bark was this effect found to be significant (t_{22}=2.253, $p<0.05$).

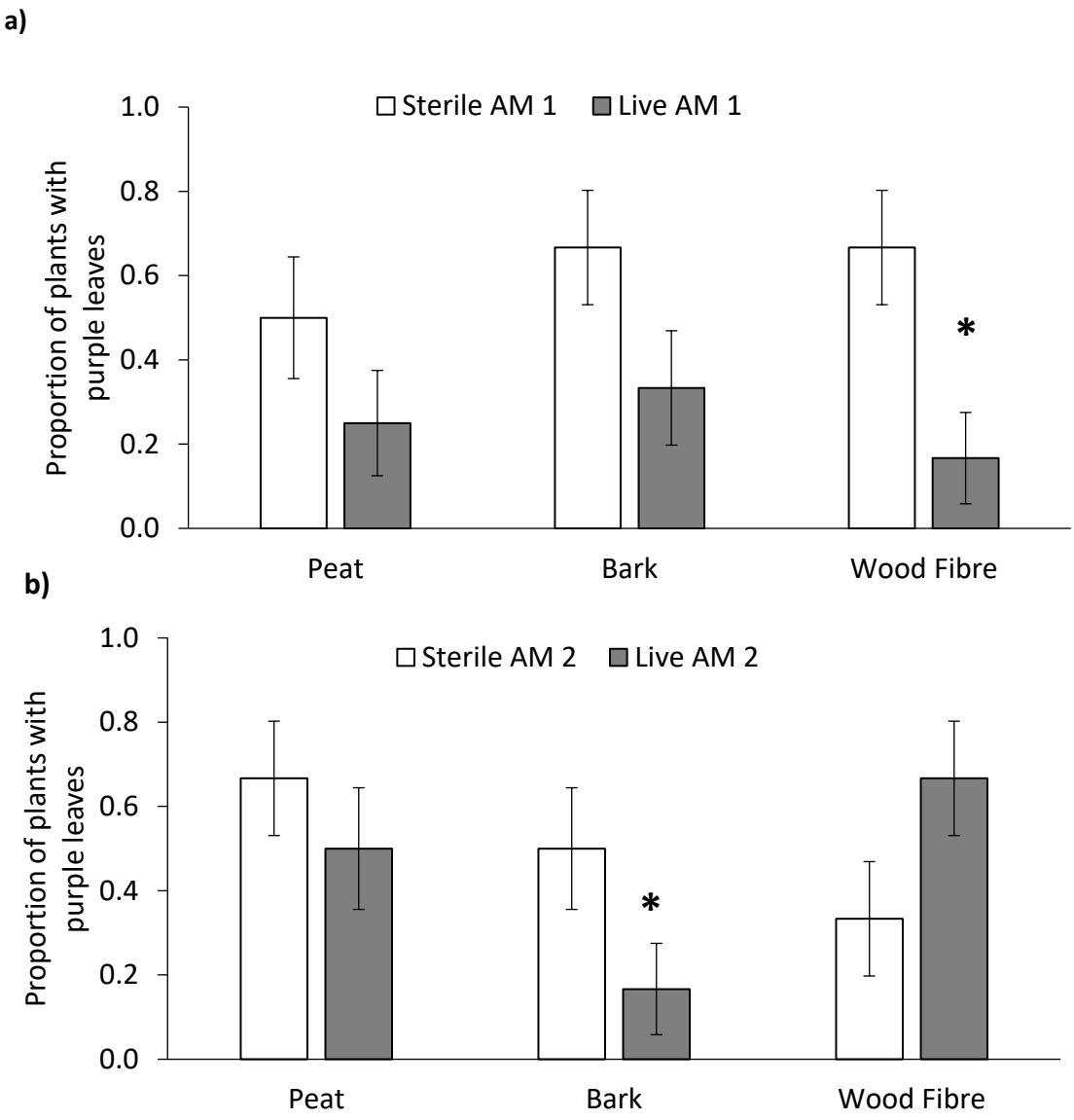


Figure 3.13 The proportion of replicate plants in each growing media and inoculum treatment that had obvious signs of stress in the form of purple leaves. a) AM1, b) AM2. Asterisk denotes statistical significance between pairs of means. n=12, bars±SE

3.4.6 Biomass

3.4.6.1 Effect of Growing Media on Biomass

There was no significant effect of adding live inoculum on the biomass of plants grown in any of the growing media across both harvests (Table 3.7). In harvest one, plants treated with AM1 grown in bark had significantly larger biomass on average compared to plants grown in peat and wood fibre (F_2= 16.534, Figure 3.14a). In both reduced peat media plants treated with live AM2 also appeared to be smaller than control plants but no significant difference were found between treatments (Table 3.7, Figure 3.14b). In harvest two plants grown in bark but treated with AM2 were shown to have significantly higher biomass than plants grown in wood fibre (F_2=5.322, Figure 3.14c).

		Harvest 1		Harvest 2	
AM 1	***d.f***	***F***	***Sig.***	***F***	***Sig.***
Media	2	16.534	**P<0.001**	1.2	P>0.05
AM	1	0.056	P>0.05	0.03	P>0.05
Media*AM	2	2.742	P>0.05	2.098	P>0.05
AM 2					
Media	2	2.174	P>0.05	5.322	**P<0.05**
AM	1	2.254	P>0.05	0.486	P>0.05
Media*AM	2	2.3	P>0.05	1.512	P>0.05

Table 3.7 Results of two-way ANOVAs on dry biomass of plants grown with each commercial inoculum at each harvest. Error degrees of freedom: Harvest 1 AM1=28, AM2=29, Harvest 2 AM1=30, AM2=29.

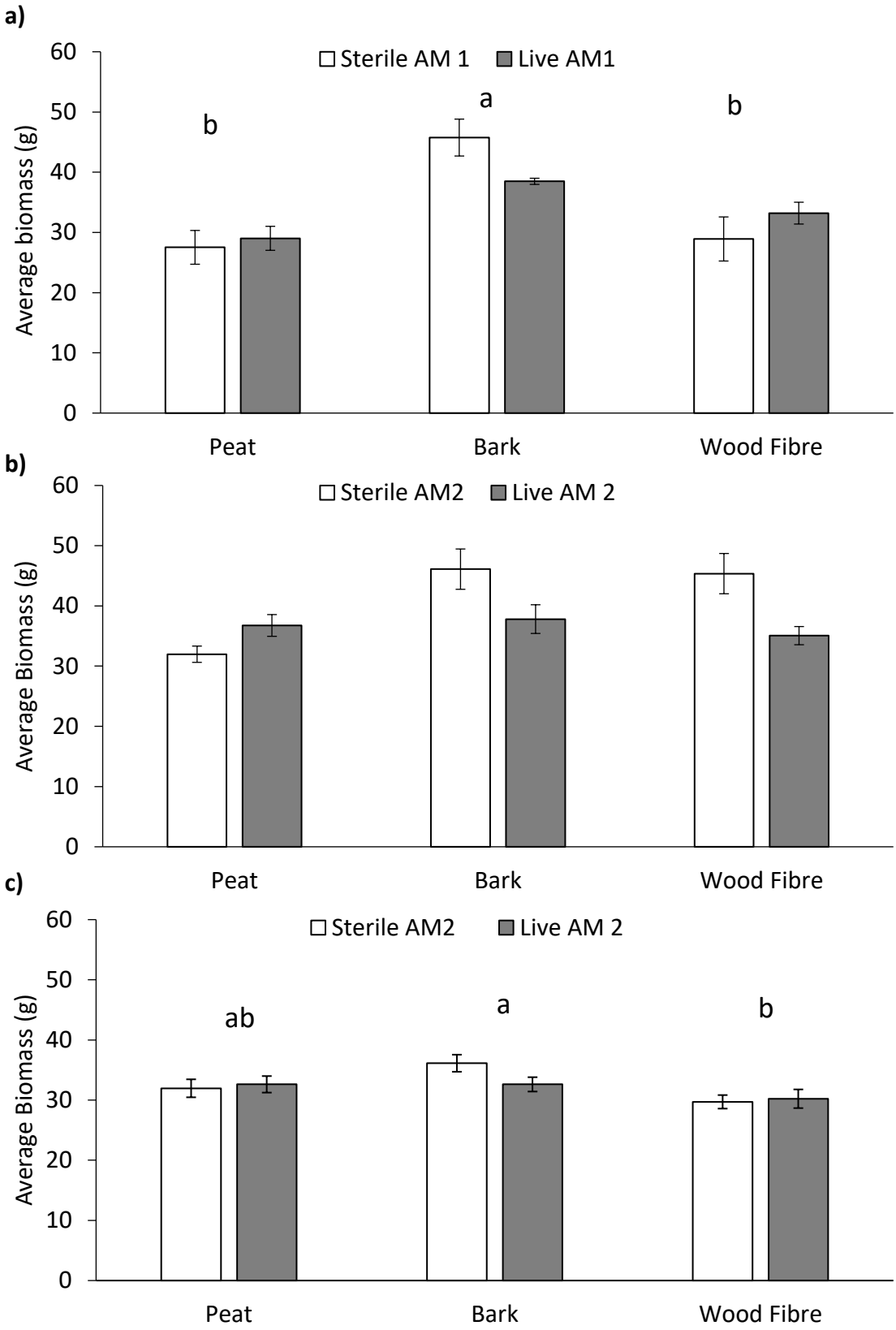


Figure 3.14 Average dry biomass of plants grown in each medium with each commercial inoculum: a) AM1, b) AM2 (both harvest 1), and c) AM2 (harvest 2). Groups of bars with different letters show statistically significant differences between growing media, p=0.05. n=6, bars±SE.

3.4.6.2 Effect of Colonisation on Biomass

The amount of root colonisation was not significantly linked to the biomass of plants with either inoculum treatment. This is an unsurprising result for AM2 inoculated plants as only five plants out of thirty-six were found to show evidence of colonisation in their roots.

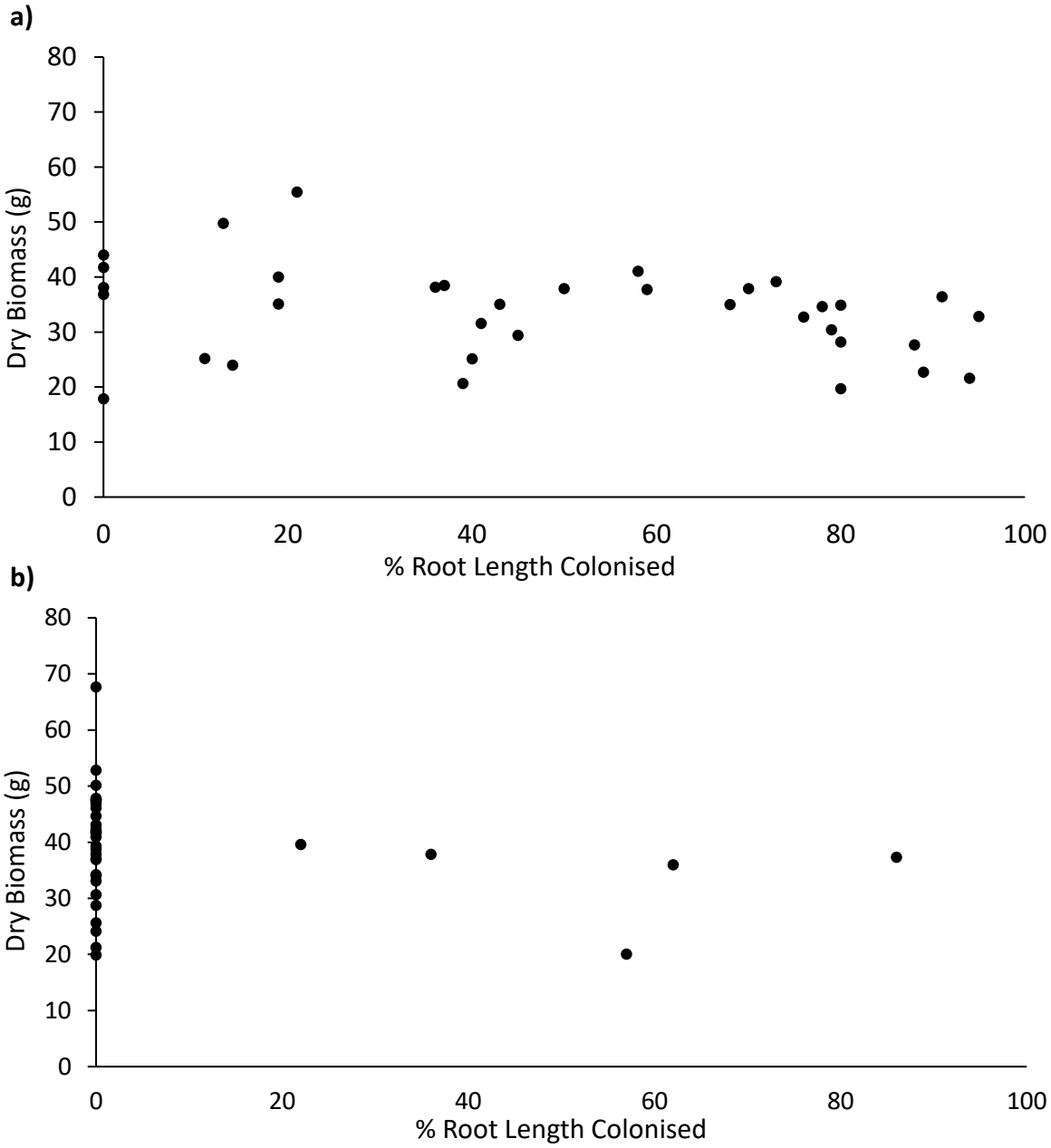


Figure 3.15 Relationship between plant dry biomass and root length colonised by hyphae for plants grown with each commercial inoculum: a) AM1, b) AM2. n=36.

3.4.6.3 Size Inequality

As no major increases or decreases in biomass were seen with addition of either inoculum, the effect of AMF colonisation on the variation in size of plants was investigated for harvest one plants only. Figure 3.16 compares the variation in biomass of sterile and live treated plants in each growing media. The results of the Z test showed the COV of biomass was significantly reduced in plants grown with AM1 in both reduced peat growing media (Figure 3.16b, c), and the 95% confidence intervals did not overlap (Table 3.8). Plants grown with AM2 in the wood fibre media did appear to be less variable in size but this was not found to be significant (Figure3.16f). In peat grown plants the addition of either inoculum did not increase size variation between plants (Figure 3.16a, 3.16d). Plants grown in the bark medium treated with live AM2 (Figure3.16e) were the only live AM group found to significantly increase in size variability when compared to those treated with sterilised AM.

AM1	**Coefficient of Variation**					
	Sterile	**95% CI**		**Live**	**95% CI**	
Peat	24.972	15.940	31.870	16.838	10.030	22.460
Bark	16.126	10.250	22.110	**3.249**	0.484	4.499
Wood Fibre	31.008	21.200	42.790	**10.908**	6.850	17.830
AM2	**Coefficient of Variation**					
	Sterile	**95% CI**		**Live**	**95% CI**	
Peat	18.445	5.460	30.550	0.198		
Bark	9.004	5.377	12.676	26.566	7.870	46.060
Wood Fibre	33.594	4.750	59.150	0.198	10.620	27.700

Table 3.8. The coefficient of variation and 95% confidence intervals calculated for each group of ten replicate plants grown in each growing media with live and sterile inoculum for both AM1 and AM2. Bold numbers indicate CIs do not overlap between live and sterile treatments. No CI was calculated for plants grown in peat with AM2 because of a missing value the sample was too small for bootstrapping.

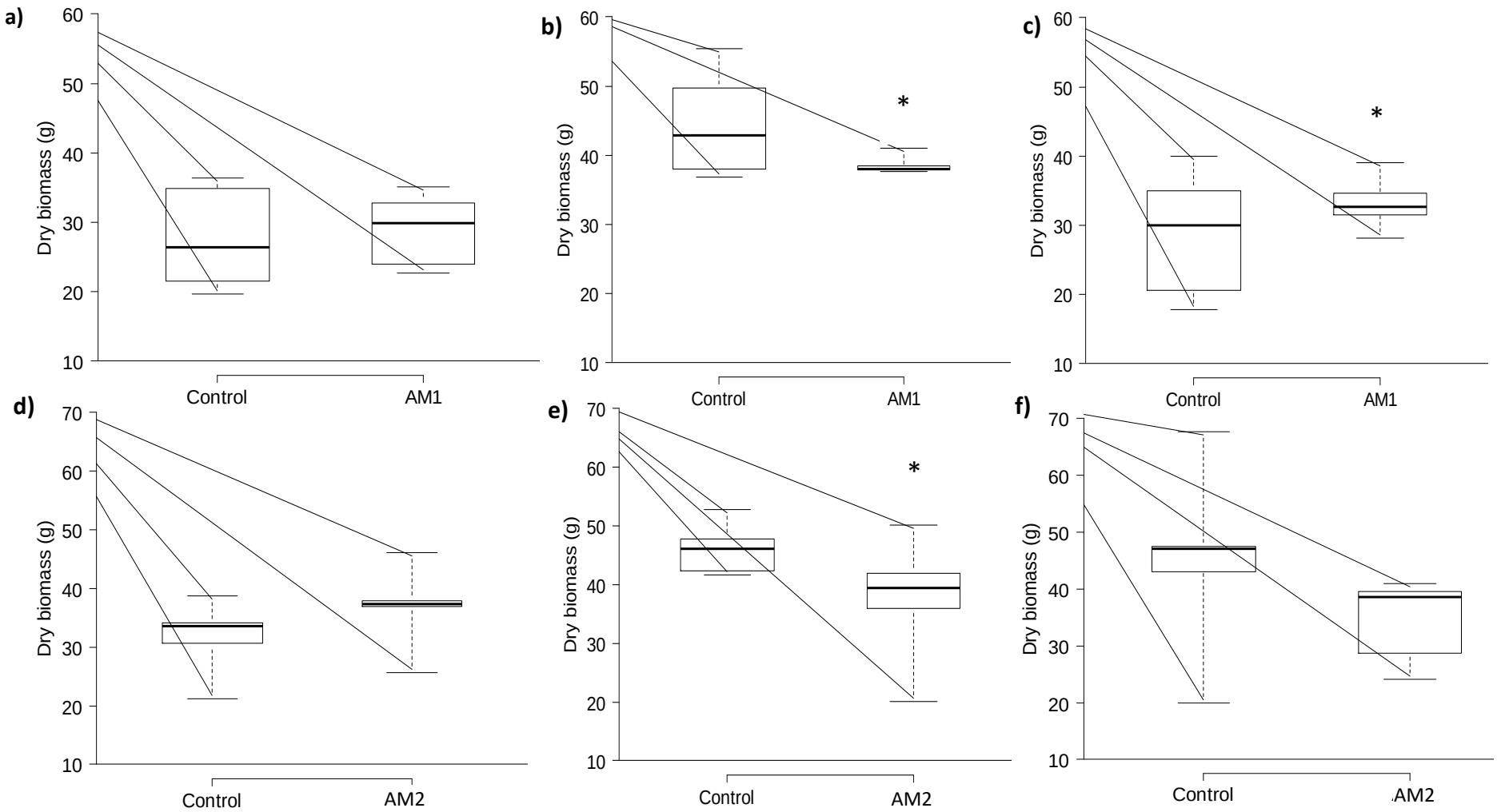


Figure 3.16. Biomass of plants grown with AM1 (a-c) and AM2 (d-f) in each growing media: a) Peat. b) Bark, Z=2.945, p<0.01. c) Wood Fibre, Z=2.056, p<0.05. d) Peat. e) Bark, Z= -2.141, p<0.05. f) Wood Fibre. Asterisks denote statistically different coefficient of variation

To examine this effect further, Lorenz curves were constructed using the cumulative percentage biomass for individuals treated with each live and sterile inoculum in each growing medium for harvest one plants. Gini and Lorenz asymmetry coefficients (LAC) were also calculated for each curve. Comparing the confidence intervals of Gini coefficients in Table 3.9 suggests that the biomass inequality was reduced in both reduced peat media with the addition of AM1. The Lorenz curves (Figure 3.17b, c) and asymmetry coefficients (Table 3.10) indicate that colonisation by mycorrhizas reduced size in equality in two different ways. Bark grown plants had a LAC greater than one which suggested the inequality of biomass is caused by the number of large plants. Comparing the Lorenz curves (Figure3.17b) and boxplots (Figure 3.16b) there were fewer large plants when compared to sterile inoculum plants. The LAC for plants grown in the wood fibre medium showed that the live AM1 treatment resulted in an LAC value less than one; this indicates that size inequality was the result of smaller plants. The graphs (Figure 3.16c, 3.17c) show that AM1 addition caused a reduction in the number of small plants grown in the medium.

The overlap of confidence intervals of the Gini coefficients for plants grown with AM2 did not indicate any differences in equality (Table 3.9), however it was shown that plants grown in bark with live inoculum were more variable in size than control plants. The LAC for plants grown in the bark medium with live AM2 being lower than one suggests that the addition of AMF inoculum was caused by the number of small plants. From the Lorenz curves (Figure 3.17e) and box plots (3.16e) it appears that there was an increase in smaller replicate plants as a result of the live inoculum treatment.

AM1	Gini Coefficient					
	Sterile	**95% CI**		**Live**	**95% CI**	
Peat	0.153	0.123	0.179	0.103	0.072	0.129
Bark	0.098	0.070	0.121	**0.016**	0.004	0.025
Wood Fibre	0.189	0.145	0.235	**0.065**	0.039	0.097
AM2	**Gini Coefficient**					
	Sterile	**95% CI**		**Live**	**95% CI**	
Peat	0.104	0.050	0.285	0.114		
Bark	0.054	0.034	0.071	0.153	0.052	0.261
Wood Fibre	0.186	0.026	0.338	0.112	0.049	0.155

Table 3.9. Gini coefficient calculated for each group of ten replicate plants grown in each growing media with live and sterile inoculum for both AM1 and AM2. Bold numbers indicate CIs do not overlap between live and sterile treatments. No CI was calculated for plants grown in peat with AM2 because of a missing value the sample was too small for bootstrapping.

	Lorenz Asymmetry Coefficient		
AM1	**Sterile AM**	**Live AM**	**Inequality**
Peat	1.223864	0.9167585	=
Bark	1.289165	1.665344	-
Wood Fibre	1.007816	0.4972716	-
AM2	**Sterile AM**	**Live AM**	**Inequality**
Peat	0.7910598	0.7352374	=
Bark	1.136362	0.9672203	+
Wood Fibre	0.7695052	0.8267164	-

Table 3.10. Lorenz Asymmetry coefficient calculated for each group of ten replicate plants grown in each growing media with live and sterile inoculum for both AM1 and AM2. Inequality symbols represent an increase (+), decrease (-) or no change (=) in size inequality with the addition of live AM (according to Lorenz curves, Gini coefficients and coefficient of variation tests).

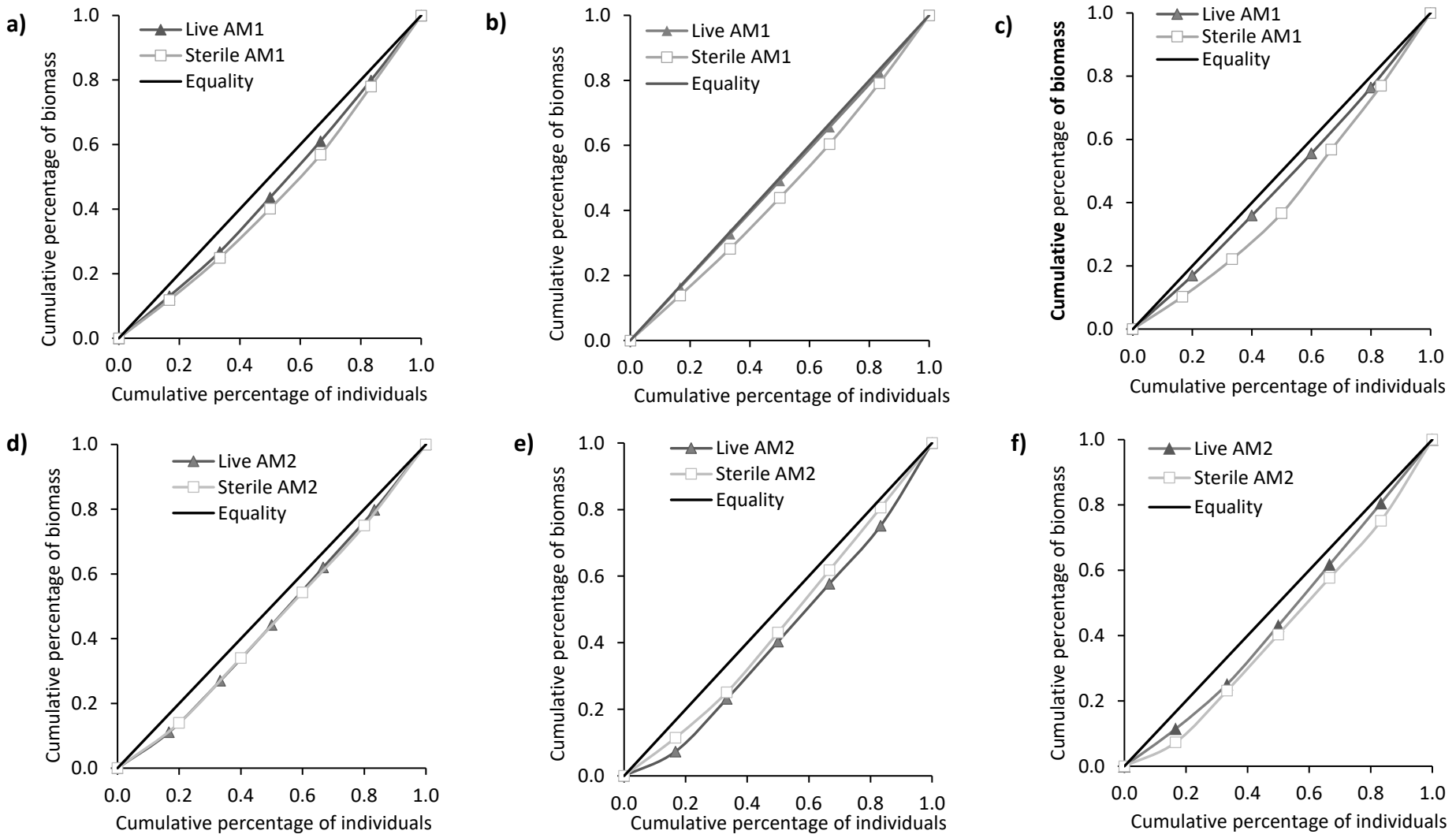


Figure 3.17 Graphical analysis of size inequality of plants using Lorenz curves plotted against lines of equality in Peat, Bark and Wood Fibre in a-c) AM1 and d-f) AM2

3.5 Discussion

3.5.1 Growing Media

For the previous experiment four different commercially available multipurpose composts were purchased from a local garden centre (section 2.2.2). After finding the variability of these commercial multipurpose composts too great, and with evidence that the low peat and wood based mixes produced the best performing plants (section 2.5.1), the media treatments were reduced to one peat control and two reduced peat, wood based mixes in this experiment. Reduced peat media, rather than peat free were used because these ingredients cannot be used in isolation. A 25% reduction is already being used as an industry standard by UK growers (Else, 2013) and this research hopes to show how the addition of AMF could facilitate an increase in this amendment. Schmilewski (2008) summed up that "*highly technical and specialised horticulture is impossible without peat*".

3.5.2 AMF Colonisation

3.5.1.1 Sterilised Controls

Presence of hyphae was recorded in control plants in this experiment with colonisation levels in peat plants treated with sterilised AM1 being higher than plants treated with live AM1. Low levels of colonisation could be expected in control plants due to small amounts of contamination through spore transfer either during the potting process or due to the pots being grown outside where they could be exposed to external sources of inoculum. A small amount of colonisation recording could also be due to the misidentification of hyphae as mycorrhizal when arbuscules or vesicles were not present. Other fungal endophytes or symbionts could be present in root material which will have been stained and the morphological differences between the hyphae of different fungal species are very small.

The high proportion of colonised roots in peat treated with sterile AM1 suggest that the sterilisation technique used in this experiment was not sufficient to deactivate the propagules in AM1. Gamma radiation is often cited (Hayman, 1974; Herrmann and Lesueur, 2013; Gosling, Jones and Bending, 2016) as the most effective method for successful sterilisation of mycorrhizal material but access to such radiation was not available for these experiments. A more effective sterilisation technique along with negative (no inoculum added) controls should be used for future experiments to ensure data can be collected from plants that have not been colonised. The sterilisation technique did appear to be effective with AM2 as no hyphal presence was recorded in sterilised inoculum treated plants in bark and peat and an average of less than three

percent was found in wood fibre. However, this inoculum performed poorly even without sterilisation, it should be noted that the large number of roots where no colonisation was recorded for this inoculum could be due to the recording method which uses a small sample (approximately 10 roots per pot).

The majority of control plants in the literature (pot based experiments) have no mycorrhizal inoculum added and they result in no colonisation (Clapperton and Reid, 1992; Klironomos and Hart, 2002; Linderman and Davis, 2003; Berruti *et al.*, 2013; Jin *et al.,* 2013) but there are some examples of sterilised inoculum or growing media resulting in colonisation. Most involve field grown plants (Ayres, Gange and Aplin, 2006; Candido *et al.*, 2015) but Ortas and Ustuner (2013) had control plants in pots which received autoclaved growing media as a control treatment and levels of colonisation in their roots ranged from 3.3-10%. When grown in peat, orange seedlings inoculated with two species of fungi (*Glomus intraradices* and *Glomus margarita*) had equal or lower levels of colonisation than control plants. Only two species out of the nine-remaining AMF species produced plants with significantly higher levels of root colonisation than the control plants and this included a mixed species commercial inoculum product like AM1 and AM2. In other trials non-inoculated control plants found to have no root colonisation by AMF at transplantation stage have been found to have low levels of colonisation (~6%) in their roots after seven weeks of growth (Sohn *et al.*, 2003), the authors suggested this was due to contamination when one treatment group was inoculated during transplanting. This could also have been the case in this experiment but the large levels in peat suggests the source of contamination from the growing media which could be due to the fact it is not sterilised. Despite evidence of colonisation in control plants in this experiment, live inoculum controls were found to have significantly higher levels of root length colonisation than sterilised controls (AM1) or no evidence of colonisation was found in sterile controls (AM2) in the reduced peat media.

3.5.1.2 Comparing AM1 and AM2

The increased performance of AM1 could be due to differences in propagules between the inocula (IJdo, Cranenbrouck and Declerck, 2011). AM1 consists of propagules made up of root fragments, hyphal fragments and spores in the granular substrate the bait plants were grown to produce the inoculum; compared to AM2 which is simply made up of spores which have been mixed with an inert powder carrier. It has been shown that, depending on the species of AMF colonisation can be successfully achieved through colonised root and hyphal fragments as well as spores but that some species can only achieve colonisation with spores (Klironomos and Hart, 2002). Inoculum made from colonised root material, hyphae spores and sometimes soil (in a similar way to AM1) has also shown to be highly effective at colonising roots and is often used

in the literature (Graham, Linderman and Menge, 1982; Son and Smith, 1988; Koide *et al.*, 1999; Gaur, Gaur and Adholeya, 2000; Sohn *et al.*, 2003; Stonor *et al.*, 2014), perhaps because isolating spores is more time consuming but perhaps because the use of a propagule mixture is more effective.

The batch of spore-only, AM2 inoculum used in previous experiments (Section 2.4.4.1) did manage to colonise plant roots successfully, so the extremely poor performance of AM2 in this experiment supports the negative opinions on the unreliability of commercial inoculum (Corkidi & Evans 2004; Tarbell & Koske 2007; Faye et al. 2013; Berruti et al. 2013). On speaking to the manufacturers, no other user of this batch of inoculum raised the same concerns this year and the transport and storage of the inoculum was not considered a reason for this low performance. A sample of AM2 used in this experiment and along with a sample from the batch used to inoculate plants in future experiments outlined in Chapters 4 and 5 was sent back to the manufacturer for testing. Unfortunately, at the time of submission no results had been provided.

3.5.1.3 Commercial Inoculum Effectiveness

The differences in performance of the two commercial inocula did allow for a direct comparison of colonisation and the effects seen in order to evaluate their effectiveness. Low levels of colonisation and large numbers of uncolonized plants in AM2 correlated to lack of significant differences seen between live and sterile inoculum treated plants for all variables. In contrast, addition with live AM1 showed consistently high levels of root length colonisation compared to controls and this resulted in significant reductions in the number of plants displaying purple leaves and in the variability of biomass of plants in both peat alternatives. There was also no difference found between the amount of colonised root tissue which contained arbuscules or vesicles between live AM1 treated plants in each growing media. This is promising as it indicates that colonised plants were undergoing nutrient exchange and that the performance of the mycorrhizae was not adversely affected by the growing media.

Higher numbers of vesicles than arbuscules was also relatively consistent, but this could be because arbuscules are more difficult to see and identify. Overall colonisation of plants in this experiment with both commercial inocula (0-95%) did not differ greatly from results seen with other non-specific commercial AMF products (Table 3.11) but results do show how variable these inocula can be. In many cases colonisation with AM1 had a greater range but had higher levels of colonisation in some plants than all products in Table 3.11.

Min %	Max %	Study	Min %	Max %	Study	Min %	Max %	Study
0.0	1.50	(Berruti *et al.*, 2013)	10.0	46.7	(Ortas and Ustuner, 2014b)	32.0	57.8	(Gaur, Adholeya and Mukerji, 1998)
20.0	30.0	(Perner, Schwarz and George, 2006)	36.0	47.0	(Yildiz, 2010)	0.0	60.0	(Faye *et al.*, 2013)
15.0	36.0	(Perner *et al.*, 2007)	0.8	50.7	(Corkidi *et al.*, 2004)	18.0	70.0	(Puschel, Rydlova and Vosatka, 2014)

Table 3.11. Minimum and maximum root length colonisation (%) recorded from plants grown with commercial mycorrhizal inoculum.

3.5.2 Weather Data

Seasonal differences in weather resulted in the two harvests experiencing different conditions at the same growth period with the weather being cooler and light levels lower for plants harvested in the second batch. Difference between harvests resulted in a significant effect of harvest on various growth measurements, overall plants grown in harvest two were smaller. Stonor et al. (2014) show that shade (low light) did reduce growth but in both mycorrhizal and non-mycorrhizal plants.

Reduced light levels (Son & Smith 1988; Johnson et al. 1997; Bever 2002; Stonor et al. 2014) and low temperatures (Hayman 1974) have been shown to reduce increases in biomass seen with mycorrhizal colonisation by having an effect on the cost: benefit relationship between mycorrhizas and plants. In this case, where harvests were analysed separately, there were few significant effects of growing media and no significant effect of inoculum treatment seen in harvest two plants for any growth parameter. This is unsurprising for AM2 treated plants as there was no difference in colonisation between control and live treatments. For AM1 however, the lack of significant benefit (increase in biomass, height etc.) or parasitic effect in harvest two plants of AMF indicating a commensalism could be the result of reduced temperature and light levels. This would have reduced the photosynthetic rate of plants which could lead to a reduction in the cost:benefit relationship with the colonised AMF which would explain the lack of differences between live and sterile treatments. However, growth depression isn't always

caused by lack of colonisation (due to low photosynthetic output) or by reduction in nutrient transfer from AMF fungi (Stonor *et al.*, 2014).

3.5.3 Plant Growth Measurements

3.5.3.1 Plant Height

Consistent patterns in the effect of each inoculum treatment within the growing media over two harvests suggests there could be factors caused by the growing media affecting the symbiosis. Studies have shown that plant height increases with AMF colonisation (Lu and Koide, 1994; Wu, Xia and Zou, 2008; Ortas and Ustuner, 2014a), however in some trials which test multiple inoculum sources or commercial products very few cause an increase in height. In two studies involving maize only one out of ten different inoculum treatments resulted in significantly taller plants when compared to controls (Corkidi *et al.*, 2004) and only in one out of three different growing media, when using commercial inoculum only two out of twelve different AMF products significantly increased the height of maize plants (Faye *et al.*, 2013). With plant height increase plants showed a similar effect where each inoculum had a significant effect on plant height in a different growing media. Plants grown in bark compost with live AM1 were significantly shorter than control plants and this was the only negative effect of AM1. Wood fibre plants treated with AM2 were significantly shorter than controls which seems to suggest this inoculum to be more compatible with the bark growing media. This was similar to results seen in the previous experiment (section 2.4.2.1) where plant height was increased in plants grown in the wood fibre compost with AM1 but no effect was seen with AM2 and the opposite for green waste compost which contained bark fines. It has been shown that substrates can have an effect on mycorrhizal colonisation and performance (Linderman & Davis 2003) and it has been suggested that this could be due to the interaction with the physical structure of the media and the AMF's ability to produce an external mycelium (Graham, Linderman and Menge, 1982) This will be discussed further in section 3.5.5.

3.5.3.2 Flower Number

The addition of live AM1 and AM2 to plants growing in bark and wood fibre did not increase the number of flowers produced. This lack of positive effect is important as reduction in flower number would not be a trait that would promote the use of AMF to gardeners or professional growers. If the reason for adding AMF is to increase the amount of nutrients a plant receives,

more nutrients should be available to the plant to make more flowers. Reduction in vegetative growth in favour of flower production towards the end of the season would be expected and studies have shown mycorrhizal colonisation increasing the number of flowers plants produce when grown in peat based substrate (Sohn *et al.*, 2003; Perner *et al.*, 2007; Berruti *et al.*, 2013; Puschel, Rydlova and Vosatka, 2014; Bona *et al.*, 2015). There is evidence of mycorrhizas having no effect on flower number in substrates containing increased levels of phosphorus (Lu and Koide, 1994), and increasing fertiliser levels has been shown to reduce flower number (despite increasing biomass) in greenhouse grown *T. patula* (Bi *et al.*, 2010). In another greenhouse experiment mycorrhizal colonisation of *Plantago lanceolata* was also found to have no effect on flower number, despite increasing foliar biomass (Ayres, Gange and Aplin, 2006).

The different growing media treatments did have a significant effect on the number of flowers produced by plants grown in harvest one and with both AM1 and AM2 bark grown plants produced more flowers than plants grown in peat. The reduced number of flowers produced by peat in this experiment is surprising. A study which looked at the performance of fuchsia plants in different potting substrates under different watering regimes showed that peat plants produced more flowers than those grown in wood fibre when given the optimum amount of water. The negative effect on plant performance was explained due to non-uniform distribution of water in pots containing wood fibre. As plants in this experiment should all have received the same irrigation the resulting flower number differences between media could also be the result of the physical properties of each substrate. Both bark and wood fibre can be used to increase air filled porosity and drainage of peat (Barrett *et al.*, 2016) so maybe it was this ability which improved flower production in both reduced peat media. Pine bark mixed with peat has been shown to have higher water holding capacity than peat alone (Hidalgo, Matt and Harkess, 2006).

3.5.4 Porosity

The measurements of air filled porosity and water holding capacity along with properties such as bulk density would normally be taken using fresh media before it is used for planting. In this experiment, in order to quantify these properties as well as the effect of the addition of each inoculum carrier, and root colonisation by AMF, a modified technique was used after the plants had been growing for three months. This method was based around the porometer method (Fonteno and Harden, no date; Bilderback, 2009). These measurements are therefore only an indication of the actual media structure as the presence of roots and hyphae will have disrupted the natural composition of each substrate.

One of the reasons peat is favoured by growers is for its air-filled porosity (Alexander *et al.*, 2008, 2009; Barrett *et al.*, 2016) and one of the problems found with alternative media is the variability in water holding capacity and drainage (Alexander *et al.*, 2009; Alexander and Williams, 2012). In order to facilitate the use of these sustainable substrates in uniform conditions, on a large commercial scale they would have to be relatively free draining but also not dry out too quickly. Bark and wood fibre both have high air holding capacity so they have been used to increase air space in peat (Bilderback and Lorscheider, 1995; Schmilewski, 2008). Wood fibre also improves the re-wetting capacity of peat but bark can be used to improve its water holding capacity (Bilderback and Lorscheider, 1995). This was demonstrated with bark and wood fibre pots having significantly higher air-filled porosity and bark pots having the highest water retention porosity.

As previously stated one of the problems seen with wood fibre is that water does not distribute evenly through the media and it struggles to retain it (Alexander, Williams and Nevison, 2014). It was thought that mycorrhizas could help improve the water filtration properties of both bark and wood fibre because of the evidence of their ability to alter the structure of soil (Rillig, Wright and Eviner, 1998; Rillig and Steinberg, 2002; Rillig and Mummey, 2006; Medina and Azcón, 2010). This ability is most likely due to the production of a hydrophobic, glycoprotein known as glomalin related soil protein (GRSP) produced by the extraradical mycelium. (Wright & Upadhyaya, 1996). This insoluble glycoprotein coating is found on the outside of hyphae and spores, it could be necessary for hyphae to be able to transport water. Using immunofluorescence glomalin has been shown to also be present in soil aggregates found around colonised plants and its concentration is positively correlated with the water stability of those aggregates. (Wright & Upadhyaya, 1998). This has led to the observation that the production of these insoluble proteins helps to form water stable aggregates which aid water infiltration of soil as well as airing of root tissue. The, as yet undescribed, gene products of AMF and fractions of them in soil are collectively grouped and known as glomalin related soil proteins (GRSPs) as extraction methods will always include a mixture but the relationship between them is still not fully clear (Rillig, et al., 2002).

Aggregate stability is a good measure of a soils ability to maintain good water infiltration, aeration and allow space for roots to grow which are essential for optimal plant growth. (Oades, 1984) While there may be a saturation point where adding more GRSP will not result in increased soil aggregate water stability, (Rillig, et al., 2002), one could hypothesise that the presence of more hyphae would increase the amount of GRSPs which could improve water filtration and drainage through substrates. Indeed, there are studies which have shown that GRSP

concentration and soil aggregate stability have been positively correlated to mycorrhizal root volume and that the size of aggregates were positively correlated with the number of AMF hyphae present in pots (Bedini *et al.*, 2009). The effect on water stable aggregates has also been shown to indirectly enhance plant growth by increasing the water retention porosity of drought stressed plants in pots (Graham, Linderman and Menge, 1982; Wu, Xia and Zou, 2008; Medina, Vassilev and Azcón, 2010).

Despite no significant differences there did appear to be an increase in water retention porosity in the reduced peat media with the addition of live AM2 inoculum. This effect could be due to the ability of mycorrhizal hyphae to increase overall porosity by creating pore spaces. This effect should be examined further in future experiments where higher levels of colonisation could allow correlation with porosity to be tested.

3.5.5 Plant Stress Response

An indication that some plants were under stress after three months of growth was shown by the discolouration of their leaves from green to purple. Purpling of the dorsal side of the leaf has been described as a sign of phosphorus deficiency (Woolley and Broyer, 1957) and gardening websites also claim that this is the reason for the purpling of leaves seen in plants (Day, 2011; Patterson, 2015). In phosphorus deficient environments, mobilisation and transport of the nutrient away from old tissues will occur, often resulting in purpling of older leaves first. It was apparent that in some plants the majority of leaves were purple at the time of harvesting, suggesting that these plants had been nutrient stressed for some time. As all plants received the same amount of nutrients and there was no difference in the number of purple plants between different growing media, plants must have been able to extract phosphorus from each growing media equally. The most striking difference was seen between live and sterile treated plants grown with AM1 in the two reduced peat media. Unsurprisingly no differences were seen between the number of purple plants in different AMF treatments in peat because the colonisation levels were the same. But the significant increase in root colonisation in live treated compared to sterilised control plants resulted in a significant reduction in the number of purple plants in both the bark and wood fibre media. This reduction can be explained by the well documented ability of AMF to provide plants with phosphorus when levels surrounding roots are low (Smith and Gianinazzi-Pearson, 1988; Smith and Read, 2002b; Marschner, 2012).

Species of AMF have been shown to differ in their ability to provide the plants with certain nutrients including phosphorus, (Mensah *et al.*, 2015) this could explain the differences in effect seen between AM1 and AM2 treated plants grown in wood fibre. However, as levels of colonisation were so low it is not surprising that little effect or a negative effect was seen in AM2 plants. This effect should be investigated using more replicates and improved colonisation as the aesthetic properties of ornamental plants is important, not only to gardeners but for the industry which relies on plants to be sold for profit. Potted plants which become visibly stressed before they are sold or soon after they are bought would not be favourable, so the presence of AMF improving this to allow for more sustainable growing media to be used would be very valuable to the industry.

3.5.6 Biomass

In harvest one, bark compost mixed with AM1 produced the largest plants compared to the other growing media and in harvest two bark compost mixed with AM2 produced significantly larger plants than wood fibre which were also comparable to peat grown plants. This is not what was hypothesised as previously (section 2.4.3) wood fibre media produced the largest plants, but increased biomass has often been shown as the result of increased water uptake (Bryla and J M Duniway, 1997; Bryla and John M. Duniway, 1997; Wu, Xia and Zou, 2008; Ortas and Ustuner, 2014b) and the water retention porosity of bark pots was significantly higher. Despite being the largest overall there were consistent (non-significant) decreases in the biomass of plants grown in bark with live AMF across both inocula and harvests, this was also seen with plant height. The consistent effect of both inocula in wood fibre also matches the patterns seen with plant height; live AM2 produced smaller plants or plants that were the same size as control plants and live AM1 produced slightly larger plants. Despite the highest level of AMF colonisation peat grown plants were the smallest which suggests a lack of benefit or negative effect on growth from the symbiosis.

Growth depressions as a result of mycorrhizal colonisation have been seen but often they are the result of high nutrient levels (Peng *et al.*, 1993; Klironomos, 2003; Koide and Mosse, 2004; Tavakkoli, Rengasamy and McDonald, 2010), as all pots should have contained similar nutrient levels, and significant growth depressions were only seen in harvest one this is probably not the cause in this case. Reduction in biomass or lack of biomass increase between mycorrhizal and non-mycorrhizal plants has also been attributed to low light levels as it reduces photosynthetic rate (Son and Smith, 1988; Smith and Read, 2008; Stonor *et al.*, 2014), this (coupled with reduced

overall biomass) could explain the lack of biomass differences seen in harvest two plants across all treatments. Small plants produced by peat and consistent growth depression in live treated bark plants could be explained by the increased presence of vesicles. AMF have been shown to produce more vesicles in summer in response to decreasing plant growth and higher temperatures, production is increased because of the approaching end of the growing season (García and Mendoza, 2008). This would make sense as vesicles are thought to be storage structures or potential propagules (sources of regeneration) for the fungus and the presence of these has been shown to decrease plant size, perhaps because more photosynthetic product is required to produce these (Jin *et al.*, 2017). Vesicles were present in nearly all roots inoculated with AM1, the highest in peat plants but there were more vesicles in live inoculated bark plants than in sterile ones.

However, this does not explain the contrasting effects of AM1 (increase) and AM2 (decrease) in wood fibre as vesicles were higher in live inoculated plants with AM1 and very few vesicles were recorded in roots of plants grown with AM2. The difference could be explained by colonisation with different AMF species which could confer different levels of plant benefit (Mensah *et al.*, 2015). This is also clear when correlating increases and decreases in biomass of plants with inoculum addition to plant size inequality and the effect of each inoculum in each growing media.

3.5.6.1 Size Inequality

It has been shown repeatedly that plant growth can be increased through colonisation by AMF, these include height (Sohn *et al.*, 2003; Rasouli-sadaghiani *et al.*, 2010; Asrar and Elhindi, 2011; Ortas and Ustuner, 2014a), shoot biomass (dry weight) (Treseder, 2013; Berruti *et al.*, 2016), fruit yield (Gagné *et al.*, 1993; Gaur, Adholeya and Mukerji, 1998; Bona *et al.*, 2015; Candido *et al.*, 2015) and flower number (Gaur, Gaur and Adholeya, 2000; Poulton *et al.*, 2002).

Investigating the effects of AMF on size variation resulted in perhaps the most interesting find of this study. Significant decreases in the coefficient of variation of biomass of plants grown in both reduced peat media treated with AM1 were supported by the reduction in their standard error, range, and Gini coefficients compared to control plants. Lack of significant effect of inoculum for peat grown plants was expected given the high levels of root colonisation but as the focus for this effect was more important in reduced peat media this was not a disappointing result, it also helped to support root colonisation as the cause of decreased variability in the other media. The unexpected result was that in bark pots treated with live AM2 plants were

significantly more variable in size. Due to half the number of replicate plants in bark and wood fibre not being colonised, any skew in size could be due to the effect of AMF on very few plants.

It has been found that plant size inequality was reduced by mycorrhizas in P*lantago lanceolata* when planted on their own in field plots (Ayres, Gange and Aplin, 2006). Ayres et al. found a similar effect seen in wood fibre grown plants here, where there were fewer small individuals and overall biomass was increased in mycorrhizal plants. Levels of mycorrhizal colonisation in controls compared to live treated plants were also similar to this experiment as complete negative controls were not used. In the same experiment Ayres et al. (2006) found that high levels of colonisation in *P.lanceolata* increased size inequality when plants were grown in large pots under glass. The theory given for this contrast in results is that the high levels of colonisation in a limited nutrient environment caused them to deplete available nutrients limiting plant growth and becoming carbon parasites.

Plants used in this experiment were grown in pots but they had sufficient nutrients to prevent them suffering nutrient depletion within their 12-week period (which was eight weeks shorter than the *P.lanceolata* in Ayres et al. (2006)). Despite seeing equally high levels of colonisation in plants, these were grown outside and not under glass therefore it is understandable that these results are similar to those seen in the field grown *P.lanceolata*. Contrasting literature has suggested that in field populations or communities of plants in containers which share a common mycorrhizal network size inequality would be increased, likely driven by below-ground competition (Weremijewicz and Janos, 2013).

The use of Lorenz asymmetry coefficient and box plots described the skew in plant size which helped to explain the differences (albeit non-significant) seen in biomass in live and sterile AM1 treated plants in each media. Where bark live treated plants had smaller biomass on average compared to sterile controls because there were fewer larger plants, and live AM1 wood fibre plants showed an increase in biomass on average because the number of smaller plants had been reduced. These differences suggest an effect of growing media on the mycorrhizal symbiosis. Recent research into 'partner selection' could begin to explain how two different inocula could result in colonisation by beneficial, mutualistic or parasitic AMF species (Kiers *et al.*, 2011; Walder and van der Heijden, 2015; Werner and Kiers, 2015). Different plants and mycorrhizal species can select each other based on factors such as host identity, nutrient availability and mycorrhizal species competition (Werner and Kiers, 2015). As a different mix of AMF species was added in each inoculum the competition dynamics and host selection choices will likely be different for AM1 and AM2, this coupled with differences in water content and

uptake between the growing media creates different environments for each plant. Differences in nutrients, water allocation and level of exchange between a single, or multiple, species of AMF in pots containing different growing media and inoculum could explain the differences in skew shown by the Lorenz asymmetry coefficients. In the wood fibre growing media, a beneficial mycorrhiza could be reducing size inequality by increasing the growth of smaller individuals through increased nutrient transfer (Sweatt and Davies, 1984; Rasouli-sadaghiani *et al.*, 2010; Ortas and Ustuner, 2014a). This would also explain the significant reduction in purple leaves seen in live inoculum treated wood fibre plants. In bark, the number of large plants being reduced could simply be due to the relationship with a less beneficial or more mutualistic AMF species. Carbon exchange with the fungus could limit plant growth, preventing plants in bark from reaching large biomasses. If plants were grown for a longer period a significant reduction in biomass could eventually occur especially if colonisation increased, this would result in the same negative effects seen in the glasshouse plants in the Ayres et al (2006) experiment.

This effect on biomass is extremely positive as amateur gardeners, but most importantly commercial growers put great value in consistency. Plants often have to meet regulation sizes set by distributors and ones that do not meet this will be wasted leading to financial loss. The use of peat free growing media has widely been rejected by growers because of the inconsistency of the plants they produce compared to peat. These results show that where size consistency in control plants in bark (AM2) and wood fibre (AM1) was higher than that of peat control plants the addition of live inoculum significantly reduced variability to lower than that of peat control plants even though peat had high levels of colonisation.

6.1 3.6 Conclusions

To summarise, both alternative growing media produced plants of comparable height, biomass and flower number when compared to those grown in peat. Plants grown in bark produced the only plants that were significantly taller and heavier with more flowers than those grown in peat with wood fibre plants not far behind.

Plants grown with AM1 showed consistently high levels of mycorrhizal colonisation across all growing media. Increased colonisation did not correlate with an increase in biomass and no significant differences in biomass were seen between live and sterilised inoculum treatments. However, treatment with live AM1 inoculum was shown to significantly reduce plant size inequality in both bark and wood fibre grown plants. This is an encouraging result as increased variability in plant performance using alternative peat free growing media is a common

complaint amongst growers and consumers. Treatment with live inoculum was also found to significantly reduce the number of plants showing signs of nutrient stress through purple leaf discolouration. Aesthetic properties and health of plants is also very important to gardeners and growers producing plants for sale.

Despite the lack of significant interaction effects between inoculum and growing media in this experiment AM1 inoculum appeared to cause different effects in bark and wood fibre. Closer investigation into what is causing this interaction, if it is due to colonisation of different AMF species in each media will help to explain these differences. As there is now more data on which species of AMF confer the most benefit to plants. If the AMF species colonising plant roots can be identified then these could be related their effects on plant performance.

There are some things to be considered and improved for future experiments. The lack of colonisation seen in AM2 should be investigated and attempts should be made to prevent it from happening again. This experiment also showed the effects of seasonality on plant growth and plant benefit of mycorrhizas, it was encouraging that this did not significantly affect all measurements and these results do not suggest the need for these experiments to be conducted under controlled conditions in the future. The results from harvest one showed the most significant effects of growing media and inoculum treatment, this was closer to the normal 'planting out' time for amateur gardeners so this schedule will be used again to try and ensure a representative result. Combining harvests in the future and increasing overall replicate number will also allow more reliable results to be obtained for porosity, leaf discoloration and plant size inequality measures. Improvements to the sterilisation technique used for inoculum controls must be made to allow for more reliable comparison of live and control treatments and to increase chances of significant effects of live inoculum treatments. Negative controls could also be used to try and guarantee low levels of colonisation as a comparison.

Chapter 4 - Bedding plants grown outdoors with commercial mycorrhizal inoculum in reduced peat media

4.1.Introduction

The overall objective of this experiment was to see if the positive effects of the commercial inocula the growth of marigolds in peat reduced substrates were repeatable over another growth season. Changes were made to allow for an increase in replicate number so that variability could be reduced for all measurements. Where possible, all other methods were kept the same to allow data to be compared between the growth seasons of two years. Repeatability of results will be important when using this data to persuade industry growers that results will be consistent year to year. The majority of experiments using AMF reported in the literature include only one season of growth which makes it hard to know if any of the effects would be consistent with repeated use. Where experiments have been repeated over seasons, they are usually field based and increases and decreases in plant growth have been related to better weather conditions (Gholamhoseini *et al.*, 2013) and increased rainfall (Nzanza, Marais and Soundy, 2011) respectively. Differences in the effect of AMF were seen between seasons, in tomato the increase of early fruit yield with AMF was decreased by almost half in the second season (Nzanza, Marais and Soundy, 2011). Some glasshouse pot based trials using commercial inocula have been repeated over two growing seasons which have also shown positive effects of AMF on flower number and fresh shoot weight disappear in the second year, along with an increase in colonisation (Aboul-Nasr, 1996; Matysiak and Falkowski, 2010). Corkidi et al. (2004) also found root colonisation increased in the second season but plant growth responses remained similar. If similar results could be shown across two growth seasons of this outdoor experiment where more variability would be expected, then growers could have more faith that these products could be used in nursery conditions and gardeners should also expect to see consistent effects when using more sustainable media.

Based on data from Chapter 3, it was hypothesised that bedding plants in both reduced peat media would match the performance of those grown in peat but that the addition of live inoculum, especially AM1, would reduce size inequality and visible signs of nutrient stress of plants compared to sterilised inoculum and non-mycorrhizal controls. It was also hoped that increased replicate number, as a result of a combined harvest, would improve the consistency of AMF effects on plant growth and porosity across growing media.

4.2. Materials and Methods

4.2.1. Growing media

The same three growing media outlined in section 3.2.1 were used for this experiment. They were sourced from the same manufacturer (Bulrush Ltd) and contained the same mix of materials and nutrients.

4.2.2. Commercial AMF

The same commercial inocula AM1 and AM2 as outlined in section 2.2.2 were used for this experiment however they were from new batches produced in 2015 as the recommended shelf life for these products does not exceed a year (as recommended by manufacturers). After extremely low colonisation scores across all experiments in 2015 we were recommended by the manufacturer, who suspected low germination rate of spores, to increase the dose to 0.8g per pot. Results on the tests of AM2 were not available before the experiment was due to start.

Following advice from manufacturers an alternative formula of AM2 was also used in 2016 experiments to try and ensure colonisation, hereafter termed AM3. It contained the same endomycorrhizal mix as AM2 but with additional symbiotic microbes: five species of a beneficial soil fungi (*Trichoderma sp.*) and three species of plant growth promoting bacteria (*Bacillus* sp.). The dosage of AM3 was adjusted to make the number of mycorrhizal spores added similar to AM2 so if necessary it could be studied as an alternative. The number of mycorrhizal spores in the mix was diluted by other additions so the dose was increased and 1.6g was added per pot as this equated to approximately 100 spores.

Sterilisation of inoculum in 2015 resulted in marigolds treated with sterilised inoculum containing high levels of hyphae in their roots. It was decided that to improve sterilisation smaller batches of AM1 would be sterilised and each inocula was autoclaved at 121˚C for one hour and then left to cool before being sealed in containers. Sterilised inoculum was used within a week of sterilisation.

4.2.2.1 Root harvest

Root samples were taken from every pot including negative (no inoculum) controls and all six pots used to measure water holding capacity and porosity in order to relate any changes to colonisation. Root samples were also taken for DNA analysis, samples from six replicates of each treatment were taken, these were then rinsed in water in a fine sieve to remove large soil particles then split into two smaller samples and placed in 1.5ml Eppendorf's. One sample was

stored in 2% CTAB buffer and the other flash frozen in liquid nitrogen before being stored at -20^{o}C.

4.2.2.2 Root staining

Root staining for visualisation of mycorrhizal fungal structures was carried out according to the method described in Chapter three (section 3.2.1.1).

4.2.3. Plant Species

French marigolds, (*Tagetes erecta L.*) were grown from seed under glass for three weeks until ready to be transferred into pots in early June where they were grown for three months. Seedlings were placed one to a pot. *T. patula* 'Bonita mixed' seeds were sourced from Thompson & Morgan.

4.2.4. Experimental Design

The three custom growing media were: peat, bark and wood fibre (section 3.2.1) these were each treated with commercial inocula AM1, AM2 and AM3 in both their live and sterilised form. Replicate number was increased to ten replicates of each of the 18 treatments. Ten replicate plants were also grown in each media without any inoculum to see the effect of adding the carrier itself as well as any effect caused by low levels of colonisation from sterilised inoculum. In total 210 pots were planted, all of these marigolds were grown for three months (June-September) and harvested together over five days in September to avoid seasonality effects as seen in Chapter 3.

4.2.5. Site and Weather Data Collection

The site set up and weather monitoring was as described in section 3.2.4.

Noticeably, over 40mm of rain fell in less than 24 hours on the 23rd of June 2016. By the end of July, a large number of marigolds across all treatments started to show signs of chlorosis and leaf purpling (Figure 4.1), as this was significantly sooner than the expected time of nutrient depletion, given the slow release nutrients available, it was thought that the heavy rainfall in late June had caused significant leakage of these nutrients from the growing media. To ensure that the plants had sufficient nutrients to complete the growing period a commercially available soluble plant food that contained a low level of phosphorus (Homebase Soluble Plant Feed – N12.5%, P0.4%, K17%) was mixed with water as per the product instructions, and 60ml of this mix was added evenly to the surface of each pot using a 100ml syringe.

Figure 4.1 a-b) Plant showing signs of leaf purpling and yellow/green leaves compared to (c) the darker green leaves of a plant grown in 2015

4.1.1. Flower Number

Cumulative flower number was recording by totalling the number of open flowers, dead flowers, and seed heads of each plant the day before the biomass was harvested. This was then average across replicate plants for each treatment.

4.1.2. Leaf discolouration scoring

In Chapter 3 there were obvious visual differences between plants, where some had a large number of purple leaves and some didn't (section 3.2.6). In order to analyse and quantify the same effect in this experiment photos taken of each plant were taken just before the biomass harvest and were used to assign scores. Previously the purpling was obvious so plants were given a score of one if plants had purple leaves and zero if their leaves were green but in this experiment purpling was not as easy to distinguish so a three-score system was used. Plants scored zero if the majority of their leaves were green (Figure 4.2a), one if less than 25 percent of their leaves were purple (Figure 4.2b) and two if more than 25 percent of their leaves were purple (Figure 4.2c). Score counts for each treatment were then analysed using the Fisher's Exact test.

Figure 4.2 a) An example of a marigold that had no purple leaves that scored 0, b) A marigold with <25% of its leaves purple which scored 1, and c) A marigold with >25% of its leaves purple which scored 2.

4.1.3. Porosity

This was measured and calculated according to methods outlined in Chapter three, section 3.2.6. All pots used for this were exposed to the same amount of water and rainfall because there were no separate harvests.

4.1.4. Biomass

The final harvest of above ground biomass was carried out after 12 weeks. Fresh weight was recorded. Plants were then placed in envelopes in an oven set at approximately 40^{o}C and dried to constant weight, which was then recorded. Replicate pots were taken once above ground biomass was removed for analysis of the physical properties of the media, including porosity and water holding capacity. Root samples were then taken and fixed for mycorrhizal colonisation analysis after these measurements had been collected.

4.2.Statistical Analysis

For flower number, porosity, biomass, and root length colonisation a two-way ANOVA was used to analyse differences between, and interactions within media and inoculum type (live or control) for AM1, AM2 and AM3. Tukey's HSD was used to perform post-hoc tests for growing media treatments. Where interactions between growing media and inoculum were found to be significant, independent sample t-tests were used to look at differences between the effect of live and sterile treatments on plants grown in each medium. To identify if plant biomass could be predicted by root length colonisation, these data for replicate plants were subjected to linear regression analysis. Where data were not normally distributed values were transformed with square roots or logarithms. As root length colonisation was measured as a percentage for each

root sample these data (hyphae, arbuscules and vesicles) were transformed using the arcsine transformation. All these analyses were conducted using IBM SPSS 21.

As a three-score system had to be used to score the degree of leaf purpling, Fisher's Exact test was used to analyse the difference in score profile between plants grown with live and sterile inoculum for each inoculum type. This test was used as it is more robust than Chi-squared when expected numbers are small and data could contain zero values.

The size inequality of plants in each growing medium treated with live and sterile inocula was analysed as described in section 3.3 using R (version 3.4.1).

4.3. Results

4.3.1. AMF Colonisation

There was no difference found between the overall levels of colonisation of hyphae or arbuscules between plants in each growing media for each inoculum type. In plants grown with AM1, live inoculum resulted in significantly higher levels of hyphal colonisation than sterile inoculum in every growing media (F_1=89.479, Figure 4.1). Colonisation of arbuscules and vesicles was only significantly increased in live inoculated plant roots compared to sterile inoculum roots in peat and bark media (Table 4.1, Figure 4.5a).

In AM2 treated plants, despite levels of hyphal colonisation in roots of live inoculum treated plants being higher than control plants, the difference was only found to be significant in peat (t_{18}=-2.919, p=0.009). Arbuscule and vesicle colonisation was not found to be significantly increased between AM treatments in peat or wood fibre-grown plants (Table 4.1), in wood fibre this was due to large standard errors caused by some plants which were found to have no structures present (Figure 4.5b). In bark, only the number of arbuscules was significantly higher in live inoculum plants.

In AM3 no differences were found between the percentage of vesicles colonising roots of live and sterile inoculated plants (F_2=2.035, Table 4.1). There was a significant interaction effect between media and inoculum treatment found in plants grown with AM3 (F_2=3.654, Table 4.1) because a significant increase in hyphal colonisation on the addition of live AM3 compared to control plants was found in wood fibre media only.

		Hyphae		Arbuscules		Vesicles	
AM1	**df**	**F**	**sig**	**F**	**sig**	**F**	**sig**
Media	2	2.260	p>0.05	0.418	p>0.05	0.270	p>0.05
AM	1	89.479	**P<0.001**	25.803	**P<0.001**	9.970	**P<0.01**
*Media*AM*	2	1.213	p>0.05	1.458	p>0.05	1.357	p>0.05
AM2							
Media	2	0.326	p>0.05	0.099	p>0.05	0.844	p>0.05
AM	1	12.805	**P<0.01**	9.708	**P<0.01**	1.798	p>0.05
*Media*AM*	2	0.114	p>0.05	0.682	p>0.05	1.281	p>0.05
AM3							
Media	2	0.220	p>0.05	0.194	p>0.05	0.114	p>0.05
AM	1	15.641	**P<0.001**	10.621	**P<0.01**	1.274	p>0.05
*Media*AM*	2	3.654	**P<0.05**	0.012	p>0.05	2.035	p>0.05

Table 4.1 Results of two-way ANOVAs on plant root length colonised by hyphae for plants grown in each inoculum. Error degrees of freedom for hyphae: AM1=49, AM2=54, AM3=53 and Arbuscules and Vesicles: AM1=18, AM2=20, AM3=24.

Evidence of colonisation was found in negative control pots (≤20% on average) which in some growing media meant negative controls had higher levels of colonisation than the sterile inoculum treated plants. This was most consistently seen with AM1 inoculum. However, no significant difference was found between the root colonisation of negative control plants and all sterile inoculum treated plants from each growing medium.

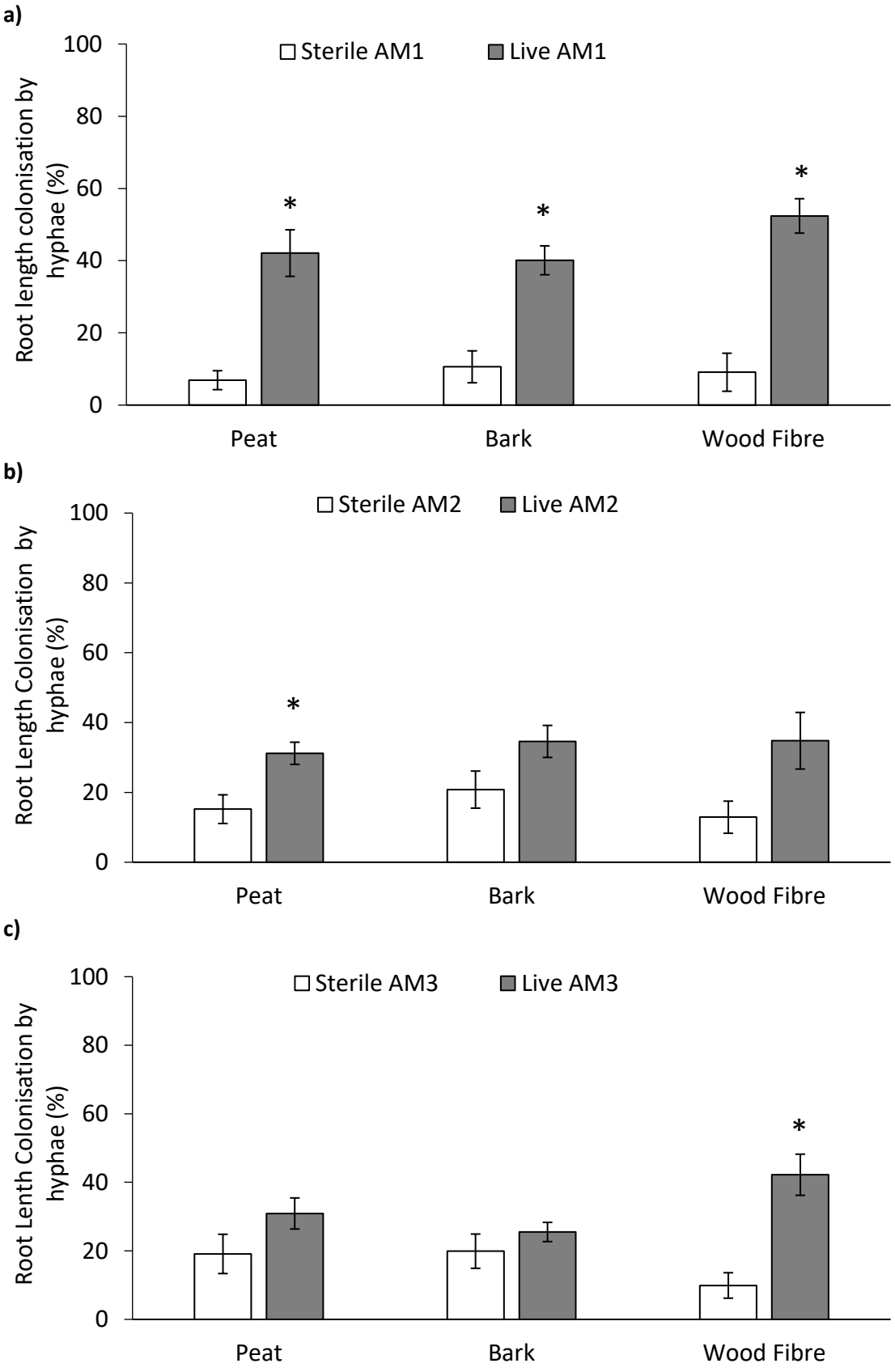


Figure 4.3 Root length colonised by hyphae in each growing medium for each inoculum: a) AM1, b) AM2, c) AM3. Asterisks denote statistical difference between mean pairs, $p<0.05$. n=10, bars±SE

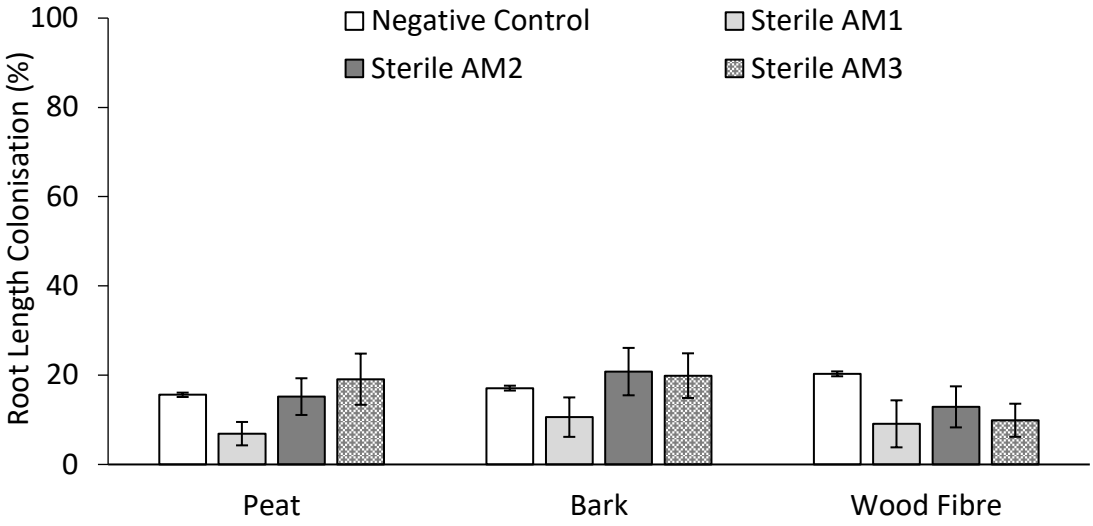


Figure 4.4 Root length colonisation by hyphae in negative control and sterile inoculum treated plants in each growing media. n=10, bars±SE

Less than 10% of the root length of negative control plants was colonised by arbuscules (Figure 4.5) and the high error bars are due to the large number plants which were not colonised by either structure. The number of vesicles and arbuscules was highest in plants treated with live AM1, but there was a consistent pattern across all three inoculum treatments that in all media the frequency of arbuscules was higher or equal to that of vesicles.

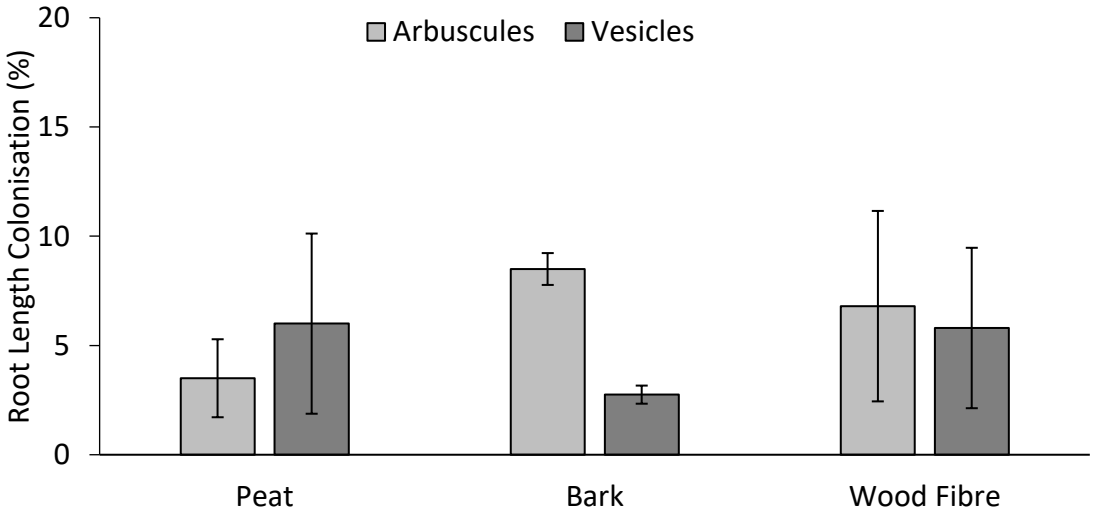


Figure 4.5 Average root length colonised by arbuscules and vesicles in negative control plants in each growing media. n=5, bars±SE

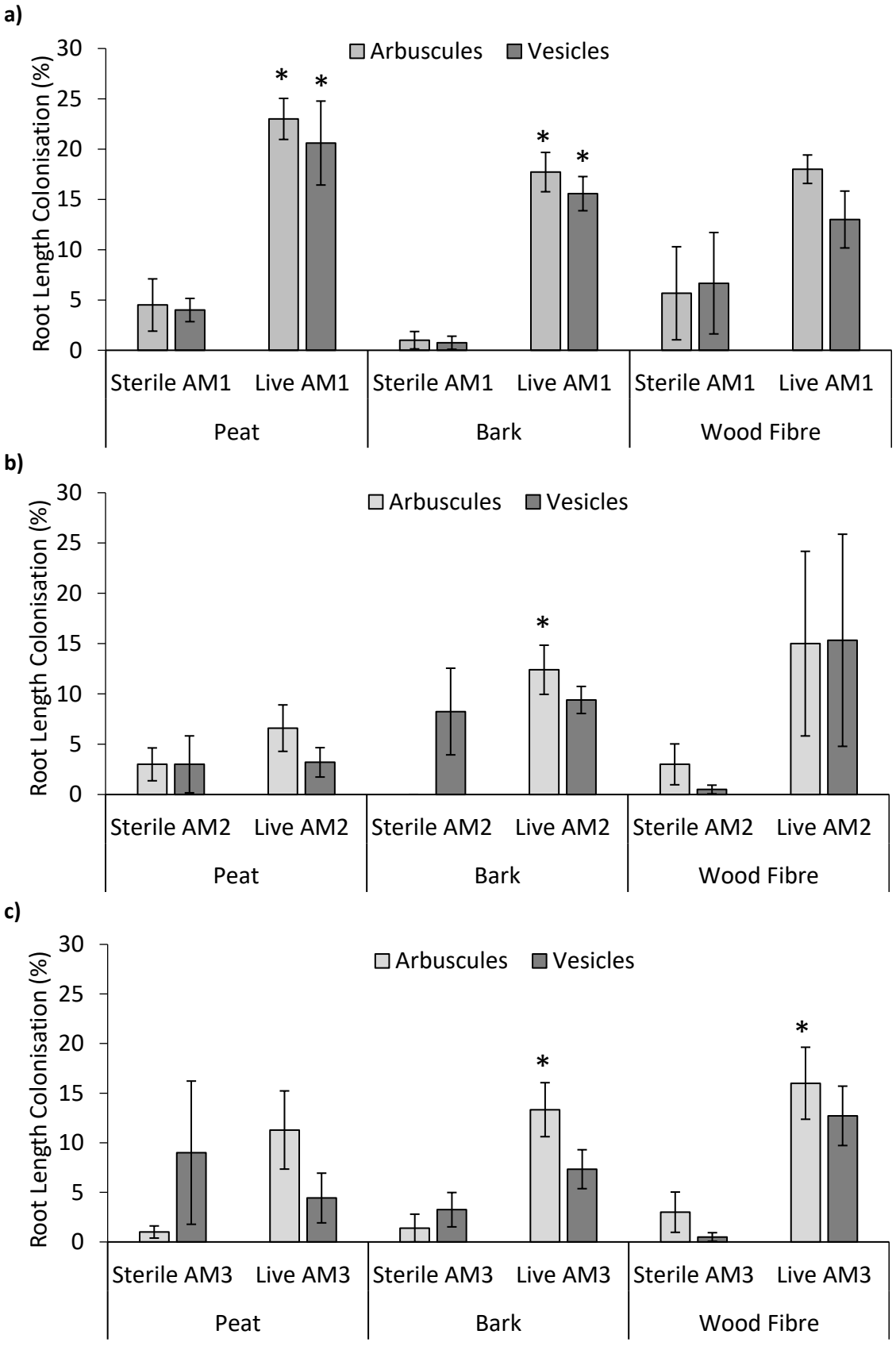


Figure 4.6 Root length colonised by vesicles and arbuscules of plants grown in each media with live and sterile inoculum: a) AM1, b) AM2, c) AM3. n=5, bars±SE. Asterisks denote statistical difference between colonisation in sterile and live treatments (p<0.05).

4.3.2. Site Weather Data

In this experiment, the opposite weather conditions occurred compared to the previous year where rainfall reduced and temperature increased throughout summer months. The rainfall in June however was significantly higher than normal with the MET Office citing it as the wettest June on record for parts of the south-east (Met Office, 2016).

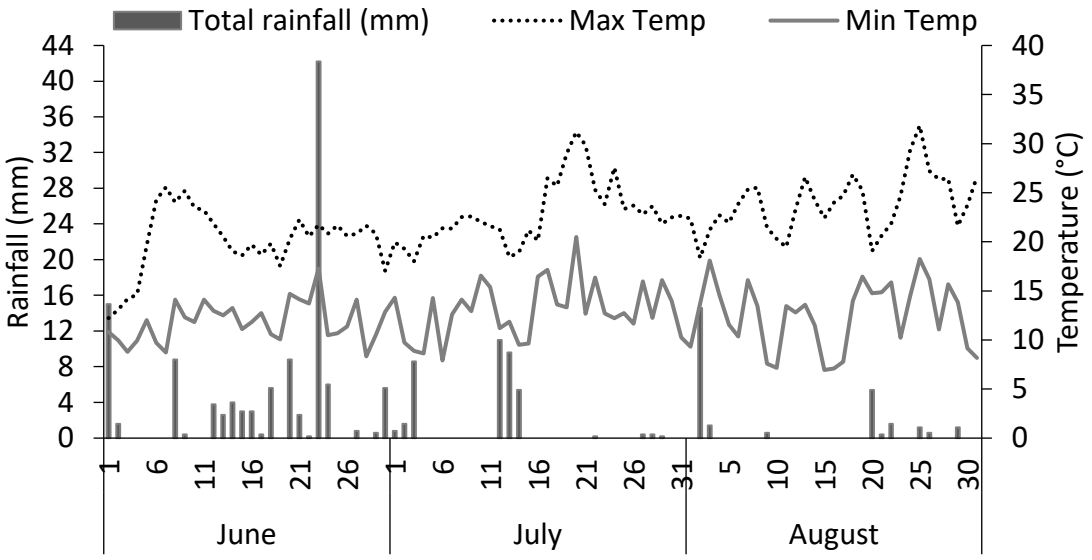


Figure 4.7 Total rainfall, minimum and maximum temperature recorded daily for the growth period at Deer's Farm Site.

Despite changeable conditions and the exposed nature of the site, control plants grown in this experiment, although smaller on average, were not found to vary significantly from control plants grown in 2015 (F_1=1.006, p=0.369).

4.3.3. Flower Number

Overall plants grown with AM2 in wood fibre had significantly fewer flowers than those grown in bark (F_2=3.438), no other media effects on flower number were seen for plants grown with AM1 or AM3 (Table 4.2).

AM 1	*d.f*	*F*	*sig*
Media	2	2.506	p>0.05
AM	1	1.068	p>0.05
Media*AM	2	0.559	p>0.05
AM 2			
Media	2	3.438	**P<0.05**
AM	1	0.306	p>0.05
Media*AM	2	2.7	p>0.05
AM 3			
Media	2	1.232	p>0.05
AM	1	0.447	p>0.05
Media*AM	2	2.151	p>0.05

Table 4.2 Results of two-way ANOVAs on flower number produced by plants grown with each commercial inoculum. Error degrees of freedom AM1=50 AM2 and AM3=52.

Consistent patterns of live inoculum addition were seen across all three inoculum treatments for the alternative medias with a slight reduction in flower number in bark grown plants and a slight increase in flower number for wood fibre grown plants (Figure 4.8). The effect of live inoculum treatment was not consistent in peat grown plants with AM1 producing an increase in flower number (Figure 4.8a) and AM2 and AM3 (Figure 4.8b-c respectively) producing a slight decrease.

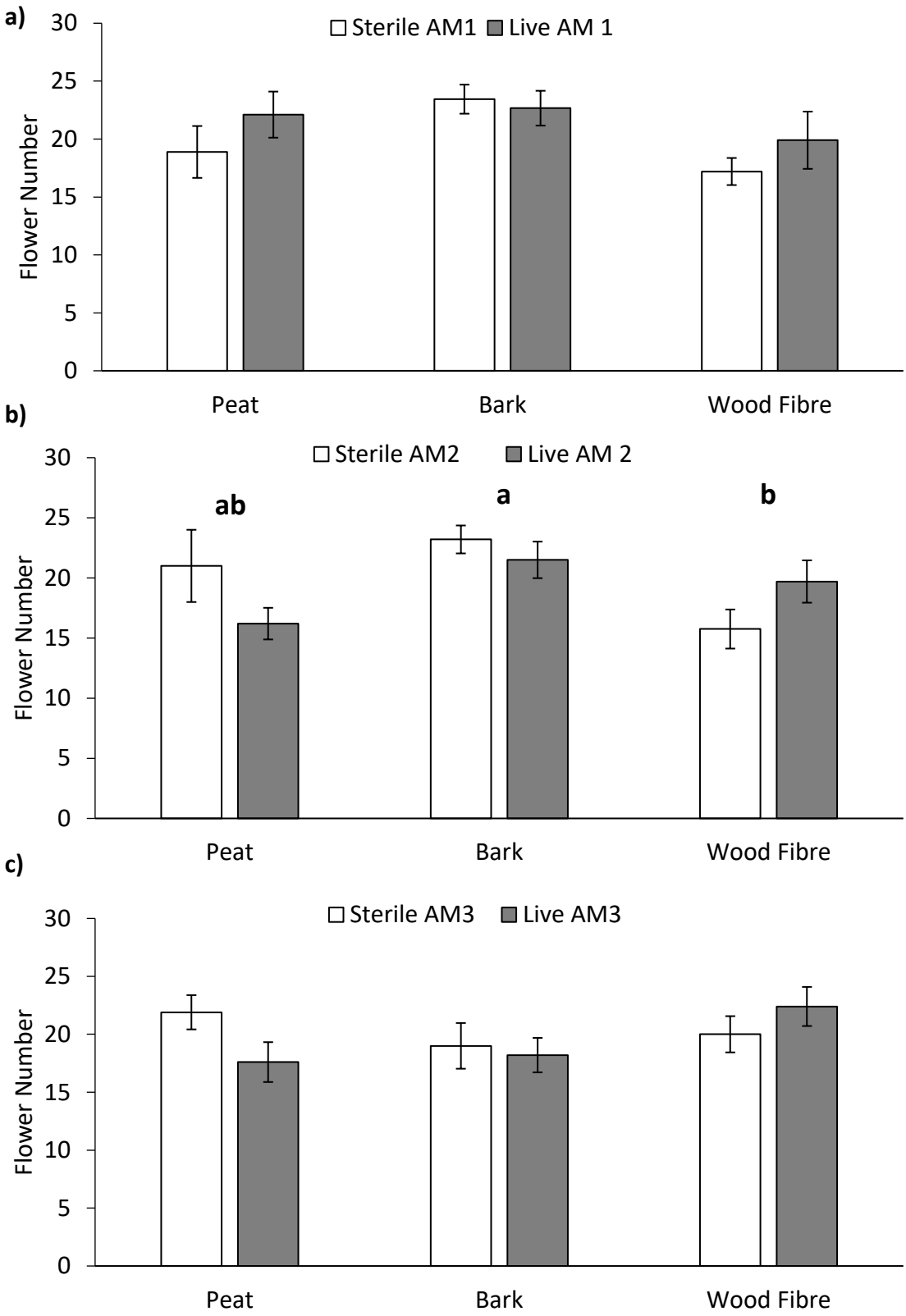


Figure 4.8 Average flower number of plants grown in each media with each inoculum: a) AM1, b) AM2, c) AM3. Groups of bars with different letters show statistical difference between growing media, p<0.05. n=10, bars±SE.

4.3.4. Leaf Discolouration

In plants treated with AM1 more than 50% of individuals in all treatments showed some degree of purpling of their leaves but there were more plants with more than 25% of their leaves purple in sterile treated plants compared to those with live inoculum. However, this decrease was only found to be significant in wood fibre pots (χ^2=9.11, p=0.01). In plants treated with AM2 all plants treated with sterile AM2 showed signs of leaf purpling but there were significantly fewer plants (χ^2=10.5, p=0.005) treated with live AM2 in wood fibre that had purple leaves. Plants treated with AM3 had the lowest number of plants with purple leaf discolouration especially in the reduced peat growing media. The addition of live AM3 significantly reduced the proportion of plants with purple leaves in peat grown plants (χ^2=6.97, p=0.031). The number of plants which had more than 25% purple leaves was reduced in live inoculum treated plants in all growing media treated with AM1, as well as in bark and peat pots treated with AM3 but these differences were not found to be significant.

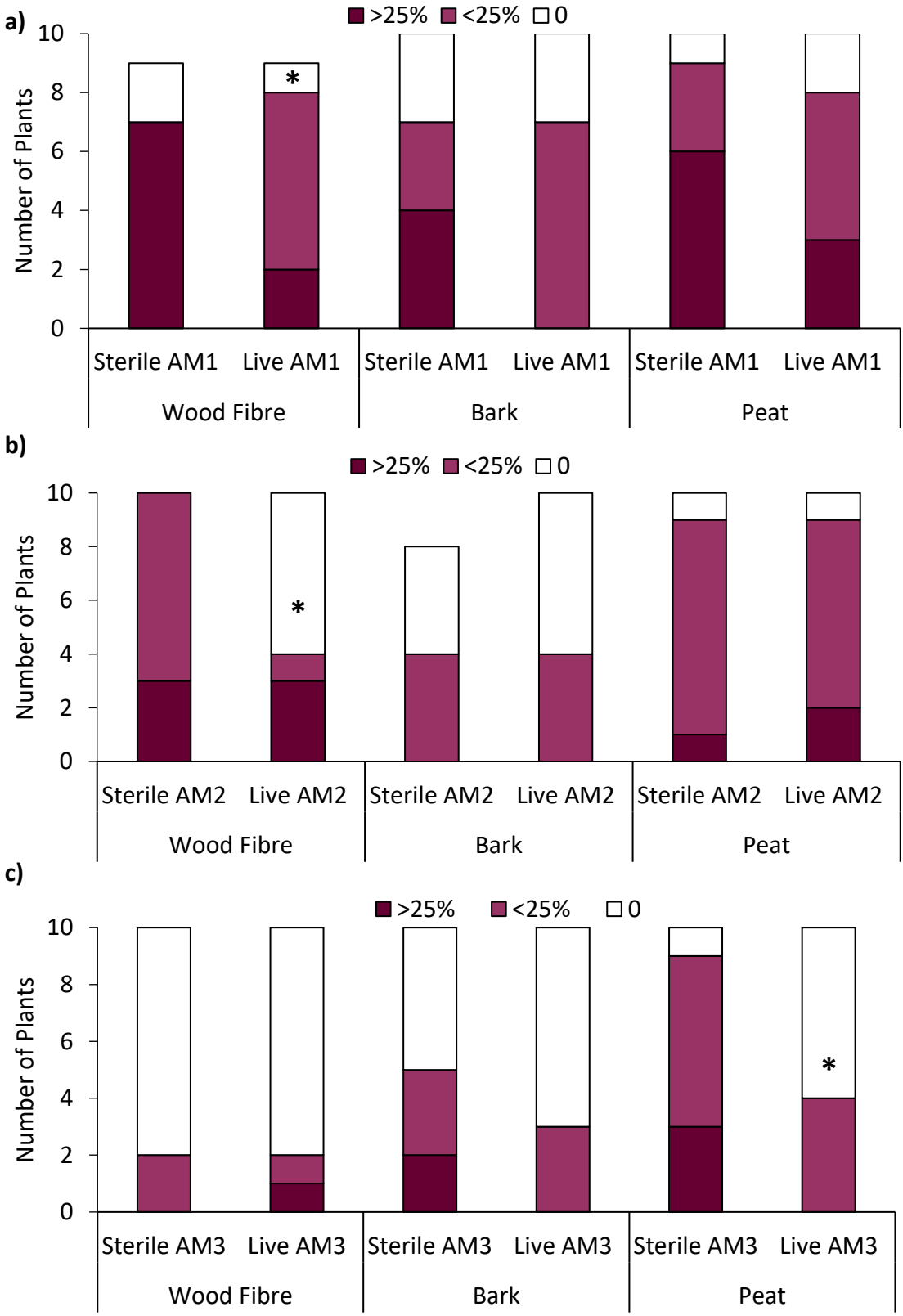


Figure 4.9 The number of replicate plants from each media and inoculum treatment that had no purple leaves, less than 25% purple leaves and more than 25% purple leaves in each growing media and inoculum treatment: a) AM1, b) AM2, c) AM3. Asterisk denotes statistical difference in proportions between inoculum pairs.

4.3.5. Porosity

Overall total porosity was found to be significantly higher in wood fibre than bark in pots that contained AM1 (F_2=4.712, Table 4.3), this could be due to increased water retention porosity which was also significantly higher in AM1 treated wood fibre pots compared to both peat and bark pots (F_2=11.662, Table 4.5). No differences were found in the air-filled porosity of each growing media and no inoculum treatment had any significant effect on the air-filled porosity (Table 4.4).

In AM1 WRP was also significantly increased in wood fibre pots treated with live AM1 compared to sterile inoculum controls (t_{10}=-2.630, p=0.025, Figure 4.13a).

AM 1	*d.f*	*F*	*sig*
Media	2	4.712	**P<0.05**
AM	1	0.692	p>0.05
Media*AM	2	2.535	p>0.05
AM 2			
Media	2	0.464	p>0.05
AM	1	1.799	p>0.05
Media*AM	2	3.295	p>0.05
AM 3			
Media	2	0.894	p>0.05
AM	1	1.221	p>0.05
Media*AM	2	2.851	p>0.05

Table 4.3 Results of two-way ANOVA on total porosity of pots for each commercial inoculum treatment. Error degrees of freedom= 30.

AM 1		*d.f*	*F*	*sig*
	Media	2	0.345	p>0.05
	AM	1	0.160	p>0.05
	Media*AM	2	0.947	p>0.05
AM 2				
	Media	2	0.509	p>0.05
	AM	1	0.166	p>0.05
	Media*AM	2	0.598	p>0.05
AM 3				
	Media	2	0.463	p>0.05
	AM	1	2.637	p>0.05
	Media*AM	2	0.443	p>0.05

Table 4.4 Results of two-way ANOVAs on air-filled porosity (AFP) of pots for each commercial inoculum treatment. Error degrees of freedom= 30

AM 1		*d.f*	*F*	*Sig*
	Media	2	11.662	**P<0.01**
	AM	1	0.681	p>0.05
	Media*AM	2	1.991	p>0.05
AM 2				
	Media	2	1.789	p>0.05
	AM	1	2.013	p>0.05
	Media*AM	2	3.341	**P<0.05**
AM 3				
	Media	2	3.388	**P<0.05**
	AM	1	0.047	p>0.05
	Media*AM	2	3.229	p>0.05

Table 4.5 Results of two-way ANOVAs on water retention porosity (WRP) of pots for each commercial inoculum treatment. Error degrees of freedom= 30

This effect of AM1 on water retention porosity in wood fibre was shown to positively correlate with root length colonisation, with water retention porosity increasing with increased colonisation of roots by arbuscular mycorrhizal hyphae. (n=31, F=6.609, p=0.016). However, despite the relationship being significant the R^2 value was low with only 19% of data being

explained by the trend line. This could be due to the number of plants which were found to have no colonisation.

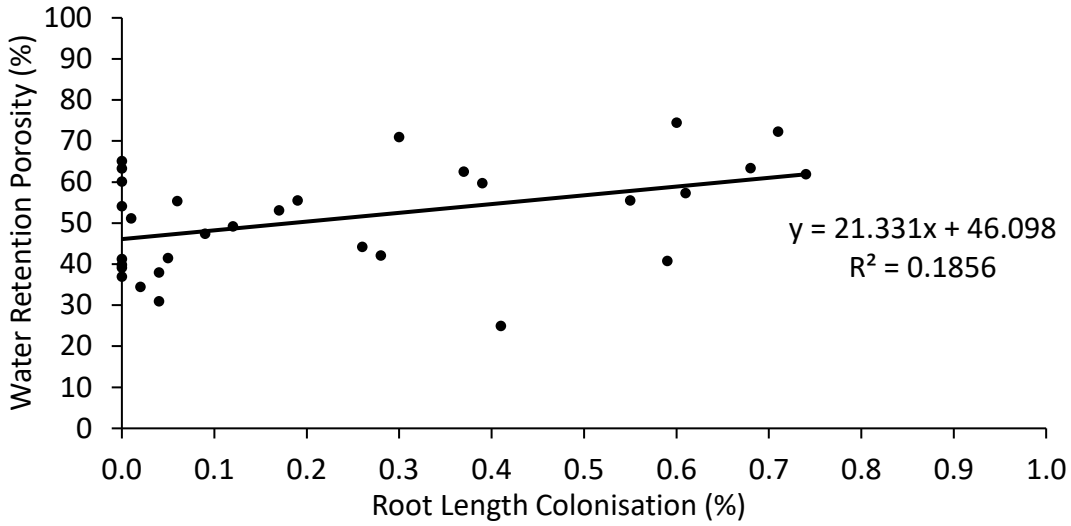


Figure 4.10 Relationship between root length colonisation by hyphae and water retention porosity of pots containing wood fibre and AM1.

No positive effects on water retention porosity were seen with AM2 or AM3. Addition of live AM2 resulted in a significant reduction in water retention porosity in bark pots ($t_{7.1}$=3.55, p=0.009), hence the interaction term in the ANOVA (Table 4.5). Bark pots inoculated with AM3 had significantly lower water retention porosity than the other media (Table 4.5, F_2=3.388). Peat pots that contained live AM3 had significantly lower water retention porosity than control plants (t_{10}=2.520, p=0.03).

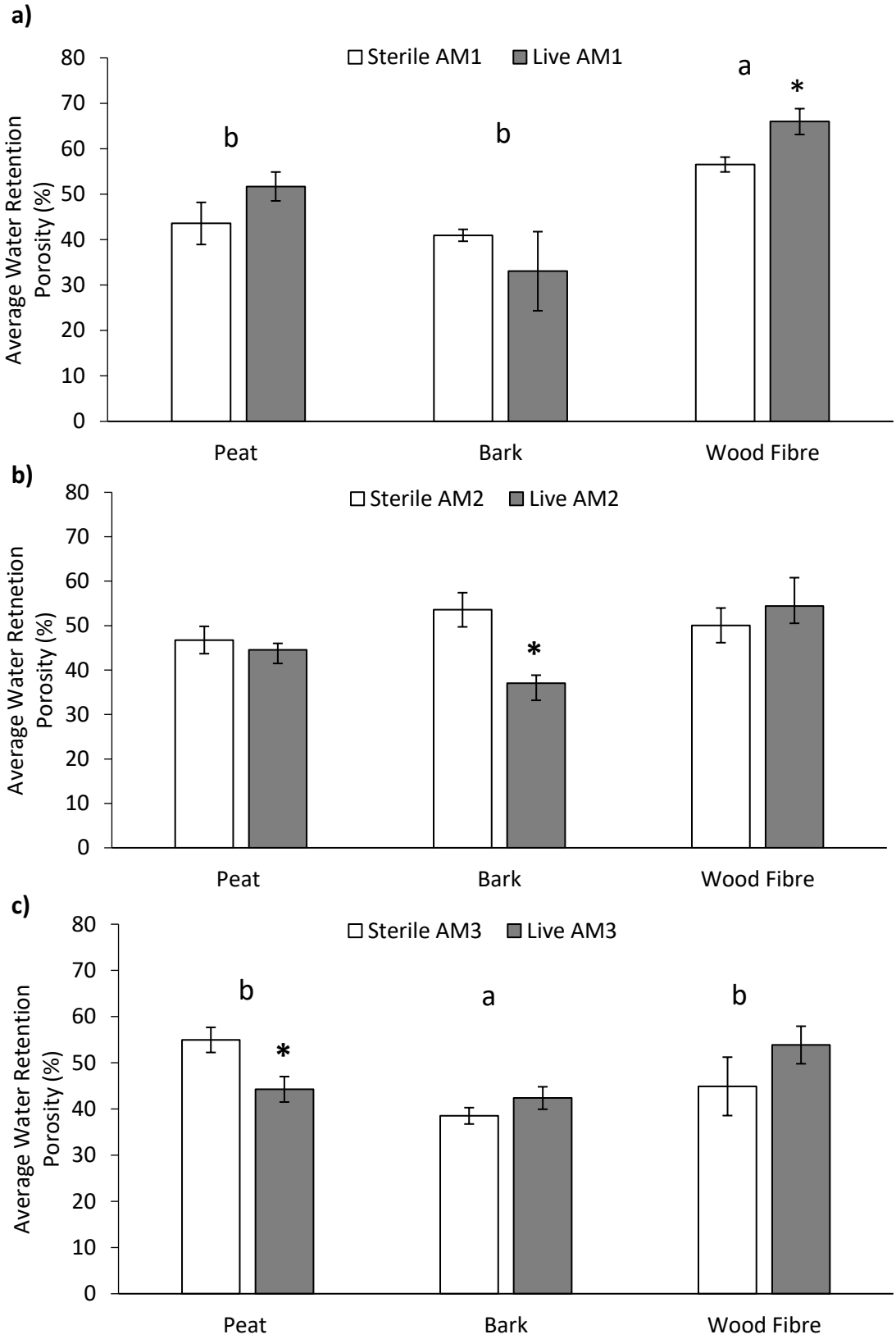


*Figure 4.11 Average water retention porosity of pots containing each growing media for each inoculum: a) AM1, b) AM2, c) AM3. Groups of bars with different letters indicate statistical difference between growing media and asterisks denote statistical significance between pairs of bars, *=p,0.05, **=p<0.01. n=6, bars±SE.*

4.3.6. Biomass

No significant relationship or correlation was found between the amount of colonisation and biomass (data not shown). In plants treated with AM1 the addition of live inoculum increased the biomass of plants significantly in both bark and wood fibre (F_1=7.968, Table 4.6). In AM2 there was a significant interaction between AM treatment and media (F_2=6.105, Table 4.6), despite an increase in biomass in both alternative media, plants with live AM2 were only found to be significantly larger in wood fibre (t_{18}=-2.994, p=0.008). No significant effect of adding live inoculum on biomass was found in plants grown with AM3. Growing media did not have any effect on biomass of plants across all inoculum treatments. The addition of live AM1 and AM2 inoculum increasing biomass was consistent in both wood fibre and bark grown plants.

AM 1	*d.f*	*F*	*Sig.*
Media	2	1.395	p>0.05
AM	1	7.968	**P<0.01**
*Media*AM*	2	0.341	p>0.05
AM 2			
Media	2	1.686	p>0.05
AM	1	0.277	p>0.05
*Media*AM*	2	6.105	**P<0.01**
AM 3			
Media	2	1.135	p>0.05
AM	1	0.065	p>0.05
*Media*AM*	2	1.323	p>0.05

Table 4.6 Results of two-way ANOVAs on dry biomass of plants grown with each commercial inoculum. Error Degrees of Freedom AM1=49, AM2 and AM3 =54.

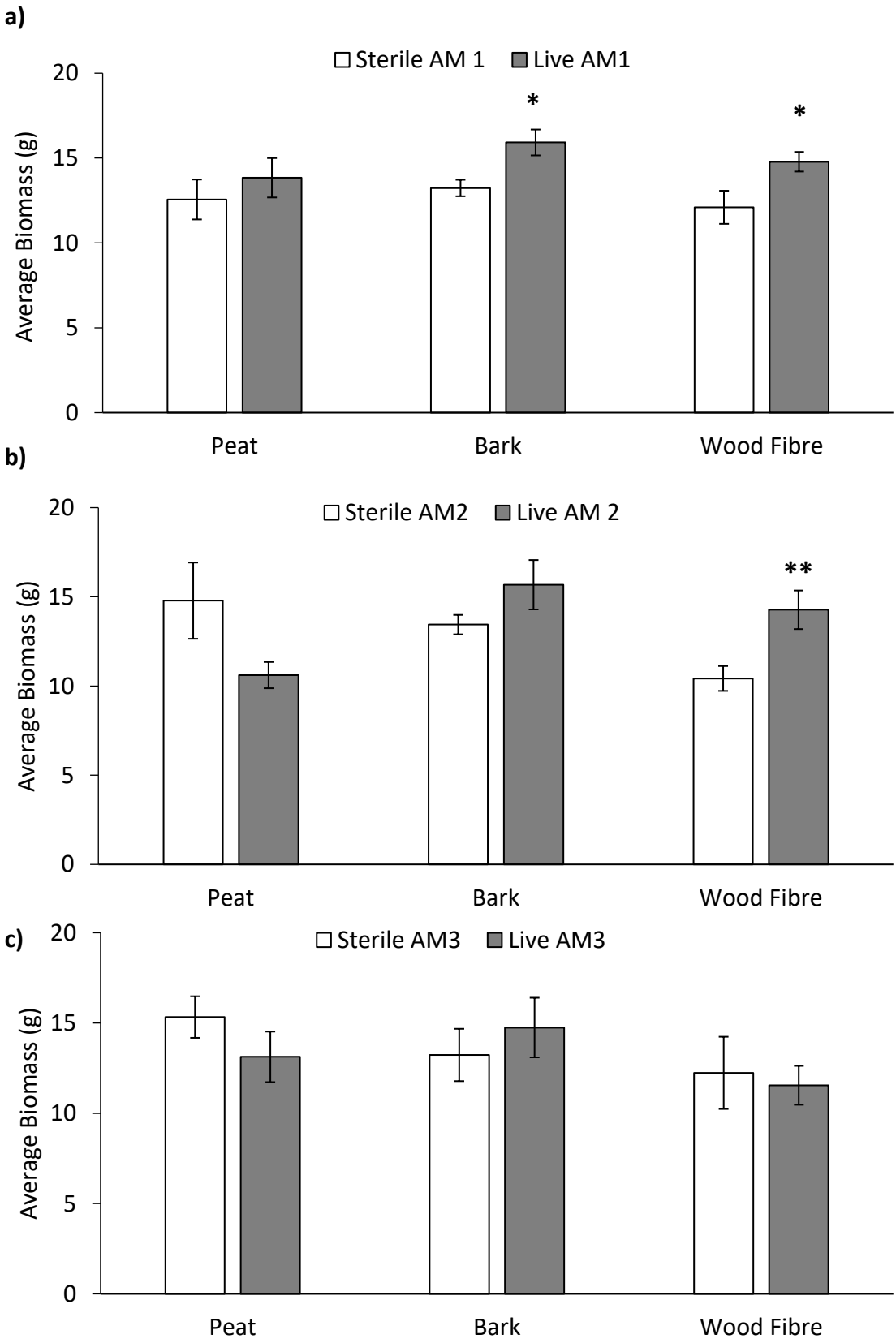


*Figure 4.12 Average dry biomass of plants grown in each medium with each commercial inoculum: a) AM1, b) AM2, c) AM3. Asterisk denotes statistical significance between pairs of bars, *=p,0.05, **=p<0.01. n=10, bars±SE.*

4.1.3.1 Coefficient of Variation of Biomass

Coefficient of variation of biomass was used in this experiment to compare the difference in biomass between plants that had no inoculum added at all (to see how the alternative growing media compared to the peat standard), to see if adding the sterilised inoculum along with the inert carrier material had any significant effect on biomass.

Despite bark producing the largest plants, both alternative media did not produce plants with a significantly different coefficient of variation of biomass compared to the peat industry standard (Figure 4.13).

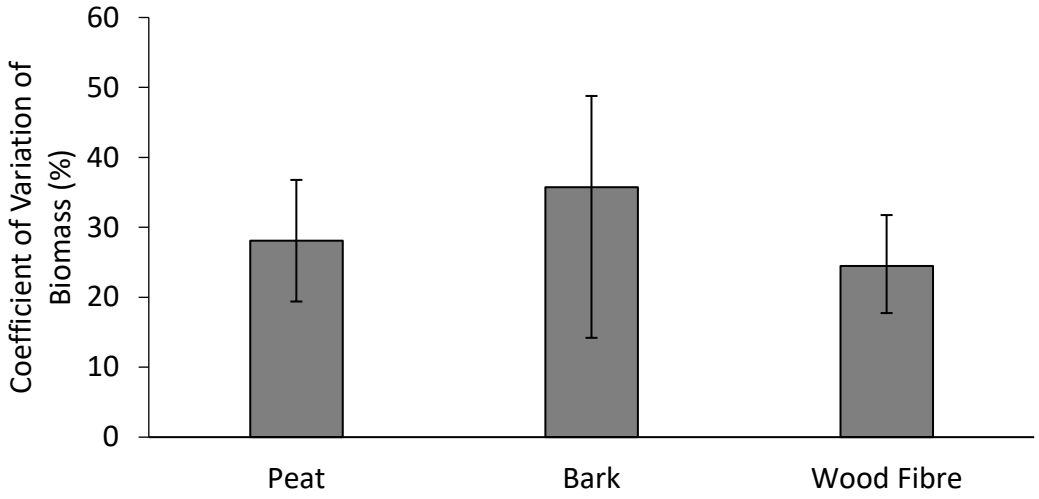


Figure 4.13 Coefficient of variation of biomass of negative control (no inoculum added) plants from each growing media. n=10, bars±95%CI

Although there was large variability (Figure 4.14) the biomass of plants grown with sterile inoculum were not found to vary significantly from the biomass of plants grown without inoculum in each growing media. The biomass of plants in wood fibre grown with AM1 were found to have a significantly lower coefficient of variation of biomass compared to the negative control plants ($Z=2.352$, $p<0.05$).

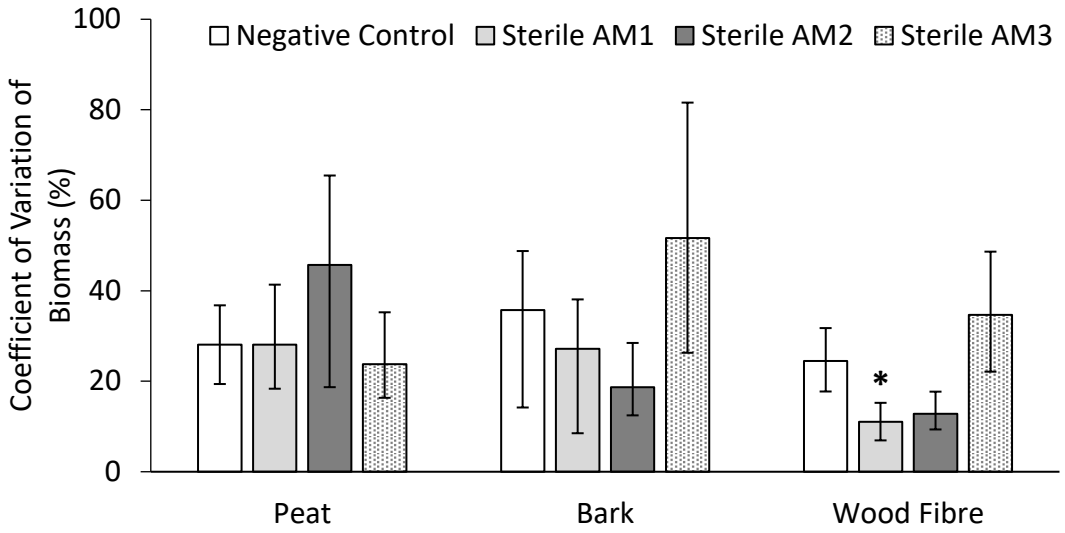


Figure 4.14 Coefficient of variation of biomass of plants for the negative and sterile control treatments for each inoculum in each growing media. n=10, bars±95% CI. Asterisk indicates significantly different CV compared to negative control.

4.1.3.2 Size Inequality

Despite seeing fewer negative effects and more significant increases in biomass as a result of adding live inoculum to plants compared to the previous experiment (section 2.4.3). Figures 4.15-4.17 (a-c) compare the variation in biomass of sterile and live treated plants in each growing media. The results of the Z test showed the coefficient of variation of biomass was significantly reduced in plants grown with live AM1 in bark media (Table 4.7, Figure 4.15b) but significantly increased in live AM2 plants grown in wood fibre (Table 4.7, Figure 4.16c). Plants grown with live AM2 in peat (Table 4.7, Figure 4.16a) and live AM3 in bark (Table 4.7, Figure 4.17b) did appear to be less variable in size than control plants but the reduction in coefficient of variation were not found to be significant. Wood fibre plants grown with live AM2 did also appear more variable in size than sterile inoculum treated plants but this was not found to be significant.

AM1	Coefficient of Variation					
	Sterile	**95% CI**		**Live**	**95% CI**	
Peat	28.084	18.350	41.350	26.648	19.580	37.700
Bark	27.203	8.510	38.090	**11.970**	8.040	15.550
Wood Fibre	11.027	6.930	15.230	21.006	14.730	29.940
AM2	**Sterile**	**95% CI**		**Live**	**95% CI**	
Peat	45.694	18.690	65.470	21.910	16.380	29.890
Bark	18.678	12.460	28.470	23.963	18.140	32.490
Wood Fibre	12.803	9.350	17.700	**32.506**	22.350	47.640
AM3	**Sterile**	**95% CI**		**Live**	**95% CI**	
Peat	23.741	16.340	35.240	28.001	19.810	37.330
Bark	51.655	26.290	81.580	26.656	19.320	33.980
Wood Fibre	34.643	22.120	48.640	35.415	25.290	48.040

Table 4.7 The coefficient of variation and 95% confidence intervals calculated for each group of ten replicate plants from each growing media with live and sterile inoculum for each AM treatment.

As in the previous experiment plants grown in peat with AM1 the addition of live inoculum did not increase size variation between plants (Figure 4.14a), this was also the case for wood fibre plants treated with AM3 (Figure 4.16c).

Lorenz curves can be seen for AM1, AM2 and AM3 treated plants in Figure 4.15-4.17 (d-f) along with the Gini coefficients calculated for each treatment in Table 4.7. These data match the patterns seen with coefficient of variation; the 95% confidence intervals for the Gini coefficients of live and sterile inoculum treated plants did not overlap for bark AM1 plants or wood fibre AM2 plants. The Gini coefficient for peat grown plants with live AM2 (0.131) was smaller than that for sterile AM2 (0.227), and also for live AM3 plants grown in bark (0.158) and sterile AM3 (0.262) suggesting that live inoculated plants were more equal in size.

AM1	Gini Coefficient					
	Sterile	**95% CI**		**Live**	**95% CI**	
Peat	0.163	0.110	0.230	0.158	0.122	0.220
Bark	0.157	0.102	0.218	**0.062**	0.047	0.088
Wood Fibre	0.062	0.039	0.085	0.126	0.091	0.173
AM2	**Sterile**	**95% CI**		**Live**	**95% CI**	
Peat	0.227	0.110	0.347	0.131	0.102	0.172
Bark	0.110	0.072	0.164	0.139	0.110	0.179
Wood Fibre	0.076	0.057	0.102	**0.187**	0.133	0.272
AM3	**Sterile**	**95% CI**		**Live**	**95% CI**	
Peat	0.137			0.165	0.123	0.213
Bark	0.262	0.136	0.429	0.158	0.123	0.193
Wood Fibre	0.193	0.128	0.267	0.206	0.154	0.268

Table 4.8. Gini coefficient calculated for each group of ten replicate plants grown in each growing media with live and sterile inoculum for both AM1, AM2 and AM3. Bold numbers indicate CIs do not overlap between live and sterile treatments.

Lorenz asymmetry coefficients (LAC) were calculated for each curve (Table 4.9) as these can help describe skews in biomass. Comparing the Lorenz curves and coefficients for plant grown in bark with AM1 (Figure 4.16e) the addition of live AM1 decreased size inequality by reducing the number of small plants (Table 4.9). The effect of colonisation by AMF from AM1 in wood fibre (Figure 4.16f) and AM2 in bark and wood fibre plants (4.17e-f) had the opposite effect as it caused an increase in size inequality by increasing the number of large plants. The size inequality of peat grown plants inoculated with live AM2 was reduced because there were fewer large plants than in the sterilised AM2 control treatments. Although reduction in size inequality was not shown to be significant in bark plants grown with live AM3 the effect of the addition live inoculum was different again because the number of large plants was reduced (Figure 4,15e). These observations can also be supported by comparing the boxplots for each inoculum and growing media combination (Figure 4.14b, 4.15c and 4.16b).

Lorenz Asymmetry Coefficient

AM1	Sterile AM	Live AM	Inequality
Peat	1.110	1.040	=
Bark	0.926	1.264	-
Wood Fibre	1.384	1.047	+
AM2	**Sterile AM**	**Live AM**	**Inequality**
Peat	1.280	1.122	-
Bark	0.865	1.245	+
Wood Fibre	0.936	1.182	+
AM3	**Sterile AM**	**Live AM**	**Inequality**
Peat	1.252	1.170	=
Bark	1.331	0.854	-
Wood Fibre	1.318	1.183	=

Table 4.9. Lorenz Asymmetry coefficient calculated for each group of ten replicate plants grown in each growing media with live and sterile inoculum for both AM1 and AM2. Inequality symbols represent an increase (+), decrease (-) or no change (=) in size inequality with the addition of live AM (according to Lorenz curves, Gini coefficients and coefficient of variation tests).

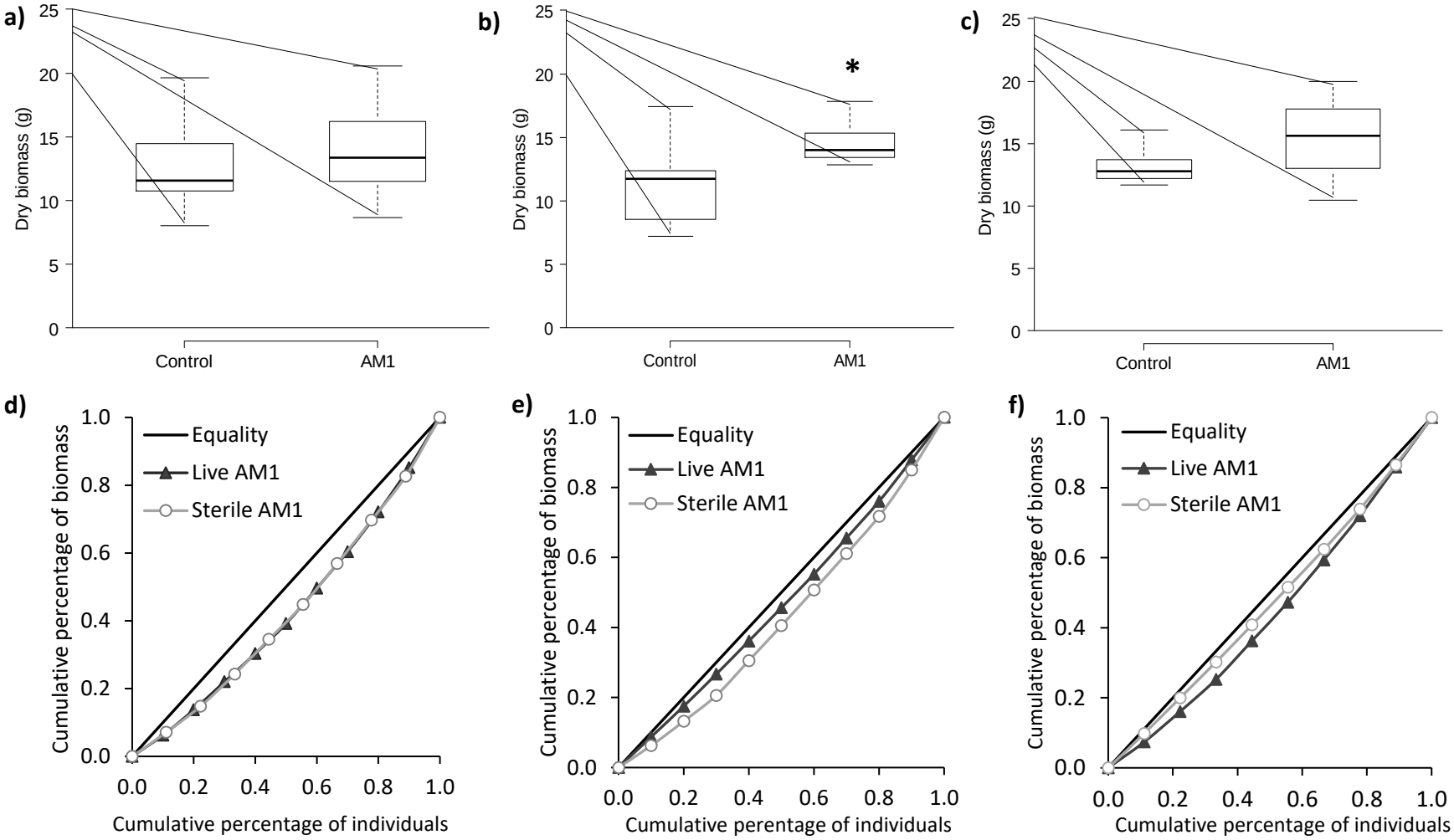


Figure 4.15 ***a-c)*** *Biomass of plants grown with AM1 in each growing media: a) Peat. b) Bark, Z=2.249, p<0.05. c) Wood Fibre. Asterisk denotes statistically different coefficient of variation* ***d-f)*** *Graphical analysis of size inequality of plants using Lorenz curves plotted against line of equality AM1 in Peat, Bark and Wood Fibre.*

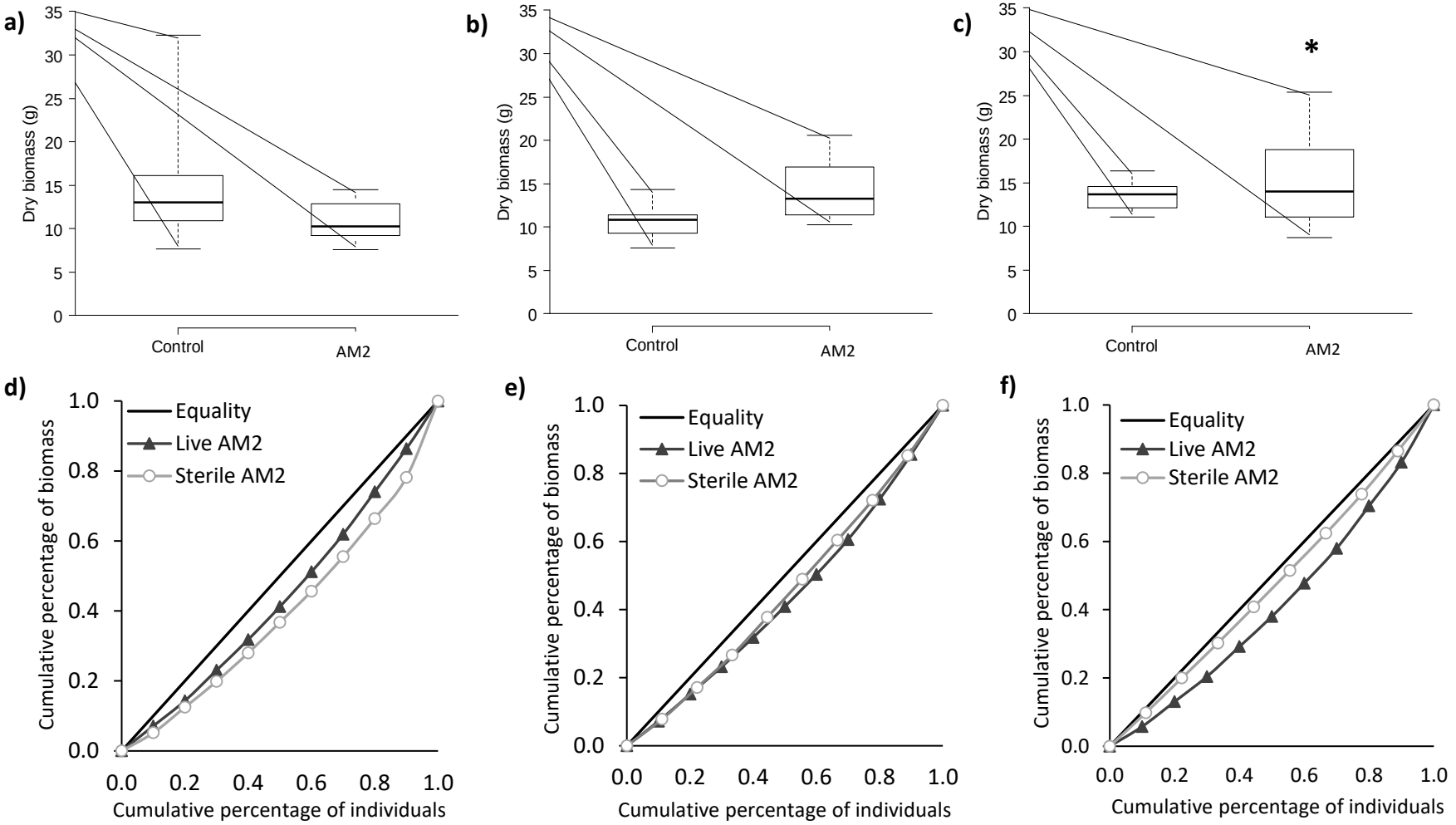


Figure 4.16 ***a-c)*** *Biomass of plants grown with AM2 in each growing media: a) Peat, b) Bark, c) Wood Fibre Z=-2.485, p<0.05. Asterisk denotes statistically different coefficient of variation.* ***d-f)*** *Graphical analysis of size inequality of plants using Lorenz curves plotted against line of equality AM2 in Peat, Bark and Wood Fibre.*

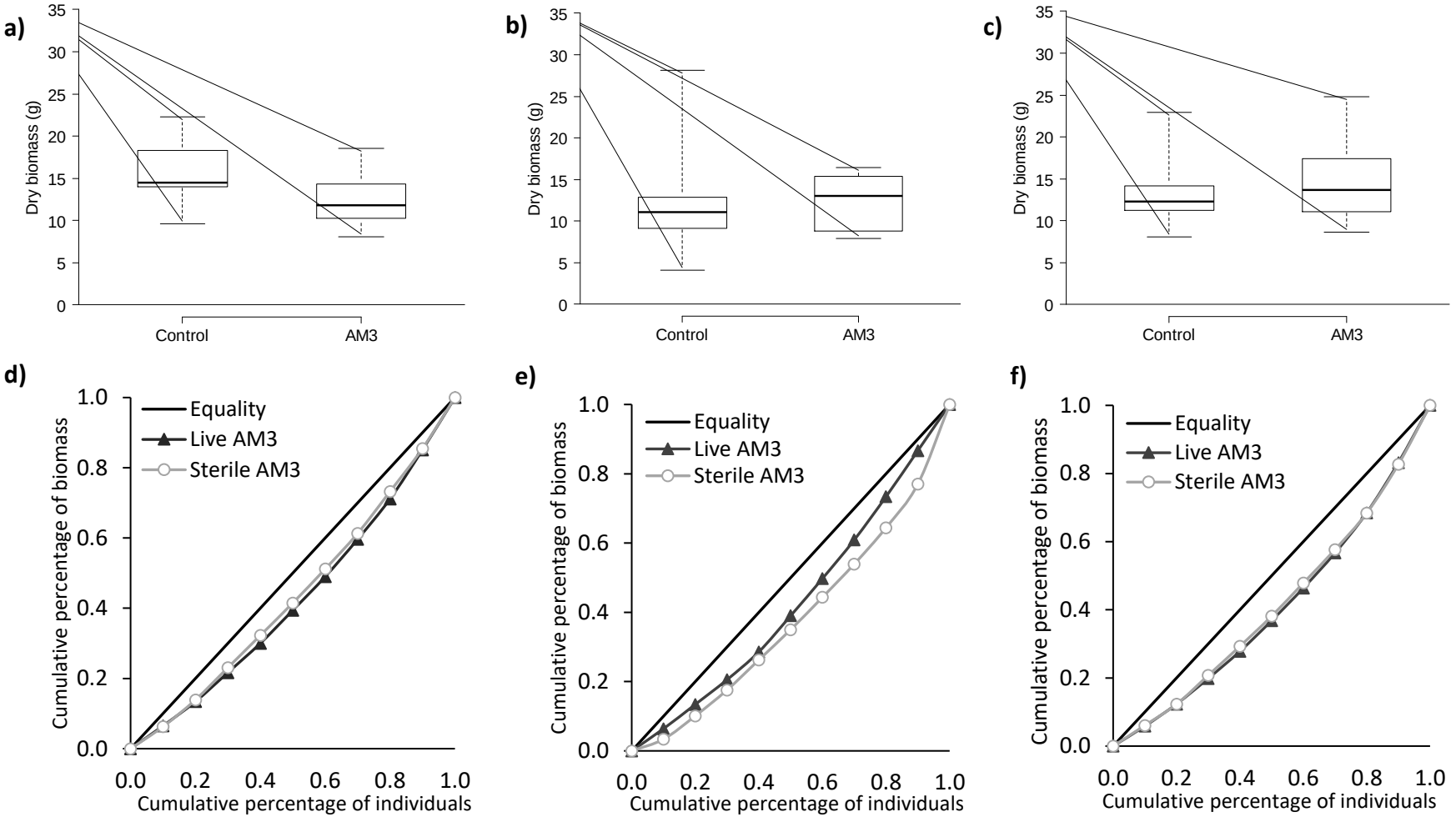


Figure 4.17 ***a-c)*** *Biomass of plants grown with AM3 in each growing media: a) Peat, b) Bark, c) Wood Fibre.* ***d-f)*** *Graphical analysis of size inequality of plants using Lorenz curves plotted against line of equality AM3 in Peat, Bark and Wood Fibre.*

4.4. Discussion

4.4.1. AMF Colonisation

4.4.1.1. Sterilised controls

Significantly reduced root colonisation in plants treated with sterilised inoculum controls for each product in this experiment shows that the use of an autoclave was more effective than heating in an oven, but it still did not manage to create controls that were completely non-mycorrhizal. However, some of the sterilised inoculum treated plants contained less mycorrhizal colonisation than the plants grown without any inoculum. The aim of using non-inoculant control treatments was to try and ensure some plants remained un-colonised however with an uncovered field trial maintaining sterile conditions is impossible. The amount of colonisation of arbuscules and vesicles in control plants suggests that it is unlikely any significant nutrient exchange was occurring between plants and any symbiotic fungi present. Due to the method used, it could also be possible that colonisation could be overestimated due to misidentification of hyphae.

There have been experiments involving field trials (and outdoor pot trials (Matysiak and Falkowski, 2010), which have non-inoculated but non-sterile control treatments which result in low levels of colonisation (Nzanza, Marais and Soundy, 2011; Gholamhoseini *et al.*, 2013; Candido *et al.*, 2015). In these cases, the increase in colonisation of inoculated plants compared to controls was measured and the differences in colonisation were enough to produce significant effects on plant growth. There are other studies, usually glasshouse trials, which use sterilised media to grow plants and non-inoculated controls to ensure control plants are not colonised. This also ensures the inoculum added is the only source of microorganisms, but for the use of commercial inoculum this would not accurately represent the environment that this product would be used in. It is unreasonable to expect home gardeners and indeed growers to maintain a sterile environment to use this product so testing the added benefits that this commercial product can provide on top of low level or 'background' colonisation is more representative. Whilst there are examples of sterilised media (Giovannetti *et al.*, 2012; Jin, Germida and Walley, 2013) and inoculum (Medina, Vassilev and Azcón, 2010) resulting in no colonisation, even sterilised media or inoculum controls in pot based, glasshouse experiments have resulted in colonisation levels similar to this experiment (Sohn *et al.*, 2003; Ortas and Ustuner, 2014a; Ziane *et al.*, 2017).

As peat is relatively low in biological activity (Schmilewski, 2008) it is not normally sterilised before use in commercial horticulture, and the potting bark used in this experiment will have gone through a composting process (Alexander and Bragg, 2014) and therefore will likely have a rich community of microorganisms present. The wood fibre media is the only substrate used in this

experiment to go through any sterilisation due to the methods of its secondary processing (Alexander and Bragg, 2014; Barrett *et al.*, 2016). Presence of mycorrhizal spores and other microorganisms within each growing media could therefore be possible. There has been recent data showing that natural communities of mycorrhizas interact with and in some cases, rely on bacteria and other microorganisms in complex networks (Mar Vázquez *et al.*, 2000; Nadeem *et al.*, 2014). Some of the studies which use mycorrhizas in conjunction with PGPR and other microorganisms (Calvet, Pera and Barea, 1993; Mar Vázquez *et al.*, 2000; Dubsky, Sramek and Vosatka, 2002) or use a non-sterile compost amendment (Perner, Schwarz and George, 2006; Ustuner *et al.*, 2009; Matysiak and Falkowski, 2010) see increases in colonisation compared to controls. The sterilisation of wood fibre could explain why plants grown in the wood fibre medium treated with all sterilised inocula had consistently lower levels of colonisation than non-inoculated controls. However, if this was the case one would expect to see a reduction in colonisation of non-inoculated control plants grown in wood fibre compared to the other growing media, which was not observed.

4.4.1.2. Differences between commercial inocula

This experiment included the use of AM3 as an alternative product. For the reasons stated in section 4.4.1.1, AM2 does not usually get sold as a commercial product, it is sold as AM3 because it includes the PGPR species and species of the *Trichoderma* fungi, which are both naturally present in most soils. It was hypothesised that the presence of these organisms would create a more natural rhizosphere environment in the pots which would help increase mycorrhizal colonisation, as well as plant benefit. However, there was no increase in colonisation between roots of plants grown with AM3 compared to AM2 and both inocula produced plants with less colonisation than AM1 treated plants, (except for AM3 treated plants grown in wood fibre). It was thought this mix would also produce greater plant benefits as *Trichoderma* species have been shown to increase plant size due to a mixture of factors. Mostly, they increase root growth which has been linked to increases in yield (Harman & Howell, et al. 2004), however this appears to have the most effect in stressful environments. Colonisation with *Trichoderma* has helped increase nutrient uptake (Yedidia *et al.*, 2001) which could explain why AM3 plants showed the least amount of leaf purpling, especially in the wood based media. In non-stressed maize plants *Trichoderma* increased growth but this was likely due to the biocontrol effects of Trichoderma species (Hale, Lindsey and Hameed, 1973; Pozo *et al.*, 2002) helping to control pathogens which reduce root growth (Harman, Petzoldt, *et al.*, 2004). Synergy has also been found between *Trichoderma harzianum* (present in AM3) and same AMF species (Datnoff, Nemec and Pernezny, 1995; Nemec, Datnoff and Strandberg, 1996) promoting mycelial growth (Calvet, Barea and Pera,

1992), increasing colonisation in *Glomus* species (Martínez-Medina *et al.*, 2009), and increasing plant growth in potted ornamental plants grown in peat (Dubsky, Sramek and Vosatka, 2002). In marigolds (*Tagetes erecta*) colonised by *F.mosseae,* dry biomass and flower size of plants grown in peat was further increased in the presence of *Trichoderma aureoviride* (Calvet, Pera and Barea, 1993). Despite the evidence for the beneficial effect of *Trichoderma spp*., in this experiment there was no significant effect on the dry weight of plants treated with live AM3 compared to control plants, and plants grown with AM3 were no larger and colonisation was not increased when compared to AM2 or AM1 treated plants. This could be because the correct combination of AMF and *Trichoderma* species did not result in compatible relationships, it has been shown that to avoid negative effects on plant or fungal growth the right pairing must be used (Martínez-Medina *et al.*, 2009). Despite the beneficial *T. harzianum* being present in AM3, *T. koningii* was also present and has been shown to reduce dry weight of plants and root colonisation of *F. mosseae* (McAllister *et al.*, 1994). Different plant species and cultivars have also shown different responses to the same *Trichoderma* species (Dubsky, Sramek and Vosatka, 2002; Tucci *et al.*, 2011) and some *Trichoderma* have been shown to inhibit spore germination of *Glomus* species (Sylvia and Schenck, 1983). Faye et al. (2013) also found that inoculation of plants with two commercial AMF mixed-species inocula containing *Trichoderma* species did not result in significant increases in plant biomass or root colonisation compared to control plants. This would suggest that specific combinations of AMF and *Trichoderma* species would be needed in order to see the benefits on plant growth.

4.4.2. Site Weather Data

The high rainfall in June and subsequent nutrient leakage early on in the growth of the plants is most likely responsible for the reduced growth of these plants compared to the previous experiments. The effect of adding fertiliser to try and prevent early onset nutrient stress and potential shortening of the experiment time was carefully considered as increasing nutrients could have affected colonisation by mycorrhizas. The addition of extra nutrients added appeared to result in a reduction in the effect of AMF on leaf purpling compared to the previous experiment (section 3.4.5).

4.4.3. Flower Number

Significant increases in colonisation in live inoculum treated plants did not result in significant increases in flower number. It has been shown that colonisation with AMF doesn't always result in a significant effect on flower number (Matysiak and Falkowski, 2010; Berruti *et al.*, 2013).

Linderman & Davis (2004) also show that inoculation with four different species of AMF did not increase flower number compared to non-inoculated control plants in four different varieties of *T. patula. Glomus etunicatum* (present in AM2) has been shown to increase flower number in potted plants grown under glass (Aboul-Nasr, 1996). Mixed inoculum with AMF and *T. harzianum* (present in AM3) increased flower number in peat grown ornamentals (Dubsky, Sramek and Vosatka, 2002) and number of flowers produced by strawberry plants was increased in the presence of AMF and plant growth promoting bacteria (Bona *et al.*, 2015) so it is surprising that plants inoculated with live AM3 did not appear to produce more flowers than AM2 plants.

As seen in 2015 plants grown in bark were more floriferous than plants grown in wood fibre but only with AM2 treatment. Perner et al. (2007) showed that the same commercial inoculum could have different effects on the flower number of plants grown in peat amended with different amounts of green waste compost: in peat amended with 20% compost only one commercial inoculum significantly increased flower number in plants compared to non-inoculated controls, but in peat mixed with 40% compost all three commercial inocula significantly increased the number of flowers compared to control plants. This could explain why an effect of growing media was only seen with one inoculum type. Growing media has also been shown to have an effect on flower number in plants (Hidalgo, Matt and Harkess, 2006), increased nutrients and flower number could be linked to higher water holding capacity of the growing media. Interestingly in the same study the majority pine bark media resulted in a reduction in flower number. Higher water holding capacity in peat amended with compost has been shown to significantly reduce flower number in one species of nursery grown shrub compared to peat with no compost added despite the compost addition increasing AMF colonisation and nutrient content of shoots (Matysiak and Falkowski, 2010).

4.4.4. Leaf Discolouration

Plants with purple leaves were found in all live treatment groups but the number or severity of purpling was always equal or less than in sterile control treatments, suggesting that levels of phosphorus in live AM plants were increased. A significant increase in the levels of root colonisation in live AM1 treated plants reduced the number of individuals with purple leaves in wood fibre and bark media (although not significantly) but this pattern was not followed with peat grown AM2 treated plants or AM3 inoculated plants in wood fibre. Although phosphorus uptake, or content of plant tissue, was not measured in this experiment the method of visual scoring of a phosphorus stress indicator correlates with previous findings. Increased P uptake as

a result of mycorrhizal colonisation has been correlated with increases in biomass (Mensah *et al.*, 2015) and significant reductions in the number of plants with purple leaves or severity of leaf purpling occurred in live treatment groups where significant increases in biomass were also seen (live AM1 in bark and wood fibre and live AM2 in wood fibre). In other studies the effects of increased P and increases in biomass were also shown to correlate with root length colonisation (Treseder, 2013).

The amount of root colonisation by arbuscules and vesicles can help to explain why significant increases in root colonisation by hyphae did not always result in beneficial effects on P uptake and biomass. In peat, plants treated with live AM2 showed no significant reduction in leaf purpling and no increase in shoot biomass despite significantly higher root colonisation by hyphae in live inoculum plants compared to controls. This was because the amount of arbuscules and vesicles colonising roots was low and similar in both treatments. In AM2 the number of arbuscules and vesicles colonising the roots of plants grown in wood fibre was significantly higher compared to the roots of plants which received sterile inoculum and these plants also saw the benefits of significantly increased biomass and reduced leaf purpling.

There were instances where no significant increase in biomass or reduction in leaf purpling was seen even though levels of root length colonisation by hyphae or arbuscules and vesicles were significantly increased in live inoculum treated plants compared to sterile inoculum addition: peat with AM1, and bark and wood Fibre with AM3. Lack of significant increases in biomass have been seen with AM colonisation and plant growth has been shown to be independent of colonisation in plants (Linderman & Davis 2003). Non-significant increases in biomass and reduced amounts of plant phosphorus (up to 40% less than controls) have been seen in plants colonised by *G. etunicatum,* which is present in AM2 (Aboul-Nasr, 1996). It has also been shown that colonisation by AMF from commercial inocula in peat did not produce significant plant growth benefits or increase P uptake (Perner, Schwarz and George, 2006) in onion. The reason the authors give is that P is in a form readily available to the plant in fertilised peat so AMF are not necessary. This does lead to the question as to why, if P is so readily available, more plants with purple leaves were seen in sterile inoculum treated peat than any other treatment combination with 90% of replicates displaying some level of purpling. The addition of organic amendments to peat has been shown to increase colonisation and the uptake of P in plants (Perner, Schwarz and George, 2006; Perner *et al.*, 2007; Matysiak and Falkowski, 2010). Hidalgo et al. (2006) showed their majority pine bark medium had higher P concentration compared to a majority peat substrate

which had the lowest nutrient content of all substrates used to grow plants. The presence of extra P in forms that are not all easily obtained by plant roots in the other two media could have resulted in more of a need for AMF and a functional relationship, which would explain the differences in effect seen with AMF in the reduced peat media compared to peat for each inoculum, with the exception of AM3 treated plants.

As previously discussed, AM3 plants were found to contain the smallest number of purple leaved individuals and this could be due to the presence of PGPR and *Trichoderma* species. The addition of these organisms which have been shown to increase nutrient uptake, including P, in plants (Gagné *et al.*, 1993; Yedidia *et al.*, 2001; Çakmakçi *et al.*, 2006; Lavakush *et al.*, 2014) could be the reason for the lack of effect as a result of mycorrhizal colonisation. Certainly, without an appropriate control to be sure of the effect of adding these organisms without AMF it cannot be claimed that this effect is due to the additional species alone but a control for the presence of the AMF species alone can be seen with AM2 plants. In AM3 inoculated plants the number of purple leaves is significantly reduced in all treatments compared to AM1 and 2, except in sterile inoculum treated peat, but live AM3 was the only treatment to reduce the number of purple leaved plants in peat suggesting the effect of something other than the AMF species that were present in AM2.

4.4.5. Porosity

In this experiment, the wood fibre medium had higher water retention porosity (WRP) than bark in AM1 and AM2. This is surprising as pine bark is known for its high WRP and has been used to improve the water holding capacity of peat (Barrett *et al.*, 2016) and the previous experiment showed that pots containing bark had higher WRP than wood fibre pots. The only significant positive effect on water retention porosity caused by root length colonisation in wood fibre plants treated with live AM1 could be explained by those plants containing the highest levels of colonisation: 52%±4.76 which were considerably higher when compared to the second highest colonisation 42.2%±6.00 also found in wood fibre grown plants but with live AM3. Increase in hyphal density has been shown to improve soil structure through increasing water stable aggregation in pot experiments using a soil substrate (Wu, Xia and Zou, 2008). So far, this effect has been shown in drought stressed plants, the evidence suggests that aggregation helps maintain water close to the roots as the soil dries (Davies, Potter and Linderman, 1992; Asrar and Elhindi, 2011; Carminati *et al.*, 2016). Growing media is soilless and is often a lot more porous and contains larger particles than soil due to the need to create a suitable amount of air space and drainage for container plants, but particle size (Gaur and Adholeya, 2000), has been shown to

affect the growth of hyphae and the production of glomalin in artificial soilless media. Rillig & Steinberg (2002) also suggest that AMF are able to detect a fine particle (non-aggregated) medium and modify it using glomalin to increase aggregation, and therefore pore space. If this effect has been shown in an artificial medium as well as in containers, then it is reasonable to suggest AMF will have the same effect in soilless growing media. Wood fibre was the only medium to show consistent increase in WRP with addition of live AMF inoculum. Improved water relations have also been linked to improved nutrient uptake (Koide 1985; Nelsen & Safir 1982) which could explain why plants grown in wood fibre were the only AM1 inoculated plants found to have significantly reduced leaf purpling. This positive effect of AMF in wood fibre is encouraging, the lightweight nature of the material would allow for reduced transport costs but this results in the negative feature of high air filled capacity and low water retention, but this could be improved with AMF which are also likely to help improve plant growth in drier conditions (Ruiz Lozano, Azcón and Gomez, 1995; Bryla and John M. Duniway, 1997; Augé, 2001b; Wu, Xia and Zou, 2008) including marigolds (Asrar and Elhindi, 2011).

The physical properties and structure of growing media is likely to have been affected and changed after such a long growth period. It is known that wood fibre in particular is prone to shrinking or 'slumping' when in containers over time (Gruda and Schnitzler, 2004) which may lead to excessive water retention (Nash and Porkorny, 1990). The above-average rainfall that plants experienced in June, could have resulted in increased compression and therefore slumping of the wood fibre media, resulting in increased WRP compared to 2015. It has been shown that plants perform better in wood fibre medium when well-watered (Alexander, Williams and Nevison, 2013, 2014). Similar increases in biomass were seen in bark and wood fibre-grown plants with live AM1 and AM2 despite their opposite effects on water holding capacity.

Higher levels of root colonisation and subsequently hyphae in wood fibre pots could be the reason for its increased WRP compared to bark and peat pots. The addition of live inoculum in bark and peat pots had the opposite effect on WRP, significantly decreasing it in bark AM2 and peat AM3 pots and a similar reduction was seen in AM1. The negative effect in AM1 was not found to be significant due to the high standard error, it is unsurprising that this method produced variable results as it is not designed to be used on growing media containing roots. The addition of composted bark to peat has been used to increase its water holding capacity (Schmilewski, 2008; Barrett *et al.*, 2016) but pine bark itself has high air-filled porosity and low water holding capacity and it depends on the amount of bark added to peat how the physical properties will be affected.

Maher (2001) showed that 25% bark and 50% bark decreased the easily available water by 2.8% and 5.4% respectively, compared to 100% peat. This supports the significant decrease in WRP seen in bark pots compared to peat in AM1 and AM3 treatments. The effect of colonisation decreasing WRP does not fit with the literature, as this effect was not seen to correlate with root length colonisation so the result could be due to an indirect effect of AMF colonisation. Roots also influence the structure and water retention of media and their presence in the growing media (especially in pore spaces) when these measurements were taken could have caused a reduction in pore space to be measured. Colonisation with AMF is widely known to increase root biomass of potted plants (Gaur et al. 1998; Linderman & Davis 2003; Wu et al. 2008; Ortas & Ustuner 2014) and after three months of growth the root density of these pots was high.

4.4.6. Biomass

The most consistent finding in these data were the increases in biomass with live inoculum treatment in both reduced peat media treated with AM1 and AM2. Although these were not all found to be significant and hyphal colonisation did not correlate with biomass it is encouraging to see a consistent effect. As with leaf purpling the significant increases in biomass in live inoculum do correlate to significant increases in root length colonisation by hyphae (AM1) and arbuscules (AM2) in live inoculated plant roots compared to sterilised control plants. The addition of AMF has been shown to increase biomass in plants (Aboul-Nasr, 1996; Asrar and Elhindi, 2011) and this effect was consistent in *T.patula* grown in peat-based substrates with different amounts of coir added (Linderman & Davis 2003). Although, increases in plant phosphorus content as a result of colonisation with AMF have been found irrespective of increases in biomass, (Perner *et al.*, 2007; Matysiak and Falkowski, 2010; Gosling, Jones and Bending, 2016) the combination of increased colonisation, biomass increase, and reduction in leaf purpling in live AM treated wood fibre-grown plants suggests that there is a causal link here.

Plants grown with AM3 were the only plants to show no effect of AM on biomass despite significantly higher levels of hyphae (wood fibre) and arbuscules (wood fibre and bark) in live inoculum treated plants compared to controls. As mentioned previously there was no control for the effect of the additional microorganisms present in the AM3 mix and therefore it is possible that the presence of these was not affected by the sterilisation method. If the levels of PGPR and *Trichoderma.* species were similar between live and sterile inoculum treatments then one would not expect biomass to differ significantly. Although the combination of some *Trichoderma* and

AMF species has been shown to have an additive effect on plant biomass (Calvet, Barea and Pera, 1992).

Variability in plant growth has been seen in response to different soilless growing media (Corkidi *et al.*, 2004; Hidalgo, Matt and Harkess, 2006) including reduced peat (Benito *et al.*, 2005; Perner, Schwarz and George, 2006), and peat free (Alexander et al. 2013, 2014) so the lack of significant differences in biomass between growing media is surprising. As both peat reduced media produced plants that were comparable to peat though, this result is positive.

Plant responses to colonisation by different AMF species have also shown to be variable and growth depression has been seen with some combinations, in their experiment Linderman and Davis (2004) found shoot biomass was significantly decreased compared to control plants with certain AMF species in three out of four varieties of *T.patula*. Significant growth depressions were not seen with any AM treatment but in peat-grown plants treated with live AM2 a significant increase in root colonisation by hyphae resulted in a notable decrease in biomass compared to control plants; this, combined with a noticeable decrease in flower number suggests a parasitic relationship with colonised mycorrhizas (Johnson, Graham and Smith, 1997; Smith and Read, 2002a; Bucher, 2007; Nouri *et al.*, 2014; Walder and van der Heijden, 2015).

4.4.6.1. Coefficient of Variation

There was no significant difference in the biomass of plants grown without inoculum in each media, this supports the lack of differences in biomass between treated plants grown in each media.

The lack of significant difference between the coefficient of variation of biomass of non-inoculum and sterile inoculum treatments shows that adding sterilised inoculum did not affect or increase biomass.

4.4.6.2. Size Inequality

The addition of live inoculum did consistently increase biomass in the reduced peat media but it did not consistently reduce size inequality. Only in the bark media were plants inoculated with live AM1 found to be more consistent in size compared to control plants and this affect was achieved by producing fewer small plants. Increases in biomass were also seen in wood fibre grown plants inoculated with live AM1 and AM2 but these did not result in significant decreases in size inequality because the number of large plants was increased which increased the size range. In wood fibre-grown plants treated with AM2 the increase in size inequality was significant.

In contrast, size inequality was decreased (albeit not significantly) in peat AM2 and bark AM3 plants but it came at a cost because those plants had smaller biomass on average compared to control plants because the number of large plants had been reduced. It is unsurprising that the ability of AMF to increase access to nutrients and water would allow plants to grow bigger thus reducing the number of small plants, but for this to decrease size inequality it needs to have a consistent effect on all plants in the population. It has been suggested (Jin et al. 2017) that in a confined space the fungi could deplete nutrients which can put limitations on plant growth, this could explain the decrease in biomass and overall reduction in size in peat AM2 and bark AM3 plants.

Ultimately, while plants with a more consistent size are desirable for growers, many of whom have to meet size regulations (e.g. 14-20cm height for potted herbs (Simon Budge (Vitacress), personal communication, 2017), if those plants are significantly reduced in size due to AMF then more plants would end up not meeting those targets. Equally if AMF were to increase the biomass of plants grown in reduced peat media unnecessarily (when plants in reduced peat media without AMF were not significantly smaller than those in peat) and also increase size inequality then they would not be favoured by growers.

4.5.Conclusions

This study has shown that consistent positive effects on biomass can be seen with different commercial AMF inocula in two reduced peat growing media. The most promising effects have been seen in the wood fibre medium which showed consistent plant biomass increases, reduced leaf purpling and high levels of colonisation with more than one commercial inoculum. High levels of colonisation also helped to improve one of the problems found with this media by increasing water retention. Future work should investigate these effects of AMF seen in wood fibre to see if they can be reproduced and if they continue to help ameliorate plant growth in increasingly reduced peat substrates.

Whilst the effects of AMF on plant growth in wood fibre are promising, these resulted in an increase in plant size inequality. Increasing the size range of plants through inconsistent effects across replicates could outweigh the benefits of adding live AMF. In future, analyses should consider whether this inequality is sufficient as to be detrimental to the production process of commercial plants. Equally, a combination of AMF and growing media which results in a reduction

in size inequality but which significantly reduces the overall size of plants would not be favoured over a combination which produces multiple positive growth effects.

To provide answers to which species in the inoculum mixes are producing such results so that these effects can be understood and replicated, perhaps with customised inocula, molecular analysis of root material is necessary. This analysis could also help to confirm the positive effects of AMF are due to increased nutrient acquisition and explain negative interactions seen with some inoculum and growing media combinations, as the beneficial nature and level of resource provision of some AMF species has been characterised.

Chapter 5 - Greenhouse trials with reduced peat media and commercial AMF inocula on potted herbs.

5.1 General Introduction

To have an impact on the reduction of peat usage in horticulture, an alternative system must be found to replace the use of raw peat with its associated addition of high levels of inorganic fertiliser, and large amounts of water used by professional growers around the UK. In 2016 basil was the second best-selling herb in the UK with over 14.5 million pots, bunches and packs sold (Vitacress, 2016). A large proportion of that is likely to be fresh pots as one of the largest supermarket suppliers of potted basil in the UK reportedly produces around 12 million pots per year (Simon Budge (Vitacress), personal communication 2016). Not only is it an important food crop in the UK but basil is considered a medicinal plant with its high antioxidant levels and essential oils which can be used in pharmaceuticals and perfumes, and the ability of AMF to enhance these properties has been widely studied (Copetta, Lingua and Berta, 2006; Rasouli-sadaghiani *et al.*, 2010; Taie, Salama and Samir, 2010; Schroeder, Gange and Stead, 2012; Mnayer *et al.*, 2014). For the potted herb industry, reporting increased health benefits of herbs as a result of AMF would be an extra incentive to reduce their levels of peat usage. The aim of this branch of the thesis was to test whether effects of AMF in reduced growing media with marigold and chives could be repeated with glasshouse-grown potted herbs. This could offer growers some data on how peat could be reduced in a large part of the horticultural industry in the UK without loss of quality, or possibly with improvements.

The experiments outlined in this Chapter represent a continued effort over four years to try and understand the conditions necessary to generate comparative results to those seen with bedding plants in the previous Chapters with potted herb species that are grown for commercial sale in the UK.

Experiments were carried out alongside those featured in Chapters 2-4 and the results of those experiments helped to influence the methods used in this Chapter, however the differences in environment (controlled vs. outdoor), timescale (9 weeks vs. 3 months) as well as species meant that the positive results seen from AMF addition in bedding plants could not always be replicated in the herbs. The short timescale and optimum environment used to grow herbs in the industry may not facilitate mycorrhizal colonisation. Plants have been found to have evidence of colonisation after as little as 3 days (Afek *et al.*, 1990) and some plants inoculated with some AMF species show colonisation earlier than when inoculated with other species. Corkidi et al. 2004) found that plants inoculated with two different commercial inocula were found to be colonised after just 2 weeks whereas colonisation of roots with three other products was only found after six weeks.

Basil is known to associate with mycorrhizas and has been shown to be colonised in greenhouse experiments (Rasouli-sadaghiani *et al.*, 2010) in less than the nine weeks growing time required for these experiments (Copetta, Lingua and Berta, 2006; Toussaint, Smith and Smith, 2007) with species of the same AMF included in the commercial inocula AM1 and AM2 mixes.

After two failed attempts at finding evidence of root colonisation in basil, possibly due to the commercial seed being treated with fungicide, uncoated chive seed was used instead. Significant levels of colonisation in chives were also not achieved in 2015 but this was rectified when chives were grown outside the following year alongside the marigold experiment outlined in Chapter 4. Once the opportunity to grow plants in good, consistent growing media in a setting similar to the industry was available both basil and chives were grown again. Due to unforeseen circumstances, the root staining for this experiment could not be completed in time to include in this thesis, however the experiment was able to give an indication of the use of these sustainable growing media in this industrial set up as well as what might be needed to be changed for the optimum results to be achieved.

Experiments are presented in chronological order, they began with trying to implement the use of reduced peat compost into the semi-automated, high throughput set up in a working commercial glasshouse that produces these plants for a number of large supermarket retailers in the UK. It became apparent that this set up, which is designed for use with peat and its favourable, consistent qualities would not be suited to experiments using reduced peat substrates. Experiments in later years were all conducted at Royal Holloway in order to control conditions to better suit the AMF and reduced peat growing media, however in the summer of 2016 an experiment was conducted at Royal Holloway in similar conditions to the industry in a controlled temperature glasshouse with supplementary lighting and a flood watering system.

5.2 Can Commercial AMF Improve Growth of Commercial Potted Basil in Reduced Peat Multi-Purpose Composts?

5.2.1 Materials and Methods

5.2.1.1 Plant Species

Ocimum basilicum L. (sweet basil) cv. Marion seeds were provided by Vitacress, the seeds were sown using the automatic system at the glasshouse. Approximately 36 seeds were distributed evenly on the surface of each pot. As standard, to prevent fungal growth and contamination in the glasshouse these seeds were supplied coated with a fungicide.

5.2.1.2 Multipurpose compost

This experiment used the same multipurpose composts used in the 2012 preliminary experiment (Edwards, 2012), two of these composts were the same brands used in the experiment outlined in Chapter 2: the coir based 'Fertile Fibre' and the low peat based 'Levington® Multipurpose Compost' (section 2.2.2). A CGW, wood fibre and peat for comparison were also used.

- Green Waste

The green or recycled waste compost used was 'Vital Earth Multi-Purpose Organic Compost' a reduced peat mix that claimed to contain mostly fine composted conifer bark, and some green compost. The 'Vital Earth' company are small and UK based, they only produce reduced peat products and all their products contain garden waste that has been directly collected and composted by the company (Vital Earth GB Ltd, 2017).

- Wood Fibre

The wood based mix used was produced by 'Westland Horticulture', based in Northern Ireland, this is a large, recognised and trusted brand in the horticultural market. 'West+ Light and Easy' is reduced peat and contains 50% 'West+ wood fibre', this product advertises that it weighs 60% less than standard bags of multipurpose compost, reduces the need for watering by 50% and has enough fertiliser to feed plants for four months (Westland Horticulture, 2017).

- Peat

For this experiment the peat used was direct from Vitacress and was the same as used for all pots and consisted of 100% imported Estonian peat. No additional fertilisers are added to this before it is used to fill pots, unlike the other multipurpose composts.

5.2.1.3 Commercial AMF Inoculum

The same commercial inocula AM1 and AM2 were used as described in section 2.2.3 of Chapter 2 of this thesis. As the pots were approximately one litre, half the recommended dose for a 2-litre pot was used for each inoculum, so 7ml of AM1 was measured in a 15ml falcon tube and 0.1g AM2 was weighed in an Eppendorf tube and added to each pot. Control pots had no inoculum added. As this experiment involved growing basil plants from seed instead of using plug plants, the pots were half filled with each compost or peat. The inoculum was then sprinkled in an even layer and this was then covered with the rest of the compost to fill the pot and the seeds were sown on the surface. This method was used to allow the roots of the basil shoots to grow through the inoculum layer where they would come in to contact with propagules and hopefully, be colonised.

5.2.1.3.1 Root Staining and analysis for AMF colonisation

Root material was harvested from each pot at the final harvest and fixed in 70% ethanol for mycorrhizal colonisation analysis. The method of staining root material was as outlined in section 2.2.3.1 of Chapter 2 with the exception that roots required only 7-10 minutes in KOH and acidified ink stain as these roots were younger and thinner than marigold roots. The method for determining root length colonisation was also as described in 2.2.3.1

5.2.1.4 Experimental Design

Twenty-one replicate pots were planted for each of the 15 treatments: the two commercial mycorrhizal products and no inoculum controls combined with each of the five multipurpose composts, in total 315 pots were planted. The replicate number was chosen to make sure all pots fitted onto one flood bench tray to make sure they could be kept separate from other research experiments in the glasshouse, as well as all receive the same amount of exposure to water and nutrients at the same time. The pots were placed randomly on the bench in diagonal rows. The replicate number also allowed for three harvests of seven replicate pots from each treatment to be completed across the growing period of nine weeks to track the growth and antioxidant content over time.

5.2.1.5 Site and Conditions

All pots received the same watering regime where they were watered as necessary via flooding of the bench for up to 3cm depth for 10-15mins. They were all grown under the same commercial conditions, including pest, temperature and light control, as they would be for the normal commercial production of this pot-grown basil. It was intended that the amount of nutrients added in the flood water in solution would be reduced to 50% of the normal rate, this was to try

and ensure that levels of nutrients, particularly phosphorus were not so high as to negatively impact the germination and colonisation ability of the mycorrhizal fungi. Unfortunately, this was not maintained for the entirety of the experiment as the bench was moved and put on the standard watering regime with 100% fertilisation by mistake. This occurred sometime between the second and third harvests.

The seeds were sown on the 26th October 2013 and grown at the Vitacress West End Nursery, (Angmering UK) for approximately 9 weeks. This is the standard growing time used for Basil at the nursery at this time of year.

5.2.1.6 Harvest of Material

105 pots were placed in boxes and transported back to Royal Holloway every 3 weeks (26th November 10th December and 27th December). It took 2-3 days to harvest all the material; for this time the plants were maintained in a controlled temperature growth room under a 12hr day (c. 20µmoles.m^{-2}.s^{-1}) at 21°C.

Every individual basil plant in each pot was cut at soil level. The weight of every seedling was measured and the above soil height measured with a 30cm ruler.

5.2.1.7 Antioxidant Analysis

Leaf material representative of the pot was harvested for antioxidant analysis from three replicate pots from each treatment batch and placed in small falcon tubes before being frozen rapidly in liquid nitrogen before being stored at -20˚C.

Individual leaves were weighed and water-soluble antioxidants extracted by grinding the leaves with 500µl of 50mM acetate buffer (pH 3.6) and a small amount of sand. The resulting sample was transferred to a microfuge tube with residue from the pestle and mortar included by washing with a further 500µl of buffer. These extracts were then stored on ice until they were centrifuged (16,000xg for 2 minutes). After centrifugation, the supernatant was transferred to clean, labelled tubes and either assayed immediately or stored at -20°C.

antioxidant activity was compared to that of ascorbic acid (c.3-1000µM) using the method modified from that of Benzie & Strain (1996). In the well of a microtitre plate 30µl of either standard (3-1000µM ascorbic acid) or sample was placed along with 300µl of FRAP reagent. Freshly prepared FRAP reagent was made up using 25ml acetate buffer (50mM, pH 3.6) containing 10mmol TPTZ (2, 4, 6-tripyridyl-s-triazine) prepared in 40mmol of HCl with 20mmol Ferric chloride. On each plate, all the samples were replicated at least 3 times and the standard a

minimum of twice. The absorbance of each sample was determined using an iEMS microtitre plate reader at 590nm and the data recorded using Ascent software for iEMS version 2.6.

5.2.2 Statistical Analysis

Two-way ANOVAs were carried out on plant height, biomass and relative antioxidant content means to examine differences between compost treatments as well as interactions of sterile and live inoculum treatments within composts. Separate analyses were carried out for each harvest and each inoculum. Where data were not normally distributed values were transformed with squares or logarithms. Percent data for root colonisation were subjected to arcsine square root transformations, prior to analysis. Means were separated with Tukey's HSD post hoc test. All standard ANOVAs and T-tests were conducted using IBM SPSS 21.

5.2.3 Results

5.2.3.1 AMF Colonisation

Analysis of sampled root material for mycorrhizal colonisation showed that no sampled roots had been colonised by AMF.

5.2.3.2 Plant Height

By the final harvest peat-grown plants were significantly taller than plants grown in all of the alternative composts but green waste and low peat plants were significantly smaller than plants grown in wood fibre compost (F_4=16.661, Table 5.1). In AM2 treated pots peat-grown plants were found to be significantly taller than those in all alternative composts (F_4=22.055, Table 5.1). No significant differences were seen between different AM treatments for either inoculum (Figure 5.1). The effect of compost or AM treatment on plant height also did not vary between harvests for either inoculum (Table 5.1).

		AM1		AM2	
	d.f	**F**	**Sig.**	**F**	**Sig.**
AM	1	2.962	p>0.05	1.768	p>0.05
Compost	4	16.661	**p<0.001**	22.055	**p<0.001**
Date	2	1035.652	**p<0.001**	1348.729	**p<0.001**
AM * Compost	4	1.805	p>0.05	2.270	p>0.05
AM * Date	2	1.511	p>0.05	2.825	p>0.05
Compost * Date	8	1.536	p>0.05	1.711	p>0.05
AM * Compost * Date	8	0.880	p>0.05	0.809	p>0.05

Table 5.1 Results of two-way ANOVAs on plant height for basil grown in all composts treated with AM1 and AM2. Error degrees of freedom: AM1=169, AM2=170.

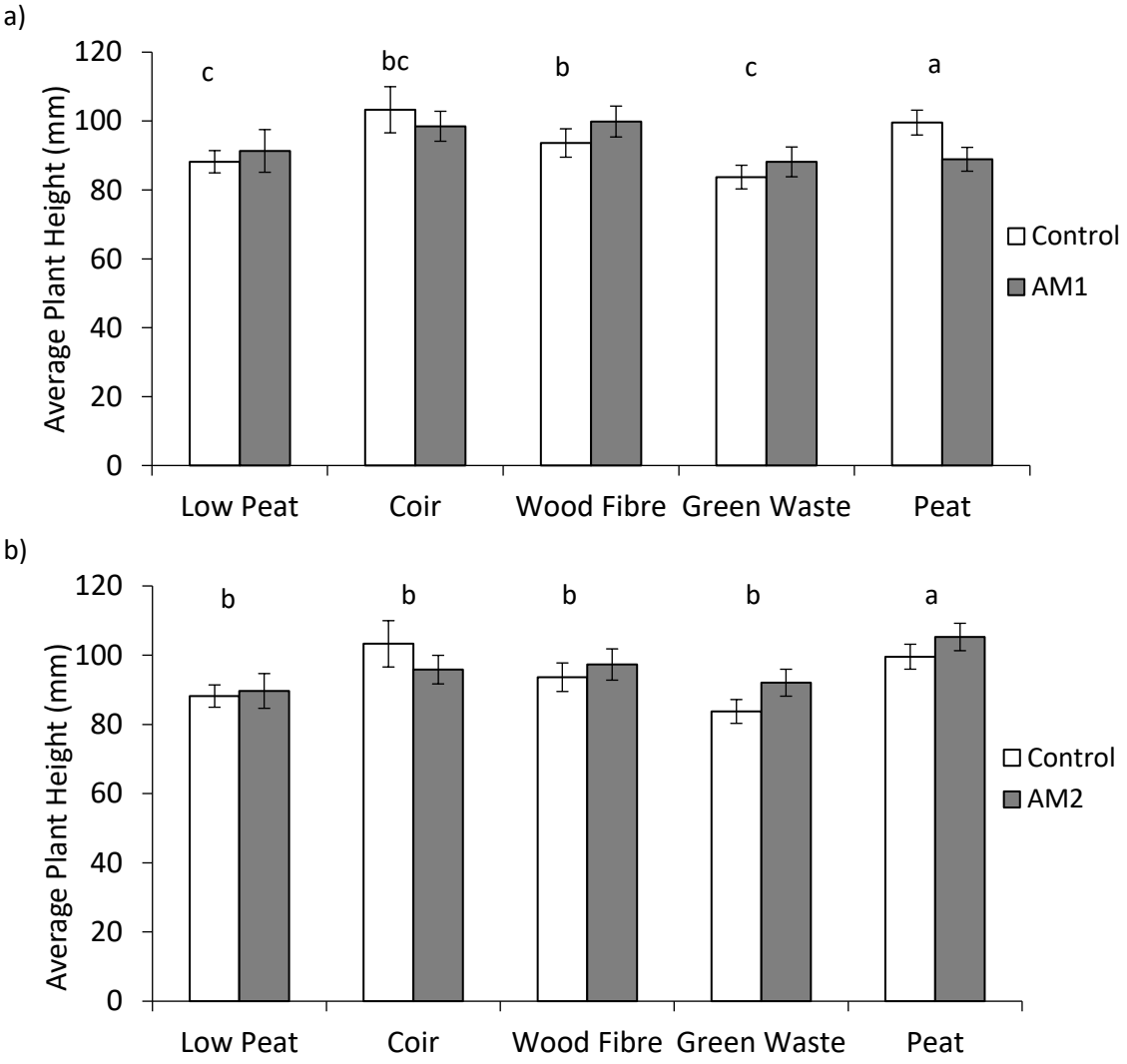


Figure 5.1 Average height of basil plants grown in each compost with non-inoculated control plants compared to a) AM1 and b) AM2 treated plants. Pairs of bars with different letters indicate significant differences between compost treatments. n=7, bars±S.E.

Figure 5.2 shows that the height of basil plants grown in peat did appear to be greater than all other composts. The pictures also demonstrate the poor performance of the green waste compost, again especially when treated with AM inoculum.

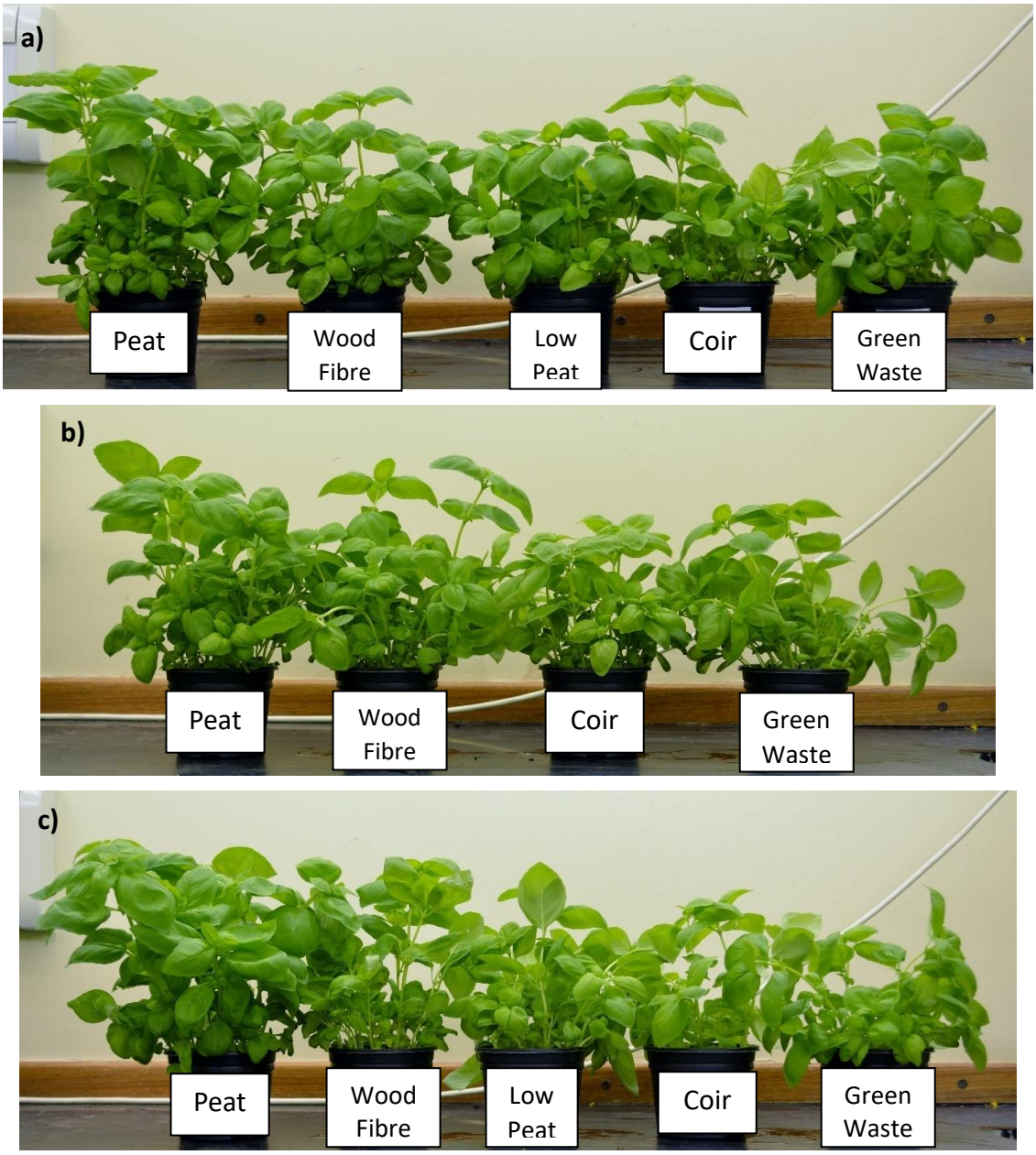


Figure 5.2 Photographs of basil plants just before the final harvest after 9 weeks of growth in each compost for each inoculum treatment: a) Non-inoculated controls, b) AM1, c) AM2.

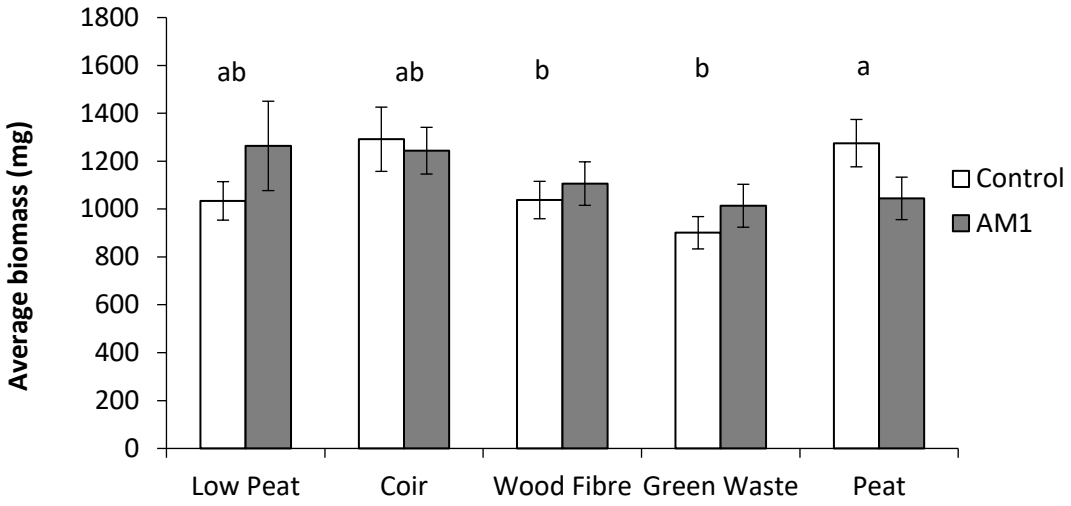
5.2.3.3 Biomass

In AM2 treated plants, biomass was significantly affected by compost and this varied with each harvest (F_8=8.450, Table 5.2). After three weeks of growth, peat-grown plants were significantly larger than those grown in all alternative composts and the biomass of plants grown in the low peat compost was also found to be significantly higher than plants grown in the green waste compost, by the final harvest (9 weeks) coir pots were the only ones to produce plants that were of a similar size to those grown in peat. Green waste was the compost that produced the smallest plants (Figure 5.3b). In AM1 biomass was found to differ between compost treatments (F_4=6.976, Table 5.2) but was consistent across harvests. Similarly, peat-grown plants were significantly larger than wood fibre and green waste grown plants but coir and the low peat compost produced plants that were of comparable size to those in peat (Figure 5.3a). Despite not seeing any evidence of colonisation there was a significant effect of inoculum treatment on biomass with AM1 and this was found to vary with harvest (F_2= 3.553, Table 5.2). Using independent t-tests it was discovered that inoculation with AM1 significantly increased biomass of control plants at early harvests in wood fibre and green waste composts (Figure 5.4), but this effect had disappeared by the final harvest (Table 5.3).

		AM1		AM2	
	d.f	**F**	**Sig.**	**F**	**Sig.**
AM	1	4.430	**p<0.05**	2.764	p>0.05
Compost	4	6.976	**p<0.001**	26.894	**p<0.001**
Date	2	602.339	**p<0.001**	1691.105	**p<0.001**
AM * Compost	4	0.063	p>0.05	1.943	p>0.05
AM * Date	2	3.553	**p<0.05**	4.756	**p<0.05**
Compost * Date	8	1.926	p>0.05	8.450	**p<0.001**
AM * Compost * Date	8	0.490	p>0.05	1.604	p>0.05

Table 5.2 Results of two-way ANOVAs on plant biomass for basil grown in all composts treated with AM1 and AM2. Error degrees of freedom: AM1=169, AM2=170.

a)



b)

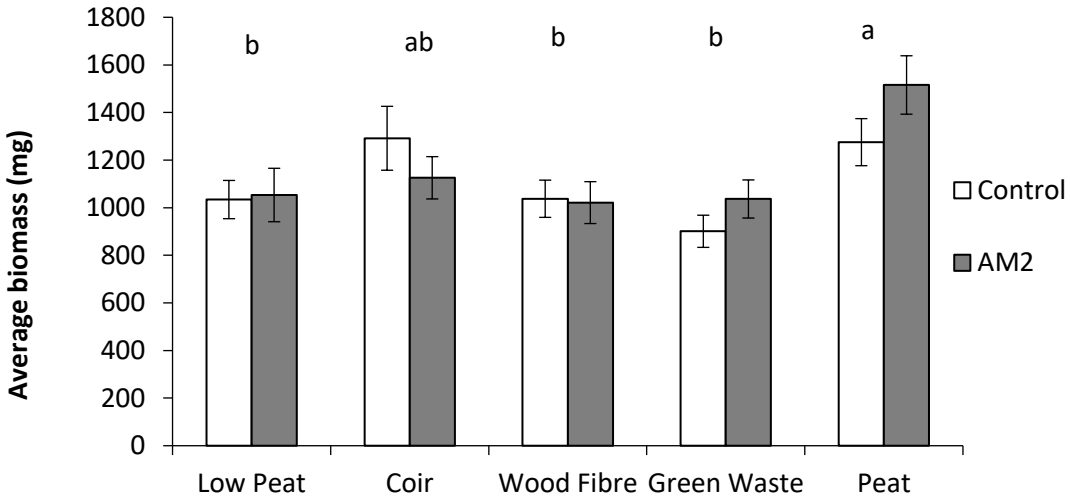


Figure 5.3 Average biomass of basil plants grown in each compost with non-inoculated control plants compared to a) AM1 and b) AM2 treated plants. Pairs of bars with different letters indicate significant differences between compost treatments. n=7, bars±S.E.

Compost		Harvest 1 (3 weeks)		Harvest 2 (6 weeks)		Harvest 3 (9 weeks)	
	d.f	***t***	***Sig.***	***t***	***Sig.***	***t***	***Sig.***
Wood Fibre	12	0.518	p>0.05	-2.733	**p<0.05**	-0.960	p>0.05
Green Waste	12	-2.621	**p<0.05**	-2.311	**p<0.05**	-1.471	p>0.05

Table 5.3 Results of independent t-tests comparing average plant biomass for basil grown in three composts with no inoculum or AM1.

Figure 5.4 The difference in biomass between non-inoculated control plants and plants treated with AM1 after 6 weeks of growth in two composts. Asterisk represents significant difference between inoculum pairs. n=7, bars±SE

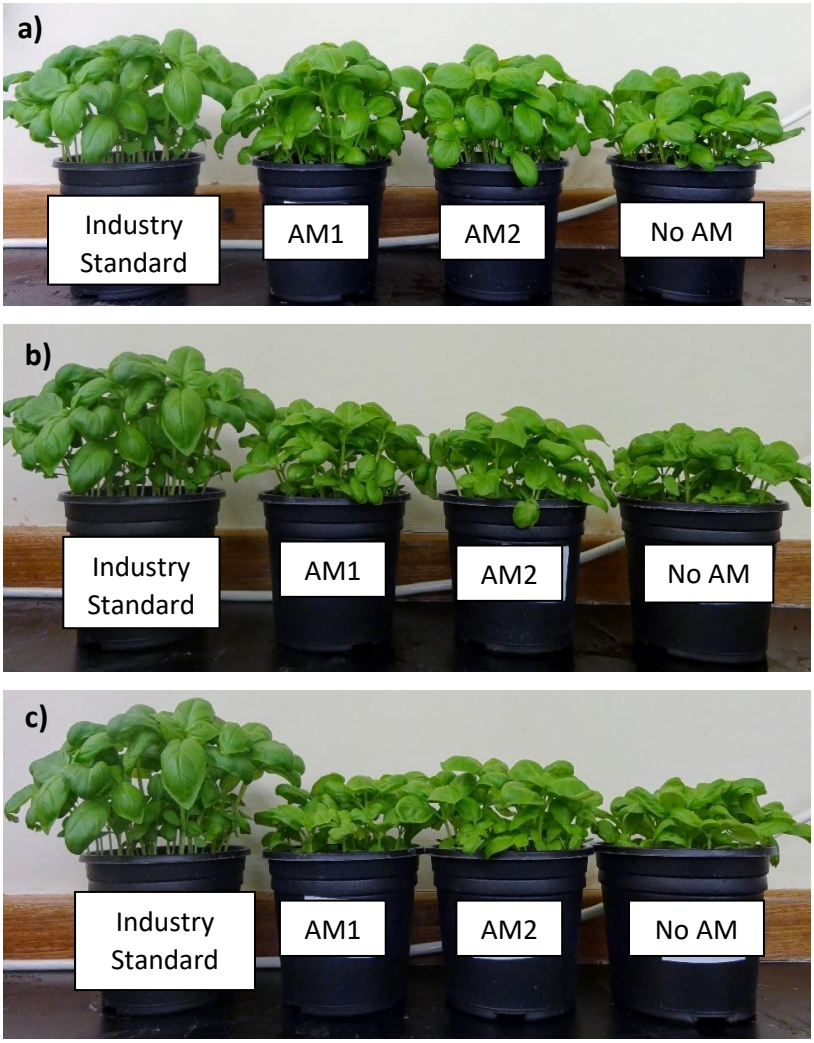


Figure 5.5 Basil plants from each inoculum treatment compared to industry standard peat-grown basil after six weeks of growth in three composts: a) peat, b) wood fibre c) green waste.

Figure 5.5 shows that plants grown in peat (a) were not significantly different in size to the industry standard after six weeks of growth but that the plants grown in wood fibre (b) and green waste (c) were much smaller. Figures 5.5b-c do support the evidence that there was a positive effect on biomass in pots treated with inoculum in the two alternative composts, however this effect was not enough to improve growth to the standard of the current commercial product after the same growing time.

Average pot biomass of plants grown in peat alternatives with both mycorrhizal treatment combinations was compared to the current biomass of plants produced by Vitacress in normal conditions in peat with full fertiliser over a 9-week growing period (Figure 5.6). It shows the positive effects of AM1 on plant biomass in low peat and coir composts and that low peat plants treated with AM1 were the only ones to achieve the same size as basil plants currently produced for sale. Basil grown in coir with AM1 was a close second.

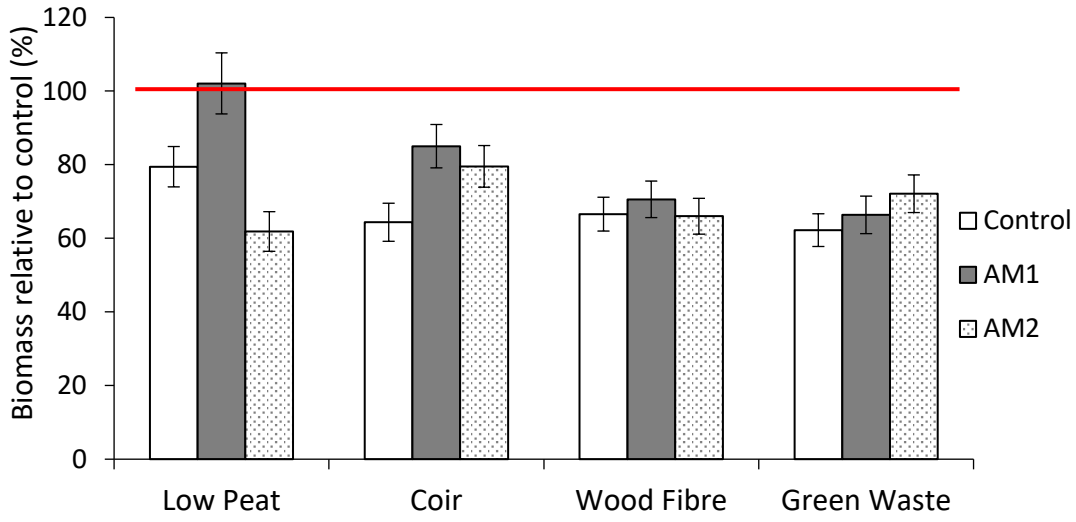


Figure 5.6 Comparison of average plant biomass in each reduced peat treatment relative to the average biomass produced by Vitacress's normal peat and full fertiliser which has been normalised to 100% (red line).

5.2.3.4 Antioxidant Content

Significantly more antioxidants were present in the leaves of plants harvested after 9 weeks compared to the two earlier harvests (F_2=25.251, P<0.001). Unsurprisingly (given the lack of colonisation) the addition of inoculum did not significantly alter the antioxidant content of leaves; however, levels of antioxidants did vary between leaves of plants grown in different composts (F_4=4.923, p <0.01). Leaves from plants grown in the green waste compost had significantly higher levels of antioxidants compared to those tested from all of the other composts; except the low peat alternative (Figure 5.7).

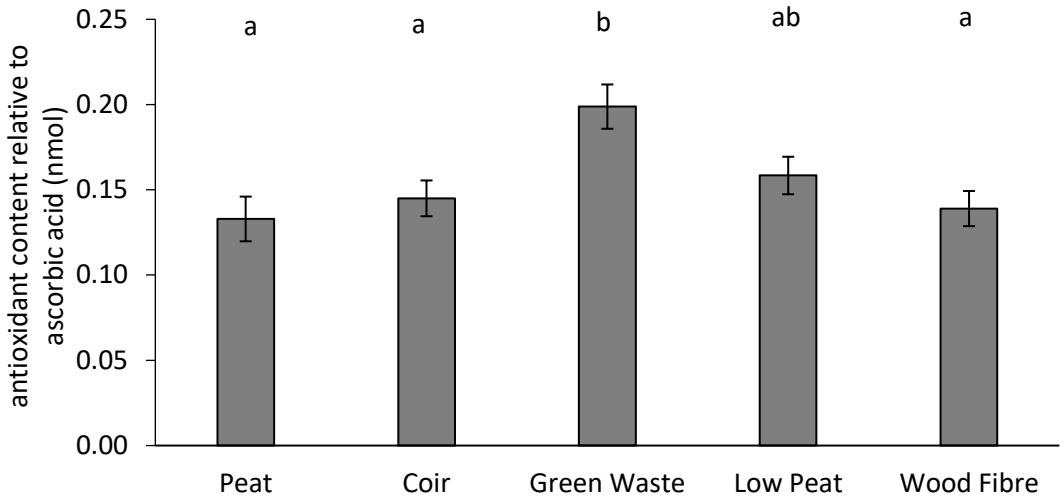


Figure 5.7 Average antioxidant content (relative to ascorbic acid) of leaves from basil plants grown in each compost type, (inoculum treatments combined as no significant effect of AM or evidence of colonisation was found). Bars with different letters have significantly different means. n=12, bars±SE.

5.2.4 Discussion

5.2.4.1 Colonisation

The lack of evidence of mycorrhizal colonisation seen in roots of plants from the final harvest was most likely caused by the addition of full fertiliser to the plants during the (later part of the) growing period because although root samples were not taken from the other harvests evidence of significant AM effects on biomass were seen at harvest two in two green waste and wood fibre composts. It has been shown that high concentrations of N and P are detrimental to AMF colonisation and the symbiotic relationship (Treseder, 2004). The decision was made to half the normal fertiliser rate because evidence has also shown that plants including herbs, perform best when inoculated with mycorrhiza under reduced but not severely limiting conditions. Previous experiments growing basil with AMF at Royal Holloway have also shown similar patterns of mycorrhizal colonisation "disappearing" or reducing after a certain growing period (Dr. T Stead, personal communication, 2014). Moreover, a reduction in colonisation has also been reported by Üstüner et al. (2009) who recorded increases in root length colonisation in chives grown in three types of organically amended sand after 1 month but colonisation then steadily decreased over the next 3 months.

Growing seeds in pots with a layer of inoculum has been shown to result in successful colonisation previously (Afek *et al.*, 1990). However, the time it takes for colonisation to establish in this set up may be longer than directly inoculating the roots of plug plants as seen with the marigolds.

Colonisation has been shown to take place after as little as three days (Afek *et al.*, 1990) or as long as six weeks (Corkidi *et al.*, 2004), depending on plant and AMF species. It is therefore perhaps not surprising that no significant effects or presence of colonisation was seen after only three weeks of growth.

As well as the fertiliser problem, it was later discovered that the commercial basil seed had been treated with a fungicide during its production. It is not known if this coating is systemic or if it would affect AMF species but it is worth noting that this could be the cause of the lack of colonisation. Fungicide is generally used to 'sterilise' or reduce AMF presence in control plots of field experiments or when making a non-mycorrhizal soil-based control treatment (Carey, Fitter and Watkinson, 1992; Ayres, Gange and Aplin, 2006).

A repeat of this experiment in more appropriate conditions with decreased levels of fertiliser added, or removing additional fertiliser entirely as there are already nutrients provided in all of the multipurpose composts, with non-fungicide coated seed should allow mycorrhizal colonisation to occur. This could then show whether the addition of AMF would allow plants in all the alternative media to reach the same size as those grown in the peat with reduced fertiliser levels and perhaps water too.

Any interaction between the different mycorrhizal treatments and the different composts could also be identified helping to determine if one combination works better than others when attempting to grow basil in a peat alternative medium.

5.2.4.2 Effect of Compost on Plant Height and Biomass

Significant differences in height and biomass between compost types were recorded. The growth rate of plants in reduced peat composts can also be compared to peat. Growth rate is important in a commercial setting as plants will all need to grow to meet saleable size within a set time period in order to meet supply demands and maintain a regular supply of product.

Overall, despite Vitacress's peat producing the tallest plants and the plants with the largest biomass they were not always significantly larger than the plants grown in the alternative media. The low peat and the coir based multipurpose composts produced plants that, on average, were not significantly smaller than those grown in 100% peat with reduced fertiliser. Low peat plants were also the only ones to reach the same size as standard peat-grown basil with full fertiliser. However, none of the pots met the saleable height limit of 13-18cm as set by the supermarkets, this is most likely due to them receiving reduced fertiliser at the start of the growing period which

reduced their growth rate. Without the benefit of AMF colonisation, the plants did not have the nutrient supply to grow to the same size as the industry standard plants on full fertiliser and this was clear in the pictures of the six-week-old plants (Figure 5.5).

Variability in compost performance was expected but this data shows that some alternative media can produce suitable basil plants (when compared to peat under the same growing conditions); even without mycorrhizal symbionts. It appears that low peat and coir performed the best in terms of producing plants with sufficient biomass and this could be because the commercial conditions favoured their more efficient drainage properties (Schmilewski, 2008; Alexander, Williams and Nevison, 2013, 2014). The poor performance of green waste and wood fibre could be explained by the watering regime used which is designed for a peat substrate, these two composts require more extreme watering regimes due to their structure. As previously discussed in Chapter 2 (section 2.5.3) which also saw the use of these composts, green waste composts are prone to waterlogging if they receive too much water as they have a high water holding capacity and the more open structure of wood fibre is prone to drying out quickly. As the majority of the bench contained peat and coir pots they would have most likely been the visual and physical test pots for the technicians to decide when to water the bench causing the green waste pots to be over watered and the wood fibre to be underwatered, thus affecting plant growth. To truly tests these alternatives, changes would have to be made to the watering system to allow for a more tailored approach, this has the potential to reduce water usage costs (especially in the case of green compost) however, if the plants are not able to reach saleable height even with tailored watering or AMF presence then growers would not be willing to make such a drastic change. It was shown in marigolds how AMF colonisation can improve the water holding capacity of wood fibre-based growing media (section 4.3.5) so if herbs could be colonised then this could reduce the need for such changes.

5.2.4.3 Effect of Compost on Antioxidant Content

Relative antioxidant levels appeared highest in leaves of plants grown in the two composts that produced the smallest plants in terms of biomass and height. The high levels of antioxidants could be explained by plants in these composts being stressed. With the recycled, green waste compost especially as it proved to have significantly higher levels of antioxidants. As previously mentioned, the watering regime used was the same for each compost and this may not have been ideal as each compost has very different water holding capacity and drainage rate. Due to the nature of the recycled waste compost being prone to waterlogging the flooding method used most likely

saturated the pots having a negative impact on the uptake of nutrients. It would also have reduced the amount of air spaces that allow oxygen to reach the roots to facilitate respiration. Increase in antioxidant enzyme activity in response to stresses caused by waterlogging has been described in multiple plant species (Lin *et al.*, 2004; Arbona *et al.*, 2008; Kumutha *et al.*, 2009). To determine whether a compost itself has a direct effect on antioxidants, future experiments should involve each compost receiving the appropriate amounts of water or being kept at the same moisture content to rule out waterlogging.

There is also evidence to suggest that recycled waste composts such as the one used in this experiment produce plants with higher levels of antioxidants compared to peat due to a high level of microbial activity. In previous studies Basil plants grown in composted green waste were found to have higher antioxidants than control plants grown in sand and the number of antioxidants was significantly increased when the compost treatment was combined with added bacteria in the form of a bio-fertiliser (Taie, Salama and Samir, 2010). The presence of beneficial bacteria has been shown to reduce biotic and abiotic stresses in plants (Dimkpa, Weinand and Asch, 2009; Sandhya *et al.*, 2010) and this could be linked to increased antioxidant levels which are known to confer resistance to pathogens (Taheri and Kakooee, 2017). Reuveni et al. (2002) showed that growth in compost induced resistance to *Fusarium* wilt in basil compared to peat and that autoclaving the compost removed the suppressive effect, thus suggesting a positive microbial effect. The second highest antioxidant content was seen in the low peat compost which also claims to contain recycled waste on its packaging.

The alternative theory is that the relative antioxidant content of the leaves of plants grown in the recycled and low peat alternatives were actually the same as those growing in the others but because their leaf size was smaller the antioxidant:leaf tissue ratio was decreased increasing the apparent concentration of antioxidants. In the larger plants with larger volumes of leaf tissue the average antioxidant concentration appeared less because it was more dilute, using leaves of similar size could also help reduce dilution effects in future.

Despite antioxidant content of herbs such as basil being considered healthier and more useful for the pharmaceutical industry (Copetta, Lingua and Berta, 2006; Taie, Salama and Samir, 2010), creating stressed plants which exhibit poor growth is not ideal for commercial sale.

5.2.5 Conclusions

Overall plants in the various alternative media did not achieve the same final biomass as those grown in peat but not all of the composts produced plants that were significantly shorter or smaller than peat-grown plants and this is promising. The alternative composts used were not suited to the current watering regime set up for peat which caused some plants to be stressed and the fertiliser levels were too high to maintain mycorrhizal colonisation, these factors must be altered in order to test the reduced peat media effectively. This experiment can be used to formulate better conditions for a repeat to allow mycorrhizal colonisation of basil so its effects on biomass and height and the interaction effects of these inocula in the various reduced peat media can be recorded. Future experiments could also generate data which would allow growers to be more informed of the optimum water and fertiliser levels required to produce successful growth of basil in reduce peat media with AMF which will hopefully also result in reduced production costs.

5.3 Can Commercial AMF or Natural Indigenous AMF Inocula Improve the Growth of Potted Chives in Reduced peat media Growing Media?

5.3.1 Objectives

The aim of this experiment was to try and increase the effectiveness of the commercial AMF on potted herbs after identifying low levels of colonisation with basil. A different species (chives) of popular potted herb sold in the UK (nearly 4 million units sold in 2016 (Vitacress, 2016)) was grown with combinations of the same commercial inocula with two peat reduced growing media mixes. These chives were found to be naturally colonised by AMF in the field, where they are usually grown, so this gave the perfect opportunity to compare the commercial inocula against an 'indigenous' or 'natural' AMF community. This comparison has often been used when experimenting with commercial AMF inocula (Gaur, Adholeya and Mukerji, 1998; Yildiz, 2010; Ortas and Ustuner, 2014b).

5.3.2 Materials and Methods

5.3.2.1 Plant Species

Allium schoenoprasum L. seed of the 'Polyvert' variety (thick leaved, fast growing) was purchased from CN seeds (Cambridge, UK). This is the variety that Vitacress receive as frozen plugs from field-grown plots in Germany; these plugs are then grown under glass in pots for three weeks before selling to supermarkets.

5.3.2.2 Growing Media

The same three custom growing media (Peat, Bark, and Wood Fibre) were used as outlined in Chapter 3, section 3.2.1.

5.3.2.3 Mycorrhizal Inoculum

Commercial inocula AM1 and AM2 were all used as described in section 3.2.1 of Chapter 3 as well as an inoculum made from highly mycorrhizal field chive roots. This last inoculum was produced in the laboratory from soil and roots which were taken from potted chives sourced to be sold in supermarkets that contained cores extracted fresh from a field, frozen and then transported to a grower (Vitacress, Runcton, UK). These pots would then usually be left to grow under glass for approximately three weeks. To ensure that the inoculum was as fresh as possible pots were used that had been thawed and grown for less than a week (Figure 5.8a). Roots were sampled from five pots and stained to check for mycorrhizal colonisation prior to making the inoculum and were

found to be highly colonised (average 84.2±2.44%). Soil and roots were then taken from multiple pots before being homogenised in a food blender. Using a levelled scoop, 10ml of the mixture was added to each pot. Inoculum was added to half-filled pots in an even layer (Figure 5.8b), the pots were then filled with the remaining growing media and seeds were sown on the top. All three types of inoculum were also added in a sterilised form, each inocula was autoclaved as outlined in section 3.2.1.

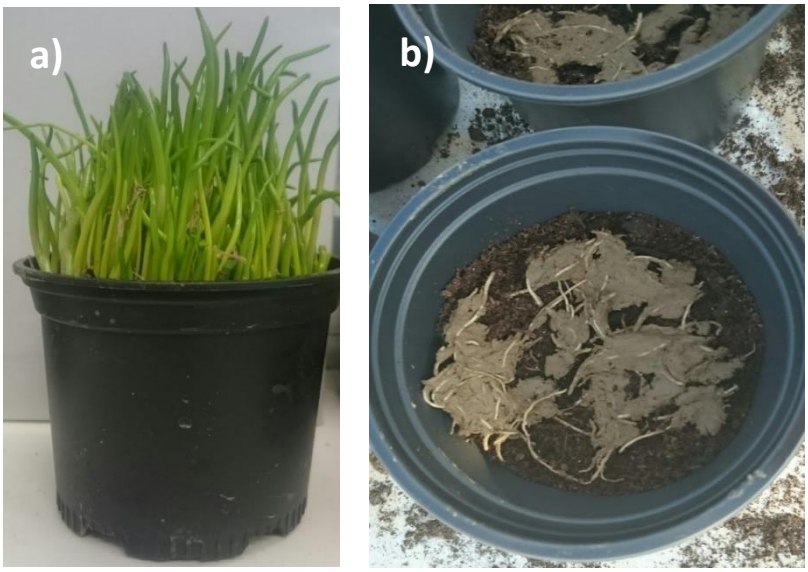


Figure 5.8 a) Field grown chives which were grown under glass at Vitacress (Runcton, UK) for less than a week. b) 10mls of Field Soil inoculum that was produced from blending roots and soil taken from pots as seen in (a) placed in a layer on top of growing media before the remaining space was filled with more of the appropriate compost.

5.3.2.3.1 Measuring AMF Colonisation

Root samples from every replicate pot were harvested after 8 weeks for staining to identify root colonisation by fungal mycorrhizae, these were fixed in 70% ethanol.

Root samples from four replicate pots of each treatment were stained with the acidified ink method outlined in Section 5.2.1.3 of this Chapter. After very low levels of colonisation were seen in the first four replicates, fixed root samples from one set of replicates already stained and the other four remaining replicates were stained using an alternative method. A trypan blue method was used to ensure that low levels of mycorrhizal colonisation were not as a result of an inefficient staining method. The method was as for acidified ink except the water bath was kept at 90°C instead of 75°C; clearing was in 10% KOH and 2% trypan blue (Sigma, UK) solution was mixed with destain solution (glycerol: lactic acid: dH2O (300:300:400ml)) at a ratio of 2:5 was used to stain

roots at 90°C for 3-5 minutes. Stained root samples were then stored in 20% glycerol at 4°C until being used to make microscope slides from which root length colonisation was recorded as described in section 3.2.1.1 of Chapter 3.

5.3.2.4 Experimental Design

Forty-eight pots were filled with each of the growing media allowing for eight replicates per mycorrhizal treatment. In total 18 treatments were used with a total of 144 pots. Approximately 60 seeds were sown into individual pots and germinated in the dark at a constant temperature room of 21°C for 10 days. After germinating the plants were then transferred to a polytunnel for ten weeks of growth. Pots were placed in trays in a random design and flood watering took place as and when plants required it, normally by feeling the weight of pots and dryness of growing media (similar to methods used at the Vitacress glasshouse). Trays were filled up to 3cm of pot height and the pots were left to soak for half an hour before the water was removed from the tray. As well as being randomly placed the plants were shuffled every week after data collection, to prevent any edge or bench placement effects.

5.3.2.5 Plant Height and Biomass

Using 30 cm ruler the height from base to tip of 20 random chive plants per pot was recorded every week for ten weeks until they reached what is considered by Vitacress as saleable size (approx. 13-18cm in height). At the end of the growth period a sample of the pot biomass was taken and the fresh weight was recorded, plants were then dried in an oven at approximately 40°C and dry weight was recorded when constant. Dry weight, relative to fresh weight was then calculated.

5.3.3 Results

5.3.3.1 AMF Colonisation

Colonisation levels were low overall (<12%) but colonisation did seem to vary between roots taken from different growing media for each inoculum (Figure 5.9). AM2 and Field Soil inoculum (FS) produced their highest levels in plants grown in bark, the field soil inoculum produced the lowest levels of colonisation and this was in peat-grown plants. Only hyphal counts were recorded as no vesicles were observed in any experimental roots (Figure 5.10).

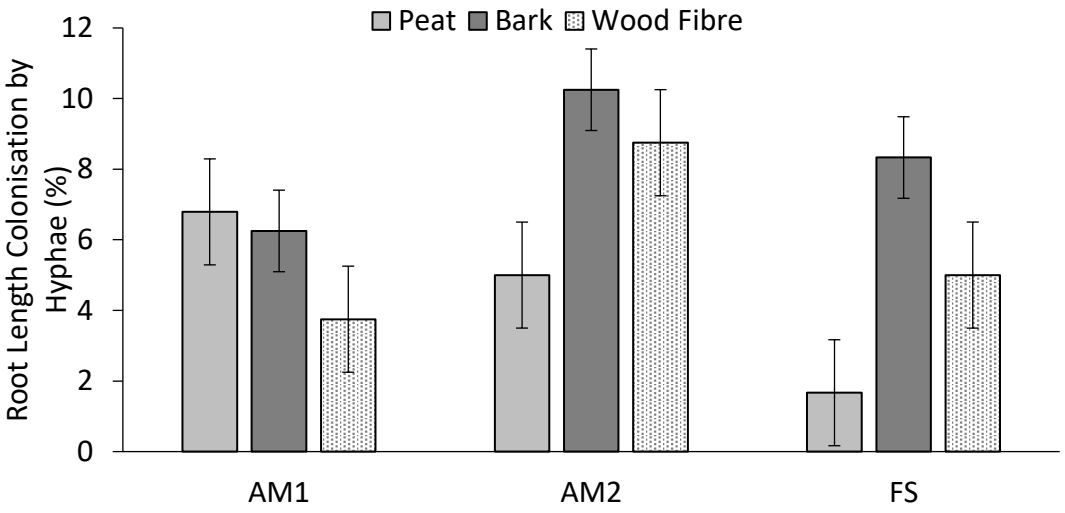


Figure 5.9 Chive root length colonised by hyphae for plants grown in each medium for each inoculum treatment. n=8, bars±S.E

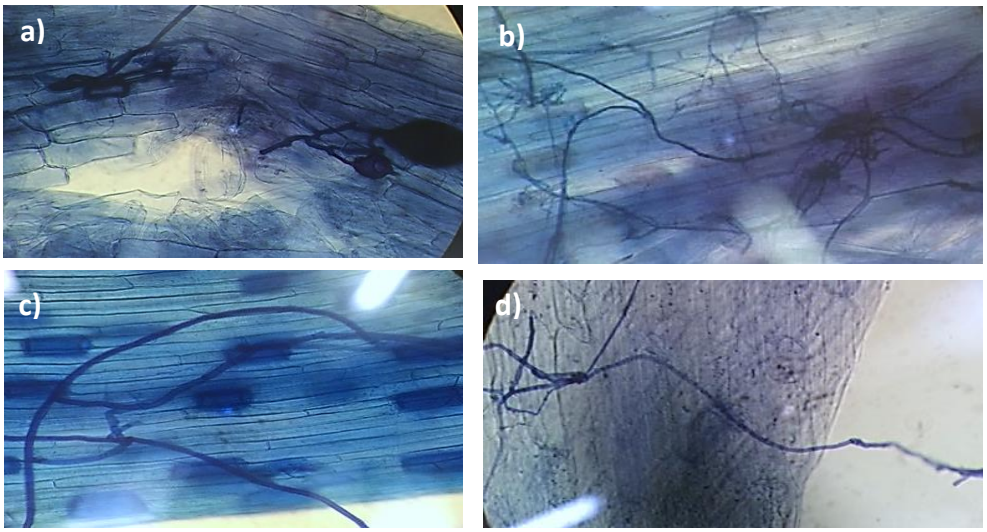


Figure 5.10 Acidified ink stained chive roots at 100x magnification. a) Sample from a field chive used to make inoculum showing vesicles. b-d) experimental chive root samples showing hyphae.

5.3.3.2 Plant Height

Addition of live AM1 had no statistically significant effect on the height of chive plants, however chives grown with AM1 were the tallest out of all inoculum treatments. Peat-grown chives with live AM1 were the tallest and outperformed all other treatments for the last three weeks of growth (Figure 5.11a). In AM2 the highest colonisation was seen in roots from chives grown in bark this correlated to the only significant effect of live AM treatment on plant height: by the final harvest plants treated with live AM2 were significantly taller on average than plants treated with

sterile AM2 (t_7=2.832, p<0.01, Figure 5.11b). In AM2 the sharp increase in growth rate of peat-grown plants can be seen from weeks 4-6, this was not as obvious in AM1 plants as growth rate was similar for all except live inoculum plants in peat (faster) and sterile inoculum treated plants in bark (slower). In all growing media, the addition of field soil inoculum (live or sterile) produced the shortest plants. Their growth rate was also not as consistent with a slower rate between weeks 2 and 4 compared to the other inocula (Figure 5.11c). As with AM2, wood fibre-grown plants were the shortest but there was more of a difference between the height of live and sterile field soil treated plants grown in wood fibre, with live field soil appearing to have a positive effect on plant height.

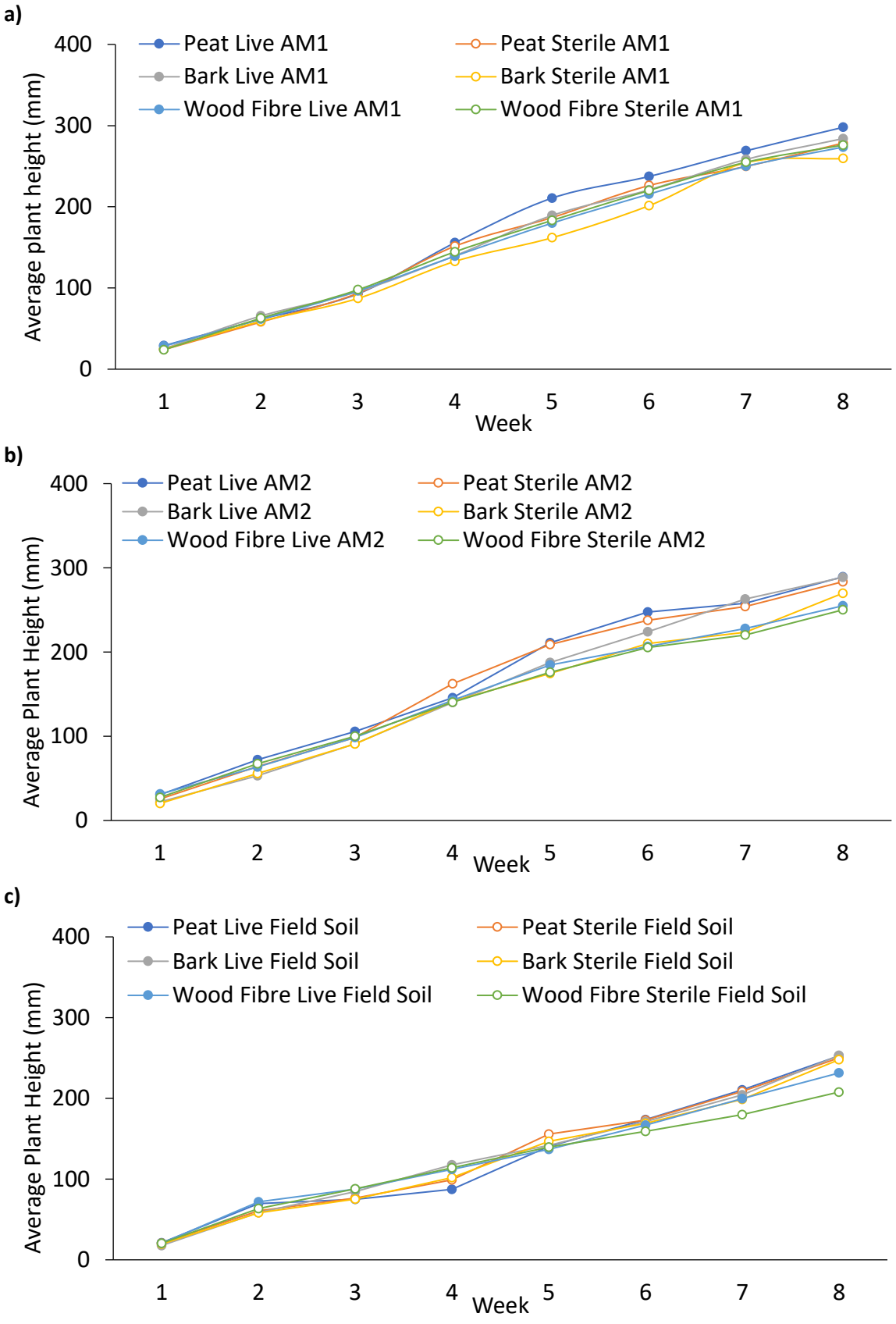


Figure 5.11. Average plant height of 20 random chive plants grown in each medium measured each week for each inoculum treatment: a) AM1, b) AM2, c) Field Soil

5.3.3.3 Biomass

No significant effect of either compost or AM treatment on plant dry biomass (relative to fresh weight) was seen in AM1 inoculated plants (Table 5.4, Figure 5.12a). In plants inoculated with AM2 bark-grown plants were shown to be significantly smaller than those in peat or wood fibre (F_2=21.312, Figure 5.12b). However, the addition of live AM2 did result in a significant increase in biomass of chive plants in bark-grown plants (t_{13}=2.199, p<0.05). In pots where Field Soil inoculum was added there was a significant interaction term between AM and media because the percentage biomass of plants appeared to be affected by the addition of live inoculum differently in different composts (F_2=3.518, Table 5.4). Plants treated with live inoculum in peat were a lot smaller than sterile inoculum-treated control plants but in bark the opposite effect was seen, neither of these differences was found to be significant when compared using an independent t-test. In wood fibre, there was no difference between the percentage biomass of live and sterile field soil inoculated plants. (Figure 5.12c).

		AM1		AM2		Field Soil	
	d.f	**F**	**Sig**	**F**	**Sig**	**F**	**Sig**
Media	2	0.704	p>0.05	21.312	**P<0.001**	0.724	p>0.05
AM	1	0.277	p>0.05	2.073	p>0.05	0.028	p>0.05
*Media*AM*	2	0.867	p>0.05	0.995	p>0.05	3.518	**P<0.05**

Table 5.4 Results from two-way ANOVAs on plant biomass for chives grown in different composts and treated with each inoculum. Error Degrees of Freedom: AM1=42, AM2=41, AM3=30.

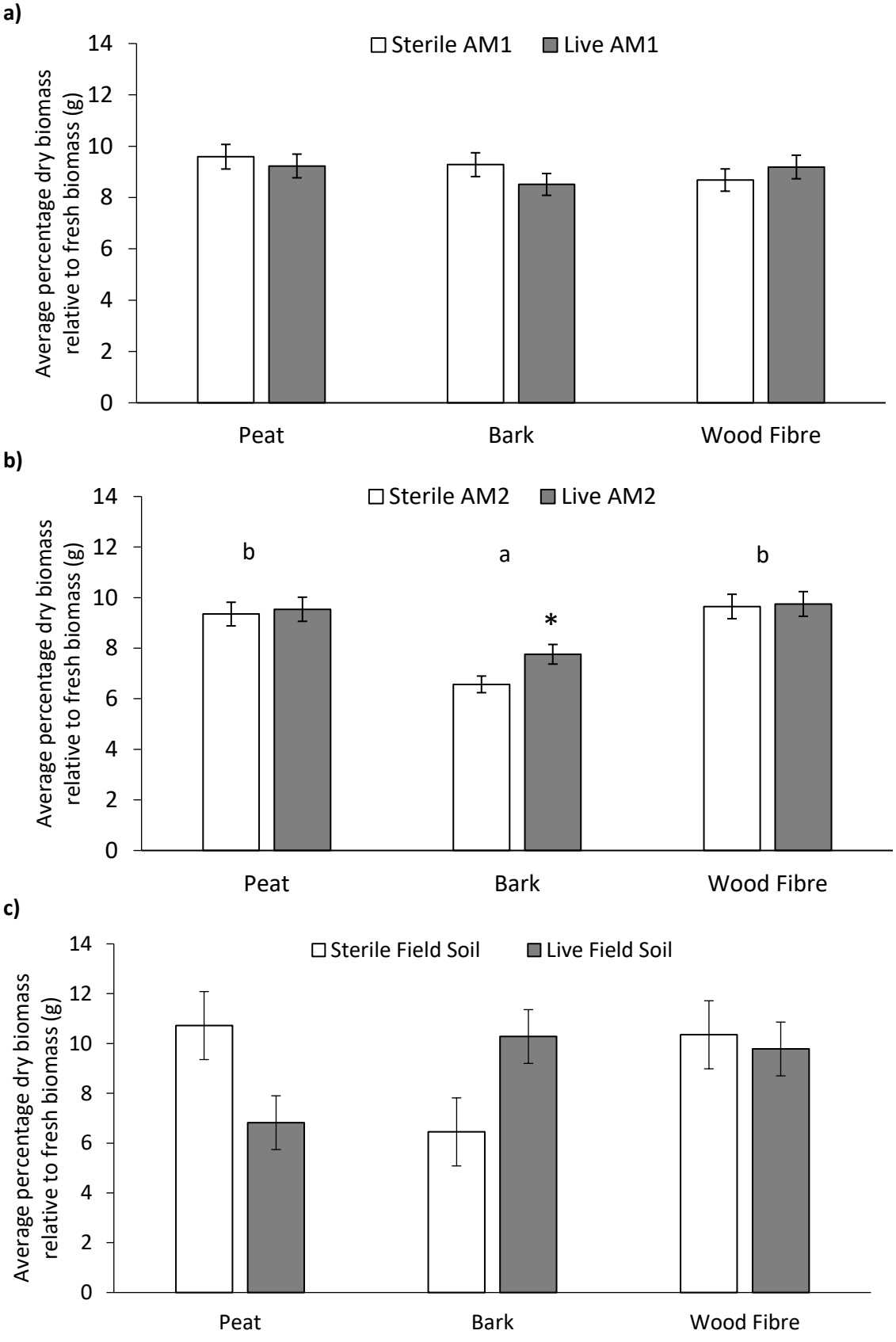


Figure 5.12 Average dry biomass (percentage relative to fresh weight) of chive pots containing peat and reduced peat growing media treated with each inoculum: a) AM1, b) AM2 n=8, bars±SE, and c) Field Soil n=5, bars ± relative SE.

5.3.4 Discussion

5.3.4.1 Mycorrhizal Inocula and Colonisation

Very low levels of colonisation were recorded and no vesicles or arbuscules were recorded in chive roots grown with any of the three inocula. Figure 5.11 also demonstrated that some hyphae recorded did not appear to be within the root cells as seen in marigolds. This evidence suggests that hyphae recorded were not from AMF species and that no symbiosis occurred. This would explain the lack of significant differences seen between live and sterile inoculum in plant height and biomass with the exception of bark-grown plants with live AM2. As previously seen in Chapters 3 and 4, the addition of AM2 has often resulted in significant effects on flower number, porosity, and biomass specifically in bark-grown plants. It has been suggested that this is because of the increased water holding capacity of bark (section 4.4.3) but it is also likely to contain a microbial community (Neil Bragg (Bulrush Ltd), personal communication, 2017) that would not be found in peat and wood fibre. Plant responses to AMF colonisation have been shown to be more positive when microbial communities are more complex (Hoeksema *et al.*, 2010) and Linderman (2008) suggests that a rich microbial community allows the AMF to recruit bacteria which will help promote plant growth and health. This 'mycorrhizosphere' idea could explain how, even with limited root colonisation plant growth is promoted in the bark plants inoculated with AM2 due to specific combinations of bacteria and AMF species present. As control plant media was not sterilised the significant increase must be the result of an additive effect of live inoculum to the active microbial community. Although the difference was not significant, the large increase in biomass in live inoculated field soil plants grown in bark also supports this theory as there may be AMF species and bacteria in the inoculum which could further enhance the microbial community in the bark media and thus increase plant growth.

As with basil, the short timing given for seeds to germinate and mature during the experiment as well as the use of seeds growing through an inoculum layer was thought to be a reason for lack of colonisation, however Yusoff (1977) showed *Allium* colonisation with arbuscules present at 3 weeks.

It was expected that the field soil inoculum would result in increased root colonisation when compared to the commercial inocula due to the high levels of root colonisation found in the field chive roots and the assumption that this 'indigenous' AMF culture would be more suited to colonising chives of this variety. Work has been done which compares indigenous soil AMF inocula (made in a similar way) that contains root fragments, spores, and soil to commercial inocula with different plant species. In some cases it did produce higher levels of root colonisation (Gaur,

Adholeya and Mukerji, 1998; Yildiz, 2010; Stonor *et al.*, 2014) but in others it produced very low levels of colonisation and it was outperformed by the commercial inocula (Faye *et al.*, 2013; Ortas and Ustuner, 2014b; Ziane *et al.*, 2017). There are a few reasons which could explain why the field soil inoculum did not produce high levels of colonisation in this case; firstly, although roots appeared to be highly colonised with hyphae, vesicles and arbuscules no measure of spore count was made, as these roots had previously been frozen and thawed there is no guarantee they would form viable propagules. However, cryopreservation of AMF spores is known to be successful and standard practice for some species (Douds and Schenck, 1990). When roots were sampled from field pots for staining roots were taken from the old growth and the new growth that had occurred in the pot since it had been growing post thawing, while the old roots were highly colonised with AMF structures the new growth was not. This could suggest that the AMF in the old roots and surrounding soil had not, or could not, colonise the new root growth, however, Douds et al. (2005) suggest that older roots previously colonised by mycorrhiza can cause more successful colonisation as an inoculum in plants than using mycorrhizal spores. Secondly, the way the inoculum was made from the material may have reduced the number of infective propagules. Klironomos & Hart (2002) suggest that root material should be cut into 60µm pieces to significantly increase the number of propagules per gram of root material. It is clear from the pictures that the root fragments chopped using a blender in this inoculum were a lot longer than that, suggesting a reduced propagule number in our inoculum. The conditions of the polytunnel may also have affected the colonisation of all inocula. In the summer months of the experiment, without suitable ventilation the polytunnel became a very hot and humid environment and the general rules for the culture and storage of AM fungi in pot culture type media suggest to keep the soil dry and cool to ensure mycorrhizas are in the optimum conditions (Douds *et al.*, 2005).

5.3.4.2 Plant Height and Biomass

Given the low levels of colonisation it is not surprising that live inoculum treatment was not found to have significantly affected plant height or biomass. However, this could have also been a result of the short time of the experiment; in onions, AMF colonisation did not significantly affect biomass until after 12 weeks when colonisation had reached over 50% (Yusoff, 1977) which is considerably higher than the root colonisation levels seen here. This is with the exception of bark and live AM2 inoculum, as previously explained this is most likely due to the specific combination of AMF species present in AM2 and the bacterial community present in the bark medium. Highest colonisation levels in bark AM2 and significant differences in plant height and biomass seen with bark AM2 pots is unlikely to be a coincidence, especially given evidence of the performance of

AM2 in bark in marigolds (Chapters 3&4). Üstüner et al. (2009) also showed that positive effects of AMF colonisation were seen only with a specific combination of organic amendment and AMF species in an inoculum mix. Similarly, negative effects of growing media on AMF colonisation and plant growth have been shown with different peat based substrates (Linderman & Davis 2003; Perner et al. 2006).

5.4 Can Co-planting Improve the Colonisation Ability and Effect of Commercial AMF Inocula on Growth of Chives?

5.4.1 Objectives

Due to low colonisation found in chive plants in the previous experiment (Section 5.3) and other herbs grown under controlled conditions but consistent colonisation found in marigolds it was decided to grow chives outdoors and alongside marigolds acting as companion plants to try and encourage colonisation. It was hypothesised that the controlled conditions previously used could be providing environments that are too favourable to allow the symbiosis to be beneficial to both organisms and marigolds had shown consistent colonisation with the same commercial inocula when grown outdoors. Previous data has also shown the positive effects on colonisation of known mycorrhizal plants with companion planting (Smith, Johnson and Cázares, 1998; Riaz and Javaid, 2017), including with *Allium* species (Kawamoto and Habte, 2011; Hage-Ahmed, Krammer and Steinkellner, 2013). Co-planting with *T. patula* has also lead to the mycorrhizal colonisation of roots of a non-mycorrhizal species (St-Arnaud *et al.*, 1997).

Based on these data it was hypothesised that the presence of mycorrhizal marigolds in close proximity would stimulate colonisation in the roots of chive plants. If this step was successful, then it was hypothesised that the colonisation of chives by AMF species would result in an increase in biomass especially in the reduced peat media.

5.4.2 Methods

5.4.2.1 Plant Species

Two species were used: French marigolds, (*Tagetes erecta L.)* and chives (*Allium schoenoprasum L. 'Polyvert'*). *Tagetes patula* 'Bonita mixed' seeds were sourced from Thompson & Morgan and again *Allium schoenoprasum L. 'Polyvert'* seeds were sourced from CN Seeds Ltd. Marigolds seeds were grown one per plug whereas a seed dispenser was used to plant approximately 40-50 chive

seeds per plug. Chive seeds were germinated in the dark for 10 days in a CT room set at 21°C. Once germinated, both species were grown under glass until ready to be transferred to two litre pots at the start of the experiment.

5.4.2.2 Growing Media

The same batch of the three custom growing media were used as in Chapter 4, the ingredients and nutrient details are outlined in section 3.2.1 of Chapter 3. To see the effect of marigold root exudates or volatiles in the soil on colonisation of chives, a set of pots were planted with a single plug of chives which contained growing media that had previously been used to grow marigolds in the experiment outlined in Chapter 3. There were three replicate pots of each growing media (peat, bark, and wood fibre) and live AM1 and AM2 inoculum treatment. Media was used from marigold pots that had been inoculated with live AM1 and live AM2 so only the same products were used to inoculate the roots of the chive plugs in their respective recycled media.

5.4.2.3 Mycorrhizal Inocula

Commercial inocula AM1, AM2 and AM3 were all used as described in sections 3.2.1 and 4.2.2 (respectively) as well as an inoculum made from highly mycorrhizal field chive roots, as described in section 5.3.1.3 of this Chapter. The average root length colonisation of roots from eight replicate pots of field grown chives used was 52%±5.04. Using a levelled scoop, 15ml of the mixture was added to each pot. As with previous experiments all inocula were added in a sterilised form for control plants. For all inocula, a well was made in each pot and each inoculum was added by sprinkling in and around the sides of the well to ensure as much contact with the plant roots as possible.

5.4.2.3.1 AMF Colonisation

Root samples were taken and fixed in 70% ethanol for mycorrhizal colonisation analysis from all pots. Staining was carried out as described in section 5.2.1.3.1. When root samples were taken from pots containing both a marigold and chive they were sorted by their morphological distinctions to try and ensure roots of different species remained separate: marigold roots were thicker, darker yellow in colour and were often branched with more root hairs; chive roots were fine, bright white and had a sheen to them with fewer root hairs. Once stained, if there was any

contamination between samples it was much easier to tell roots from different species apart due to the difference in size, root cell shape and staining pattern (Figure 5.13).

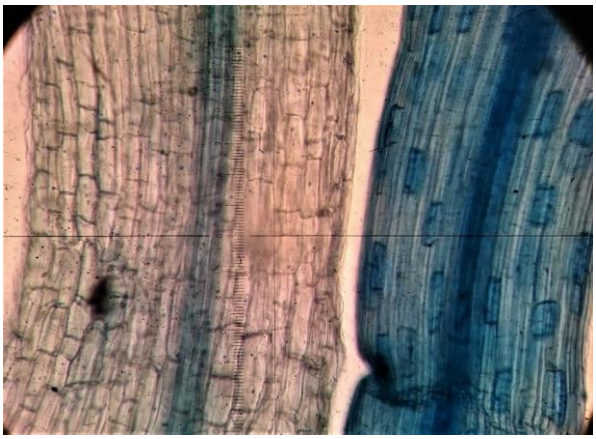


Figure 5.13 Stained roots at 100x magnification. Thicker, lighter blue root tissue with clearly defined blocks of cells on the left is from a marigold root and the thinner, darker stained tissue with faint lines of cells and individual darker stained blocks is from a chive root.

5.4.2.4 Experimental Design

All four inocula were added in their live and sterile forms to each growing media. Five pots of each of the 24 treatments were planted with either two plugs: one chive and one marigold as a companion plant (each plant was placed at the edges of the pot leaving a gap between them) or just one plug of chives (placed in the centre). Due to the increased number of plants and treatments replicate number had to be kept at five single species and five double species pots per treatment. The total number of pots was 240.

5.4.2.5 Site

The same site and set up was used as outlined in Chapter 4, section 4.2.5.

5.4.2.6 Biomass

Final harvest of above ground biomass was carried out after 12 weeks of growth. Fresh weight was recorded. Plants were then placed in envelopes in an oven set at approximately 40^{o}C and dried to constant weight, which was then recorded.

5.4.3 Results

5.4.3.1 AMF Colonisation

There was no significant difference between the levels of root colonisation between chives and marigolds grown in the same pot, in fact the root length colonisation of marigolds was found to be a significant predictor of root length colonisation in chives and *vice versa* (F_{99}=63.347, p<0.001), although the R^2 value was only 0.39 (Figure 5.14).

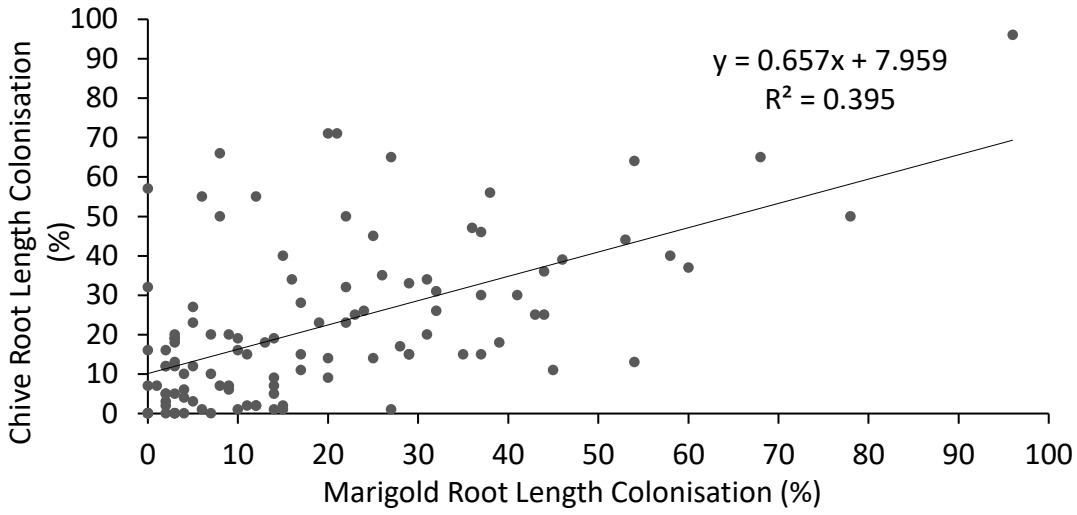


Figure 5.14 The relationship between root length colonisation by hyphae of chive and marigold plants grown in the same pot across treatments. n=100.

In chive plants grown alone treated with AM1, bark was the only media shown to have a significant effect on colonisation (F_2=4.720, Table 5.5) with live inoculum treated plants having significantly higher levels of root length colonisation compared to sterile inoculum controls (t=-3.273, p<0.05). In AM3 treated plants bark-grown plants had significantly lower levels of root length colonisation when compared to peat-grown plants (F_2=3.411, p=0.05). Overall field soil inoculum produced plants with the lowest levels of root colonisation (**Error! Reference source not found.**5).

		Chives Alone		Co-planted Chives	
AM1	**d.f**	**F**	**Sig.**	**F**	**Sig.**
Media	2	4.720	**p<0.01**	2.428	p>0.05
AM	1	3.600	p>0.05	11.196	**p<0.05**
Media*AM	2	0.324	p>0.05	2.503	p>0.05
AM2					
Media	2	2.933	p>0.05	6.524	**P<0.05**
AM	1	0.256	p>0.05	0.405	p>0.05
Media*AM	2	0.350	p>0.05	2.766	p>0.05
AM3					
Media	2	3.411	**p<0.05**	3.265	p>0.05
AM	1	0.228	p>0.05	0.175	p>0.05
Media*AM	2	2.106	p>0.05	1.518	p>0.05
Field Soil					
Media	2	1.091	p>0.05	1.111	p>0.05
AM	1	0.723	p>0.05	4.165	p>0.05
Media*AM	2	1.072	p>0.05	1.454	p>0.05

Table 5.5 Results from two-way ANOVAs on root length colonisation of chives with and without companion planting with marigolds grown with each commercial inoculum.

In companion planted chives there was a significant effect of AM on colonisation found with AM1 because sterilised inoculum treated control plants were found to have significantly reduced colonisation in bark ($t=-2.44$, $p<0.05$) and wood fibre pots ($t=-2.795$, $p<0.05$) but not in peat (Figure 5.15). There was also a significant increase in root length colonisation using live inoculum compared to sterilised inoculum treated plants grown with AM2 in bark ($t=-2.488$, $p<0.05$) although bark containing pots also produced plants with the lowest root colonisation levels with AM2 ($F_2=6.524$, Table 5.5). Although there were similarly large differences seen between them colonisation was not found to be significantly increased when using live inoculum compared to controls in peat-grown plants with AM2, as well as peat and wood fibre-grown plants with AM3 and Field Soil (Figure 5.16).

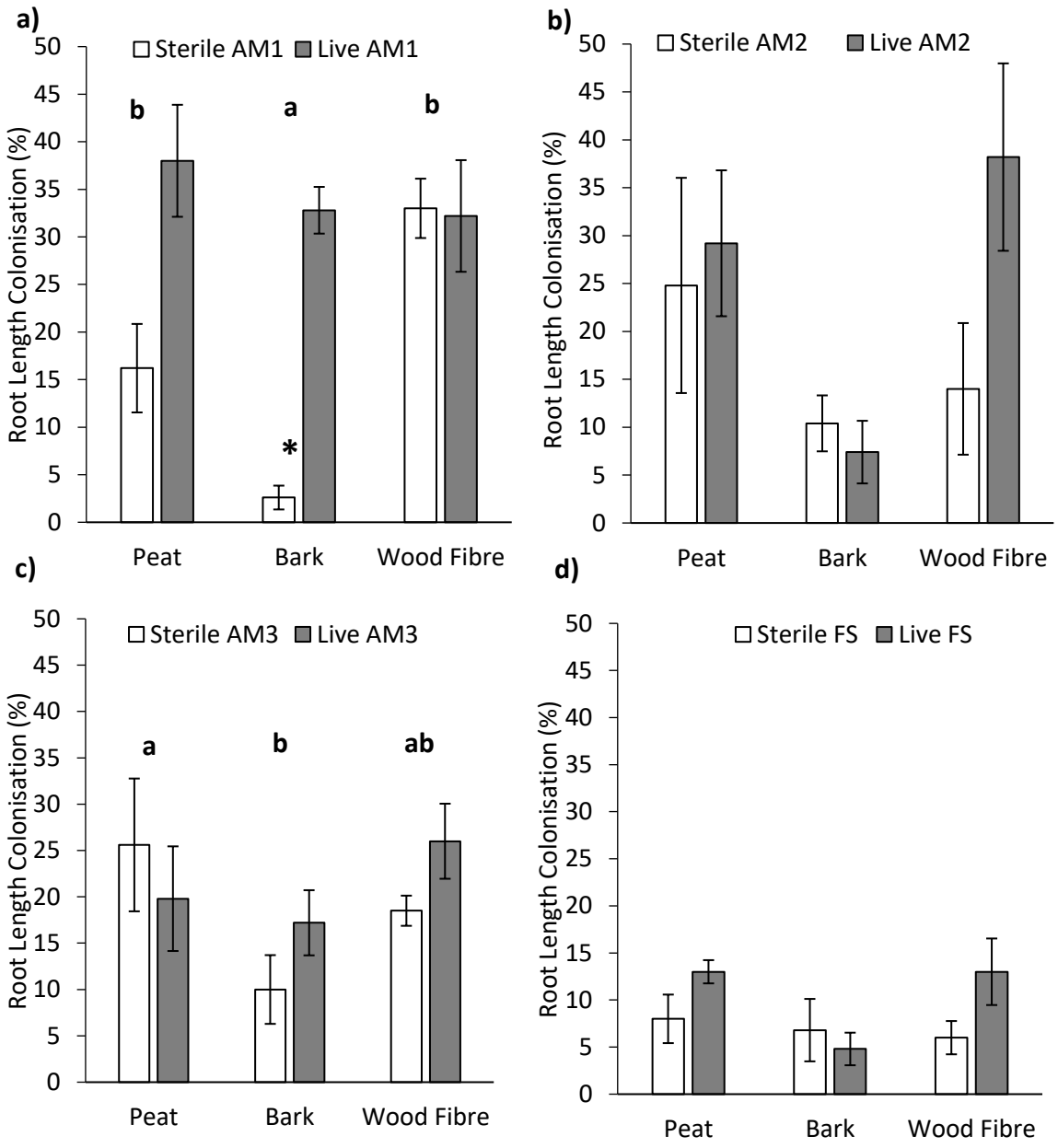


Figure 5.15 Average root length colonised by hyphae of chive plants grown alone in each media for each inoculum: a) AM1, b) AM2, c) AM3, d) Field Soil. Letters indicate statistical differences between growing media, asterisks indicate statistical differences between pairs of bars. n=5, bars±S.E No a,b,c on figures

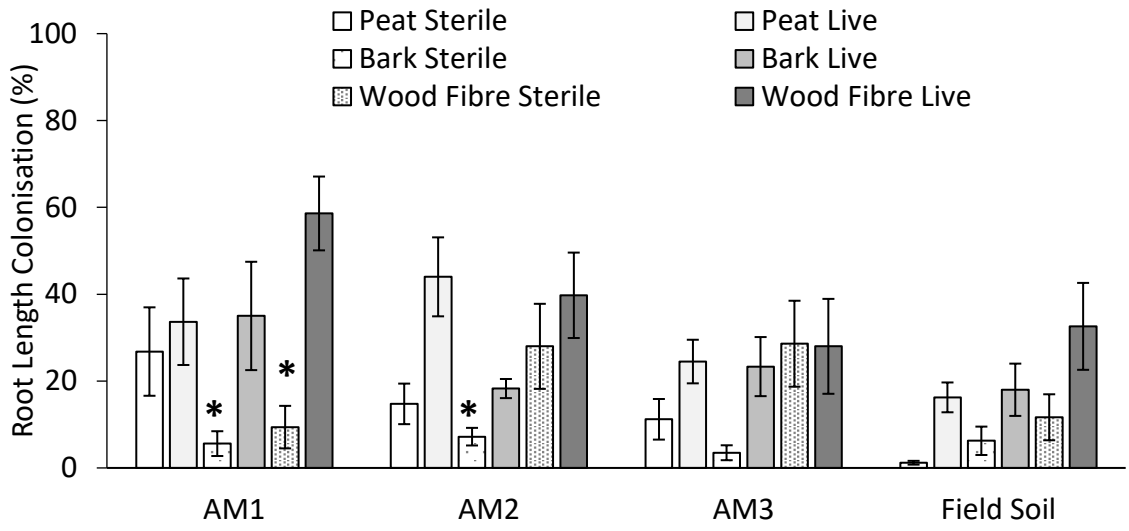


Figure 5.16 Average root length colonised by hyphae of chive plants grown with marigolds in each media for each inoculum. Asterisks indicate statistical differences between live and sterile inoculum bars for each growing media. n=5, bars±S.E

When comparing the root length colonisation of chives grown alone to chives that were grown with marigolds (Figure 5.17), higher levels of colonisation were seen in live inoculum treated co-planted chives compared to chives alone only in the reduced peat media; in bark pots treated with AM2 and Field Soil (Figure 5.17b and d) and in wood fibre pots treated with AM1 and Field Soil (Figure 5.17a and d). However only in field soil treated plants grown in wood fibre was this difference found to be significant (t_8=2.820, p=0.035).

Single plugs of chives were grown in media that was used to grow marigolds in the experiment in 2015 (Chapter 3). Figure 5.18 shows the differences in colonisation found between roots of plants grown in the fresh media with and without a marigold compared to those grown in the recycled media. In peat pots treated with fresh AM1 (Figure 5.18a), plants were found to have higher levels of colonisation than both the co-planted chives (t_9=3.705, p<0.01) and chives grown alone (t_9=3.764, p<0.01). In bark and wood-fibre pots treated with AM1 (Figure 5.18a) chives grown in the recycled media were only significantly more colonised than chives grown alone ($t_{9.3}$=2.443, p<0.05 and $t_{9.1}$=6.233, p<0.001 respectively). In AM2 treated pots (Figure 5.18b), only the bark media showed significant differences in colonisation of plants grown in recycled media but only with chives which were grown alone in the fresh media ($t_{9.2}$=3.341, p<0.01).

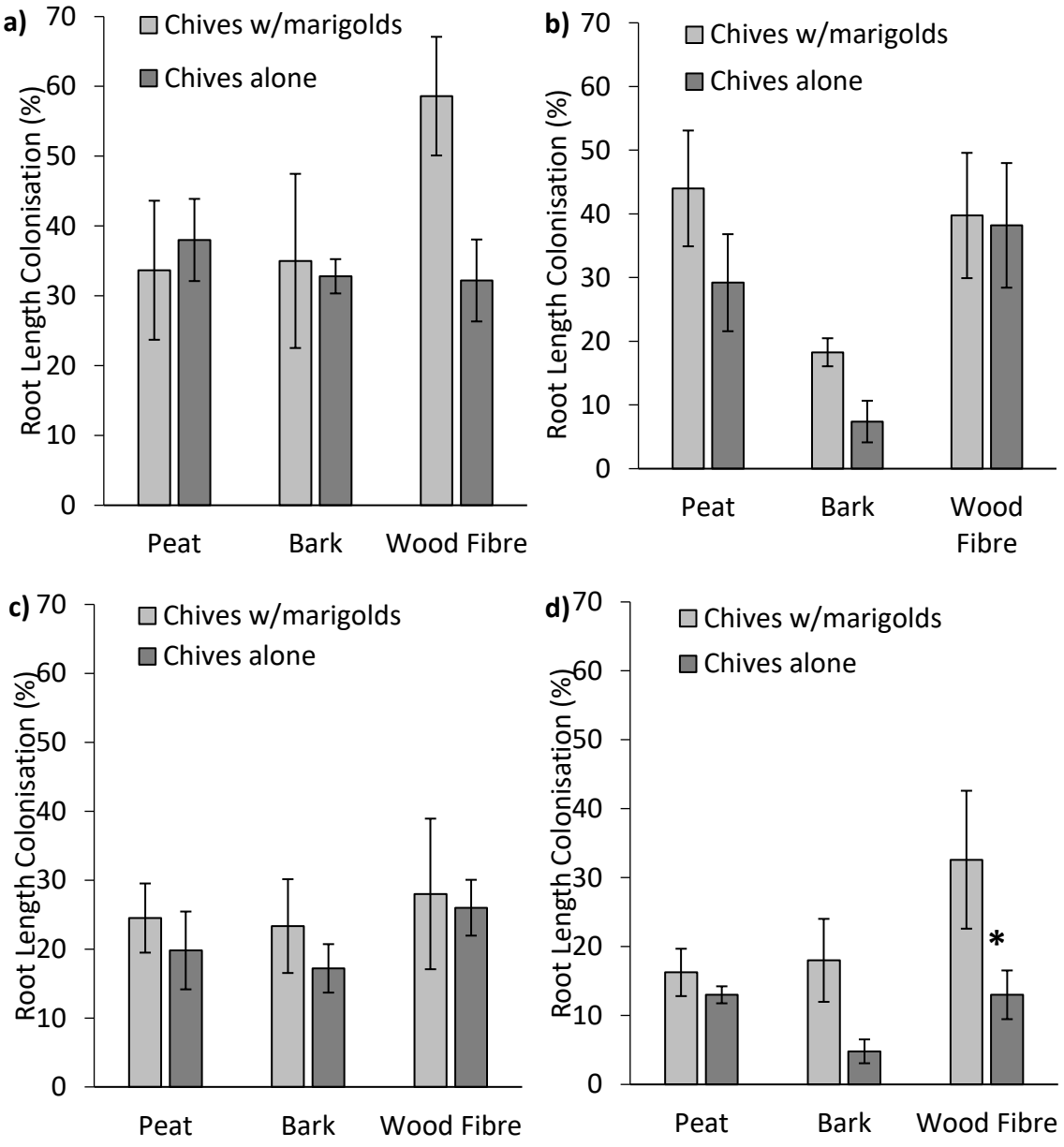
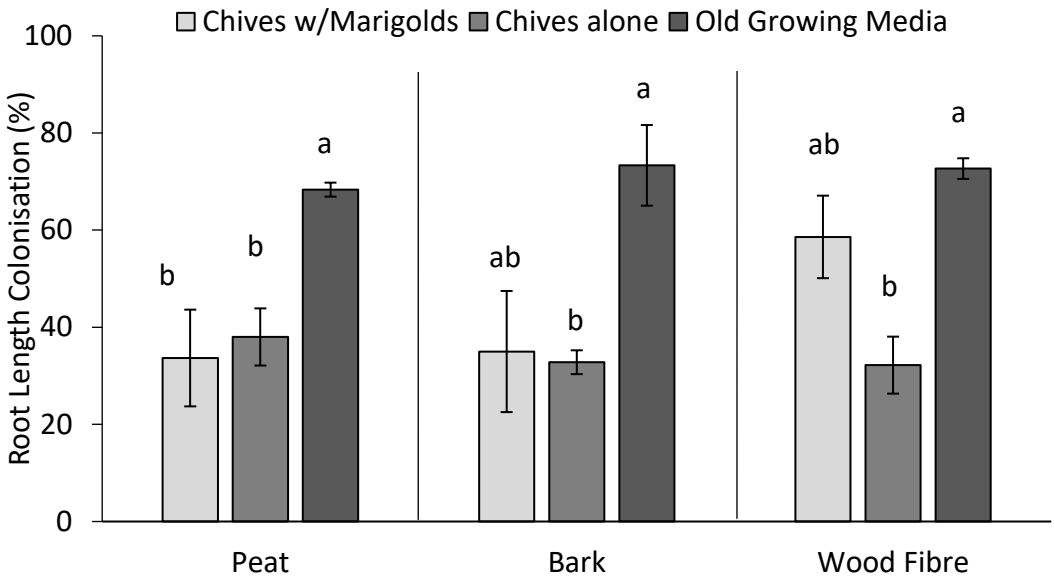


Figure 5.17 Average root length colonisation of chives grown alone and those co-planted with marigolds in each media for each inoculum: a) AM1, b) AM2, c) AM3, d) Field Soil. Asterisk denotes statistical difference between chives grown alone and with marigolds. n=5, bars±SE

a)



b)

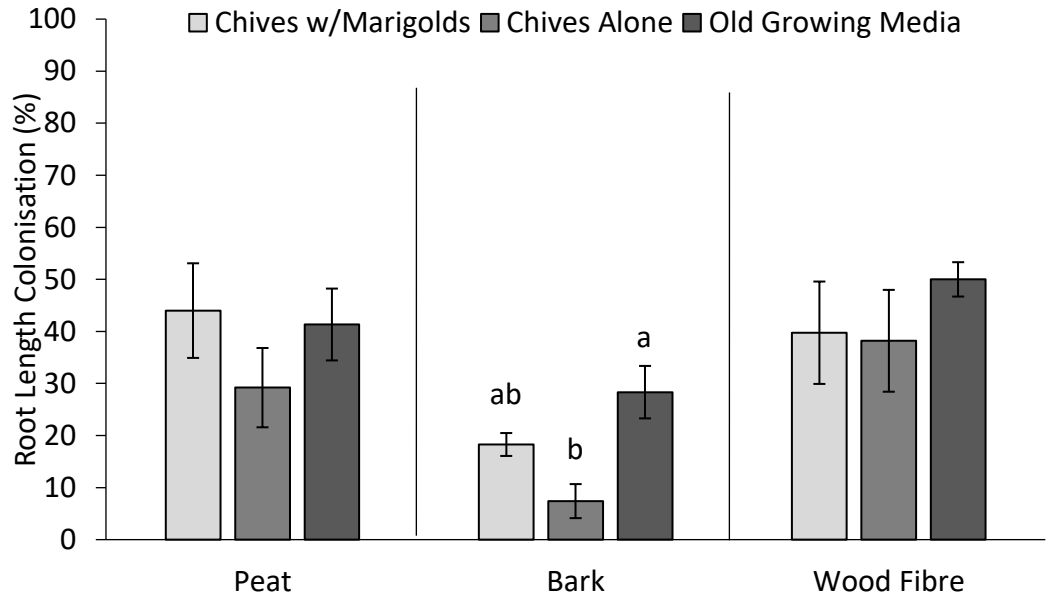


Figure 5.18 Average root length colonisation of chive plants grown with and without marigolds in fresh media along with chives grown in media that had previously been used to grow marigolds for each inoculum: a) AM1 and b) AM2. n=5, bars±S.E

The differences in colonisation and significant increases in colonisation in the roots of chives grown in the recycled media were also seen with root colonisation by arbuscules and vesicles. In AM1 (Figure 5.19a), the frequency of colonisation by both AMF structures was significantly higher in chives grown in the recycled media than in the fresh media (Arbuscules: t_9=7.654, p<0.001. Vesicles: t_9=4.577, p<0.001). In AM2 (Figure 5.19b), there was no significant difference in the

frequency of occurrence by vesicles (t_9=0.622, p>0.05) but there was a significant increase in the number of arbuscules in roots of plants grown in the recycled media (t_9=2.477, p<0.05).

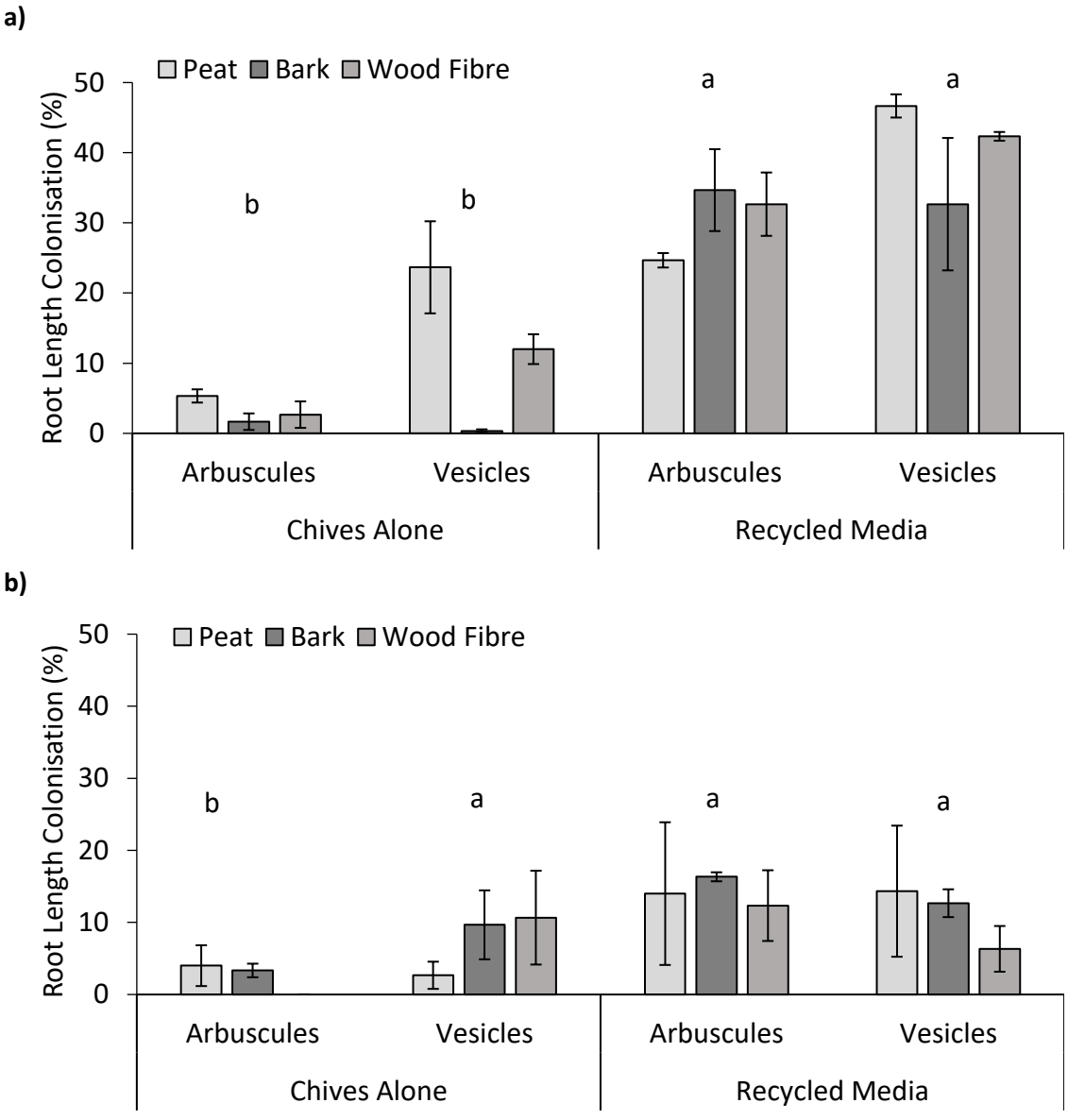


Figure 5.19 Average root colonisation by arbuscules and vesicles of chives grown alone in fresh media compared to chives grown in the recycled media for each growing media and inoculum: a) AM1, b) AM2. n=3, bars±SE

5.4.3.2 Biomass

There was no significant effect of live inoculum or growing media on the percentage dry weight of chives grown alone (Figure 5.20) or with marigolds (Figure 5.21).

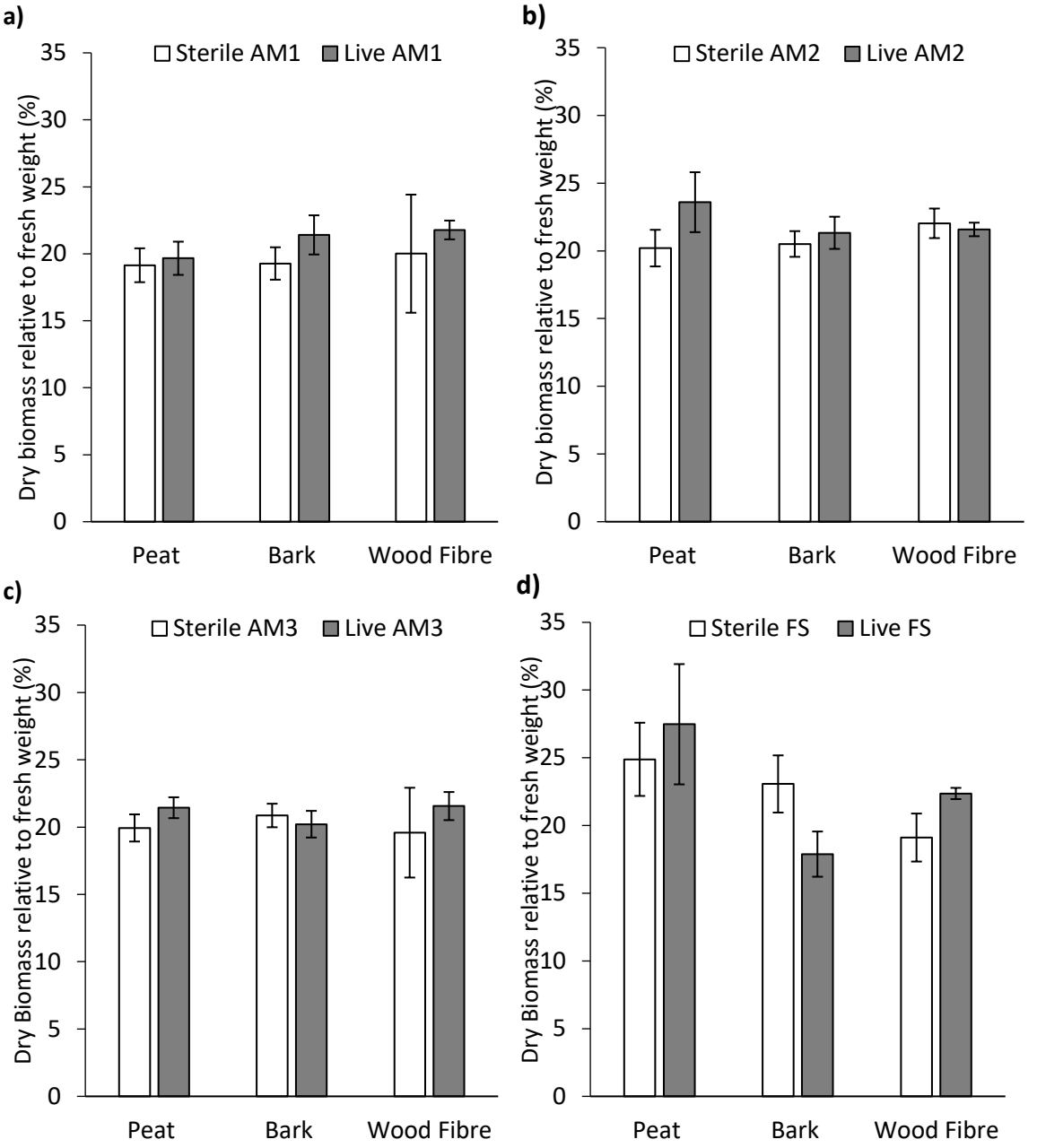


Figure 5.20 Average dry biomass as a relative percentage of fresh biomass of chives grown alone in each growing media for each inoculum treatment: a) AM1, b) AM2, c) AM3, d) Field Soil. n=5, bars±S.E (relative).

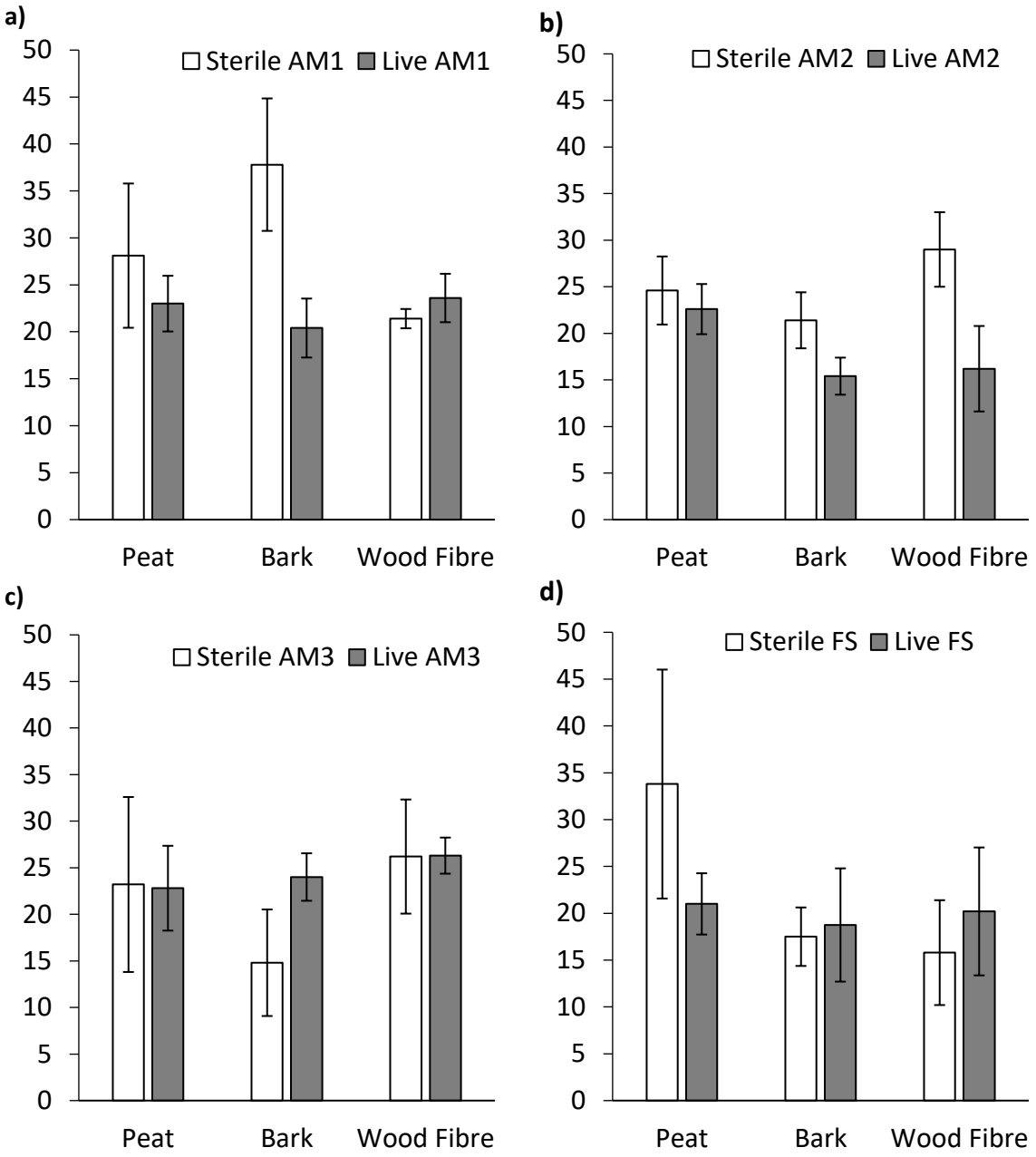


Figure 5.21 Average dry biomass as a relative percentage of fresh biomass of co-planted chives in each growing media for each inoculum treatment: a) AM1, b) AM2, c) AM3, d) Field Soil. n=5, bars±S.E (relative).

5.4.4 Discussion

5.4.4.1 Mycorrhizal Colonisation

The main aim of this experiment was to investigate the effect of co-planting with marigolds on colonisation of chives with the different inocula (commercial and naturally occurring) in peat reduced media. Whilst the co-planting did appear to increase colonisation in chives (especially with AM1 in wood fibre and AM2 in bark) although not significantly, colonisation still occurred in chives grown alone. The levels of colonisation in chives grown alone was a lot higher and a lot more consistent across treatments than seen in the previous experiment (section 5.3.2.1). There are two differences between the experiments that could account for this. The first is using chive plants as plugs instead of growing them from seed and the second is growing the plants for an extended period of time, three months instead of 9 weeks.

Similar levels of successful root colonisation of *Allium* species has been achieved using the method of adding inoculum below germinating seeds (Afek *et al.*, 1990), sowing seeds into a substrate that inoculum has been dispersed through by mixing (Linderman & Davis 2003), as well as with transplanting seedlings in a similar method to the one used in this chapter (Perner, Schwarz and George, 2006). In these published data, there was no noticeable difference between the levels of root colonisation using the three different methods but they were all obtained from plants which were grown under glass not outside.

Higher levels of colonisation could have been due to the increased length of time taken to grow the plants. Although colonisation has been seen within 3 days (Afek *et al.*, 1990) significant increases in colonisation have also been seen in plants left to grow for an extended time. Plants grown for nine weeks, have exhibited low levels of colonisation ranging from: no colonisation (Smith, Johnson and Cázares, 1998), 5% (Nelsen and Safir, 1982), up to 30% (Perner *et al.*, 2007) and in some cases up to 76% (Toussaint, Smith and Smith, 2007); compared to plants that have been left to grow for more than two months exhibited colonisation ranging from 70% after 4 months (Giovannetti *et al.*, 2012), and 60-77% after five months (Tawaraya, Hirose and Wagatsuma, 2012), showing that colonisation can increase the longer the plants are allowed to grow.

By growing chives in this experiment outdoors they were also potentially exposed to external sources of colonisation which could have meant them coming into contact with an AMF species that they are more compatible with than the ones provided in the commercial and field soil inocula. Faye et al. (2013) found AMF species present in pots which were not found in any of the commercial products used to inoculate plants. Clapperton & Reid (1992) showed that although

colonisation increases with added inoculum it increases only in plants where that species of AM is the preferred species. Functional diversity of AMF species has been demonstrated and there is evidence plants can actively favour certain AMF species which are seen as more compatible over others because they will provide an increased benefit (Mensah *et al.*, 2015; Werner and Kiers, 2015; Gosling, Jones and Bending, 2016).

Colonisation of chive roots grown alone was only found to be significantly reduced compared to roots of chives grown with marigolds in one growing media and inoculum treatment combination. Given this lack of consistent and significant increases in chive colonisation in the direct presence of marigolds in co-planted chives, the significant increase in colonisation in chives grown in the recycled media is not likely to be due to any marigold 'presence' remaining in the media. It is most likely the increased number of propagules due to remaining inoculum or colonised root fragments in the media is the cause. The method of recycling pot media containing propagules to grow plants is used when producing pot culture inoculum (Samaei, Asghari and Aliasgharzad, 2015). Bever (2002) used recycled pot cultured inoculum to reinoculated the same species of annual *Allium* species for a second generation of growth and the number of spores produced increased in the second generation. If addition of AMF inocula were to be implemented in industry then a recycled pot culture could produce higher colonisation levels than a commercial mix, however as Bever (2002b) has identified, it may be more beneficial to use a different plant species as negative feedback can exist between mutualistic AMF and plant species. So, in order to promote plant growth an AMF species which naturally associates with a different plant species may be more beneficial. The benefits and cost effectiveness of growing extra plants for this method would have to be balanced against purchasing commercial inocula.

5.4.4.2 Biomass

Even though there was an increase in colonisation levels by hyphae in live inoculum treated chive roots in this experiment, compared to the previous experiment (section 5.3.3.1), the colonisation levels of arbuscules and vesicles recorded was still lower than those seen in marigolds (Figure 4.5, Chapter 4). This could explain the lack of differences in biomass seen between the sterilised and live inoculum treatments, although (with chives grown alone) similar levels of colonisation in plants treated with sterilised and live inoculum could also be to blame. Increases in biomass of chives with AMF colonisation has been demonstrated; Üstüner et al. (2009) showed that in the presence of low and high phosphorus fertilisation, colonisation with two different mixes of AMF significantly increased leaf number and dry biomass per plant in chives. In the 2009 study,

different levels of compost amendment also had no effect on dry biomass but a specific combination of an AMF mix and compost was shown to produce the highest increase in biomass. Similarly, in the present study there was no significant effect of growing media on plant biomass in either co-planted or single grown chives in the current experiment. Whilst this supports the findings of Üstüner et al. (2009) it also shows that chives could be grown in the reduced peat media without any detrimental effects to their growth. In this experiment, the growing media used contained slow release nutrients, including phosphate (section 3.2.1), both studies show how fertilisation with phosphate can still result in colonisation by AMF which is also positive as significantly reducing fertilisation would not be an option for growing potted herbs for sale as plants would not achieve the size required, as seen with basil in the first experiment of this Chapter (section 5.2.3.3). As outlined in section 4.2.5 heavy rainfall at the end of the first month of growth caused the plants to be topped up with soluble fertiliser half way through the experiment and although this had a low concentration of phosphorus (0.4%) it is encouraging to see that this did not have a significant impact on colonisation but it may have resulted in a lack of significant increase in growth as it has been shown that more positive effects of AMF on growth occur when plants are suffering from stress and low levels of fertilisation are enough for a plant not to show a benefit as a result of the symbiosis (Jin *et al.*, 2017). Perner et al. (2007) used similar commercial inoculum and found that although phosphorus uptake was increased in mycorrhizal plants no significant increase in biomass was correlated with AMF colonisation.

5.5 Can Reduced peat media Growing Media Be Used to Grow Commercial Potted Herbs?

5.5.1 Aims and Objectives

The aim of this experiment was to examine the effect of the reduced peat growing media and commercial AMF interaction on herbs in a more industrial set up. It was important to test these growing media in a fully controlled environment with flood watering and supplementary lighting in conditions very similar to the Vitacress glasshouses used in the first experiment. For commercial growers, it will be vital to know how these mixes perform in such conditions so they can be advised how to adjust their systems to accommodate them and how AMF can improve their effect on plant performance.

5.5.2 Materials and Methods

5.5.2.1 Plant Species

Ocimum basilicum L. (sweet basil) and *Allium schoenoprasum L.* seeds of the 'Polyvert' variety were purchased from CN seeds. A seed dispenser was used to plant approximately 30-40 seeds per plug in reduced peat, wood based multipurpose compost Sylva Grow. Seeds were germinated in the dark for 10 days in a CT room set at 21°C. Once germinated, both species were grown under glass until ready to be transferred to one litre pots at the start of the experiment.

5.5.2.2 Growing Media

The same batch of three custom growing media were used as in Chapter 4, the ingredients and nutrient remained as outlined in of Chapter 3.

5.5.2.3 Commercial AMF Inoculum

The same commercial inocula AM1, AM2 and AM3 were used as described in section 2.2.2 and 4.22 of Chapter 2 respectively. Appropriate controls using sterilised inocula, (autoclaved as outlined in section 4.2.2), were used as well as live inocula. As the pots were approximately one litre, half the recommended dose for a 2-litre pot was used for each inoculum, so 7ml of AM1 was measured in a 15ml falcon tube and 0.3 grams of AM2 was weighed in an Eppendorf tube and added to each pot. The AM3 dose was 0.8g per pot.

5.5.2.3.1 Root Staining and analysis for AMF colonisation

Root material was harvested from each pot after nine weeks of growth and fixed in 70% ethanol for mycorrhizal colonisation analysis. The method of staining root material was as outlined in section 5.2.1.3.1. The method for determining root length colonisation was as described in section 2.2.3.1. Stained root samples were then stored in 20% glycerol at 4°C until being used to make microscope slides.

5.5.2.4 Experimental Design

Three growing media were used and each media had AM1, AM2 or AM3 added in either their live or sterile form. Six replicate pots of each treatment were planted with basil plugs and six with chive plugs. In total 216 pots (108 of each species) were planted. They were randomly distributed and spaced across two benches in the RHUL temperature controlled glasshouse and grown for 10 weeks from the 9th August 2016. Data were collected from three randomly selected pots for each treatment for basil and all 6 chive replicates were analysed.

5.5.2.5 Site and Conditions

All pots received the same watering regime where they were watered as necessary (approximately once a day) via flooding of the bench for up to 3cm depth for 20 minutes. They were all grown under similar conditions, including pest, temperature, and supplementary light control, as they would be for the normal commercial production of these pot-grown herbs.

5.5.2.6 Biomass

All plants were harvested after 10 weeks of growth once they had reached approximate saleable size (~15cm) as determined by the herb producers: Vitacress. Plants were cut using a razor blade at media level and the number of plants was counted before recording the total fresh weight of the above ground material per pot was recorded. Plants were then dried to constant weight in an oven maintained at 40°C. Using the number of plants, the average fresh and dry biomass per plant could be calculated for each replicate pot.

5.5.2.7 Growing Media Water Relations

To monitor how each growing media performed under the same flood-watering regime currently used to grow these plants in industry A WET-2 Sensor attached to an HH2 Moisture Meter (Delta T, place) was used. This recorded the water content and pore water conductivity (conductivity of

the pore water available to the plant) in three replicate pots of each growing media which all contained sterilised inoculum (so as not to measure AMF effects). This was repeated for both species. Measurements were taken just before watering, after watering and at specific time intervals in between watering.

5.5.3 Results

5.5.3.1 Growing Media Water Content

Basil pots conformed to a pattern which saw peat consistently absorbing and retaining the most water with wood fibre a close second and bark having the lowest water content (Figure 5.22a). Fifty minutes after watering on Day 1 it was shown that the bark filled pots had not taken up as much water as either wood fibre or peat; they also lost water more rapidly than wood fibre and peat-containing pots. Seventy-two hours post-watering on day 6 (Figure 5.22a), the water content of bark pots was considerably lower than peat pots (t4=3.037, p=0.039) and subsequently the water content of pots was again significantly lower than peat pots after only 80 minutes post-watering.

Chives showed the same patterns as seen in basil but bark was found to have significantly lower water content than peat throughout the six-day watering cycle not just after the extended dry period between day 3 and 6 (Figure 5.22b). Bark pots started off significantly drier than peat and wood fibre pots (t_4=-20.25, p=0.001) but 50 minutes after initial watering all pots had the same water content. However, within half an hour the water content of bark pots had significantly reduced to lower than peat pots (t_4=5.428, p=0.0055) when the water content of wood fibre pots remained slightly higher than peat. This same trend was seen 24 (t_3=3.4517, p=0.041) and 48 hours (t_4=2.912, p=0.043) after watering with a steeper decline in the water content of bark pots compared to peat and wood fibre which retained more water. On day six, 72 hours after watering bark pots were significantly drier than peat (0:t_4=3.924, p=0.017, 10: t_4=7.456, p=0.001). Bark pots planted with chives were drier after this 72-hour dry period (12.7±2.7%) than bark plants containing basil (19±2.9%).

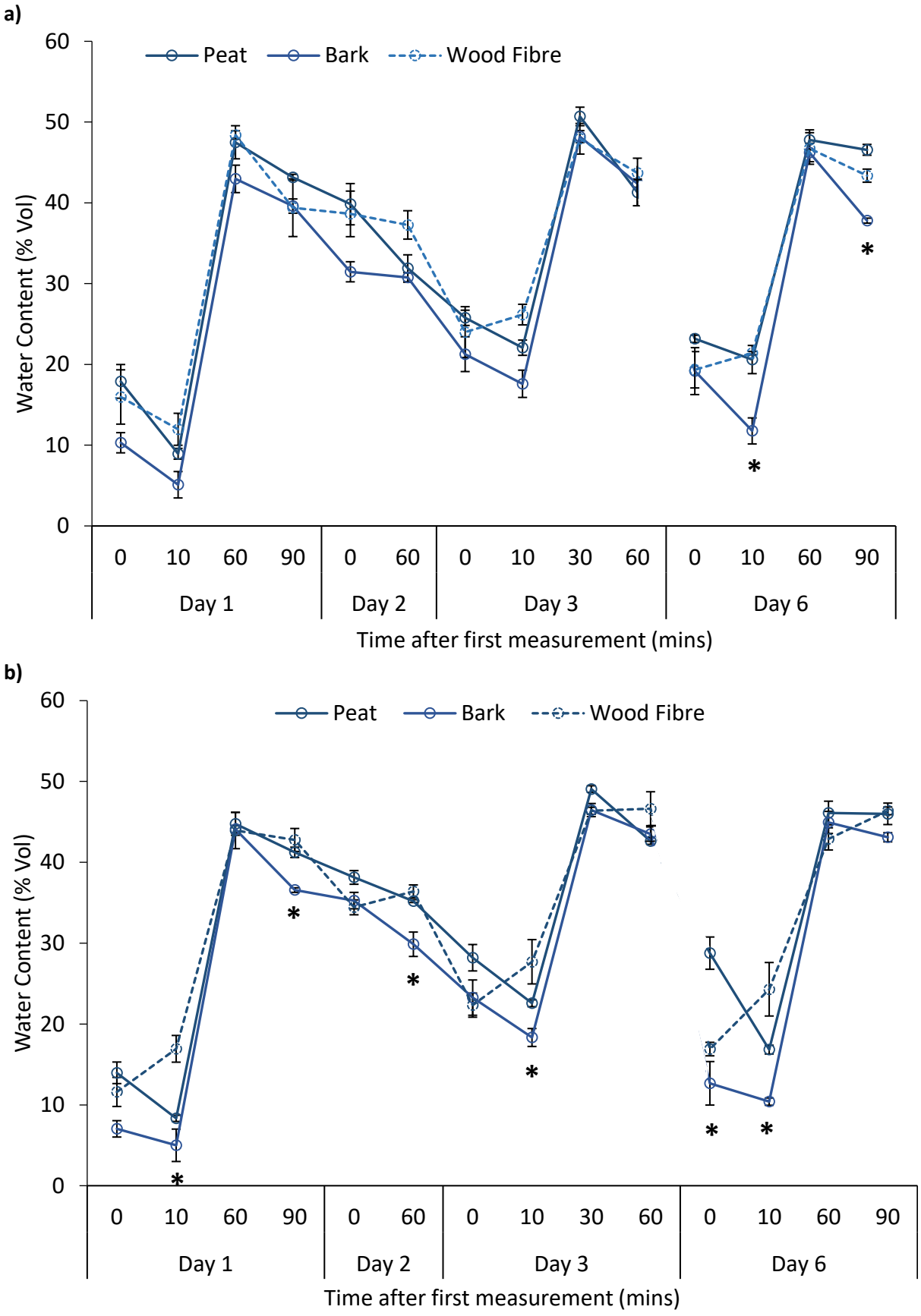


Figure 5.22 The average water content of different growing media measured over 6 days of watering. a) pots contained basil, b) pots contained chives. Flood watering occurred at 10 mins on days 1,3,6. Asterisk denotes significant difference to peat pots. n=3, bars ± SE.

5.5.3.2 Biomass

There was no statistical difference found between biomass per plant when comparing live and sterile inoculum in any of the inoculum treatments (Table 5.6), despite seemingly large differences in bark and wood fibre treated with AM2 (Figure 5.23b) which could have resulted in the significant interaction term (F_2=6.728, Table 5.6). In AM1 basil plants in wood fibre were found to be significantly smaller than those grown in bark (F_2=4.665, Table 5.6) and in AM2 it was peat-grown plants which were found to be significantly smaller than bark-grown plants (F_2=7.742, Table 5.6). In both cases, sterilised control plants in bark had a much greater biomass than plants from all other media and AM treatments (Figure 5.23a and b).

AM1	*d.f*	*F*	*Sig.*
Media	2	4.665	**P<0.05**
AM	1	3.285	p>0.05
Media*AM	2	1.196	p>0.05
AM2			
Media	2	7.742	**P<0.01**
AM	1	0.616	p>0.05
Media*AM	2	6.728	**P<0.05**
AM3			
Media	2	1.765	p>0.05
AM	1	2.643	p>0.05
Media*AM	2	0.130	p>0.05

Table 5.6 Results from two-way ANOVAs on biomass per plant of basil grown in different growing media with each inoculum treatment. Error degrees of freedom: AM1=12 and AM2 and AM3=16.

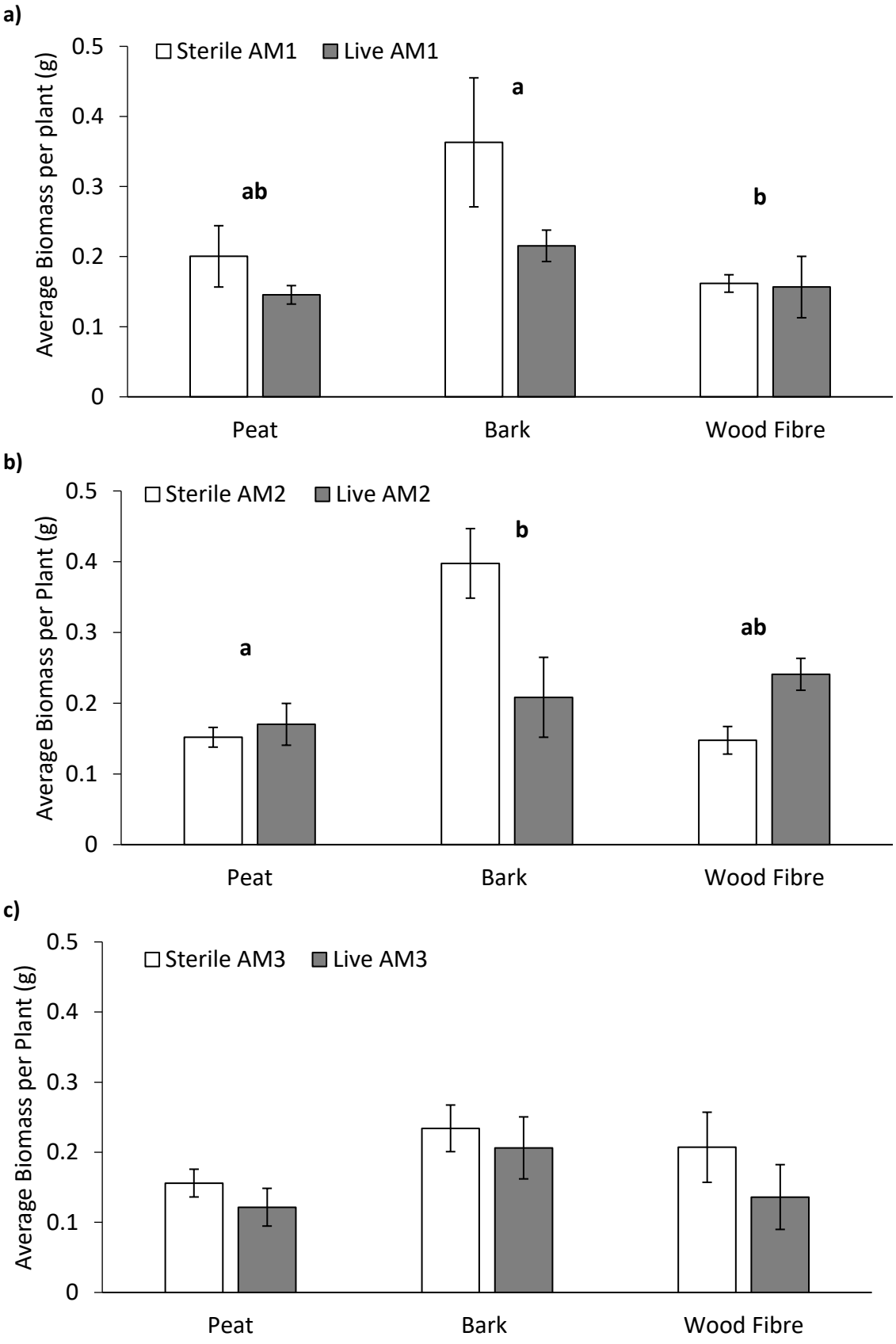


Figure 5.23 Average biomass of basil plants grown in different growing media with each inoculum treatment: a) AM1, b) AM2, c) AM3. Asterisk denotes statistical significance between inoculum pairs and pairs of bars with different letters represent significant differences between growing media means. n=3, bars ± SE.

In Chives, there was little difference in biomass between the live and sterile inoculum controls in any inoculum or compost treatment. In AM3 wood fibre plants live AM treated chives appeared to be a lot smaller than those in sterile inoculum but this difference was not found to be significant (Figure 5.24c). No difference was found between the size of chive plants grown in different media with AM1 or AM2 (Table 5.7). Chives grown in bark and treated with AM3 were found to be significantly larger overall when compared to both plants grown in peat and wood fibre (F_2=10.498, Table 5.7, Figure 5.24c).

AM1	*d.f*	*F*	*Sig.*
Media	2	1.167	p>0.05
AM	1	0.372	p>0.05
Media*AM	2	0.425	p>0.05
AM2			
Media	2	2.413	p>0.05
AM	1	0.188	p>0.05
Media*AM	2	0.016	p>0.05
AM3			
Media	2	11.719	**P<0.001**
AM	1	0.032	p>0.05
Media*AM	2	1.929	p>0.05

Table 5.7 Results from two-way ANOVAs on biomass per plant of chives grown in different growing media with each inoculum treatment. Error degrees of freedom: AM1=29, AM2=28, and AM3=30.

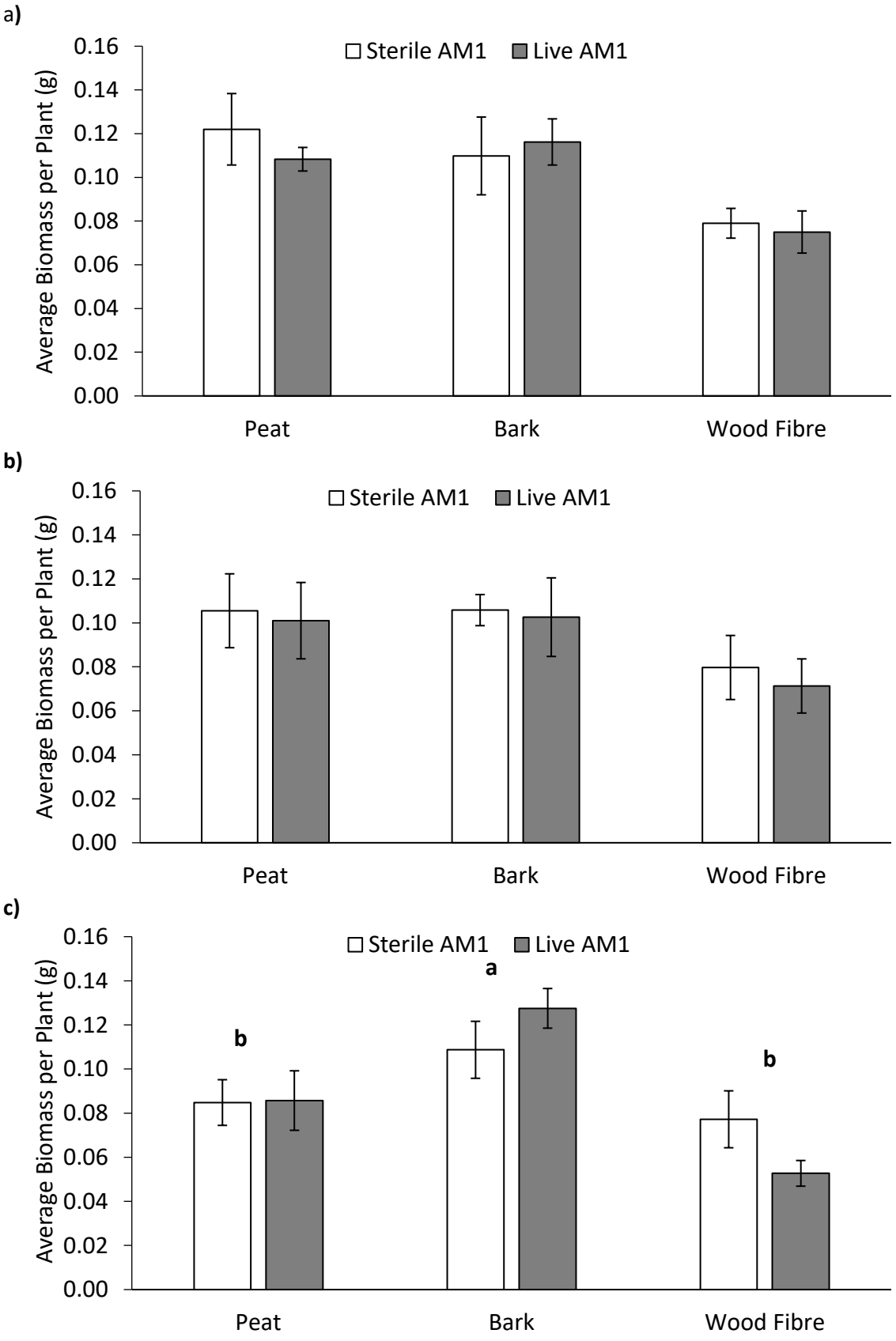


Figure 5.24 Average biomass of chive plants grown in different growing media with each inoculum treatment: a) AM1, b) AM2, c) AM3. Pairs of bars with different letters have significantly different means. n=6, bars ± SE

5.5.4 Discussion

5.5.4.1 Colonisation

Lack of significant differences in biomass between live and sterile AM treated plants in all inoculum treatments was not unusual, given that similar results were seen with other experiments using both of these herbs in this Chapter. This is despite increases in biomass of basil (Copetta, Lingua and Berta, 2006; Rasouli-sadaghiani *et al.*, 2010) and chives (Ustuner *et al.*, 2009) with AMF having been shown in previous studies. However, as these results show, this does not necessarily mean that colonisation was absent just that if these inocula resulted in colonisation it did not have any significant effect on plant biomass. This is supported by similar effects seen in marigolds in Chapters 3 and 4.

AMF colonisation of plants has been shown to affect plant water relations (Bryla & Duniway 1997; Augé 2001; Augé 2004; Querejeta 2017), including increasing plants adaptability to different soil moisture conditions to make sure enough is taken up or preventing water loss (Lazcano, Barrios-Masias and Jackson, 2014). This is surprisingly at odds with the significant decrease in water content of live AM treated basil in peat (AM2) and wood fibre (AM1) pots which were shown to have the higher water content. However, as this cannot be directly linked to colonisation it cannot be confirmed that it was the cause. Also, as only sterile AMF treated pots were measured it is not possible to correlate the water content of those pots with the water content of plants from live inoculated pots which may have been less. Effects of colonisation on the porosity and water holding capacity of these media has only been recorded in marigolds (section 4.3.5) after they had been growing for three months, the reduced time of this experiment may not have been sufficient for the AMF (if present) to have a significant effect on the surrounding substrate.

5.5.4.2 Media Water Content

The water content of peat and wood fibre-filled pots being comparable across both species is surprising given their different properties. Previous work has shown that plants grown in wood fibre required more water to maintain comparable growth and quality to peat-grown plants (Alexander, Williams and Nevison, 2013). Wood fibre has been shown to have re-wetting and water distribution problems (Alexander, Williams and Nevison, 2013). Another surprising result was bark-containing pots having significantly lower water content than peat, as previously discussed (section) substrates that contain bark have been shown to have high water holding capacity and bark fines have been added to peat to improve its water holding capacity (Bilderback and Lorscheider, 1995; Barrett *et al.*, 2016). It could be argued that as bark has greater porosity (Bilderback and Lorscheider, 1995; Barrett *et al.*, 2016), water was more available to the plant so

more water was taken up by plants. This was the case as the average pore water content measured along with % Vol of water content using the WET-2 sensor (data not shown) of peat and wood fibre pots was very similar (124.0±9.16 $mS.m^{-1}$ and 125.7±9.82 $mS.m^{-1}$ respectively) but pore water was a lot higher in bark pots (144.3±10.27 $mS.m^{-1}$). An increase in water uptake of plants in bark could explain the reduction in water volume of media in the pots as well as the increased biomass of plants in the bark media. This result is promising as greater available plant water would allow a reduction in water to be used to grow plants in the bark medium and the wood fibre showed promising results as plants were able to cope in a similar watering regime currently used for peat, however plants from both species grown in this medium were the smallest overall.

5.5.4.3 Biomass

Previously the only significant effect of these growing media on chive growth was seen in AM2 treated chives in section (5.3.3.3) where bark-grown chives were found to be significantly smaller than those produced in peat and wood fibre. With the other inoculum treatments, and when chives were grown outside, and with marigolds no differences were found between plants grown in the different substrates. Ustuner et al. (2009) also found no effect of compost amendment on biomass of chives, even with AMF colonisation in some treatments. In the present study, bark pots treated with every inoculum treatment produced plants that were significantly larger than the other media with basil (AM1 and AM2) and with chives (AM3). Wood fibre pots did seem to produce the smallest plants which is not consistent with results seen with marigolds in the previous Chapters (sections 3.2.8 and 4.3.6) but is consistent with other bedding plant experiments where wood fibre plants performed poorly in size and quality when compared to peat (Alexander, Williams and Nevison, 2013, 2014). These contrasts are interesting as it highlights the effect a controlled environment with a flood watering regime can have on the different responses of plants to each growing media.

Increased soil moisture and water uptake in bark pots would also have facilitated increased nutrient uptake; available nutrients and plant nutrient content have been shown to increase in moist soil (Sweatt and Davies, 1984; Mendoza, Escudero and García, 2005). Drought tolerance induced by AMF colonisation has been linked to increased nutrient uptake (Nelsen and Safir, 1982; Bryla and John M. Duniway, 1997), particularly in drought stressed plants which are often lacking in nutrients (Nelsen and Safir, 1982; Gholamhoseini *et al.*, 2013) and have smaller biomasses (Sandhya *et al.*, 2010). The data showing that bark-grown plants were able to take up

more water as a result of the increased porosity of the media supports the theory that these plants also were able to take up more nutrients as well and combined, these factors significantly increased the biomass of plants in bark compared to the other media.

5.6 Conclusions

This series of experiments have been grouped as overall, they each contribute to the aim of this part of the thesis but due to problems with colonisation and uncontrollable factors requiring frequent change in experimental site and conditions these projects did not produce replicated, consistent results and few effects of AMF addition can be determined.

This series of experiments has highlighted the difficulties of interpreting inconsistent data obtained when testing in different environments. Whilst colonisation and positive effects on biomass have been seen in both chives and basil in other experiments, similar encouraging results were not consistently achieved here. More work must be done to identify incompatibility between the species and the inoculum mixes or indeed, the conditions and the inoculum mixes. It is clear that colonisation of AMF was greatest in outdoor experiments and its positive effects most consistent in outdoor experiments where plants may be experiencing abiotic stresses. The industry cannot rely on plants to be stressed in order to see the benefits of AMF but reductions in water and fertiliser use, along with an increase in peat-free amendment to industry mixes could (would be beneficial to the industry thus further work to elucidate the relationships between plant species, AMF species and the use of different growing media would seem worthwhile) allow for this set up to be used in potted herb production companies such as Vitacress.

Despite the results of these experiments AMF do still have the potential to provide the herb growing industry with a successful biological answer to enable the reduction of fertiliser usage as well as for ornamental production (Gianinazzi *et al.*, 2010; Vosátka *et al.*, 2012). Other benefits of adding AMF to peat reduced media such as essential oil (Copetta, Lingua and Berta, 2006; Rasouli-sadaghiani *et al.*, 2010; Mnayer *et al.*, 2014) and antioxidant production (Toussaint, Smith and Smith, 2007; Taie, Salama and Samir, 2010) as well as disease resistance (Reuveni *et al.*, 2002) in basil and chives could also be explored to improve plant health and quality using organic methods.

Chapter 6 - Analysis of AMF DNA extracted from roots of different plant species

6.2 Introduction

The use of commercial AMF inocula has produced low (Corkidi *et al.*, 2004; Berruti *et al.*, 2013; Faye *et al.*, 2013; Ortas and Ustuner, 2014a) or variable (Gaur, Adholeya and Mukerji, 1998) levels of colonisation and their general, non-specific nature has been criticised. However, there have been some success stories in both field (Ceballos *et al.*, 2013) and pot trials (Carpio, Davies and Arnold, 2003; Perner *et al.*, 2007; Puschel, Rydlova and Vosatka, 2014). Work has been done to look into the effect of production, carrier, and transport (Vosátka *et al.*, 2012; Herrmann and Lesueur, 2013) on inoculum effectiveness and this will need to be carefully designed if AMF are to be introduced on a large commercial scale. The differences in production, carrier, and propagule type (section 2.2.3) between commercial products could be the reason for the poor performance in terms of colonisation of AM2 compared to AM1 throughout this thesis, demonstrating the unreliability of certain commercial inocula.

In this thesis, high levels of colonisation were seen with AM1 in marigolds and these correlated to significant plant benefits. Before claiming these as successes of AM1 as a product, the positive effects or interactions of the plants, or growing media, must be related directly to the inoculum treatment and mycorrhizal colonisation of root tissue from species present in the inocula should be confirmed using DNA analysis. While AMF presence in root tissue can be confirmed through staining by identifying arbuscules there is no guarantee that hyphae (which make up the majority of positive counts on slides) are the result of colonisation by arbuscular mycorrhizal species, as species cannot be identified or separated morphologically. By extracting the DNA of fungal symbionts, it is possible to identify which AMF species has colonised root material. Lee et al. (2008) claimed to have developed primers which have better specificity to an increased range of AMF groups than any used previously and which they have shown exclude sequences from other fungal organisms as well as higher plants.

The objectives of this branch of the thesis were to use these primers to amplify AMF sequences from root samples of two plants species (*Allium schoenoprasum L.* and *Tagetes erecta L.*) which had the presence of fungal structures confirmed through staining, and identify AMF species present by comparison to known AMF sequences. This would help to determine if the colonising species were those from the inoculum or external sources, as well as to see if the same species of AMF always colonises a particular plant species or if the process is random.

Identifying the species and the number of species present in plant roots will also help to determine how the inoculum is interacting with the plant species. It has been shown that increasing the species diversity of inocula past three species does not increase the benefit to the

plant (Gosling, Jones and Bending, 2016). If a similar result is seen here it would suggest that restricting each inoculum mix to a small number of species could be beneficial. This is important as it would reduce the cost of producing inocula if they were to be used on similar plant species in commercial settings and increase the number of effective propagules per ml of inoculum used.

The success of AMF and their wide reaching ability to colonise 80% of all vascular plant species on earth originally lead to the belief that they displayed little host specificity, working together to create common networks which would colonise a range of plant species in a community (Chiariello, Hickman and Mooeny, 1982; Klironomos, 2000; Weremijewicz and Janos, 2013). However, evidence has been building of functional variability and diversity amongst species (Klironomos, 2000; Hoeksema *et al.*, 2010), including in marigolds (Linderman and Davis, 2004). This functional diversity can also be context dependent, often driven by nutrient composition of soils (Gange and Ayres, 1999), growing media composition (Linderman & Davis 2003) as well as the presence of other microbial species (Calvet, Pera and Barea, 1993; Linderman, 2008). It was expected, therefore, that despite the generic, non-specific nature of commercial AMF mixes they would result in different plant-AMF species combinations and responses in the different inocula and growing media treatments, as well as between plant species. As these mixes contain AMF species which could, (in theory) all colonise the same plant species, differences within treatments may also be expected.

Work has also been done investigating differences in functional diversity between and within AMF species (Burleigh, Cavagnaro and Jakobsen, 2002; Jones and Smith, 2004; Smith, Grace and Smith, 2009; Hoeksema *et al.*, 2010; Walder and van der Heijden, 2015) which groups them according to the amount of benefit they offer the host plant (Kiers *et al.*, 2011; Mensah *et al.*, 2015; Werner and Kiers, 2015). If these species could be identified, then differences in significant effects of plant growth seen in marigolds in Chapters 4 and 5 may be attributed to the relationship with the colonised species.

The aim of this work was to try and identify which AMF species had colonised the roots of each plant. Due to variations in plant growth, porosity and stress responses seen in the same media between highly colonised plants and plants with low levels of colonisation, as well as with plants treated with the different mycorrhizal inocula, the following hypotheses were made:

1. In each pot, more than one AMF species will have colonised the roots of the same plant.
2. Different AMF species will be present in the roots of plants from the same species grown with different inocula.
3. Growing media has had an effect on which AMF species colonised the plants from each inoculum.

6.3 General Methods

6.3.1 Collection of Plant Material

Chive and marigold roots were harvested from *Allium schoenoprasum L.* and *Tagetes erecta L.* individuals that were grown in pots together, for three months. The full experiment is described in section 5.4 of Chapter 5. Material from this experiment was used so that molecular details of colonisation could be analysed in two plant species, across three different growing media and (two) inoculum treatments and their combinations. Separating the roots of each species taken from each pot to ensure there was no contamination between samples was difficult but there were some obvious differences used to sort roots, as described in Chapter 5 (section 5.4.2.3.1). Roots of each species from at least three replicate pots of nine different treatments were harvested and rinsed well in a sieve to remove as much soil as possible. Some samples were then put straight through DNA extraction, others were stored in 2% CTAB or flash frozen in liquid nitrogen and stored at -20°C to give a range of storage methods, both short and long term respectively.

6.3.2 DNA Extraction

6.3.2.1 Trial Method

Unstained fresh chive root tissue from field grown plants that were known to have high concentrations of mycorrhizal colonisation (Section 5.3.2.3) was used to trial two adaptations of the van Tuinen et al (1998) method: an increased volume but reduced percentage (60µL of 10% w/v) of Chelex® was added to account for more material being used than in the original paper (2-3 root pieces instead of 1) and two different buffers were tried, one with TE (10mM Tris-HCl, pH 8, 1mM EDTA) and one with 10mM Tris-HCl. Six root samples taken from the same pot of field grown chives were used to test the methods with DNA from three replicate samples extracted with each buffer. Yield was measured with the NanoDrop and the samples were put through the first round of the nested PCR using the protocol outlined in section 6.2.4.

6.3.2.2 Final Method

Marigold and chive roots which had been stored in CTAB buffer were rinsed well to remove any solution. Roots were placed in a clean, labelled tissue cassette (M486 - Histosette® II, Simport® Scientific). Roots were rinsed in the cassette with sterile water and checked to make sure all visible soil particles had been removed. Approximately 8-20mg of root material (2-3 1cm root pieces) were placed in an autoclaved (120°C for 20 minutes), labelled 1.5ml Eppendorf tube.

Liquid nitrogen was used to flash freeze CTAB-stored root samples in Eppendorf tubes and then liquid nitrogen was added to each Eppendorf so that root samples could be ground to a fine powder, using an ice-cold micro pestle, before the TE buffer was added. The method was similar to the one used by Manian et al. (2001) who identified the inconsistency with amplification of standard Chelex® methods. Using an autoclaved micropestle (one for each tube) roots were crushed vigorously in 40µl of TE buffer before being placed on ice. 60µl of 10% (w/v) Chelex® 100 Resin was then added to each tube and they were gently vortexed for 10 seconds. Tubes were incubated at 95˚C for 10 mins and then placed on ice for 5 minutes before being centrifuged at 12000xg for 5 min.

Approximately 50µl of supernatant was transferred to a new tube. To try and reduce DNA and contaminant concentration, the supernatant of each extract was diluted to create aliquots of 1/10 and 1/50 with RNAse free water. Where possible the supernatant was used straight away for PCR reactions, otherwise it was stored at -20˚C.

This method was used to extract DNA from root tissue samples taken from the same replicate pots and the extracts were amplified using PCR (section 6.2.4). This process was repeated on all stored root material of both species including, up to three technical replicates of each biological replicate (where possible).

6.3.3 PCR Primers

A nested PCR protocol was used as outlined by Lee et al. (2008) using the universal eukaryotic primers NS1 and NS4 (NS1: GTAGTCATATGCTTGTCTC, NS4: CTTCCGTCAATTCCTTTAAG) and their new primer pair AML1 and AML2 (AML1: ATCAACTTTCGATGGTAGGATAGA, AML2: GAACCCAAACACTTTGGTTTCC) to amplify partial small sub-unit gene fragments. The authors built on the work of Helgason et al. (1998) and specifically designed these primers to amplify AMF sequences from a wider range of orders including the *Paraglomerales* and *Archaeosporales* so as to improve AMF community analysis.

6.3.4 PCR Protocol

The nested PCR protocol was carried out using a TECHNE Flexigene FG05TUD thermal cycler. A robust PCR mix was sourced from PCR Biosystems Ltd (London). PCRBIO Ultra Mix Red was used for all PCR reactions. Each PCR was carried out according to the PCR BIO Ultra Mix Red protocol

(Table 6.1). The reaction ran as follows: initial denaturation at 95˚C for 2 minutes, followed by 40 cycles at 95˚C for 15 seconds, 50˚C for 15 seconds, and 72˚C for 25 seconds. This produced an amplicon of 1500bp. The first PCR product was diluted 1/100 with nuclease free H_2O and used as template DNA in a second PCR reaction performed using the AMF specific primers AML1 and AML2 (Lee, Lee and Young, 2008) as follows: initial denaturation at 95˚C for 2 minutes, followed by 40 cycles at 95˚C for 15 seconds, 60˚C for 15 seconds, and 72˚C for 15 seconds. This produced an amplicon of 800bp. Sixteen of the 21 original chive DNA extracts (section 6.3.5) were re-amplified using the PCR Bio Ultra Mix Red protocol.

COMPONENT	VOLUME PER SAMPLE (ML)	FINAL CONCENTRATION
2x PCRBIO Ultra Mix Red	25	1x
Forward Primer (10µm)	2	400nM
Reverse Primer (10µm)	2	400nM
Template DNA	2	<250ng/µl
Nuclease Free Water	19	

Table 0.2 Reagent components for PCR (PCR Biosystems, 2016)

6.3.5 Visualising DNA

0.3g of agarose was added to 30ml of 1x TBE buffer (Tris base 89mM, 89mM boric acid and 2mM EDTA) to make a 1% agarose gel. The solution was heated until the agarose dissolved. 3µl of Invitrogen™ SYBR™ Safe DNA Gel Stain was added to the solution, which was then mixed, poured into a sealed gel tray and left to cool and solidify with a comb inserted. Once the gel had solidified it was transferred to a gel tank and the comb and seals removed. 1X TBE was added to the gel tank so that the wells were covered. Samples were prepared on ice. For PCR products using the PCRBIO Ultra mix (Section 6.3.5) 5 µl of product was directly pipetted into wells as the PCR mix contained loading dye. The template ladder used was always HyperLadder™ 1kb, (H1, Bioline) and 5µl of this was also used. Gels were run at 80V for 40-45 minutes.

6.4 PCR Clean-up, Sequencing and Identification

PCR products using the first pair of primers (NG1 and NG4) were obtained from 43 out of 48 samples. Using the second pair of primers (AML1 and AML2) PCR products were obtained from 37 samples.

All final PCR products that produced visible bands for AMF DNA were cleaned up using the Wizard® SV Gel and PCR Clean-Up System (Promega, 2017). An equal volume of Membrane Binding Solution was added to each PCR amplification and the mixture was placed in an SV Minicolumn to incubate for 1 minute at room temperature. The SV Minicolumn and collection tube were centrifuged at 16,000xg for 1 minute. The liquid was then discarded from the collection tube. The column was then washed with 700µl of Membrane Wash Solution (previously diluted with 95% ethanol) and centrifuged for 1 minute at 16,000xg. The collection tube was emptied and the wash step was repeated with 500µl of 80% ethanol to reduce primer-dimer carryover, and the centrifuge step was increased to 5 minutes at 16,000 × g. The collection tube and flow through were discarded being careful not to wet the column. The column was dried and any remaining ethanol evaporated by re-centrifuging it in an open 1.5ml Eppendorf for 1 minute. The clean PCR product was eluted using 35µl of RNase-Free Water (Sigma) which was added directly to the centre of the column and left to incubate at room temperature for 1 minute before being centrifuged at 16000xg for 1 minute. Eluted DNA was then stored at 4°C.

Cleaned samples were then run on agarose gels (Section 6.2.3) to estimate DNA concentration based on absorbance compared to the intensity of the bands of the DNA ladder.

Cleaned PCR products were diluted to 5ng/µl of DNA and were mixed with primer AML1 to a total volume of 17 µl: 15 µl + 2 µl primer (10 µM) in autoclaved 1.5ml screw top Eppendorfs. This was in accordance with the instructions set by Eurofins Genomics (Eurofins Scientific, Ebersberg, Germany). These were then sent for sequencing using either the Value Read or Mix2seq service.

NCBI nucleotide BLAST database was then used to identify returned sequences for each sample. In total 62% of the samples sent off matched AMF fungal sequences the rest were identified as matching *Allium* sequences (Appendix II).

6.5 DNA Extraction Results

6.5.1 DNA Extraction Trial – Fresh Field Chive Roots

Out of the two variations of the modified method DNA yield was found to be higher when using the Tris method but the quality was poorer than with the TE method (Table 6.2) as the 260/230 ratio result was within the range for pure nucleic acid (1.8-2.2). It was apparent that only two out of three technical replicates with the Tris method were successful (Figure 6.1). After the first round of PCR to amplify eukaryotic DNA bands of nucleic acid from extracts using the TE buffer appeared brighter and clearer on the agarose gel (Figure 6.1), TE buffer was therefore chosen to be used for subsequent extractions.

BUFFER	YIELD (ng/µL)	260/280	260/230
TE	94.2	1.4	2.24
Tris-HCl	152.4	1.42	0.68

Table 6.2 Yield of DNA extracted from fresh chive roots using the modified method with TE and Tris-HCl buffers.

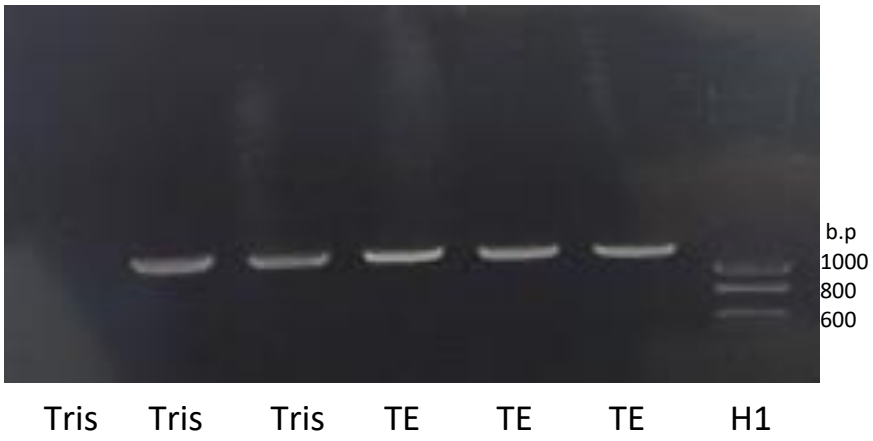


Figure 6.1 Agarose gel showing visible bands of DNA extracted from fresh chive roots with the modified method and amplified with NS1/NS4 primers. Lane 1 shows no DNA was amplified.

6.5.2 Chives

Sixteen undiluted extracts were amplified and some samples were successful, however, there were still seven samples not amplified (Figure 6.2a). These samples were diluted with RNAse free water and were re-run in the first round of PCR, it was found that with some samples 1/50 was too dilute to produce a result (C13, Figure 6.2b) but in others both dilutions were effective (C6, Figure 6.2b). Five more samples were extracted and amplified with NS1 and NS4 primers (Figure 6.2d)

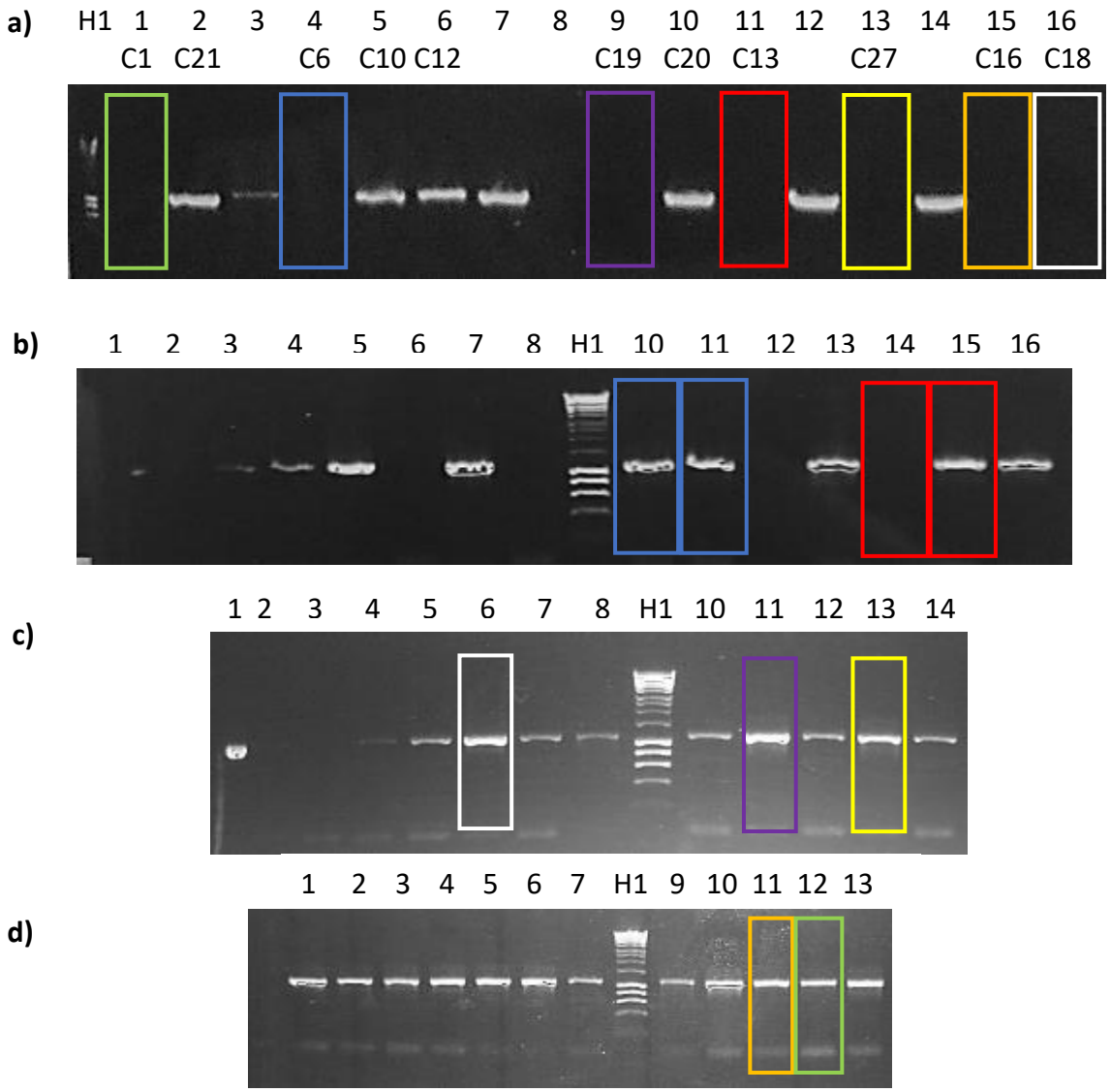
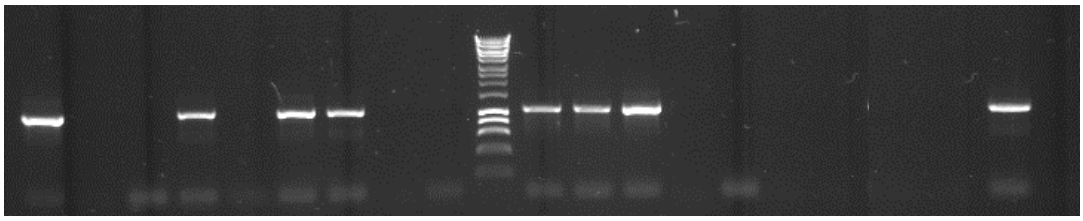


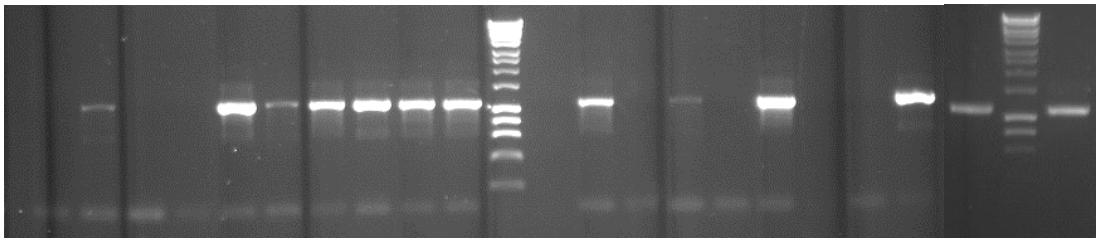
Figure 0.2 All agarose gel pictures show the results of chive root extracts ran with NS1/NS4 primers and PCR Bio Ultra mix red. Lanes with the same colour outline are results from root tissues from the same replicate plant. ***a)*** *undiluted crude extracts of chives* ***b)*** *The effect of diluting crude extracts. Lanes 10 and 11 show 1/50 and 1/10 dilution of C6. Lanes 14 and 15 show 1/50 and 1/10 dilution of C11.* ***c-d)*** *Root samples which were re-extracted.*

6.5.3 Marigolds

Figure 6.3 shows the result of amplifying some of the undiluted marigold DNA extracts alongside a 1/10 and 1/50 dilution of said extracts. None of the undiluted extracts were successfully amplified. Most of the successful marigold DNA samples came from amplifying diluted DNA extracts, in some cases both dilutions produced a positive result: 6.5, 11.1 (Figure 6.3a) and 15.5 (Figure 6.3b). With sample 15.1 although both dilutions produced bands on the gel there was an obvious difference in the concentration of DNA where the 1/10 dilution produced a fainter band compared to the 1/50 dilution (Figure 6.3b). Seven samples had only one dilution produce a successful result but the successful dilution rate was not always the same, in the majority it was the 1/50 dilution, but in sample 11.2 it was the 1/10 which produced a visible band. Sample 20.6 did not produce a successful amplification with either dilution and some samples only had one dilution represented in Figure 6.3. If no result was seen with one dilution the other was tried and this method was repeated on all remaining marigold root extracts and PCR products from all the successful amplifications were then further amplified with the second round of PCR and the AMF primers.



Sample	FC	M22			M23			M24		H1	M10		M11		M12	M8		M13	M27
Dilution	**0**	0	10	**50**	0	**10**	**50**	0	10		**10**	**50**	**10**	50	10	0	10	10	**10**



Sample	M3		M24	M6		M7		M8	M9		H1	M19		M20		M13		M15		M25		M26	H1	M27
Dilution	10	**50**	50	10	**50**	**10**	**50**	**50**	**10**	**50**		10	**50**	10	**50**	10	**50**	10	50	**10**		**10**		**50**

Figure 6.3. Agarose gel pictures showing PCR product from original marigold DNA extractions and dilutions of them amplified with NS1 and NS4 primers using the improved PCR protocol. Bold text indicates band was visible.

6.6 Results Analysis

6.6.1 Effect of Root Colonisation on AMF PCR Results

Chives inoculated with AM1 produced the most replicate samples of DNA successfully amplified with the AMF primers AML1/AML2, this corresponded to higher levels of root length colonisation in these plants (45.13%±9.59). In total 19 PCR products were sent off for sequencing (Figure 6.4) with the smallest number coming from field soil inoculated plants, these plants also had the lowest levels of colonisation by AMF recorded in stained root material (17.4%±4.47).

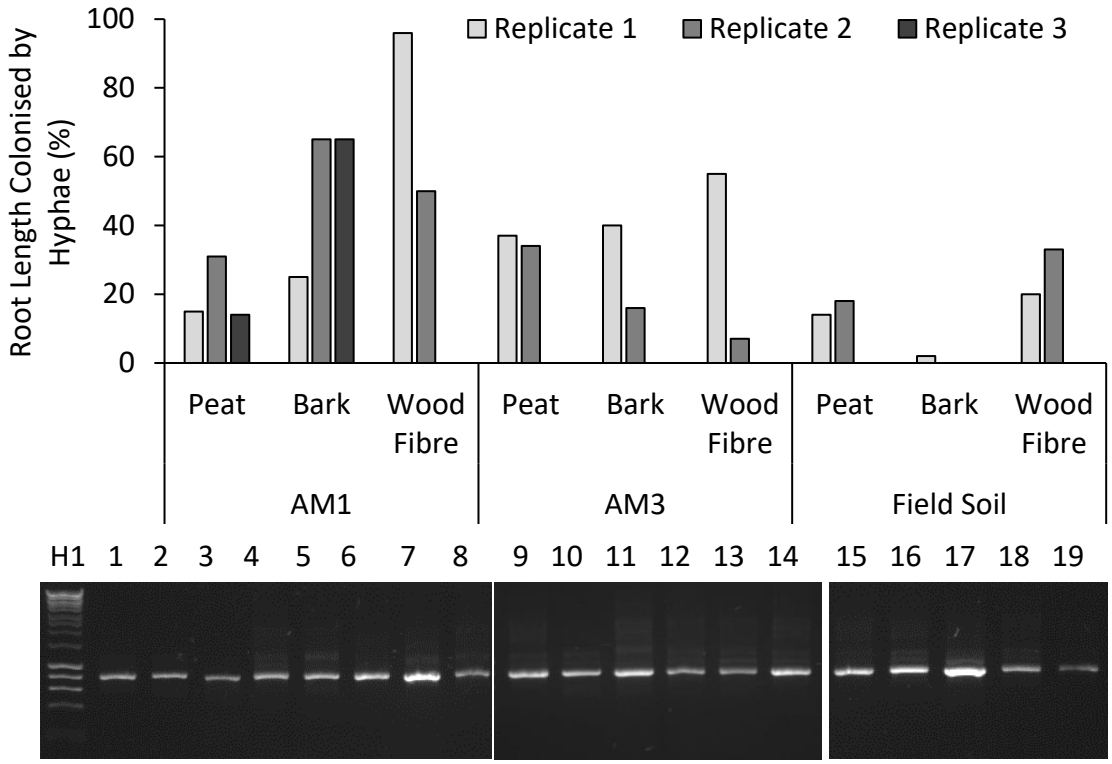


Figure 6.4 Graph: Average root length colonisation by AMF hyphae recorded in chive roots from each replicate pot. Agarose gel picture showing AMF DNA amplified from roots from the corresponding replicate pots. H1 ladder in the first well. Lanes 1-3: peat AM1, lanes 4-6: bark AM1, lanes 7-8: wood fibre AM1, lanes 9-10 peat AM2, lanes 11-12: bark AM2, lanes 13-14: wood fibre AM2, lanes 15-16 peat FS, lane 17: bark FS and lanes 18-19: wood fibre FS.

In total eighteen clean PCR products, which showed successful amplification of AMF DNA extracted from marigold roots were sent off for sequencing. AM1 and AM2 inoculated plants produced an equal number of successful samples with more replicates for each growing media and Field Soil inoculated roots had the fewest successful DNA extractions (Figure 6.5). This did not correspond to colonisation levels as well as with chives, although with AM1 the highest colonisation on average (43.29%±9.69) did produce more consistent results, with at least two

replicates samples per media treatment resulted in amplified AMF DNA. With AM2 only one replicate was produced for peat grown samples, this could be the result of lower levels of colonisation than in AM1 (21.29%±5.47), as plant roots were less likely to contain AMF DNA. Again, the lowest levels of colonisation in FS inoculated plants (14%±2.16) resulted in the fewest samples produced, no DNA from roots of wood fibre grown plants was successfully amplified with the AMF primers.

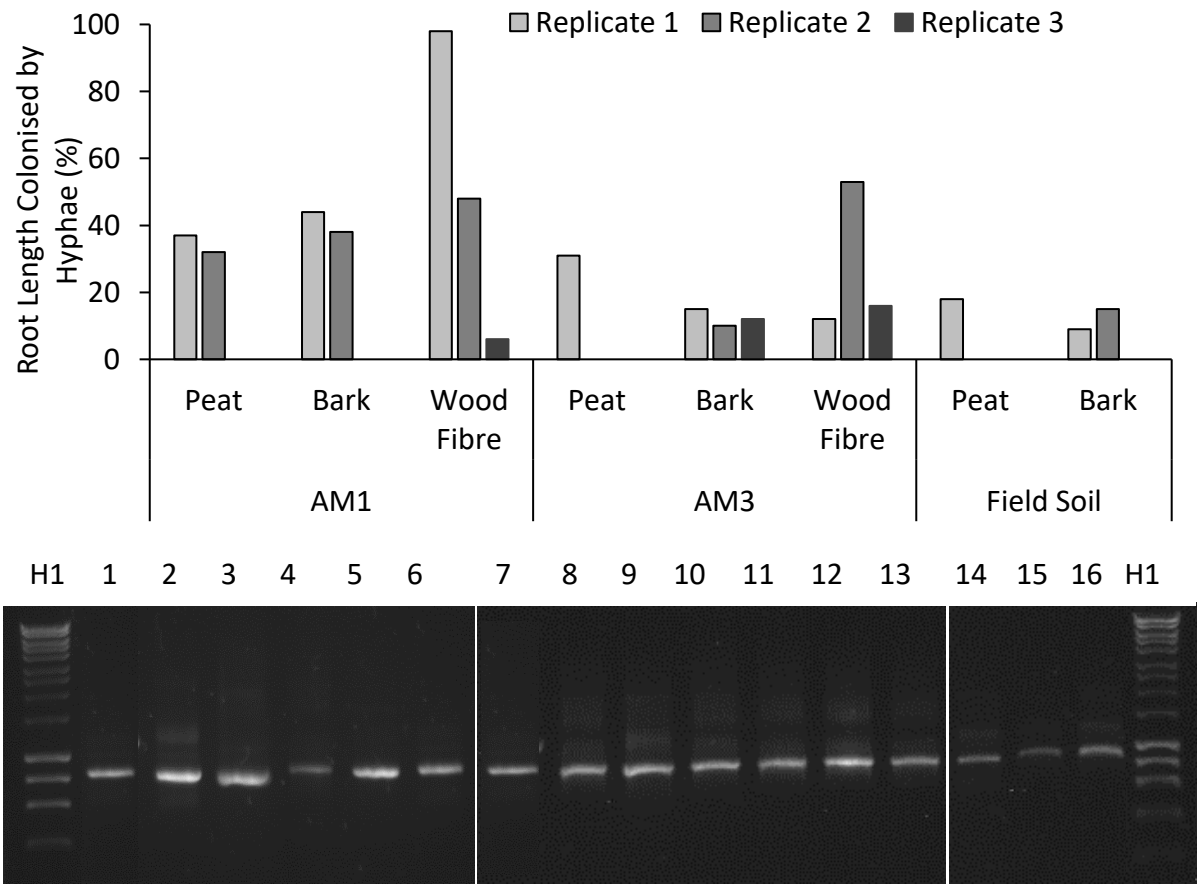


Figure 6.5 Graph shows colonisation by AMF hyphae in marigold roots from each replicate pot and agarose gel picture showing AMF DNA extracted from roots from the same replicate pots.

6.6.2 Plant Species effect on Commercial Inocula

Despite using AMF specific primers AML1 and AML2 a large number of PCR product sequences returned as matching chive (*Allium fistulosum)* 18S ribosomal RNA when blasted (See tables in Appendix I). This included marigold root samples which must have been contaminated with chive DNA as they were grown in the same pot, this could also be due contamination with chive roots during harvesting. As a result of this, only 19 mycorrhizal fungi sequences were identified from root DNA across both plant species and all nine treatments (AM1, AM3 and Field Soil in each of the growing media). The BLAST alignment data can be seen in Table 6.5, the full BLAST alignments can be found in Appendix III.

Fungal species were only sequenced from roots of marigolds grown with AM1 inoculum and only three extracts were found to contain mycorrhizal sequences, however the colonisation of roots of marigolds and chives in those pots did match closely and the sequence of AMF was found to be the same in one pot and in the other two the species of AMF were the same but just swapped around for each species in each pot (Table 6.3). When sequences were obtained from multiple technical replicates (roots from the same pot) they always matched to the same AMF species, there were three examples of this in C1, C13 and C21. The only sequences from technical replicates that did not match up occurred when PCR product from some replicates returned plant sequences, no technical replicates from the same species and pot were found to contain different AMF species DNA.

		Marigold		Chives	
Pot Number	**Treatment**	**Colonisation (%)**	**Sequence**	**Colonisation (%)**	**Sequence**
M/C-2	Peat AM1	32	*Claroideoglomus claroideum*	31	*Paraglomus occultum*
M/C-14	Wood Fibre AM1	48	*Paraglomus occultum*	50	*Claroideoglomus claroideum*
M/C-15	Wood Fibre AM1	96	*Rhizophagus irregularis*	96	*Rhizophagus irregularis*

Table 6.3 The average root length colonisation by hyphae of each species of plant from the same pot and the AMF sequence identified from DNA extraction and PCR amplification of a sample of roots taken from each species per pot.

Chive Samples

Sample	BLAST Homolog species	Sequence ID	Expect value	Identity %	Score	Gaps %	Strand
Peat AM1	*Paraglomus occultum*	KC666034.1	0.0	89	429	2	Plus/Plus
Peat AM1	*Rhizophagus irregularis*	HF968844.1	0.0	95	641	1	Plus/Plus
Bark AM1	*Glomus sp*	FR693419.1	$6x10^{-100}$	77	201	4	Plus/Plus
Bark AM1	*Rhizophagus irregularis*	HF968850.1	0.0	99	748	0	Plus/Plus
WF AM1	*Rhizophagus irregularis*	HF968834.1	0.0	99	741	0	Plus/Plus
WF AM1	*Claroideoglomus sp.*	KP988474.1	$5 x10^{-161}$	86	311	1	Plus/Plus
Peat AM3	*Rhizophagus sp.*	HG004476.1	0.0	99	717	0	Plus/Plus
Peat AM3	*Rhizophagus sp.*	KX462854.1	0.0	93	591	2	Plus/Plus
Bark AM3	*Paraglomus occultum*	KC666034.1	0.0	87	387	3	Plus/Plus
Bark AM3	*Funneliformis mossae*	FR750227.1	$1x10^{-111}$	78	222	7	Plus/Plus
WF AM3	*Funneliformis mossae*	KU136433.1	$6x10^{-100}$	77	201	4	Plus/Plus
WF AM3	*Rhizophagus irregularis*	HF968834.1	0.0	86	410	3	Plus/Plus
Peat FS	*Glomus sp*	KF386333.1	0.0	89	506	2	Plus/Plus
Peat FS	*Rhizophagus irregularis*	HF968850.1	0.0	99	744	0	Plus/Plus
WF FS	*Archaeospora sp.*	FN869851.1	$2x10^{-164}$	81	317	5	Plus/Plus
WF FS	*Rhizophagus irregularis*	HF968850.1	0.0	87	423	6	Plus/Plus

Marigold Samples

Sample	BLAST Homolog species	Sequence ID	Expect value	Identity %	Score	Gaps %	Strand
Peat AM1	*Claroideoglomus claroideum*	KX879058.1	0.0	87	448	0	Plus/Plus
WF AM1	*Paraglomus occultum*	KC666034.1	0.0	93	502	2	Plus/Plus
WF AM1	*Rhizophagus irregularis*	HF968850.1	0.0	99	739	0	Plus/Plus

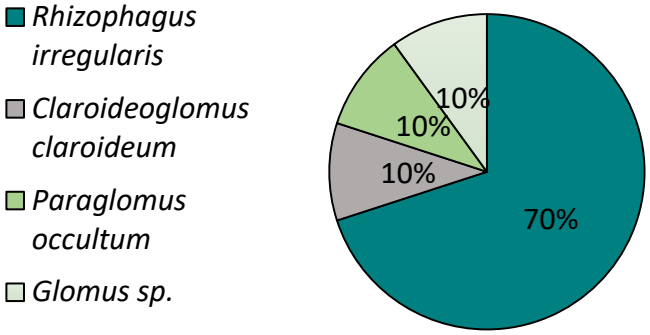
Table 6.4 Alignment data for PCR product samples which were found to be highly similar to mycorrhizal fungi sequences. All were identified using the NCBI BLAST database, full alignments can be found in Appendix III. WF = Wood Fibre.

6.6.3 Effect of Inocula on AMF Species Colonisation

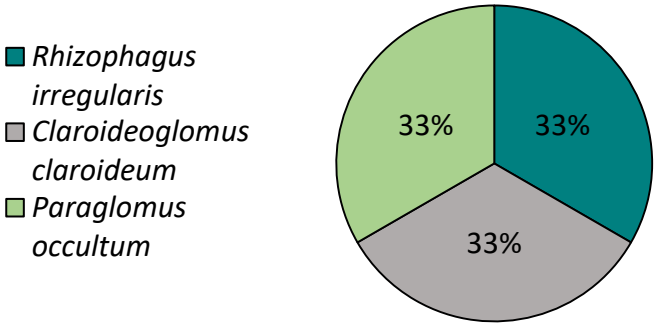
Figure 6.6 shows the percentage of root samples from plants treated with each inoculum that were found to contain sequences matching each fungal sequence. *Rhizophagus irregularis* was present in both commercial inoculum mixes and was found to be present in roots from plants treated with all inoculum types including field soil as well as both species of plant. In chive roots, it was the most common species identified in roots from all inocula. Whilst *R. irregularis* was common to all inoculum treatments, every inoculum also resulted in colonisation by one species not found in roots from the other treatments: *Claroideoglomus claroideum* (AM1), *Funneliformis mosseae* (AM3) and *Archaeospora sp*. (Field Soil).

C. claroideum, is known to be present only in the AM1 inoculum and therefore was only found in plants inoculated with AM1 and it was found in both marigold and chive roots. Interestingly, *F. mosseae,* like *R. irregularis,* was known to be present in both AM1 and AM3, however it was only found in roots of AM3 treated plants. Some sequences were not identified to species level but the presence of representatives of the genus *Glomus sp.* was not surprising as the commercial inocula contained *Glomus* species, such as *Glomus microaggregatum* in AM1. The field soil inoculum resulted in plants that were colonised with an AMF genus not present in either commercial inoculum mix: *Archaeospora sp*. There was also a species not known to be present in either inocula but was found in roots of plants inoculated with both AM1 and AM3 and in both chives and marigolds: *Paraglomus occultum.*

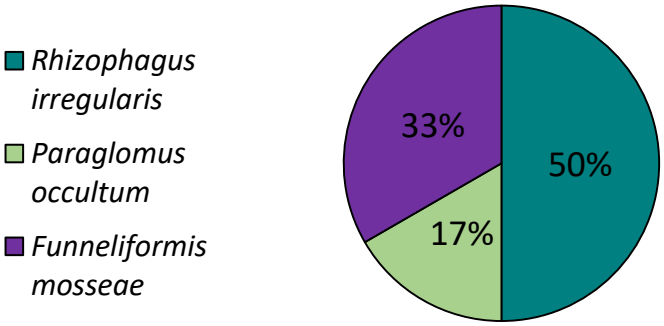
a)



b)



c)



d)

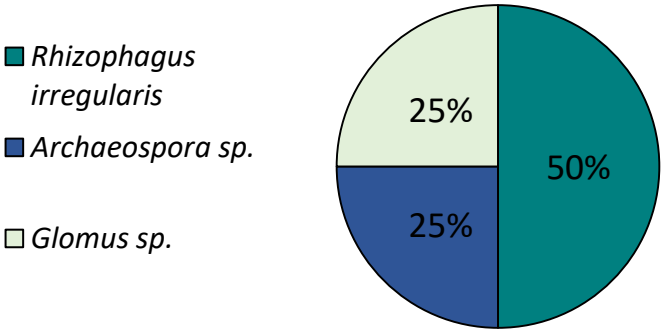


Figure 6.6 Proportion of each AMF species found in roots of plants grown with each inoculum. a) Chives grown with AM1, b) Marigolds grown with AM1, c) Chives grown with AM3, d) Chives grown with Field Soil inoculum.

6.6.4 Effect of Growing Media on AMF Species Colonisation

Figure 6.7 shows the number of each species recorded for roots of plants grown with each inoculum in each growing media. Peat and bark grown plants had four AMF species present in the roots and wood fibre produced slightly higher diversity with five but overall the number of species identified in the roots of plants did not appear to be affected by growing media treatment. The dominant species in both peat and wood fibre was *R. irregularis,* this was present in plants grown with every inoculum and was the only species identified in the root material of AM2 treated plants grown in peat. This was a contrast to bark grown plants where *R. irregularis* was only found in AM1 grown plants and bark grown plants treated with AM3 were the only plants not found to be colonised by *R. irregularis*. It should be noted that also in bark grown plants, no fungal species were identified in the roots of plants grown with Field Soil inoculum. Plants inoculated with AM1 produced the most replicate samples which were successfully amplified with PCR and it was the only inoculum to result in more than two species of AMF being identified in peat and wood fibre grown plant roots, these were also found to be the same three species (*R. irregularis, P. occultum*, and *C. claroideum*) in each medium. Other than this the profiles of the number and type of species present in each growing media and inoculum combination were different.

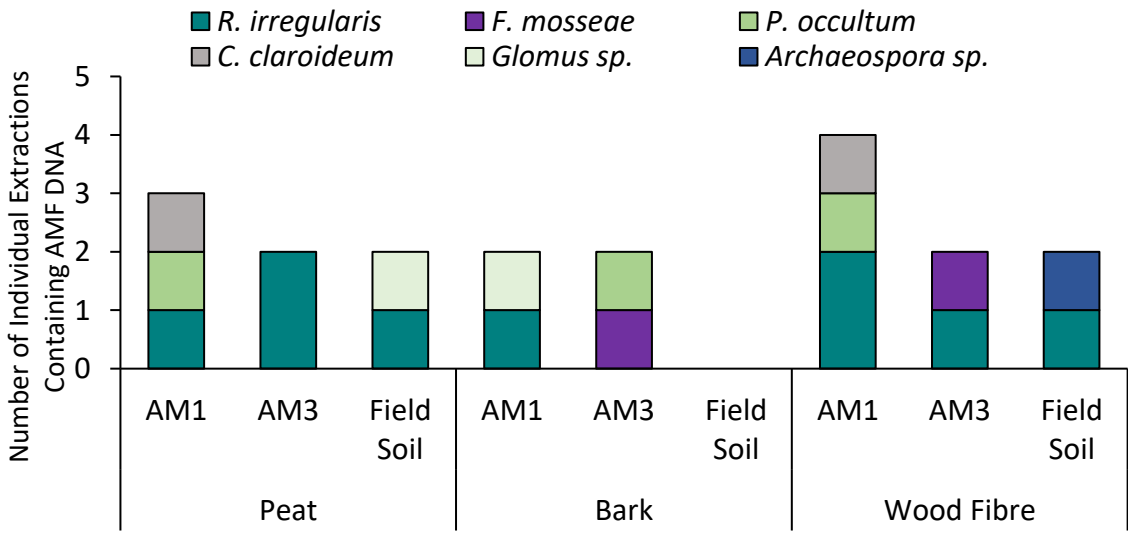


Figure 6.7 Frequency of each AMF species recorded in roots of plants grown in each media and with each inoculum. No AMF species were sequenced from DNA extracted from roots grown in bark with Field Soil inoculum.

6.7 Discussion

6.7.1 DNA Extraction Methods

The method of diluting extracts may have been more successful if it was tried on the original crude supernatants earlier when they had not been stored for so long. The Chelex® method, (despite yielding high concentrations of DNA) results in samples which are designed to be used immediately and not to be stored for lengths of time, even at -20°C, due to the amount of contaminants in the extract (van Tuinen *et al.*, 1998; Manian, Sreenivasaprasad and Mills, 2001). In future, a combination of the improved liquid nitrogen method and dilution of supernatants before storage should be used on all samples to improve yield and quality of DNA extracts.

Replicate numbers for all of the trial methods had to be kept low to avoid using up stored tissue however other methods have used similar replicate numbers (Turnau *et al.*, 2001; Bainard, Klironomos and Hart, 2010; Verbruggen *et al.*, 2012) when cloning sequences for identification. In future, now a reliable extraction method has been identified, cloning should be used along with more replicate samples in order to maximise the number of different AMF sequences identified.

6.7.2 PCR Clean-up, Sequencing, and Identification

Despite the nested PCR method producing high concentrations of amplified DNA using the AML1/AML2 primers the sequences returned from those samples were mostly of chive origin, even in marigold roots. This shows that the primers were not specific enough to avoid amplifying plant DNA, *Allium* or related species were not on the list of 14 plants tested in the original paper. Although Lee et al. (2008) claim the amplification of non-AMF species had been reduced with these primers compared to the previous set, they did see faint amplification of two out of 14 plant species tested. Despite this, the same primers have been used for successful AMF community analysis (Alguacil *et al.*, 2011; Francini *et al.*, 2014; Liu *et al.*, 2014; Grilli *et al.*, 2015). As the two orders *Paraglomerales* and *Archaeosporales* were not represented in either AM1 or AM2 mixes it was not necessary to use these newly designed primers but they would allow for amplification of colonised species from external contamination in the outdoor grown plants as well as the unknown AMF species present in field chive root samples.

Alguacil et al. (2011) also found that a large number of their sequences amplified with AML1 and AML2 (34.1%) returned as matching plant species. Kohout et al. (2014) and Liu et al. (2011) tested the use of AML2 in combination with the universal eukaryotic primer NS31 and found that this improved the problem of amplifying plant sequences but that bacterial sequences were

still amplified in some cases. Similarly Kohout et al. (2014) and Appoloni et al. (2008) found that when fungi was absent AMF primers amplified non-AMF DNA.

In this case the presence of *Allium* DNA even in marigold root sample extracts suggests that these samples could have been contaminated with misidentified chive roots but as plants were grown in the same pot, cells lost from the root caps of chives could have contaminated the sample and, in the absence of fungal DNA, were amplified instead.

Careful primer selection and the use of highly colonised and consistently colonised root material should be attempted with future work in this area.

6.7.3 Effect of Root Colonisation on AMF PCR results

Successful extraction of DNA from stained roots has been demonstrated (van Tuinen *et al.*, 1998; Jacquot *et al.*, 2000; Ishii and Loynachan, 2004) and had this method worked it would have been ideal as the amount of root colonisation could then be directly related to the extracted DNA and therefore the species identified. Highly colonised root material could also have been selected to try and ensure large yield of fungal DNA within the extracts to be amplified. When colonisation levels were lower or inconsistent across replicate plants fewer root samples were found to contain fungal DNA, this was the case in AM3 and Field Soil treated plants in all growing media. Also, without knowing the average colonisation of roots from each pot, roots were harvested from three replicate pots at random which meant that samples being used to extract DNA came from pots where the average colonisation may have been very low. Bark grown plants inoculated with field soil are a good example of this as the average root length colonisation by hyphae of the replicate pots used to sample roots for DNA extraction was only between 2 and 7%, it is therefore unsurprising that all sequences extracted from these roots returned as matching *A.fistulosum*.

Of all the samples (including technical replicates) only 22% of sequences from DNA extracts from Field Soil inoculated roots and only 50% of sequences identified from PCR products from AM3 inoculated roots were identified as mycorrhizal, compared to 85% of sequences from AM1 inoculated root samples. In AM1 treated chives the only samples which were returned as matching chive sequences were technical replicates from pots which had already had root samples return showing AMF sequences so the success rate for the number of AMF sequences identified from PCR products from AM1 inoculated chives was actually 100%.

In marigolds, this result was a lot lower (42%), but the success of AM1 inoculated chives could explain why only AM1 treated marigold roots were found to contain mycorrhizal sequences. In Chapter 5 (section 5.4.3.1) root length colonisation of marigolds was shown to significantly effect that of chives grown in the same pot.

As previously discussed, AM1 appears to have consistently higher colonisation performance than AM2 and even native or "indigenous" inocula and the increased number of mycorrhizal sequences between AM1 and the other inocula here seem to support the idea that AM1 is the most effective AMF inoculum. This also supports the idea of inconsistent colonisation across replicate plants treated with AM2 and AM3 which could also explain variations in biomass between plants in the same treatment, in particular the size inequality seen in marigolds treated with AM2 and AM3 in Chapter 4 (Section 4.1.3.2).

6.7.4 Plant Species effect on Commercial Inocula

As previously mentioned AMF exhibit wide functional diversity between species and, in nature, it is not uncommon for AMF communities to differ between plant species (Veresoglou and Rillig, 2014), and within plant species, depending on their neighbouring community (Mummey, Rillig and Holben, 2005) and nutrient availability (Liu *et al.*, 2014). These simple qualitative results show that when given the same mix of AMF species two different species of plant roots were shown to contain the same three species of AMF fungi, although these seemed to vary between growing media. Without data on the AMF species that colonised marigolds with the other inoculum and with so few replicates from marigolds treated with AM1, robust conclusions cannot be drawn on the different ways the AMF species in each of these inocula interacted with the different plant species or different growing media. A more in-depth, focussed study would be necessary in future; however, these results can provide an indication that despite a mixture of five AMF species only three were found in the roots of both plant species inoculated with the product. This could support evidence that no more than three species are required to have an effect on plant growth (Gosling, Jones and Bending, 2016) and that there will be functional diversity which will affect which AMF species dominates when this inoculum mixed is used with different species.

The three different AMF species: *C. claroideum, P. occultum* and *R. irregularis* which have occurred together in each pot might have been favoured by the marigold and chive plants for different reasons when grown in the peat or wood fibre medium. These three species have been identified by Mensah et al. (2015) as having different levels of performance when it comes to

benefiting plant growth through increased P and N uptake. Isolates of *C. claroideum* were identified as low-medium performance species, isolates of *P. occultum* were present in low, medium and high-performance categories and the *R. irregularis* isolates were found to be low performing. Despite *R. irregularis* having little effect on the growth rate of plants (hence its low performance rating) it was shown to significantly increase the concentration of phosphorus in both shoots and roots compared to control plants and plants inoculated with high performance isolates (Mensah *et al.*, 2015). This could explain why this species dominated across all treatments and could explain high levels of colonisation with limited effect on plant growth increase (Chapter 3, sections 3.4.1 and 3.4.6). Kiers et al. (2011) have shown how the benefits of different AMF species can result in plants manipulating the relationship with colonised species. As a potentially high-performance species *P. occultum* could have outcompeted *C. claroideum* in chive plants by receiving a greater reward for its ability to significantly increase both P and N compared to medium performance species. This change in AMF species could also have been affected by the growing media, how each plant species obtained nutrients in peat and wood fibre could have caused a change in their preference for each AMF species, but much more data is needed to reach a conclusion on this.

Given the small number of replicate samples providing this data these are just examples of how the different plant species may have interacted with the AM1 inoculum to produce these results. It could also be possible that all three of these AMF species was present in the roots of each plant in all three pots and by chance, root samples containing a larger amount of fungal material of each AMF species were sampled from each plant. The emphasis that Mensah et al. (2015) put on the high variability between isolates of the same AMF species should also not be ignored as it cannot be guaranteed that colonisation by the same species of AMF would produce the same results.

6.7.5 Effect of Inocula on AMF Species Colonisation

It is unfortunate that low colonisation was seen with both AM3 and the Field Soil inoculum as it did appear that increased root colonisation resulted in an increase in amplified fungal DNA, which in turn led to an increased number of successfully identified fungal sequences with AM1. The inconsistency in root colonisation of the other two inocula could have caused underrepresentation of the actual number of species present due to lower numbers of successful replicate samples sequenced. The use of mixed inoculum and general AMF primers without cloning prior to sequencing is also likely to have reduced the number of sequences

amplified and identified as only the species that dominated the extracted DNA would have been detected. Chromatogram outputs for each sample indicated some very low-level background peaks being present in the amplified samples, there were also very few instances where more than one band was present after the second round of PCR which supports the idea that one AMF species dominated each sample.

It is unsurprising that differences were seen between inocula for species such as *C. claroideum* because it was only present in the AM1 mix, this also helps with confirming lack of contamination between inoculum treatments and that the species identified to be colonising plants are there as a result of the inoculum treatment. The presence of unidentified *Glomus* sequences is also unsurprising given that they made up a large proportion of the species in the inoculum mixes and that it is the largest genus of the AMF (Schwarzott, Walker and Schussler, 2001), so its presence in the natural field soil was expected. Species being identified in roots grown with the Field Soil inoculum which were not present in either commercial inoculum such as *Archaeospora sp.* was also expected as this inoculum should represent a specific, natural community of species which have been growing with a single species of plant. The aim of commercial inocula is not to replicate specific environments but to provide common AMF species that are good at colonising a range of host plants, for example *R. irregularis* has been extensively studied and was shown to be one of only two species, (the other being *F. mosseae)* when mixed with up to seven species to have a positive effect on shoot dry weight (Gosling, Jones and Bending, 2016). The low colonisation ability of the field soil inoculum, especially in chives, suggests that only a small proportion of the propagules were viable or that not enough were added to the pot and as a result the community of AMF which could be provided by this inoculum was not properly represented in these data.

The main unexpected, and therefore interesting, differences between the results of the number of replicate species colonising plants from different inocula come with the presence of *F. mosseae* in only roots inoculated with AM2 and *P. occultum* being identified as present in roots of plants treated with both commercial inocula despite it not being present in either mix.

The quality control processes in the production of AM1 inoculum that involves the combination of separate species would aim to ensure that infective propagules of *F. mosseae* are present and that there is an equal number of propagules of all species represented in the mix. This would also be assumed of AM2. The differences between the inocula that could result in a different performance of *F. mosseae* in the same growing media and the same species are the other species in the mix including the number and also the type because AM3 also contains bacteria

and *Trichoderma* species. The number and type of AMF could result in competition with other AMF species within the pot environment and if a more beneficial AMF species is present it could be selected for by the plant (Werner and Kiers, 2015). This is unlikely given that the AMF species that dominated in AM1 inoculated plants (*R. irregularis*) was also present in AM3. A lot of studies have used AMF and *Trichoderma* species in combination and where *F. mosseae* (formerly *Glomus*) was used, the presence of *Trichoderma* was shown to increase its colonisation (Calvet, Pera and Barea, 1990) including in marigolds (Calvet, Barea and Pera, 1992; Calvet, Pera and Barea, 1993). Calvet et al. (1992) suggest this is the result of volatile compounds produced by *Trichoderma* which stimulate mycelial growth. The species of *Trichoderma* used by Calvet et al. (1992 and 1993) is not present in AM3 and in field experiments using tomato other species of *Trichoderma* which are present in the commercial mix were shown to have no effect on colonisation by *F. mosseae* (Nzanza, Marais and Soundy, 2011). When comparing root length colonisation in roots of plants treated with AM2 (which contains no *Trichoderma* species) to AM3 in bark pots, colonisation was higher (AM2=7.4±3.27% and AM3=17.2±3.51%, section 5.4.3.1). These observations indicate the positive effect that other microorganisms, such as *Trichoderma* can have on the effectiveness of commercial inocula by improving the colonisation of certain species.

Paraglomus occultum is part of a family of AMF species which have physiological features that are indistinguishable from *Glomus* individuals under the microscope, as such they have only been identified as a divergent group at the molecular level through 18s rDNA (Redecker, 2000; Redecker, Morton and Bruns, 2000; Morton and Redecker, 2001). They are also very difficult to stain in order to record their presence (Morton and Redecker, 2001). This means that this species could be present in the commercial inoculum without being picked up by either producer unless they use molecular methods as part of their quality control process, something that the producers of AM1 do not do. When testing different commercial inocula in sterilised soil in a greenhouse study. Faye et al. (2013) found evidence of AMF species which were not listed in any of the commercial inocula including one which was present in over half the inoculants tested. As this study was conducted in the field it is also possible that the presence of *P. occultum* in roots was the result of external colonisation, however although this species was present in all growing media, (peat AM1 chives, bark AM2 chives and wood fibre AM1 marigolds) it was not found to be present in roots from all three inoculum treatments and was not as common as *R. irregularis*.

6.7.6 Effect of Growing Media on AMF Species Colonisation

It was hypothesised that the difference in AMF species identified in roots of plants from different growing media was only seen with AM1 in bark where roots were not shown to contain *C. claroideum* or *P. occultum* as seen in roots of plants grown in peat and wood fibre. This could have been due to higher levels of bacteria present as a result of the composted bark material. Although the presence of other microbial species has been considered necessary to enhance the performance of AMF by replicating the natural environment of these fungi in soilless potting media (Linderman, 2008), the presence of certain bacterial and fungal species have been shown to inhibit growth or have a negative effect on AMF colonisation. Presence of contaminating fungi has been shown to negatively affect AMF spore germination under some conditions (Sylvia and Schenck, 1983). The reduced colonisation of AM3 and specifically Field Soil inoculum in plants grown in the bark medium resulted in fewer AMF sequences identified for those treatments, this means it is also possible that AMF species were missed due to lack of AMF DNA present in samples in these treatments and it is not the fault of spore inhibition as a result of fungal contaminants.

Sequences of *F. mosseae* were only identified in AM3 inoculated plants grown in the wood based mixes and not peat. Peat has been shown to inhibit colonisation in some species of AMF (Linderman and E. A. Davis, 2003; Ma, Yokoyama and Marumoto, 2007; Puschel, Rydlova and Vosatka, 2014) and Calvet et al. (1992) showed that composted pine bark medium resulted in significantly higher spore production in *F. mosseae* than found in peat. If the properties of the wood based media combined with the presence of *Trichoderma* species (as discussed in section 6.7.5) increased colonisation of roots by *F. mosseae* then that would explain its increased representation in roots samples compared to other species in the inoculum. As *Trichoderma* species contribute to the degradation and breakdown of wood and bark material (Błaszczyk *et al.*, 2016) it could be assumed that species could be present in both wood mixes, but particularly the bark medium due to its compost processing stage. *F. mosseae* isolates were shown to be consistently high performing (providing more nutrients to plants) in the study by Mensah et al. (2015), if this species thrives in the wood based mixes (due to the presence of *Trichoderma* species) then it could be responsible for the consistent increases seen in biomass in the wood based mixes in marigolds inoculated with AM1, AM2 and AM3 in Chapter 4.

6.8 Conclusions

The first conclusions to be drawn are that whilst improved methods exist for extracting AMF DNA from root samples, some method development is still required to improve these further and optimise them for use with specific plant materials. Likewise, PCR modifications should be made in tandem with extraction methods so that both are optimised for the material being used and extracts being produced.

The hypotheses identified at the start of this chapter were not all confirmed or refuted due to the limitations imposed by the methods employed, however these results did show that more than one species of AMF was able to colonise the roots of both marigolds and chives. The data also suggested that colonising species differed in plants of the same species treated with different inocula and grown in different growing media but more work needs to be carried out to confirm this.

This data confirmed that to successfully isolate and identify the AMF species colonising the roots of any given plant it appears to be necessary to have a high level of colonisation across multiple root samples, as shown with AM1.

For future work, the evidence of increased AMF colonisation could be quantified using q-PCR techniques and, with more time and an increase in the number of successful fungal DNA extractions, functional diversity within AMF species present in each inoculum could be described more accurately, and related to effects of growing media through improved PCR amplification and cloning of PCR products for sequencing.

Due to the functional diversity of AMF symbioses more detailed analysis of how these inocula are interacting with the growing media and their effect on plant growth is required. In this way, customised commercial inocula could be produced for use in the industry, just as all variables affecting growth have been currently optimised for all commercially produced plant species.

Chapter 7 - General Discussion

The aim of this PhD research was to investigate the effects of arbuscular mycorrhizal fungi on plant performance in reduced peat substrates, with a view to implementing their use in the horticultural industry in order to facilitate an increase in the use of peat alternatives. This is in order for the industry to fulfil the government's target to eliminate the use of peat in the UK by 2030.

From the first experiment where two types of AMF inocula were applied to four different peat alternative base materials it was apparent that the differing effects of each growing medium on the mycorrhizal relationship was going to determine the choice of growing media going forward. This theme of functional diversity continued throughout the experiments and was confirmed through molecular techniques. This will be an important factor to consider when making recommendations for the application of these growing media and AMF combinations.

The outcomes of the main research questions in are summarised for each Chapter in Table 7.1.

The main findings from the different branches of results which emerged from the studies described in Chapters 2-6 and their effect on peat use in the horticultural industry will now be discussed.

Research Question	Chapter	Outcome
Were there significant differences in the growth of plants in peat reduced substrates compared to those in full peat?	2,5	Yes, green waste and coir composts produced significantly smaller marigold and basil plants compared to peat but wood fibre and low peat composts produced plants that were not significantly smaller than those grown in peat.
	3,4	No, marigolds grown in wood-based reduced peat media were not found to be significantly smaller than those grown in peat.
	5	No, basil and chives grown in wood-based reduced peat media were not found to be significantly smaller than those grown in peat.
Did the effects of AMF colonisation on biomass differ between reduced peat substrates?	2,5	Yes, AM treatment significantly reduced biomass but only in the green waste compost.
	3	No, AMF did not significantly affect biomass in either wood-based reduced peat media.
	4	No, colonisation increased biomass consistently in both wood-based reduced peat media.
Were benefits of AMF colonisation other than increased biomass recorded?	2,5	No,
	3,4	Yes, reduced size inequality, leaf purpling and an increase in water retention porosity of growing media, but these effects were not consistent across all treatment combinations.
Did both commercial AMF products produce the same effects?	2-6	No, overall AM2 resulted in lower and more inconsistent levels of colonisation compared to AM1 which resulted in a reduction in the number of significant effects of AM2 across growing media treatments. However, more significant results were seen in the bark reduced peat medium with AM2 than with the wood fibre reduced peat medium.
Did the same AMF species colonise roots of different species?	6	Yes, more than one species of AMF was able to colonise the roots of both marigolds and chives
Did the AMF species colonising the root differ between inoculum and growing media?	6	Maybe, data suggests that there was a difference between the AMF species which colonised plants in different growing media and inoculum but too few replicates were obtained to be conclusive.

Table 7.1 Research questions and associated outcomes listed by Chapter.

7.1. Performance of Reduced Peat Growing Media

7.1.1. Initial results and their effect on substrate choice for this research

In the first experiment, it was encouraging to see that the wood fibre compost produced large plants which were not significantly different when compared to the size of peat grown plants and that this was helped by an increase in biomass seen with the addition of AM1.

The negative responses of control plants (i.e. those without added AMF) in some composts were expected as the preliminary 2012 experiment demonstrated growth depression in plants in green waste and coir based multipurpose composts compared to low peat and wood based mixes. The repeat of poor plant growth in non-mycorrhizal controls and evidence of significant reductions in plant growth (green waste composts) and root length colonisation (coir) in inoculated pots in the first experiment of this thesis made it easy to rule out green waste and coir composts as substrates to investigate further. This is despite coir being commonly used as a peat replacement in the industry and showing that it can produce bedding plants of the same quality as peat in similar trials (Alexander, Williams and Nevison, 2013, 2014). The processes producing coir as a waste product in India and Sri Lanka and the subsequent supply chain for coir to the UK horticultural market was assessed in 2012 as part of the remit of The Sustainable Growing Media Task Force (SGMTF) and they identified areas of the supply chain that were significant sustainability issues which need to be addressed, such as water consumption and pollution (Drewe, 2012). Coir and green waste are also popular peat alternatives in the amateur horticulture market. As a locally produced waste product, green waste compost would make the perfect sustainable alternative to peat, however the unreliability and variability of its nutritional, microbial, and physical properties (e.g. pH or water holding capacity) and its performance in these experiments did not recommend it for industrial use.

7.1.2. Sustainability of wood fibre and bark chip as peat amendments

It has been said that in order for peat replacements to be successful they must be shown to add value without extensive costs and to be sourced locally (Alexander *et al.*, 2009). The reasons for choosing wood based peat alternatives to study were clearly demonstrated by plant and AMF performance in Chapter 2, however the sustainability of these alternatives had to be taken into consideration to make sure that any recommendations made to growers would be reasonable and therefore successful.

A working group of the SGMTF was tasked with trying to identify what “sustainable” growing media is and, during this research, they developed a system which enabled the assessment and comparison of the sustainability of different growing media materials. For each category, a material could achieve a score out of 20, with 20 being the most sustainable. Wood fibre and bark chip were assessed as case studies and although they both scored low for energy (5 and 9 respectively) and wood fibre scored low for water (9) because of the requirements for mechanical steam extrusion, they both had very high sustainability scores for continuity of supply (16, 18), renewability (17, renewable within 5 years), habitat biodiversity (18) and social compliance (20) (Alexander and Bragg, 2014).

These positive results for both materials justified the use of these as locally sourced and renewable products for the present research. It can also be used to highlight the sustainability of these resources to professional growers and in turn, they can use such measurements to try and improve the sustainability of their supply chains. The increased cost of wood based substrates could be justified by the added benefits to the plant such as water retention but also reduction in haulage costs due to its light weight as well as a reduction in the amount of wetting agent needed (Alexander and Bragg, 2014).

7.1.3. Influence of this research on peat usage in the ornamental and herb growing industries

Despite large efforts being made in the retail sector with positive increases in the production and sale of peat free and peat reduced composts to gardeners, the uptake by professional growers is still slow. Whilst many undertake research with sustainable materials there is no indication that any move to actually using these is on the horizon because the integration into current systems would require a lot of changes and the risk to product quality is too great. The data collected in this thesis could provide professional growers with the confidence to focus research and trials on wood based substrates.

From the performance of the peat reduced substrates with herbs in the controlled environments in Chapter 5 it is easy to see how integrating these growing media into set-ups (like the Vitacress production greenhouses) which have been specifically optimised for peat, is a difficult process. Even altering the physical properties of peat with a 30% reduction was enough to cause significant changes in growth of basil and chives. The results here do indicate that, with a change in the watering regime the bark-amended peat substrate could be used in a such a set up successfully as growth changes were positive.

For nursery stock and other ornamentals produced for commercial sale, the comparable size of plants grown in the peat free wood fibre multipurpose and the industrial bark and wood fibre amended peat across three different seasons of growth experiments should instil confidence in these materials as reliable alternatives. The consistent positive effect of the bark media on increasing flower number, when compared to peat, should also be an incentive to use the peat amended growing media as this was achieved without the use of increased fertilisers. The effect of having a composted material as a peat amendment which likely contained a microbial community was often attributed to the many positive direct effects on plant growth and effects via AMF colonisation seen in bark-grown plants.

These effects were achieved with all pots receiving the same watering regime and method, despite the physical differences between wood fibre and bark themselves and the amended mixes difference to 100% peat, including water retention porosity. It has already been demonstrated that specific watering regimes must be optimised for peat reduced and peat free substrates in outdoor nursery conditions and that the amount of water required for peat reduced and peat free substrates is significantly lower than that required to maintain peat at the optimum level (Else, 2013). In future, with physical data from this thesis and other water use experiments such as those carried out by Alexander (2013, 2014) and Else (2013) as part of the ongoing aim of the SGMTF, it is hoped that growers will be more informed and therefore able to tailor watering to improve the performance of these media which could also allow for an increase in the wood material component of the mixes.

7.2. Effects of AMF on Plant Performance in Reduced Peat Growing Media

7.2.1. Effect of AMF Colonisation on Plant Growth

The effect of mycorrhizal colonisation on biomass was not consistent, increasing colonisation did not ever correlate with increasing plant biomass and significant increases in biomass followed inconsistent patterns. This was not surprising as growth depression and inconsistent responses of plants to AMF colonisation have been recorded, due to the functional diversity of AMF species and the changeable nature of the symbiosis depending on nutrient availability, plant/fungal species combination and substrate (Smith, Grace and Smith, 2009; Hoeksema *et al.*, 2010; Mensah *et al.*, 2015; Gosling, Jones and Bending, 2016). Significant growth reductions were rarely seen and never in both reduced peat media treated with the same commercial inoculum product. Reduced biomass averages, or lack of increase in biomass in live inoculated

plants were often the result of an underlying effect of colonisation on size consistency (which will be discussed later in this chapter).

All experiments used peat as a benchmark in order to determine if the AMF treatment could improve plant performance in the reduced peat media to match that currently achieved by peat. However, plants treated with sterilised inoculum grown in the reduced peat substrates across the experiments in Chapters 3-5 were not found to have significantly reduced biomasses compared to peat. Therefore, in these cases, it was not entirely necessary for the AMF to significantly increase the biomass of inoculated plants so that it matched those grown in peat, but significant growth reductions as a result of colonisation would not be acceptable.

Significant increases in root colonisation of marigolds did result in significant increases in biomass in the reduced peat media. However, this was only seen in the final bedding plant experiment outlined in Chapter 4 and only consistently with AM1 inoculated plants. The colonisation levels of AM2, AM3 and Field Soil inocula were not high or consistent enough across plant replicates to produce a reliable pattern of effect on biomass. However, other consistent effects of colonisation on plant health, size as well as physical properties of growing media were seen in the bedding plant experiments especially when focusing on specific inoculum and growing media combinations.

7.2.2. Other Benefits of AMF Colonisation

For both seasons of bedding plant experiments differences between live and sterile inoculated plants were identified, quantified and in most cases the additional benefits could be attributed to root length colonisation by AMF. The effect of AMF colonisation on plant size consistency, leaf discolouration as a result of stress and water retention capacity of the growing media are all novel findings which demonstrate support for the use of AMF as an essential part of a sustainable horticultural industry.

7.2.2.1. Size Consistency

The effect of AMF colonisation on size inequality has only been studied in the context of AMF community research. It has focused on the effect of the underground network and the AMF community on size inequality and plant competition. Very few experiments detail the effects of AMF colonisation on size inequality in potted trials and they still include multiple species per pot. In the experiment outlined in Chapters 3 inoculation with live AM1 was shown to

significantly reduce size inequality in a group of individual potted marigolds in both reduced peat media and the same effect was seen in bark with live AM2 treatment. Reductions in variability were also seen in peat plants grown with live AM1 and wood fibre with live AM2 but these were not found to be significant. Interestingly, the AMF appeared to have different mechanisms for reducing variability in the different peat alternatives. Lorenz curves and asymmetry coefficients provided data which showed that in bark plants treated with live AM1 there was a reduction in the number of large plants and therefore an overall decrease in the average plant size but in wood fibre the number of small plants was reduced resulting in an overall increase in the average plant size. This difference in effect was thought to stem from an equilibrium between AMF colonisation facilitating plants to grow larger through increased nutrition, thus reducing the number of small plants; balanced with the presence of AMF in the pot using up nutrients themselves, combined with plants having to provide their symbiont with carbon which reduced the number of very large plants.

Inconsistencies across inoculum treatments were seen when colonisation across replicate pots was inconsistent, where some pots in the replicate group showed little to no colonisation the data was skewed. Equally when contamination resulted in control and live inoculated plants both having consistent colonisation no reduction in size inequality was seen (as in peat AM1, Figures 3.16 and 4.16). In peat, however the coefficient of variation of plants was not as low as that of colonised plants in the peat alternatives, with plants able to access more nutrients in peat this is not surprising.

In Chapter 4 similar effects on size inequality were seen with significant reductions in bark pots treated with live AM1 and AM3. The effects were not significant or as consistent as those in Chapter 3 but this could have been due to these plants receiving extra nutrients during the experiment so they were not limiting by the end, this correlates with a reduction in leaf discolouration as a stress response to low nutrients. In Chapter 3 purple scored plants had the majority of their leaves purple whereas in Chapter 4 a scale was used because very few plants had more than 25% of their leaves purple.

Plant size consistency is very important in the horticultural industry and is one of the main reasons that peat has been favoured by professional growers for decades, its properties allow for hundreds of pots to be grown at the same time which will all produce consistently sized plants within specific growth parameters (often set by distributors such as supermarkets). The best example of this is the Vitacress production glasshouse (Figure 7.1). If potted herbs do not meet the height requirement outlined by the supermarkets then it will be wasted and the

company will be charged, it is understandable therefore that risking product wastage and the incurring fine on a more sustainable growing media which produces inconsistent sizes is not an option. Transportation of plants may also require them to be of a certain height. Consistency is also important when growing ornamentals for commercial product or as a hobby. For display purposes or for sale plants should be of consistent size or it could affect the perceived value or quality of the batch of plants. If consistent, high enough levels of colonisation can be achieved in every pot then the addition of AMF could facilitate the use of reduced peat growing media by reducing size inequality of individuals.

Figure 7.1 Photograph showing uniformity of potted basil plants growing on flood benching at the Vitacress production glasshouse, Angmering, UK.

7.2.2.2. Plant Stress Response font and line

The ability of AMF to ameliorate or buffer the effect of abiotic stresses on plants is well documented, particularly for drought (Nelsen and Safir, 1982; Allen and Boosalis, 1983; Ruiz Lozano, Azcón and Gomez, 1995; Bryla and John M. Duniway, 1997; Asrar and Elhindi, 2011; Gromberg *et al.*, 2015; Ruiz-Lozano *et al.*, 2016); and salinity stress (Giri, Kapoor and Mukerji, 2003; Porras-Soriano *et al.*, 2009; Evelin and Kapoor, 2014) tolerance. Although increased nutrient uptake and nutrient content of AMF colonised plants is also not a new concept, the visible effects of these benefits on a plant's aesthetic quality is very important when dealing with ornamental plants for sale. Whilst leaf purpling of marigolds cannot be exclusively attributed to low phosphorus or nitrogen levels, in these experiments because plant material was not

subjected to nutrient analysis, the nature of the AMF symbiosis and the reduction of this effect in Chapter 4 experiments when extra nutrition was provided gave enough evidence to support this theory. Purpling of leaves has also been attributed to drought stress in plants (Hughes and Lev-Yadun, 2015) but this cause can be ruled out as plants were well watered with both rainfall and supplementary overhead sprinklers. The reduction of leaf purpling again appeared to rely on consistent, high levels of root colonisation as it was only seen in wood fibre and bark in AM1 treated plants in Chapter 3 although there was also a large reduction in bark plants treated with AM2 which did show the highest number of colonised individuals of any AM2 pots. In Chapter 4, only wood fibre plants treated with live AM1 and AM2 showed significant decreases in the number of purple leaves per plant, however there was a considerable lack of purpling in plants grown in bark with live AM2 again. Given the later molecular work which revealed the presence of *Funneliformis mosseae* only in the roots of bark-grown plants inoculated with live AM2 and its positive association with *Trichoderma* species likely to be present in the composted wood the consistent results in bark AM2 pots are unsurprising. All plants grown with AM3 showed reduced amounts of leaf purpling which also suggested the possible additive influence of the presence of plant growth promoting bacteria (PGPR) on increased nutrient acquisition by plants.

The ability of AMF in this study to prevent plant stress responses by increasing nutrient uptake demonstrates the important biofertiliser role AMF could play in a sustainable horticulture system. Using reduced peat and alternative peat substrates will run the risk of decreasing the amount of nutrients plants can access due to the properties of each mix. Increasing plant nutrition with the addition of composted materials to peat has been shown to be successful and in some cases the combination of composted amendment and AMF inocula can produce plants which are comparable to highly fertilised substrate (Ustuner *et al.*, 2009). Again, this result was achieved with a specific AMF and growing media combination much like AM2 and bark. For effects such as nutrient stress reduction, which involve a delicately balanced symbiosis, careful selection of AMF species, substrate and microbial input will be required but the result could mean a significant reduction in the use of chemical fertilisers. A cost:benefit analysis comparing benefit from AMF and other microbes with any increased costs compared to fertiliser would have to be undertaken by growers before deciding to adopt this method however.

7.2.2.3. Water Retention Porosity

The influence of AMF hyphal networks on soil structure, stability, and water relations covers a large area of mycorrhizal research which includes the positive effects AMF could have in the

agricultural sector (Rillig, Wright and Eviner, 1998; Rillig and Mummey, 2006; Querejeta, 2017). Similar results of AMF hyphae and their protein exudates increasing the number and size of soil aggregates in pots leading to changes in porosity, has been demonstrated with different types of soil (Wu, Xia and Zou, 2008; Samaei, Asghari and Aliasgharzad, 2015) but the patterns in Chapter 3 which were then confirmed with the results in wood fibre pots treated with AM1 in Chapter 4 are the first example (to the authors knowledge) of this effect on soilless growing media.

When analysing and comparing different substrates for horticultural use, bulk-density, air-filled porosity, and water holding capacity are all evaluated to create an optimum for plant growth. The particle size of soilless substrates means they often have a lot more of an open structure compared to soil and therefore pore space is already higher than that of soils. Wood fibre and bark were both known for their high air filled capacity but wood fibre has lower water retention porosity and is prone to slumping and drying out quickly (Schmilewski, 2008; Alexander, Williams and Nevison, 2013). Bark is added to increase air filled capacity but also water holding capacity of peat (Bilderback and Lorscheider, 1995; Barrett *et al.*, 2016). As demonstrated in the final experiment in Chapter 5 where plants in the bark pots had higher water content and there was increased pore water availability than in peat and wood fibre pots.

It has been demonstrated that peat free substrates containing wood fibre need to be well watered in order to maintain plant quality comparable to peat (Alexander, Williams and Nevison, 2013). The significant relationship between increasing root length colonisation and water retention porosity in wood fibre pots treated with AM1 is a significant result because it indicates that colonisation with AMF can not only improve plant performance in reduced peat media directly through the symbiosis but indirectly by improving the growing media itself. This also could mean that increasing the amount of wood fibre amendment and reducing the amount of peat could be done without compromising the physical status of the growing media. Interestingly the opposite effect was seen in bark with a significant reduction in water retention porosity in live AM2 pots.

7.3. Reliability and Efficacy of Commercial AMF Inocula

The performance of different commercial inocula has been tested in various environments in the literature and the results have been varied, as seen in these experiments. However the amount of studies using commercial inocula have been low, in a publication search from 2001 to 2015 only 15% of studies stated that the inoculum origin was a commercial product (Berruti

et al., 2016). The effect of different propagule types, production methods, carrier materials, transport methods, storage methods, shelf-life and species mixes have all been discussed and evaluated and the general consensus is that different products will produce different results depending on the plant species and substrate used (Linderman & Davis 2003; Perner et al. 2007; Puschel et al. 2014).

Corkidi et al. (2004) showed that some commercial AMF inoculum products demonstrated complete failure to colonise, but plants inoculated with these showed positive growth effects compared to controls which suggested the presence of nutrient additives in the carrier material or other organisms (PGPR, *Trichoderma* etc.) present in the inoculum mix may be responsible.

Berruti et al. (2016) showed that across AMF studies single and native or indigenous inocula were found to significantly outperform mixed species and commercial products when it came to effects on shoot biomass. Although, as demonstrated here, increasing biomass is not always the best outcome of an AMF symbiosis. These studies were also largely based around improving plant resistance to some form of stress where increased biomass would have been a positive result when the stress condition or pathogen was absent. Corkidi et al. (2004) also demonstrated that some commercial products (three out of twelve) increased colonisation compared to an indigenous soil inoculum as was also seen with the chives in Chapter 5 of this thesis.

The production method and propagule type of the two inocula in this thesis appear to be the main reasons for the difference in performance. AM1 is produced using bait plants in an inert carrier in the UK, there are multiple fungal species but no more than five and they are not grown in competition with each other. It also contains all kinds of propagules including root fragments which may contain vesicles which have been shown to be a primary source of regrowth for certain AMF species (Biermann and Linderman, 1983b). The method of production for AM2 is completely different and based on large scale, *in vitro*, monoaxenic-based production similar to that outlined by Adholeya et al. (2005). The lack of visible root fragment propagules such as those found in AM1 as well as the larger number of species in the mix (which caters to a global market) maybe the reason for the poor colonisation performance compared to AM1. The formulation of the carrier may also be a factor in this, the large open granular structure of AM1 may allow for an increase in different types of propagules which can grow through the porous granules whereas the ground, very fine dust-like substance of AM2 is prone to clumping when moist and does not appear to include larger fragments which may have been ground down. AM2 is also produced in the USA and is transported for distribution to the UK and this could affect its viability.

The inconsistent performance of AM2 between batches (years e.g. 2015-2016) in these experiments highlight the risks taken when using commercial inocula. In order to confirm colonisation and relate effects to the AMF growers would have to conduct some quality control or send root samples away to be checked to ensure products work in their system which may be too time consuming to carry out in practice.

Improvements to the molecular methods outlined in Chapter 6 of this thesis would allow more effective quality control and investigation of how AMF inocula were functioning in different plant species and substrates. This could include reducing the number of fungal species in the inoculum to cut costs associated with using unnecessary species in the inoculum mix. Chapter 6 indicated that less species were found in roots than were present in the inoculum and it has been suggested that increasing the species density of AMF to any more than three will not increase the amount of benefit the plant receives (Gosling, Jones and Bending, 2016). But producing specialised AMF products for different crops will be expensive thus the Symbio approach where they hope to have a 'one size fits all' type product.

7.4. Final Conclusions and Impact of this Study

This work has provided evidence of three positive, (including two novel) effects of AMF colonisation in plants grown in peat reduced growing media. Improved size consistency and decreased nutrient stress in plants along with improvements to the water holding capacity of a growing medium are all effects which directly support the use of AMF to improve plant performance in peat reduced, and hopefully, peat free growing media.

As direct results of adding AMF inoculum these improvements can be used as positive indicators to growers, particularly of plants in nursery conditions, that AMF can reduce the negative effects of decreasing peat content in substrates. By reducing size inequality and increasing plant access to nutrients and water (in notoriously poor water holding media) AMF allow plants to grow just as successfully in reduced peat substrates as in full peat with added benefits which may prevent a change in commercial set ups for fertiliser and water provision. The use of AMF may even allow for a reduction in both water and fertiliser use which could reduce production costs, along with the benefits of using more sustainable materials to comply with government guidelines. It is hoped that future work will show that AMF will provide these benefits even in media with increased reductions in peat and even peat free mixes so that the transition to a peat free horticultural industry in the UK will be possible without sacrificing yield and quality of produce.

7.5. Future Work

7.5.1. Future Experiments

The main aims of future work should be trying to see if the positive benefits of AMF colonisation on plant performance in reduced peat growing media can be replicated in different plant species and in growing media with a further reduced peat content.

Plant stress responses and overall plant aesthetic quality could be visually assessed and scored to take into account industry and consumer standards on plant performance. This could be combined with testing the effect of AMF on water retention porosity in wood fibre in a challenging environment such as a hanging basket. This could be combined with a more detailed analysis on the change in growing media looking at aggregates or presence of AMF hyphal proteins such as glomalin reactive soil protein.

Another main aim of future work should be attempting to achieve successful colonisation of basil or other potted herb species in an industrial environment because this industry is a significant contributor to the importation and use of peat in this country. This could be achieved by perhaps using other commercial inoculum mixes, natural inocula or different basil varieties. An experiment where replicates could be constantly checked for colonisation throughout the growth cycle may allow for colonisation to be monitored in case it fluctuates during plant growth. This could be achieved by taking cores or small samples of growing media from pots and separating the roots so that colonisation can be confirmed through staining and molecular techniques; while the growing media material could be subjected to protein analysis to look for GRSP (glomalin related soil protein) which would have been produced by AMF hyphae. This work will be essential if AMF is to be given the opportunity to produce similar results seen in bedding plants and help facilitate the reduction in the use of peat in the potted herb industry.

The development and improvement of more robust sterilisation techniques to avoid contamination and colonisation in control plants as well as the use of microbial filtrate to rule out the positive effects of PGPRs should be included in future experiments. For outdoor nursery-style experiments, non-inoculated controls which contain low levels of “background colonisation” are acceptable as addition of any inoculum must be shown to significantly increase AMF colonisation and plant performance compared to any normal colonisation seen as a result of external contamination (as seen in Chapter 4). For controlled indoor environments, such as glasshouses, control plants should be free from AMF as contamination from external sources is highly unlikely, the effect of introducing AMF and other microbes to plants in this environment must be investigated.

7.5.2. Impact of Future Work on the Horticultural Industry

This study has highlighted the benefits of combining physiological and molecular data when it comes to analysing the effects of AMF. The specific AMF species and number colonising plants can give a greater insight into the causes of the variable plant growth responses to AMF across different treatments which aren't just related to different amounts of root colonisation. With more work producing data on the different effects of specific AMF species on plant growth and performance through their nutrient providing abilities, it is important to know the species of AMF colonising plants so that their benefit or cost to the plant can be explained. With that in mind, it has been interesting to use DNA to identify species and unravel how the species in different mixed commercial inocula interact with different plants and growing media.

Now that a method has been established which produces sufficient DNA from root material further modification to the method using more specific primers and sequence cloning could be used to make more robust conclusions about the interactions of commercial inocula with different species and growing media. This should allow for DNA from all AMF species present in root material to be sequenced and not just that of a dominant species.

If it is confirmed that most AMF species in commercial mixes play no part in the colonisation of different plant species, then it would support the idea of using custom commercial mixes where the species number is reduced. Despite one species appearing to be dominant in certain plant roots there were still different species colonising the roots of plants in the same treatments so inocula with combinations of three, two and one AMF species should be tested to see if the presence of other species has an interactive or additive effect.

Going forward this non-destructive method could be used to investigate the patterns seen here to decipher if such diverse mixtures are necessary and if the "one size fits all" approach of commercial AMF products is needed when using them in industrial set ups. Eventually a system could be in place which could directly recommend an inoculum mix and sustainable growing media combination to growers for their desired plant species which will maximise the plant benefits. If the number of species is reduced in these specialised inocula then the price will also be lower for growers.

For home gardeners, the number of species could still be reduced but not as much as they will still want to be able to use this on a range of plant species. This data could still have an effect on the retail market for the hobby AMF products though, as if it is confirmed that growing media does have an effect on AMF species and their colonisation effects then more detailed usage

recommendations should be provided when these products are sold, perhaps each inoculum could be sold alongside a suitable sustainable growing media or compost product.

References

Aboul-Nasr, A. (1996) 'Effects of vesicular-arbuscular mycorrhiza on Tagetes erecta and Zinnia elegans', *Mycorrhiza*, 6(1), pp. 61–64. doi: 10.1007/s005720050107.

Adholeya, A., Tiwari, P. and Singh, R. (2005) 'Large-Scale Inoculum Production of Arbuscular Mycorrhizal Fungi on Root Organs and Inoculation Strategies', *In Vitro Culture of Mycorrhizas*, 4, pp. 315–338. doi: 10.1007/3-540-27331-X_17.

Afek, U., Rinaldelli, E., Menge, J. A., Johnson, E. L. V and Pond, E. (1990) 'Mycorrhizal Species, Root Age, and Position of Mycorrhizal Inoculum Influence Colonization of Cotton, Onion, and Pepper Seedlings', 115(6), pp. 938–942.

Al-Karaki, G. N. (2000) 'Growth of mycorrhizal tomato and mineral acquisition under salt stress', *Mycorrhiza*, 10(2), pp. 51–54. doi: 10.1007/s005720000055.

Alexander, P. and Bragg, N. (2014) 'Defining sustainable growing media for sustainable UK horticulture', *Acta Horticulturae*, 1034, pp. 219–226. doi: 10.17660/ActaHortic.2014.1034.26.

Alexander, P., Bragg, N., Meade, R., Padelopoulos, G. and Watts, O. (2008) 'Peat in horticulture and conservation: the UK response to a changing world.', *Mires & Peat*, 3, pp. 1–11.

Alexander, P., Bragg, N., Meade, R., Padelopoulos, G. and Watts, O. (2009) 'What future for peat in horticulture?', *The Plantsman*, pp. 23–27.

Alexander, P. and Williams, R. (2012) 'Consumer attitudes to peat-free media', *The Plantsman*, pp. 44–47.

Alexander, P., Williams, R. H. and Nevison, I. M. (2013) 'Improving gardeners' understanding of water management in peat and peat-free multi-purpose growing media: An assessment with fuchsia', *Acta Horticulturae*, 1013, pp. 257–264.

Alexander, P., Williams, R. and Nevison, I. (2014) 'An experimental comparison of growing media, petunia quality and amount of water applied - an opportunity for water saving?', *Acta Horticulturae*, pp. 211–218.

Alguacil, M. M., Torres, M. P., Torrecillas, E., Díaz, G. and Roldán, A. (2011) 'Plant type differently promote the arbuscular mycorrhizal fungi biodiversity in the rhizosphere after revegetation of a degraded, semiarid land', *Soil Biology and Biochemistry*, 43(1), pp. 167–173. doi: 10.1016/j.soilbio.2010.09.029.

Allen, M. F. and Boosalis, M. G. (1983) 'Effects of two species of a mycorrhizal fungi on drought

tolerance of winter wheat', *New Phytologist*, 93(1), pp. 67–76. doi: 10.1111/j.1469-8137.1983.tb02693.x.

Appoloni, S., Lekberg, Y., Tercek, M. T., Zabinski, C. and Redecker, D. (2008) 'Molecular community analysis of arbuscular mycorrhizal fungi in roots of geothermal soils in Yellowstone National Park (USA)', *Microbial Ecology*, 56(4), pp. 649–659. doi: 10.1007/s00248-008-9384-9.

Arbona, V., Hossain, Z., López-Climent, M. F., Pérez-Clemente, R. M. and Gómez-Cadenas, A. (2008) 'Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus', *Physiologia Plantarum*, 132(4), pp. 452–466. doi: 10.1111/j.1399-3054.2007.01029.x.

Asrar, A. W. A. and Elhindi, K. M. (2011) 'Alleviation of drought stress of marigold (Tagetes erecta) plants by using arbuscular mycorrhizal fungi', *Saudi Journal of Biological Sciences*. King Saud University, 18(1), pp. 93–98. doi: 10.1016/j.sjbs.2010.06.007.

Augé, R. M. (2001a) 'Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis', *Mycorrhiza*, pp. 3–42. doi: 10.1007/s005720100097.

Augé, R. M. (2001b) 'Water relations , drought and vesicular-arbuscular mycorrhizal symbiosis', *Mycorrhiza*, 11, pp. 3–42.

Augé, R. M. (2004) 'Arbuscular mycorrhizae and soil / plant water relations', *Canadian Journal of Soil Science*, 84(4), pp. 373–381. doi: 10.4141/S04-002.

Ayres, R. L., Gange, A. C. and Aplin, D. M. (2006) 'Interactions between arbuscular mycorrhizal fungi and intraspecific competition affect size, and size inequality, of Plantago lanceolata L.', *Journal of Ecology*, 94(2), pp. 285–294. doi: 10.1111/j.1365-2745.2006.01103.x.

Baath, E. and Spokes, J. (1989) 'The effect of added nitrogen and phosphorus on mycorrhizal growth response and infection in Allium schoenoprasum', *Canadian Journal of Botany*, 67(11), pp. 3227–3232.

Bain, C. G., Bonn, A., Stoneman, R., Chapman, S., Coupar, A., Evans, M., Gearey, B., Howat, M., Joosten, H., Keenleyside, C., Labadz, J., Lindsay, R., Littlewood, N., Lunt, P., Miller, C. J., Moxey, A., Orr, H., Reed, M., Smith, P., Swales, V., Thompson, D. B. A., Thompson, P. S., Van de Noort, R., Wilson, J. D. and Worrall, F. (2011) *IUCN UK Commission of Inquiry on Peatlands*. Edinburgh.

Bainard, L. D., Klironomos, J. N. and Hart, M. M. (2010) 'Differential effect of sample preservation methods on plant and arbuscular mycorrhizal fungal DNA', *Journal of Microbiological Methods*, 82(2), pp. 124–130. doi: 10.1016/j.mimet.2010.05.001.

Balzergue, C., Chabaud, M., Barker, D. G., Bécard, G. and Rochange, S. F. (2013) 'High phosphate reduces host ability to develop arbuscular mycorrhizal symbiosis without affecting root calcium spiking responses to the fungus.', *Frontiers in plant science*, 4(October), p. 426. doi: 10.3389/fpls.2013.00426.

Barrett, G. E., Alexander, P., Robinson, J. S. and Bragg, N. (2016) 'Achieving environmentally sustainable growing media for soilless plant cultivation systems – A review', *Scientia Horticulturae*. Elsevier B.V., 212, pp. 220–234. doi: 10.1016/j.scienta.2016.09.030.

Baum, C., El-Tohamy, W. and Gruda, N. (2015) 'Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review', *Scientia Horticulturae*, pp. 131–141. doi: 10.1016/j.scienta.2015.03.002.

Bedini, S., Pellegrino, E., Avio, L., Pellegrini, S., Bazzoffi, P., Argese, E. and Giovannetti, M. (2009) 'Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species Glomus mosseae and Glomus intraradices', *Soil Biology and Biochemistry*. Elsevier Ltd, 41(7), pp. 1491–1496. doi: 10.1016/j.soilbio.2009.04.005.

Benito, M., Masaguer, A., De Antonio, R. and Moliner, A. (2005) 'Use of pruning waste compost as a component in soilless growing media', *Bioresource Technology*, 96(5), pp. 597–603. doi: 10.1016/j.biortech.2004.06.006.

Berruti, A., Borriello, R., Della Beffa, M. T., Scariot, V. and Bianciotto, V. (2013) 'Application of nonspecific commercial AMF inocula results in poor mycorrhization in Camellia japonica L.', *Symbiosis*. Springer Netherlands, 61(2), pp. 63–76. doi: 10.1007/s13199-013-0258-7.

Berruti, A., Lumini, E., Balestrini, R. and Bianciotto, V. (2016) 'Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes', *Frontiers in Microbiology*. doi: 10.3389/fmicb.2015.01559.

Bever, J. D. (2002a) 'Host-specificity of AM fungal population growth rates can generate feedback on plant growth', *Plant and Soil*, 244(1–2), pp. 281–290. doi: 10.1023/A:1020221609080.

Bever, J. D. (2002b) 'Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit', in *Proceedings of the Royal Societey London*, pp. 2595–2601. doi: 10.1098/rspb.2002.2162.

Bi, G., Evans, W. B., Spiers, J. M. and Witcher, A. L. (2010) 'Effects of Organic and Inorganic Fertilizers on Marigold Growth and Flowering', *HortScience*, 45(9), pp. 1373–1377.

Biermann, B. and Linderman, R. G. (1983a) 'Effect of Container Plant Growth Medium and Fertiliser Phosphorus on Establishment and Host Growth Response to Vesicular-Arbuscular Mycorrhizae', *Journal of American Horticultural Science*, 108(6), pp. 962–971.

Biermann, B. and Linderman, R. G. (1983b) 'Use of Vesicular-Arbuscular Mycorrhizal Roots, Intraradical Vesicles and Extraradical Vesicles as Inoculum', *New Phytologist*. Blackwell Publishing Ltd, 95(1), pp. 97–105. doi: 10.1111/j.1469-8137.1983.tb03472.x.

Bilderback, T. (2009) 'A Nursery Friendly Method for Measuring Air Filled Porosity of Container Substrates', *SNA Research Conference*, 54, pp. 28–34.

Bilderback, T. and Lorscheider, M. R. (1995) 'Physical Properties of Double-Processed Pine Bark: Effects on Rooting', *Acta Horticulturae*, 401, pp. 77–84.

Błaszczyk, L., Strakowska, J., Chełkowski, J., Gąbka-Buszek, A. and Kaczmarek, J. (2016) 'Trichoderma species occurring on wood with decay symptoms in mountain forests in Central Europe: genetic and enzymatic characterization', *Journal of Applied Genetics*, 57(3), pp. 397–407. doi: 10.1007/s13353-015-0326-1.

Bona, E., Lingua, G., Manassero, P., Cantamessa, S., Marsano, F., Todeschini, V., Copetta, A., D'Agostino, G., Massa, N., Avidano, L., Gamalero, E. and Berta, G. (2015) 'AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels', *Mycorrhiza*, 25(3), pp. 181–193. doi: 10.1007/s00572-014-0599-y.

Breuillin, F., Schramm, J., Hajirezaei, M., Ahkami, A., Favre, P., Druege, U., Hause, B., Bucher, M., Kretzschmar, T., Bossolini, E., Kuhlemeier, C., Martinoia, E., Franken, P., Scholz, U. and Reinhardt, D. (2010) 'Phosphate systemically inhibits development of arbuscular mycorrhiza in Petunia hybrida and represses genes involved in mycorrhizal functioning', *Plant Journal*. Blackwell Publishing Ltd, 64(6), pp. 1002–1017. doi: 10.1111/j.1365-313X.2010.04385.x.

Bryla, D. R. and Duniway, J. M. (1997) 'Effects of mycorrhizal infection on drought tolerance and recovery in safflower and wheat', *Plant and Soil*, 197(1), pp. 95–103. doi: 10.1023/A:1004286704906.

Bryla, D. R. and Duniway, J. M. (1997) 'Growth, Phosphorus Uptake, and Water Relations of Safflower and Wheat Infected with an Arbuscular Mycorrhizal Fungus', *Source: The New Phytologist New Phytol*, 136(136), pp. 581–590.

Bucher, M. (2007) 'Functional biology of plant phosphate uptake at root and mycorrhizal

interfaces', *New phytol*, 173, pp. 11–26.

Burleigh, S. H., Cavagnaro, T. and Jakobsen, I. (2002) 'Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition.', *Journal of experimental botany*, 53(374), pp. 1593–1601. doi: 10.1093/jxb/erf013.

Çakmakçi, R., Dönmez, F., Aydin, A. and Şahin, F. (2006) 'Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions', *Soil Biology and Biochemistry*, 38(6), pp. 1482–1487. doi: 10.1016/j.soilbio.2005.09.019.

Calvet, C., Barea, J.-M. and Pera, J. (1992) 'In vitro interactions between the vesicular-arbuscular mycorrhizal fungus glomus mosseae and some saprophytic fungi isolated from organic substrates', *Soil Biology and Biochemistry*, 24(8), pp. 775–780. doi: 10.1016/0038-0717(92)90252-S.

Calvet, C., Pera, J. and Barea, J.-M. (1990) 'Interactions of trichoderma spp. with Glomus mosseae and two wilt pathogenic fungi', *Agriculture, Ecosystems and Environment*, 29(1–4), pp. 59–65. doi: 10.1016/0167-8809(90)90255-C.

Calvet, C., Pera, J. and Barea, J.-M. (1993) 'Growth response of marigold (Tagetes erecta L.) to inoculation with Glomus mosseae, Trichoderma aureoviride and Pythium ultimum in a peat-perlite mixture', *Plant and Soil*, 148(1), pp. 1–6. doi: 10.1007/BF02185378.

Candido, V., Campanelli, G., D'Addabbo, T., Castronuovo, D., Perniola, M. and Camele, I. (2015) 'Growth and yield promoting effect of artificial mycorrhization on field tomato at different irrigation regimes', *Scientia Horticulturae*, 187, pp. 35–43. doi: 10.1016/j.scienta.2015.02.033.

Carey, P. D., Fitter, A. H. and Watkinson, A. R. (1992) 'A field study using the fungicide benomyl to investigate the effect of mycorrhizal fungi on plant fitness', *Oecologia*. Springer-Verlag, 90(4), pp. 550–555. doi: 10.1007/BF01875449.

Carminati, A., Zarebanadkouki, M., Kroener, E., Ahmed, M. A. and Holz, M. (2016) 'Biophysical rhizosphere processes affecting root water uptake', *Annals of Botany*, (1990), p. mcw113. doi: 10.1093/aob/mcw113.

Carpio, L. A., Davies, F. T. and Arnold, M. A. (2003) 'Effect of commercial arbuscular mycorrhizal fungi on growth, survivability, and subsequent landscape performance of selected container grown nursery crops.', *Journal of Environmental Horticulture*, 21(4), pp. 190–195.

Ceballos, I., Ruiz, M., Fernández, C., Peña, R., Rodríguez, A. and Sanders, I. R. (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(8). doi: 10.1371/journal.pone.0070633.

Chiariello, N., Hickman, J. C. and Mooeny, H. A. (1982) 'Endomycorrhizal Role for Interspecific Transfer of Phosphorus in a Community of Annual Plants', *Science*, 217(September), pp. 941–943.

Clapperton, M. J. and Reid, D. M. (1992) 'A Relationship between Plant Growth and Increasing VA Mycorrhizal Inoculum Density', *New Phytologist*. Elsevier B.V., 10(3), pp. 39–50. doi: 10.1111/j.1469-8137.1977.tb04835.x.

Clapperton, M. J. and Reid, D. M. (1992) 'A Relationship between Plant Growth and Increasing VA Mycorrhizal Inoculum Density', *New Phytologist*, 120(2), pp. 227–234. doi: 10.1111/j.1469-8137.1992.tb05658.x.

Colla, G., Rouphael, Y., Cardarelli, M., Tullio, M., Rivera, C. M. and Rea, E. (2008) 'Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration', *Biology and Fertility of Soils*, 44(3), pp. 501–509. doi: 10.1007/s00374-007-0232-8.

Copetta, A., Lingua, G. and Berta, G. (2006) 'Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in Ocimum basilicum L. var. Genovese', *Mycorrhiza*, 16(7), pp. 485–494. doi: 10.1007/s00572-006-0065-6.

Corkidi, L., Merhaut, D., Allen, M. F., Downer, J., Bohn, J., Evans, M., Allen, E., MF, A., J, D., J, B., M, E. and B, A. E. (2004) 'Assessing the infectivity of commercial mycorrhizal inoculants in plant nursery conditions.', *Journal of Environmental Horticulture*, 22(September), pp. 149–154.

Damgaard, C. and Weiner, J. (2000) 'Describing size inequality in plant size or fecundity', *Ecology*, 81(April), pp. 1139–1142. doi: 10.1890/0012-9658(2000)081[1139:DIIPSO]2.0.CO;2.

Datnoff, L. E., Nemec, S. and Pernezny, K. (1995) 'Biological control of Fusarium crown and root rot of tomato in Florida using Trichoderma harzianum and Glomus intraradices', *Biological Control*, 5(3), pp. 427–431. doi: 10.1006/bcon.1995.1051.

Davies, F. T., Potter, J. R. and Linderman, R. G. (1992) 'Mycorrhiza and Repeated Drought Exposure Affect Drought Resistance and Extraradical Hyphae Development of Pepper Plants Independent of Plant Size and Nutrient Content', *Journal of Plant Physiology*. Urban & Fischer, 139(3), pp. 289–294. doi: 10.1016/S0176-1617(11)80339-1.

Day, J. (2011) *Problem with Purple Leaves Caused by Phosphorus Deficiency*, *Today's*

Homeowner. Available at: https://www.todayshomeowner.com/problem-with-purple-leaves-caused-by-phosphorus-deficiency/ (Accessed: 1 August 2017).

Dimkpa, C., Weinand, T. and Asch, F. (2009) 'Plant-rhizobacteria interactions alleviate abiotic stress conditions', *Plant, Cell and Environment*, 32(12), pp. 1682–1694. doi: 10.1111/j.1365-3040.2009.02028.x.

Dixon, P. M., Weiner, J., Mitchell-olds, T., Woodley, R., Dixon, P. M., Weiner, J., Mitchell-olds, T. and Woodley, R. (2016) 'Bootstrapping the Gini Coefficient of Inequality', 68(5), pp. 1548–1551.

Douds, D., Nagahashi, G., Pfeffer, P., Kayser, W. and Reider, C. (2005) 'On-farm production and utilisation of arbuscular mycorrhizal fungus inoculum', *Canadian Journal of Plant Science*, 85(1), pp. 15–21.

Douds, D. and Schenck, N. C. (1990) 'Cryopreservation of Spores of Vesicular Arbuscular Mycorrhizal Fungi', *New Phytologist*, (115), pp. 667–674.

Drewe, L. (2012) *Final Report SP1214 Coir : a sustainability assessment*.

Dubsky, M., Sramek, F. and Vosatka, M. (2002) 'Inoculation of cyclamen (Cyclamen persicum) and poinsettia (Euphorbia pulcherrima) with arbuscular mycorrhizal fungi and Trichoderma harzianum', *Rostlinna Vyroba*, 48(2), pp. 63–68.

Edwards, L. (2012) *Do commercial mycorrhizal inoculants enhance plant growth?*

Else, M. A. (2013) *Developing optimum irrigation guidelines for reduced peat, peat-free and industry standard substrates*, *Agriculture and Horticulture Development Board Final Report*.

Evelin, H. and Kapoor, R. (2014) 'Arbuscular mycorrhizal symbiosis modulates antioxidant response in salt-stressed Trigonella foenum-graecum plants', *Mycorrhiza*, 24(3), pp. 197–208. doi: 10.1007/s00572-013-0529-4.

Faye, A., Dalpé, Y., Ndung'u-Magiroi, K., Jefwa, J., Ndoye, I., Diouf, M. and Lesueur, D. (2013) 'Evaluation of commercial arbuscular mycorrhizal inoculants', *Canadian Journal of Plant Science*. Canadian Science Publishing, 93(6), pp. 1201–1208. doi: 10.4141/cjps2013-326.

Fonteno, W. C. and Harden, C. T. (no date) 'Procedures for Determining Physical Properties of Horticultural Substrates Using the NCSU Porometer', *North*.

Francini, G., Männistö, M., Alaoja, V. and Kytöviita, M.-M. (2014) 'Arbuscular mycorrhizal fungal community divergence within a common host plant in two different soils in a subarctic Aeolian sand area', *Mycorrhiza*. Springer Berlin Heidelberg, 24(7), pp. 539–550. doi: 10.1007/s00572-

014-0573-8.

Gagné, S., Dehbi, L., Le Quéré, D., Cayer, F., Morin, J. L., Lemay, R. and Fournier, N. (1993) 'Increase of greenhouse tomato fruit yields by plant growth-promoting rhizobacteria (PGPR) inoculated into the peat-based growing media', *Soil Biology and Biochemistry*, 25(2), pp. 269–272. doi: 10.1016/0038-0717(93)90038-D.

Gange, A. C. and Ayres, R. (1999) 'On the Relation between Arbuscular Mycorrhizal Colonization and Plant "Benefit"', *Oikos*, 87(3), pp. 615–621. doi: 10.2307/3546829.

García, I. V. and Mendoza, R. E. (2008) 'Relationships among soil properties, plant nutrition and arbuscular mycorrhizal fungi-plant symbioses in a temperate grassland along hydrologic, saline and sodic gradients', *FEMS Microbiology Ecology*, 63(3), pp. 359–371. doi: 10.1111/j.1574-6941.2008.00441.x.

Gaur, A. and Adholeya, A. (2000) 'Effects of the particle size of soil-less substrates upon AM fungus inoculum production', *Mycorrhiza*, 10(1), pp. 43–48. doi: 10.1007/s005720050286.

Gaur, A., Adholeya, A. and Mukerji, K. G. (1998) 'A comparison of AM fungi inoculants using Capsicum and Polianthes in marginal soil amended with organic matter', *Mycorrhiza*. Springer-Verlag, 7(6), pp. 307–312. doi: 10.1007/s005720050197.

Gaur, A., Gaur, A. and Adholeya, A. (2000) 'Growth and flowering in Petunia hybrida, Callistephus chinensis and Impatiens balsamina inoculated with mixed AM inocula or chemical fertilizers in a soil of low P fertility', *Scientia Horticulturae*, 84(1–2), pp. 151–162. doi: 10.1016/S0304-4238(99)00105-3.

Gholamhoseini, M., Ghalavand, A., Dolatabadian, A., Jamshidi, E. and Khodaei-Joghan, A. (2013) 'Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress', *Agricultural Water Management*, 117, pp. 106–114. doi: 10.1016/j.agwat.2012.11.007.

Gianinazzi, S., Gollotte, A., Binet, M., van Tuinen, D., Redecker, D. and Wipf, D. (2010) 'Agroecology: The key role of arbuscular mycorrhizas in ecosystem services', *Mycorrhiza*, 20(8), pp. 519–530. doi: 10.1007/s00572-010-0333-3.

Giovannetti, M., Avio, L., Barale, R., Ceccarelli, N., Cristofani, R., Iezzi, a., Mignolli, F., Picciarelli, P., Pinto, B., Reali, D., Sbrana, C. and Scarpato, R. (2012) 'Nutraceutical value and safety of tomato fruits produced by mycorrhizal plants', *British Journal of Nutrition*, 107(2), pp. 242–251. doi: 10.1017/S000711451100290X.

Giri, B., Kapoor, R. and Mukerji, K. G. (2003) 'Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of Acacia auriculiformis', *Biology and Fertility of Soils*, 38(3), pp. 170–175. doi: 10.1007/s00374-003-0636-z.

Gosling, P., Jones, J. and Bending, G. D. (2016) 'Evidence for functional redundancy in arbuscular mycorrhizal fungi and implications for agroecosystem management', *Mycorrhiza*. Springer Berlin Heidelberg, 26(1), pp. 77–83. doi: 10.1007/s00572-015-0651-6.

Graham, J. ., Linderman, R. G. and Menge, J. . (1982) 'Development of External Hyphae by Different Isolates of Mycorrhizal Glomus SPP . in Relation to Root Colonization and Growth of Troyer Citrange Author (s): J . H . Graham , R . G . Linderman and J . A . Menge Published by : Wiley on behalf of the New Ph', *New Phytologist*, 91, pp. 183–189.

Grilli, G., Urcelay, C., Galetto, L., Davison, J., Vasar, M., Saks, Ü., Jairus, T. and Öpik, M. (2015) 'The composition of arbuscular mycorrhizal fungal communities in the roots of a ruderal forb is not related to the forest fragmentation process', *Environmental Microbiology*, 17(8), pp. 2709–2720. doi: 10.1111/1462-2920.12623.

Gromberg, B. C., Urcelay, C., Shroeder, M. A., Vargas-Gil, S. and Luna, C. M. (2015) 'The role of inoculum identity in drought stress mitigation by arbuscular mycorrhizal fungi in soybean', *Biology and Fertility of Soils*, 51(1). doi: 10.1007/s00374-014-0942-7.

Gruda, N. and Schnitzler, W. H. (2004) 'Suitability of wood fiber substrate for production of vegetable transplants: I. Physical properties of wood fiber substrates', *Scientia Horticulturae*, 100(1–4), pp. 309–322. doi: 10.1016/j.scienta.2003.10.001.

Hage-Ahmed, K., Krammer, J. and Steinkellner, S. (2013) 'The intercropping partner affects arbuscular mycorrhizal fungi and Fusarium oxysporum f. sp. lycopersici interactions in tomato', *Mycorrhiza*, 23(7), pp. 543–550. doi: 10.1007/s00572-013-0495-x.

Hale, M. G., Lindsey, D. L. and Hameed, K. M. (1973) 'Gnotobiotic culture of plants and related research', *The Botanical Review*, 39(3), pp. 261–273. doi: 10.1007/BF02860119.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M. (2004) 'Trichoderma species--opportunistic, avirulent plant symbionts.', *Nature reviews. Microbiology*, 2(1), pp. 43–56. doi: 10.1038/nrmicro797.

Harman, G. E., Petzoldt, R., Comis, A. and Chen, J. (2004) 'Interactions Between Trichoderma harzianum Strain T22 and Maize Inbred Line Mo17 and Effects of These Interactions on Diseases Caused by Pythium ultimum and Colletotrichum graminicola.', *Phytopathology*, 94(2), pp. 147–

153. doi: 10.1094/PHYTO.2004.94.2.147.

Hart, M. M. and Forsythe, J. A. (2012) 'Using arbuscular mycorrhizal fungi to improve the nutrient quality of crops; nutritional benefits in addition to phosphorus', *Scientia Horticulturae*, 148, pp. 206–214. doi: 10.1016/j.scienta.2012.09.018.

Hayman, D. S. (1974) 'Plant Growth Responses To Vesicular- Arbuscular Mycorrhiza Vi. Effect of Light and Temperature', *New Phytol.*, 73, pp. 71–80. doi: 10.1111/j.1469-8137.1974.tb04607.x.

Helgason, T., Daniell, T. J., Husband, R., Fitter, A. H. and Young, J. P. W. (1998) 'Ploughing up the wood-wide web?', *Nature*. Nature Publishing Group, 394(6692), pp. 431–431. doi: 10.1038/28764.

Herrmann, L. and Lesueur, D. (2013) 'Challenges of formulation and quality of biofertilizers for successful inoculation', *Applied Microbiology and Biotechnology*. Springer Berlin Heidelberg, pp. 8859–8873. doi: 10.1007/s00253-013-5228-8.

Hidalgo, P. R., Matt, F. B. and Harkess, R. L. (2006) 'Physical and Chemical Properties of Substrates Containing Earthworm Castings and Effects on Marigold Growth', *HortScience*, 41(16), pp. 1474–1476.

Hoeksema, J. D., Chaudhary, V. B., Gehring, C. A., Johnson, N. C., Karst, J., Koide, R. T., Pringle, A., Zabinski, C., Bever, J. D., Moore, J. C., Wilson, G. W. T., Klironomos, J. N. and Umbanhowar, J. (2010) 'A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi', *Ecology Letters*. Blackwell Publishing Ltd, pp. 394–407. doi: 10.1111/j.1461-0248.2009.01430.x.

Hughes, N. M. and Lev-Yadun, S. (2015) 'Red/purple leaf margin coloration: Potential ecological and physiological functions', *Environmental and Experimental Botany*. Elsevier B.V., 119, pp. 27–39. doi: 10.1016/j.envexpbot.2015.05.015.

IJdo, M., Cranenbrouck, S. and Declerck, S. (2011) 'Methods for large-scale production of AM fungi: Past, present, and future', *Mycorrhiza*. Springer-Verlag, pp. 1–16. doi: 10.1007/s00572-010-0337-z.

Ishii, S. and Loynachan, T. E. (2004) 'Rapid and reliable DNA extraction techniques from trypan-blue-stained mycorrhizal roots: Comparison of two methods', *Mycorrhiza*, 14(4), pp. 271–275. doi: 10.1007/s00572-004-0316-3.

Jacquot, E., van Tuinen, D., Gianinazzi, S. and Gianinazzi-pearson, V. (2000) 'Monitoring species of arbuscular mycorrhizal fungi in planta and in soil by nested PCR: Application to the study of

the impact of sewage sludge', *Plant and Soil*, 226(2), pp. 179–188. doi: 10.1023/A:1026475925703.

Jin, H., Germida, J. J. and Walley, F. L. (2013) 'Impact of arbuscular mycorrhizal fungal inoculants on subsequent arbuscular mycorrhizal fungi colonization in pot-cultured field pea (Pisum sativum L.)', *Mycorrhiza*. Springer-Verlag, 23(1), pp. 45–59. doi: 10.1007/s00572-012-0448-9.

Jin, L., Wang, Q., Wang, Q., Wang, X. and Gange, A. C. (2017) 'Mycorrhizal-induced growth depression in plants', *Symbiosis*. Symbiosis, 72(2), pp. 81–88. doi: 10.1007/s13199-016-0444-5.

Johnson, N. C., Graham, J. H. and Smith, F. A. (1997) 'Functioning of mycorrhizal associations along the mutualism – parasitism continuum *', *New Phytologist*. Royal Holloway, University of London, (135), pp. 575–585. doi: 10.1046/j.1469-8137.1997.00729.x.

Jones, M. D. and Smith, S. E. (2004) 'Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms?', *Canadian Journal of Botany*, 82(8), pp. 1089–1109. doi: 10.1139/b04-110.

Kawamoto, I. and Habte, M. (2011) 'Enhancement of arbuscular mycorrhizal fungal status of an established ginger crop through a mycorrhizal onion companion crop', *Soil Science and Plant Nutrition*, 57(5), pp. 659–662. doi: 10.1080/00380768.2011.609130.

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J., Franken, O., Verbruggen, E., Fellbaum, C., Kowalchuk, G., Hart, M., Bago, A., Palmer, T., West, S., Vandenkoornhuyse, P., Jansa, J. and Bucking, H. (2011) 'Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis', *Science*, 333, pp. 880–882.

Klironomos, J. N. (2000) 'Host-specificity and functional diversity among arbuscular mycorrhizal fungi', in Bell, C. R., Brylinsky, M., and Johnson-Green, P. (eds) *Microbial Biosystems: New Frontiers*. Halifax Canada: Atlantic Canada Society for Microbial Ecology, pp. 845–851.

Klironomos, J. N. (2003) 'Variation in plant response to native and exotic arbuscular mycorrhizal fungi', *Ecology*. Ecological Society of America, pp. 2292–2301. doi: 10.1890/02-0413.

Klironomos, J. N. and Hart, M. M. (2002) 'Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum', *Mycorrhiza*, 12(4), pp. 181–184. doi: 10.1007/s00572-002-0169-6.

Kohout, P., Sudová, R., Janoušková, M., Čtvrtlíková, M., Hejda, M., Pánková, H., Slavíková, R., Štajerová, K., Vosátka, M. and Sýkorová, Z. (2014) 'Comparison of commonly used primer sets for evaluating arbuscular mycorrhizal fungal communities: Is there a universal solution?', *Soil*

Biology and Biochemistry, 68, pp. 482–493. doi: 10.1016/j.soilbio.2013.08.027.

KOIDE, R. (1985) 'THE NATURE OF GROWTH DEPRESSIONS IN SUNFLOWER CAUSED BY VESICULAR-ARBUSCULAR MYCORRHIZAL INFECTION', *New Phytologist*. Blackwell Publishing Ltd, 99(3), pp. 449–462. doi: 10.1111/j.1469-8137.1985.tb03672.x.

Koide, R. T., Landherr, L. L., Besmer, Y. L., Detweiler, J. M. and Holcomb, E. J. (1999) 'Strategies for mycorrhizal inoculation of six annual bedding plant species', *HortScience*, 34(7), pp. 1217–1220.

Koide, R. T. and Li, M. (1989) 'Appropriate controls for vesicular arbuscular mycorrhizal research', *New Phytologist*, 111, pp. 35–44.

Koide, R. T. and Mosse, B. (2004) 'A history of research on arbuscular mycorrhiza.', *Mycorrhiza*, 14(3), pp. 145–63. doi: 10.1007/s00572-004-0307-4.

Kumutha, D., Ezhilmathi, K., Sairam, R. K., Srivastava, G. C., Deshmukh, P. S., Physiology, P., Agricultural, I. and Delhi-, N. (2009) 'Waterlogging induced oxidative stress and antioxidant activity in pigeonpea genotypes', *Plant Cell*, 53(1), pp. 75–84. doi: 10.1007/s10535-009-0011-5.

Lavakush, Yadav, J., Verma, J. P., Jaiswal, D. K. and Kumar, A. (2014) 'Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (Oryza sativa)', *Ecological Engineering*. Elsevier B.V., 62, pp. 123–128. doi: 10.1016/j.ecoleng.2013.10.013.

Lazcano, C., Barrios-Masias, F. H. and Jackson, L. E. (2014) 'Arbuscular mycorrhizal effects on plant water relations and soil greenhouse gas emissions under changing moisture regimes', *Soil Biology and Biochemistry*, 74, pp. 184–192. doi: 10.1016/j.soilbio.2014.03.010.

Lee, J., Lee, S. and Young, J. P. W. (2008) 'Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi', *FEMS Microbiology Ecology*, 65(2), pp. 339–349. doi: 10.1111/j.1574-6941.2008.00531.x.

Lin, K. H. R., Weng, C. C., Lo, H. F. and Chen, J. T. (2004) 'Study of the root antioxidative system of tomatoes and eggplants under waterlogged conditions', *Plant Science*, 167(2), pp. 355–365. doi: 10.1016/j.plantsci.2004.04.004.

Linderman, R. G. (2008) 'The mycorrhizosphere phenomenon', *Mycorrhiza Works*, pp. 341–355.

Linderman, R. G. and Davis, E. a. (2003) 'Arbuscular mycorrhiza and growth responses of several ornamental plants grown in soilless peat-based medium amended with coconut dust (coir)',

HortTechnology, 13(3), pp. 482–487.

Linderman, R. G. and Davis, E. A. (2003) 'Soil Amendment with Different Peatmosses Affects Mycorrhizae of Onion', *HortTechnology*, 13(2), pp. 285–289.

Linderman, R. G. and Davis, E. A. (2004) 'Varied response of marigold (Tagetes spp.) genotypes to inoculation with different arbuscular mycorrhizal fungi', *Scientia Horticulturae*, 99(1), pp. 67–78. doi: 10.1016/S0304-4238(03)00081-5.

Liu, Y., He, J., Shi, G., An, L., Öpik, M. and Feng, H. (2011) 'Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau', *FEMS Microbiology Ecology*, 78(2), pp. 355–365. doi: 10.1111/j.1574-6941.2011.01163.x.

Liu, Y., Mao, L., Li, J., Shi, G., Jiang, S., Ma, X., An, L., Du, G. and Feng, H. (2014) 'Resource availability differentially drives community assemblages of plants and their root-associated arbuscular mycorrhizal fungi', *Plant and Soil*. Springer International Publishing, 386(1–2), pp. 341–355. doi: 10.1007/s11104-014-2261-z.

Lu, X. and Koide, R. T. (1994) 'The effects of mycorrhizal infection on components of plant growth and reproduction', *New Phytologist*, 128, pp. 211–218.

Ma, N., Yokoyama, K. and Marumoto, T. (2007) 'Effect of peat on mycorrhizal colonization and effectiveness of the arbuscular mycorrhizal fungus Gigaspora margarita', *Soil Science and Plant Nutrition*. Blackwell Publishing Asia, 53(6), pp. 744–752. doi: 10.1111/j.1747-0765.2007.00204.x.

Manaf, H. H. and Zayed, M. S. (2015) 'Productivity of cowpea as affected by salt stress in presence of endomycorrhizae and Pseudomonas fluorescens', *Annals of Agricultural Sciences*, 60(2), pp. 219–226. doi: 10.1016/j.aoas.2015.10.013.

Manian, S., Sreenivasaprasad, S. and Mills, P. r. (2001) 'DNA extraction method for PCR in mycorrhizal fungi', *Letters in Applied Microbiology*, 33(4), pp. 307–310. doi: 10.1046/j.1472-765X.2001.01001.x.

Mar Vázquez, M., César, S., Azcón, R. and Barea, J.-M. (2000) 'Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (Azospirillum, Pseudomonas, Trichoderma) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants', *Applied Soil Ecology*, 15(3), pp. 261–272. doi: 10.1016/S0929-1393(00)00075-5.

Marschner, H. (2012) '15.3 Mycorrhiza', in *Marschner's Mineral Nutrition of Higher Plants*. Third. Elsevier Ltd, pp. 373–386.

Martínez-Medina, A., Pascual, J. A., Lloret, E. and Roldán, A. (2009) 'Interactions between arbuscular mycorrhizal fungi and Trichoderma harzianum and their effects on Fusarium wilt in melon plants grown in seedling nurseries', *Journal of the Science of Food and Agriculture*, 89(11), pp. 1843–1850. doi: 10.1002/jsfa.3660.

Matysiak, B. and Falkowski, G. (2010) 'Response of three ornamental plant species to inoculation with arbuscular mycorrhizal fungi depending on compost addition to peat substrate and the rate of controlled release fertilizer', *Journal of Fruit and Ornamental Plant Research*, 18(2), pp. 321–333.

McAllister, C., García-Romera, I., Godeas, A. and Ocampo, J. (1994) 'Interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*: effects on plant growth, arbuscular mycorrhizas and the saprorhyte inoculants.', *Soil biology and biochemistry*, 26(10), pp. 1363–1367.

McMillen, B. G., Juniper, S. and Abbott, L. K. (1998) 'Inhibition of hyphal growth of a vesicular-arbuscular mycorrhizal containing sodium chloride of infection from fungus in soil limits the spread spores', *Soil Biology & Biochemistry*, 30(13), pp. 1639–1646. doi: 10.1016/s0038-0717(97)00204-6.

Medina, A. and Azcón, R. (2010) 'Effectiveness of the Application of Arbuscular Mycorrhiza Fungi and Organic Amendments To Improve Soil Quality and Plant Performance Under Stress Conditions', *Journal of soil science and plant nutrition*, 10(3), pp. 354–372. doi: 10.4067/S0718-95162010000100009.

Medina, A., Vassilev, N. and Azcón, R. (2010) 'The interactive effect of an AM fungus and an organic amendment with regard to improving inoculum potential and the growth and nutrition of Trifolium repens in Cd-contaminated soils', *Applied Soil Ecology*, 44(2), pp. 181–189. doi: 10.1016/j.apsoil.2009.12.004.

Melcourt Industries (no date) *Sustainable Growing Medium*. Available at: http://www.melcourt.co.uk/product/sustainable-growing-medium/ (Accessed: 1 August 2017).

Mendoza, R., Escudero, V. and García, I. (2005) 'Plant growth, nutrient acquisition and mycorrhizal symbioses of a waterlogging tolerant legume (Lotus glaber Mill.) in a saline-sodic soil', *Plant and Soil*, 275(1–2), pp. 305–315. doi: 10.1007/s11104-005-2501-3.

Mensah, J. A., Koch, A. M., Antunes, P. M., Kiers, E. T., Hart, M. and Bücking, H. (2015) 'High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences

in phosphate and nitrogen uptake and fungal phosphate metabolism', *Mycorrhiza*, 25(7), pp. 533–546. doi: 10.1007/s00572-015-0631-x.

Met Office (2016) *UK Climate Weather Summaries June 2016*. Available at: http://www.metoffice.gov.uk/climate/uk/summaries/2016/june (Accessed: 2 August 2017).

Miller, G. E. (1991) 'Asymptotic test statistics for coefficients of variation', *Communications in Statistics-Theory and Methods*, 20, pp. 2251–2262.

Mnayer, D., Fabiano-Tixier, A. S., Petitcolas, E., Hamieh, T., Nehme, N., Ferrant, C., Fernandez, X. and Chemat, F. (2014) 'Chemical composition, antibacterial and antioxidant activities of six essentials oils from the Alliaceae family', *Molecules*, 19(12), pp. 20034–20053. doi: 10.3390/molecules191220034.

Morton, J. B. and Redecker, D. (2001) 'Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera Archaeospora and Paraglomus, based on concordant molecular and morphological characters', *Mycologia*, 93(1), pp. 181–195. doi: 10.2307/3761615.

Mummey, D. L., Rillig, M. C. and Holben, W. E. (2005) 'Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis', *Plant and Soil*, 271(1–2), pp. 83–90. doi: 10.1007/s11104-004-2066-6.

Nadeem, S. M., Ahmad, M., Zahir, Z. A., Javaid, A. and Ashraf, M. (2014) 'The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments', *Biotechnology Advances*. Elsevier Inc., 32(2), pp. 429–448. doi: 10.1016/j.biotechadv.2013.12.005.

Nash, M. A. and Porkorny, F. A. (1990) 'Shrinkage of Selected Two-component Container Media', *HortScience*. The Society, 25(8), pp. 930–931.

Nelsen, C. E. and Safir, G. R. (1982) 'Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition', *Planta*, 154(5), pp. 407–413. doi: 10.1007/BF01267807.

Nemec, S., Datnoff, L. E. and Strandberg, J. (1996) 'Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetables and citrus', *Crop Protection*, 15(8), pp. 735–742. doi: 10.1016/S0261-2194(96)00048-8.

Noble, R. and Roberts, S. J. (2004) 'Eradication of plant pathogens and nematodes during composting: A review', *Plant Pathology*, 53(5), pp. 548–568. doi: 10.1111/j.1365-

3059.2004.01059.x.

Nouri, E., Breuillin-Sessoms, F., Feller, U. and Reinhardt, D. (2014) 'Phosphorus and Nitrogen Regulate Arbuscular Mycorrhizal Symbiosis in <italic>Petunia hybrida</italic>', *PLoS ONE*. Edited by B. E. Dutilh. Public Library of Science, 9(3), p. e90841. doi: 10.1371/journal.pone.0090841.

Nzanza, B., Marais, D. and Soundy, P. (2011) 'Response of tomato (Solanum lycopersicum L.) to nursery inoculation with Trichoderma harzianum and arbuscular mycorrhizal fungi under field conditions', *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science*, 62(3), pp. 209–215. doi: 10.1080/09064710.2011.598544.

Okalebo, J. R., Gathua, K. W. and Woomer, P. L. . (1993) *Laboratory Methods of Soil and Plant Analysis: A Working Manual.* 2nd edn. Kenya: TSBF-CIAT and SACRED Africa.

Olsen, S. R., Cole, C. V, Watanabe, F. S. and Dean, L. A. (1954) 'Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate', *U. S Department of Agriculture CIrcular No. 939*.

Ortas, I. and Ustuner, O. (2014a) 'Determination of different growth media and various mycorrhizae species on citrus growth and nutrient uptake', *Scientia Horticulturae*, 166, pp. 84–90. doi: 10.1016/j.scienta.2013.12.014.

Ortas, I. and Ustuner, O. (2014b) 'The effects of single species, dual species and indigenous mycorrhiza inoculation on citrus growth and nutrient uptake', *European Journal of Soil Biology*. Elsevier B.V., 63, pp. 64–69. doi: 10.1016/j.ejsobi.2014.05.007.

Patterson, S. (2015) *Plant Deficiencies: Why Are Leaves Turning Reddish Purple In Colour*, *Gardening Know How*. Available at: https://www.gardeningknowhow.com/plant-problems/environmental/leaves-turning-reddish-purple.htm (Accessed: 1 August 2017).

Peng, S., Eissenstat, D. M., Graham, J. H., Williams, K. and Hodge, N. C. (1993) 'Growth Depression in Mycorrhizal Citrus At High-Phosphorus Supply - Analysis of Carbon Costs', *Plant Physiology*. American Society of Plant Biologists, 101(3), pp. 1063–1071. doi: 10.1104/pp.101.3.1063.

Perner, H., Schwarz, D., Bruns, C., Mäder, P. and George, E. (2007) 'Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of pelargonium plants', *Mycorrhiza*, 17(5), pp. 469–474. doi: 10.1007/s00572-007-0116-7.

Perner, H., Schwarz, P. and George, E. (2006) 'Effect of mycorrhizal inoculation and compost supply on growth and nutrient uptake of young leek plants grown on peat-based substrates',

HortScience. The Society, 41(3), pp. 628–632.

Porras-Soriano, A., Soriano-Martín, M. L., Porras-Piedra, A. and Azcón, R. (2009) 'Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions', *Journal of Plant Physiology*, 166(13), pp. 1350–1359. doi: 10.1016/j.jplph.2009.02.010.

Poulton, J. L., Bryla, D. R., Koide, R. T. and Stephenson, A. G. (2002) 'Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato', *New Phytologist*, 154(1), pp. 255–264. doi: 10.1046/j.1469-8137.2002.00366.x.

Pozo, M. J., Cordier, C., Dumas-Gaudot, E., Gianinazzi, S., Barea, J.-M. and Azcón-Aguilar, C. (2002) 'Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to Phytophthora infection in tomato plants.', *Journal of experimental botany*, 53(368), pp. 525–534. doi: 10.1093/jexbot/53.368.525.

Puschel, D., Rydlova, J. and Vosatka, M. (2014) 'Can mycorrhizal inoculation stimulate the growth and flowering of peat-grown ornamental plants under standard or reduced watering?', *Applied Soil Ecology*. Elsevier B.V., 80, pp. 93–99. doi: 10.1016/j.apsoil.2014.04.001.

Querejeta, J. I. (2017) *Soil Water Retention and Availability as Influenced by Mycorrhizal Symbiosis: Consequences for Individual Plants, Communities, and Ecosystems*, *Mycorrhizal Mediation of Soil*. Elsevier Inc. doi: 10.1016/B978-0-12-804312-7.00017-6.

Rasouli-sadaghiani, M., Hassani, A., Barin, M., Danesh, Y. R. and Sefidkon, F. (2010) 'Effects of arbuscular mycorrhizal (AM) fungi on growth, essential oil production and nutrients uptake in basil', *Journal of Medicinal Plants Research*, 4(21), pp. 2222–2228. doi: 10.5897/JMPR10.337.

Redecker, D. (2000) 'Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots', *Mycorrhiza*, 10(2), pp. 73–80. doi: 10.1007/s005720000061.

Redecker, D., Morton, J. B. and Bruns, T. D. (2000) 'Ancestral lineages of arbuscular mycorrhizal fungi (Glomales)', *Molecular Phylogenetics and Evolution*, 14(2), pp. 276–284. doi: 10.1006/mpev.1999.0713.

Reuveni, R., Raviv, M., Krasnovsky, A., Freiman, L., Medina, S., Bar, A. and Orion, D. (2002) 'Compost induces protection against *Fusarium oxysporum* in sweet basil', *Crop Protection*, 21(7), pp. 583–587. doi: 10.1016/S0261-2194(01)00149-1.

Riaz, T. and Javaid, A. (2017) 'Mixed cropping effects on agronomic parameters and mycorrhizal status of Gladiolus grandiflorus Hort . and Narcissus papyraceus Ker-Gawl', *Bangladesh Journal*

of Botany, 46(March), pp. 133–138.

Rillig, M. C. and Mummey, D. L. (2006) 'Mycorrhizas and soil structure', *New Phytologist*, 171, pp. 41–53.

Rillig, M. C. and Steinberg, P. D. (2002) 'Glomalin production by an arbuscular mycorrhizal fungus: A mechanism of habitat modification?', *Soil Biology and Biochemistry*, 34, pp. 1371–1374. doi: 10.1016/S0038-0717(02)00060-3.

Rillig, M. C., Wright, S. F. and Eviner, V. T. (1998) 'The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species', *Plant and Soil*, 238, pp. 325–333.

Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., Pascale, S. De, Bonini, P. and Colla, G. (2015) 'Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops', *Scientia Horticulturae*, pp. 91–108. doi: 10.1016/j.scienta.2015.09.002.

Ruiz-Lozano, J. M., Aroca, R., Zamarreño, A. M., Molina, S., Andreo-Jiménez, B., Porcel, R., García-Mina, J. M., Ruyter-Spira, C. and López-Ráez, J. A. (2016) 'Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato', *Plant, Cell and Environment*, 39(2), pp. 441–452. doi: 10.1111/pce.12631.

Ruiz Lozano, J. M., Azcón, R. and Gomez, M. (1995) 'Effects of arbuscular-mycorrhizal Glomus species on drought tolerance: physiology and nutritional plant responses', *Appl Environ Microbiol*, 61(61 (2)), pp. 456–460.

Samaei, F., Asghari, S. and Aliasgharzad, N. (2015) 'The effects of two arbuscular mycorrhizal fungi on some physical properties of a sandy loam soil and nutrients uptake by spring barley', *Journal of Soil Environment*, 1, pp. 1–9.

Sandhya, V., Ali, S. Z., Grover, M., Reddy, G. and Venkateswarlu, B. (2010) 'Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress', *Plant Growth Regulation*, 62(1), pp. 21–30. doi: 10.1007/s10725-010-9479-4.

Schmilewski, G. (2008) 'The role of peat in assuring the quality of growing media', *Mires and Peat*, 3, pp. 1–8. doi: 10.1016/j.jenvman.2015.10.017.

Schroeder, V., Gange, A. C. and Stead, A. D. (2012) 'Underground networking: The potential for improving yield and quality of pot-grown herbs with mycorrhizas', *Journal of the Science of Food and Agriculture*, 92(2), pp. 203–206. doi: 10.1002/jsfa.4648.

Schwarzott, D., Walker, C. and Schussler, A. (2001) 'Glomus, the Largest Genus of the Arbuscular Mycorrhizal Fungi (Glomales), Is Nonmonophyletic', *Molecular Phylogenetics and Evolution*, 21(2), pp. 190–197.

Smith, F. A., Grace, E. J. and Smith, S. E. (2009) 'More than a carbon economy: Nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses', *New Phytologist*. Blackwell Publishing Ltd, pp. 347–358. doi: 10.1111/j.1469-8137.2008.02753.x.

Smith, J. E., Johnson, K. A. and Cázares, E. (1998) 'Vesicular mycorrhizal colonization of seedlings of Pinaceae and Betulaceae after spore inoculation with Glomus intraradices', *Mycorrhiza*, 7(6), pp. 279–285. doi: 10.1007/s005720050193.

Smith, S. E. and Read, D. J. (2002a) 'Growth and carbon economy of VA mycorrhizal plants', in *Mycorrhizal Symbiosis (Second Edition)*. Second. Elsevier Ltd, pp. 105–125. doi: http://dx.doi.org/10.1016/B978-012652840-4/50005-X.

Smith, S. E. and Read, D. J. (2002b) 'Uptake, translocation and transfer of nutrients in mycorrhizal symbioses', in *Mycorrhizal Symbiosis (Second Edition)*. Second. Elsevier Ltd, pp. 379–408. doi: http://dx.doi.org/10.1016/B978-012652840-4/50015-2.

Smith, S. E. and Read, D. J. (2008) *Mycorrhizal Symbiosis*. Academic Press. doi: 10.1097/00010694-198403000-00011.

Smith, S. and Gianinazzi-Pearson, V. (1988) 'Physiological Interactions Between Symbionts in Vesicular-Arbuscular Mycorrhizal Plants', *Annual Review of Plant Physiology Plant Molecular Biology*, 39, pp. 221–244.

Sohn, B. K., Kim, K. Y., Chung, S. J., Kim, W. S., Park, S. M., Kang, J. G., Rim, Y. S., Cho, J. S., Kim, T. H. and Lee, J. H. (2003) 'Effect of the different timing of AMF inoculation on plant growth and flower quality of chrysanthemum', *Scientia Horticulturae*, 98(2), pp. 173–183. doi: 10.1016/S0304-4238(02)00210-8.

Son, C. L. and Smith, S. E. (1988) 'Mycorrhizal growth responses: interaction between photon irradiance and phosphorus nutrition', *New Phytologist*, 108, pp. 305–314.

St-Arnaud, M., Vimard, B., Fortin, J. A., Hamel, C. and Caron, M. (1997) 'Inhibition of Fusarium oxysporum f . sp . dianthi in the non-VAM species Dianthus caryophyllus by co-culture with Tagetes patula companion plants colonized by Glomus intraradices', *Canadian Journal of Botany*, 75(6), pp. 998–1005. doi: 10.1139/b97-110.

Stonor, R. N., Smith, S. E., Manjarrez, M., Facelli, E. and Andrew Smith, F. (2014) 'Mycorrhizal

responses in wheat: Shading decreases growth but does not lower the contribution of the fungal phosphate uptake pathway', *Mycorrhiza*, 24(6), pp. 465–472. doi: 10.1007/s00572-014-0556-9.

Sweatt, M. R. and Davies, F. T. J. (1984) 'Mycorrhizae Water Relations Growth And Nutrient Uptake Of Geranium Grown Under Moderately High Phosphorus Regimes', *Journal of the American Society for Horticultural Science*, 109(2), pp. 210–213.

Sylvia, D. M. and Schenck, N. C. (1983) 'Germination of Chlamydospores of Three Glomus Species as Affected by Soil Matric Potential and Fungal Contamination', *Mycologia*, 75(1), pp. 30–35.

Taheri, P. and Kakooee, T. (2017) 'Reactive oxygen species accumulation and homeostasis are involved in plant immunity to an opportunistic fungal pathogen', *Journal of Plant Physiology*. Elsevier, 216(March), pp. 152–163. doi: 10.1016/j.jplph.2017.04.018.

Taie, H. A. A., Salama, Z. and Samir, R. (2010) 'Potential activity of basil plants as a source of antioxidants and anticancer agents as affected by organic and bio-organic fertilization', *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 38(June), pp. 119–127. doi: 10.1074/jbc.M300931200.

Tarbell, T. J. and Koske, R. E. (2007) 'Evaluation of commercial arbuscular mycorrhizal inocula in a sand/peat medium', *Mycorrhiza*. Springer-Verlag, 18(1), pp. 51–56. doi: 10.1007/s00572-007-0152-3.

Tavakkoli, E., Rengasamy, P. and McDonald, G. K. (2010) 'High concentrations of Na+ and Cl- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress', *Journal of Experimental Botany*, 61(15), pp. 4449–4459. doi: 10.1093/jxb/erq251.

Tawaraya, K., Hirose, R. and 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', *Biology and Fertility of Soils*, 48(7), pp. 839–843. doi: 10.1007/s00374-012-0669-2.

Team, R. C. (2017) 'R: A language and environment for statistical analysis'.

Tognetti, C., Mazzarino, M. J. and Laos, F. (2005) 'Composting vs. Vermicomposting: A Comparison of End Product Quality', *Compost Science and Utilization*, 13(1), pp. 6–13. doi: 10.1016/j.biortech.2006.04.025.

Toussaint, J. P., Smith, F. A. and Smith, S. E. (2007) 'Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition', *Mycorrhiza*, 17(4), pp. 291–297. doi: 10.1007/s00572-006-0104-3.

Treseder, K. K. (2004) ‘A Meta-Analysis of Mycorrhizal Responses to Nitrogen, Phosphorus, and Atmospheric CO 2 in Field Studies’, *The New Phytologist*, 164(2), pp. 347–355.

Treseder, K. K. (2013) ‘The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content’, *Plant and Soil*, 371(1–2), pp. 1–13. doi: 10.1007/s11104-013-1681-5.

Tucci, M., Ruocco, M., De Masi, L., De Palma, M. and Lorito, M. (2011) ‘The beneficial effect of Trichoderma spp. on tomato is modulated by the plant genotype’, *Molecular Plant Pathology*, 12(4), pp. 341–354. doi: 10.1111/j.1364-3703.2010.00674.x.

van Tuinen, D., Jacquot, E., Zhao, B., Gollotte, A. and Gianinazzi-Pearson, V. (1998) ‘Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S-rDNA-targeted nested PCR’, *Molecular Ecology*, 7, pp. 879–887.

Turnau, K., Ryszka, P., Gianinazzi-Pearson, V. and Van Tuinen, D. (2001) ‘Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland’, *Mycorrhiza*, 10(4), pp. 169–174. doi: 10.1007/s005720000073.

Ustuner, O., Wininger, S., Gadkar, V., Badani, H., Raviv, M., Dudai, N., Medina, S. and Kapulnik, Y. (2009) ‘Evaluation of different compost amendments with AM fungal inoculum for optimal growth of chives’, *Compost Science and Utilization*, 17(4), pp. 257–265. doi: 10.1080/1065657X.2009.10702432.

Verbruggen, E., Kuramae, E. E., Hillekens, R., de Hollander, M., Kiers, E. T., Röling, W. F. M., Kowalchuk, G. a. and van der Heijdena, M. G. a (2012) ‘Testing potential effects of maize expressing the Bacillus thuringiensis cry1ab endotoxin (Bt maize) on mycorrhizal fungal communities via DNA- and RNA-based pyrosequencing and molecular fingerprinting’, *Applied and Environmental Microbiology*, 78(20), pp. 7384–7392. doi: 10.1128/AEM.01372-12.

Veresoglou, S. D. and Rillig, M. C. (2014) ‘Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis’, *Plant and Soil*, 377(1–2), pp. 395–406. doi: 10.1007/s11104-013-2008-2.

Vierheilig, H., Coughlan, A. P., Wyss, U. and Piche, Y. (1998) ‘Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi’, *Appl. Envir. Microbiol.*, 64(12), pp. 5004–5007.

Vitacress (2016) *Not just a garnish - parsley sales hit record high.*, *Vitacress News Centre, Vitacress.com*. Available at: http://www.vitacress.com/news-centre/parsley-sales-hits-record-high/ (Accessed: 30 August 2017).

Vital Earth GB Ltd (2017) *About Vital Earth*. Available at: http://www.vitalearth.tv/aboutvitalearth.html (Accessed: 18 May 2017).

Vosátka, M., Látr, A., Gianinazzi, S. and Albrechtová, J. (2012) 'Development of arbuscular mycorrhizal biotechnology and industry: Current achievements and bottlenecks', *Symbiosis*, 58(1–3), pp. 29–37. doi: 10.1007/s13199-012-0208-9.

Walder, F. and van der Heijden, M. (2015) 'Regulation of resource exchange in the arbuscular mycorrhizal symbiosis', *Nature Plants*. Macmillan Publishers Limited, 1(November), pp. 1–7. doi: 10.1038/NPLANTS.2015.159.

Warton, D. I. and Hui, F. C. K. (2011) 'The arcsine is asinine: the analysis of proportions in ecology', *Ecology*, 92(1), pp. 3–10.

Weremijewicz, J. and Janos, D. P. (2013) 'Common mycorrhizal networks amplify size inequality in Andropogon gerardii monocultures', *New Phytologist*, 198(1), pp. 203–213. doi: 10.1111/nph.12125.

Werner, G. D. and Kiers, E. T. (2015) 'Partner selection in the mycorrhizal symbiosis/mutualism', *New Phytologist*, 205, pp. 1437–1442. doi: 10.1111/nph.13113.

Westland Horticulture (2017) *Gro-Sure Peat Free All Purpose Compost*. Available at: https://www.gardenhealth.com/product/grosure-peat-free-compost.

Wiberg, A., Koenig, R. and Cerny-Koenig, T. (2006) 'Variability in plant growth in retail potting media', *HortTechnology*, 16(1), pp. 7–12.

Woolley, J. . and Broyer, T. . (1957) 'Foliar Symptoms of Deficiencies of Inorganic Elements in Tomato', *Plant Physiology*, 32(2), pp. 148–151.

Wu, Q. S., Xia, R. X. and Zou, Y. N. (2008) 'Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress', *European Journal of Soil Biology*, 44(1), pp. 122–128. doi: 10.1016/j.ejsobi.2007.10.001.

Yedidia, I., Srivasta, A. K., Kapulnik, Y. and Chet, I. (2001) 'Effect of *Trichoderma harzianum* on microelement concentration and increased growth of cucumber plants', *Plant and Soil*, 235, pp. 235–242.

Yildiz, A. (2010) 'A native Glomus sp. from fields in Aydın province and effects of native and commercial mycorrhizal fungi inoculants on the growth of some vegetables', *Turk J Biol*, 34, pp. 447–452. doi: 10.3906/biy-0901-7.

Yusoff, M. (1977) *Studies on Vesicular-Arbuscular Mycorrhiza in Allium Species*. Canterbury Christchurch New Zealand.

Ziane, H., Meddad-Hamza, A., Beddiar, A. and Gianinazzi, S. (2017) 'Effects of arbuscular mycorrhizal fungi and fertilization levels on industrial tomato growth and production', *International Journal of Agriculture and Biology*, 19(2), pp. 341–347. doi: 10.17957/IJAB/15.0287.

Appendix I

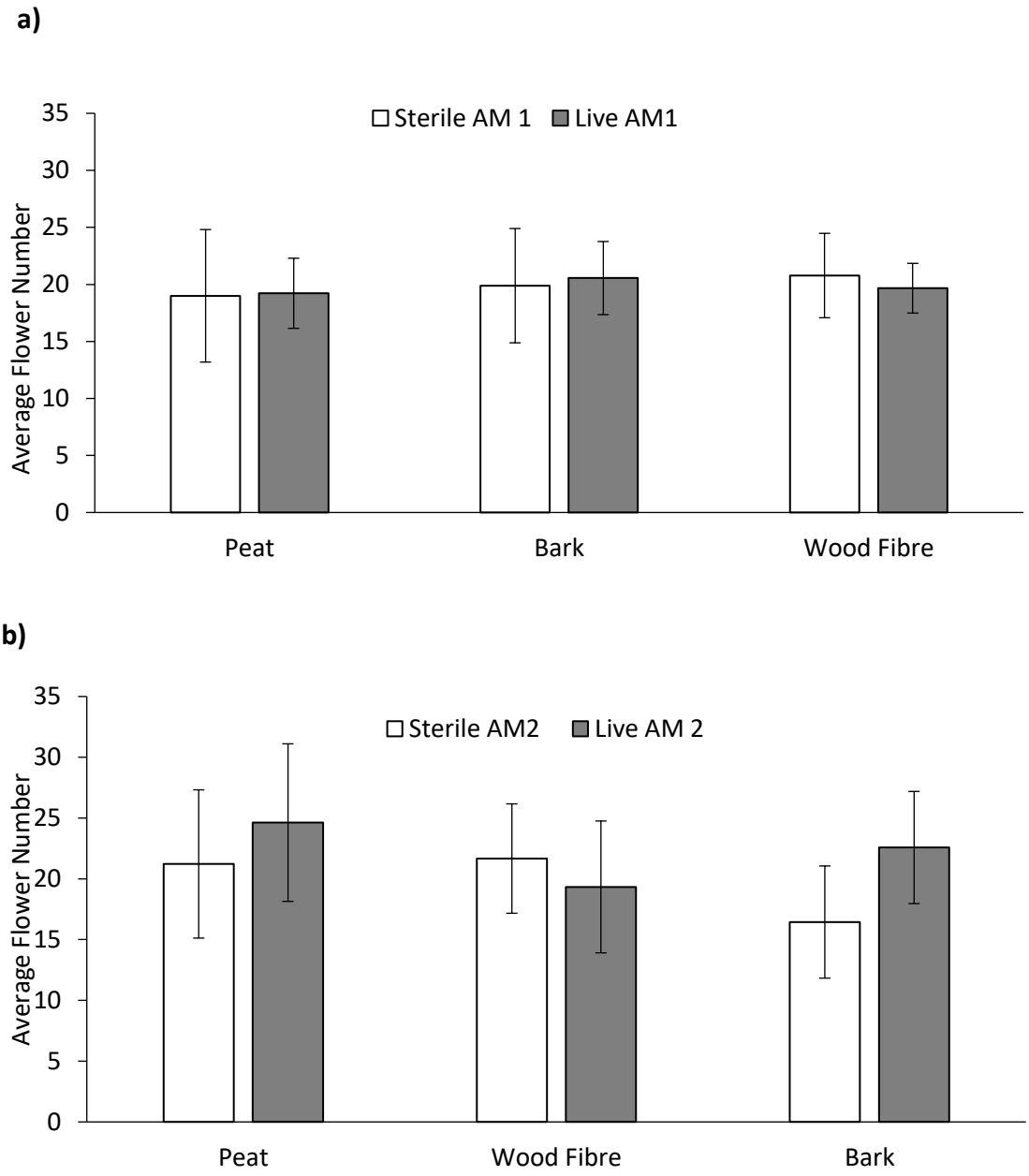


Figure 1. Average flower number of plants grown in each media with each commercial inoculum from harvest 2: a) AM1 and b) AM2.

Appendix II

Marigolds	Label	Colonisation (%)	PCR1: NS1/NS4	PCR2: AML1/AML2	Sequence Match
Peat AM1	2.1	37	Y	Y	*Allium fistulosum*
Peat AM1	2.3	32	Y	Y	*Claroideoglomus claroideum*
Peat AM1	2.8	22	Y	N	*NA*
Bark AM1	11.1	44	Y	N	*NA*
Bark AM1	11.2	68	Y	Y	***Not sequenced***
WF AM1	20.1	96	Y	Y	*Allium fistulosum*
WF AM1	20.2	48	Y	Y	*Paraglomus occultum*
WF AM1	20.3	25	Y	Y	***Not Sequenced***
WF AM1	20.4	6	Y	Y	*Rhizophagus irregularis*
Peat AM3	6.4	31	Y	Y	*Allium fistulosum*
Peat AM3	6.5	20	N	NA	NA
Peat AM3	6.6		N	NA	NA
Bark AM3	15.1	15	Y	Y	*Allium fistulosum*
Bark AM3	15.4	10	Y	Y	*Allium fistulosum*
Bark AM3	15.5	12	Y	Y	***Not sequenced***
WF AM3	24.1	12	Y	Y	*Allium fistulosum*
WF AM3	24.3	53	Y	Y	*Allium fistulosum*
WF AM3	24.4	16	Y	Y	*Allium fistulosum*
Peat FS	8.5	18	Y	Y	**Not sequenced**
Bark FS	17.2	9	Y	Y	*Allium fistulosum*
Bark FS	17.5	15	Y	Y	*Allium fistulosum*
WF FS	26.4	17	N	NA	NA

Table 1. Sequences extracted from marigold roots grown with chives

Treatment	Replicate	Colonisation (%)	PCR1: NS1/NS4	PCR2: AML1/AML2	Sequence
Peat AM1	C1	15	Y	Y	*Rhizophagus irregularis*
Peat AM1	C2	31	Y	Y	*Paraglomus occultum*
Peat AM1	C3	14	Y	Y	*Rhizophagus irregularis*
Bark AM1	C10	25	Y	Y	*Rhizophagus irregularis*
Bark AM1	C11	65	Y	Y	*Rhizophagus irregularis*
Bark AM1	C12	38	Y	Y	*Rhizophagus irregularis*
WF AM1	C13	96	Y	Y	*Rhizophagus irregularis*
WF AM1	C14	50	Y	Y	*Claroideoglomus claroideum*
WF AM1	C15	47	Y	N	NA

Table 2. Sequences extracted from chive roots grown with marigolds treated with AM1

Treatment	Replicate	Colonisation (%)	PCR1: NS1/NS4	PCR2: AML1/AML2	Sequence
Peat AM3	C21	37	Y	Y	*Rhizophagus irregularis*
Peat AM3	6.3	27	Y	Y	*Pinus sylvestris*
Peat AM3	6.5	9	Y	Y	*Allium fistulosum*
Bark AM3	C7	40	Y	Y	*Paraglomus occultum*
Bark AM3	C8	16	Y	Y	*Funneliformis mossae*
WF AM3	24.1	55	Y	Y	*Funneliformis mosseae*
WF AM3	24.5	7	Y	Y	*Rhizophagus irregularis*
WF AM3	24.4	30	Y	Y	*Allium fistulosum*

Table 3. Sequences extracted from chive roots grown with marigolds treated with AM3

Treatment	Replicate	Colonisation (%)	PCR1: NS1/NS4	PCR2: AML1/AML2	Sequence
Peat FS	C4	14	Y	Y	*Glomus sp*
Peat FS	C5	14	Y	Y	*Allium fistulosum*
Peat FS	C6	18	Y	Y	*Rhizophagus irregularis*
Bark FS	C19	7	Y	Y	*Allium fistulosum*
Bark FS	C20	2	Y	Y	*Allium fistulosum*
WF FS	C16	4	Y	Y	*Allium fistulosum*
WF FS	C17	20	Y	Y	*Archaeospora sp.*
WF FS	C18	33	Y	Y	*Rhizophagus irregularis*

Table 4. Sequences extracted from chive roots grown with marigolds treated with Field Soil Inoculum

Appendix III

Peat AM1

Paraglomus occultum isolate WDG40 18S ribosomal RNA gene, partial sequence

```
Query  27   GATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCACATCCAAGGAAGGCA  86
            ||||||||||||||||||||||||||||||||||| ||||||||||||| |||||||||
Sbjct  40   GATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCC-AGGAAGGCA  98

Query  87   GCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACAATAAATAACAATATC  146
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  99   GCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACAATAAATAACAATACC  158

Query  147  GGGCTCTTACAGTTTGGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGATCCAT  206
            |||||| |  ||| |||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  159  GGGCTCCTCGAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGATCC-T  217

Query  207  TGGAGGGCAAGTCTGGGGCCAGCAGCCGCGGTAATTCCCGCTCCCATAGCGTAAATTTAA  266
            |||||||||||||||| |||||||||||||||||||| ||| |||||||||| | ||||
Sbjct  218  TGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC-GCT-CCATAGCGTATA-TTAA  274

Query  267  GTTGTTGCAGTTAAAAAGCTCGTAATTGGACTTTGGGTTTGGTCGTCAGGTCCGCCTTT-  325
            |||||||||||||||||||||||| |||||| ||||| | || || | ||||||||
Sbjct  275  GTTGTTGCAGTTAAAAAGCTCGTAGTTGGACCTTGGGCTGGGCCGGCCGGTCCGCCCCAC  334

Query  326  GACGAGCACCTGACGTCCTGTCCCTTTTGCCGACGTTACGAATCTGGCCTTAATTGGTTG  385
            |  | ||||| | | ||| ||||||| ||||| || | ||   ||| |||||| |||  |
Sbjct  335  GGTGTGCACCGGCCTTCCCGTCCCTTCTGCCGGCGATGCGCTCCTGTCCTTAACTGGACG  394

Query  386  GGTCGTGCCTTCGGCGTTGTTACTTTGAAGAAATTATAGTGCTCATAGCAAGCCTACGCT  445
            |||||||||| |||||  |||||||||||||||| |||||||| |||||||| |||||
Sbjct  395  GGTCGTGCCTCCGGCGCCGTTACTTTGAAGAAATTAGAGTGCTCAAAGCAAGCCCACGCT  454

Query  446  TTGTATACATTATCATGGGATAACATCACATGATTTCGGTCCTATTGTGTTGGCCTTCGG  505
             || |||||||| |||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  455  CTGGATACATTAGCATGGGATAACATCACAGGATTTCGGTCCTATTGTGTTGGCCTTCGG  514
```

```
Query  506  GATC-GGAGTAATGATTAACAAGGACAGTCGGGGGCATTCGTATTTCATAGTCAGAGGTG  564
            ||||  |||||||||||||| | ||||||  ||||||||||||||||||||||||||||||
Sbjct  515  GATCGGGAGTAATGATTAAGAGGGACAGCCGGGGGCATTCGTATTTCATAGTCAGAGGTG  574

Query  565  AAATTCTT-GGATTTATGAAAGACGAACAACTGCGAAAGCA-TTTGCCACGG-ATGTTTT  621
            ||||||||  ||||||||||||||||||  ||||||||||| |||||||  ||  |||||||
Sbjct  575  AAATTCTTGGGATTTATGAAAGACGAACCACTGCGAAAGCAGTTTGCCAAGGAATGTTTT  634

Query  622  GCATT-AATCAAGAACGAAAG-TTGGGGG-C-TCGAA-GAC  657
              ||||  |||||  |||||||||  |||||||  |  |||||  |||
Sbjct  635  TCATTAAATCAGGAACGAAAGGTTGGGGGGCCTCGAAAGAC  675
```

Peat AM1

Rhizophagus irregularis partial 18S rRNA gene, strain DAOM229456, isolate spore 1, clone 03S2_3_10

```
Query  7     ATGTGTGGGTACGGGT-GCGGGGTGTTAGGGCACGACTATCCGCGAGAGGGAGCCTGAGA  65
             |||  ||||  ||||||  |||||||||||||||||||  |  |||  ||||||||||||||||
Sbjct  310   ATG-GTGGTAACGGGTAACGGGGTGTTAGGGCACGAC-A-CCG-GAGAGGGAGCCTGAGA  365

Query  66    AACGAGCTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGG  125
             ||||  ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  366   AACG-GCTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGG  424

Query  126   GAGGTAGTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTA  185
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  425   GAGGTAGTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTA  484

Query  186   CAATTTAAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGT  245
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  485   CAATTTAAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGT  544

Query  246   AATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATT  305
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  545   AATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATT  604
```

```
Query  306   TCGGGGTGAGTAGGTTGGTCATGCCTCTGGTATGTACTGATCTCACTGATTCCTCCTTCC  365
             ||||||| |||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  605   TCGGGGTTAGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCC  664

Query  366   TTATGAACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAA  425
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  665   TTATGAACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAA  724

Query  426   AATTAGAGTGTTTAAAGCAGGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGG  485
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  725   AATTAGAGTGTTTAAAGCAGGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGG  784

Query  486   ACGTTCGATCTTATTTTGTT-GTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGG  544
             |||||||||||||||||| |||||||||||||||||||||||||||||||||||||||
Sbjct  785   ACGTTCGATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGG  844

Query  545   GGGCATTAGTATTCAATTGTCAGAGGTGAATTTCTTGGATTTATTGAAGACAACCTACTG  604
             |||||||||||||||||||||||||||| |||||||||||||||||| | ||||||
Sbjct  845   GGGCATTAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTG  904

Query  605   CGAAAGCATTTGCCA-GGATGTTTTC-TTAATCAAGAACGAAT-TTGGGGGATCGAACAC  661
             ||||||||||||||| |||||||||| |||||||||||||||  || |||||||||| ||
Sbjct  905   CGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGAC  964

Query  662   TATCAGATACCGTCATACTCTTC-CCAGC-ACTACGCCGACGAGGGATCGGATGATGC-A  718
              |||||||||||| || ||||  |||   |||| |||||| ||||||||||||||  |
Sbjct  965   GATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTA  1024

Query  719   A-TTTTTAATGACTCATTCGGCGCCTTACG  747
             | ||||||||||||||||||||||||||||
Sbjct  1025  ATTTTTTAATGACTCATTCGGCGCCTTACG  1054
```

Bark AM1
Uncultured *Glomus* partial 18S rRNA gene, clone G+I4-15

```
Query  12   GTGACGGGTGACGGAG-TGTAGGGTCTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTAC  70
            |||||||||||||||   |||||| |||||||||||||||||||||||||||| ||||||
Sbjct  39   GTGACGGGTGACGGAGAATTAGGGT-TCGATTCCGGAGAGGGAGCCTGAGAAACGGCTAC  97

Query  71   CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA  130
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  98   CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGA  157

Query  131  CAATAAATAACAATACCGGGCTCTTACAGATC-GCTAATTGGAAAGGATACCATCTAAAT  189
            |||||||||||||||||||||| | || || | ||||||||| |  ||| ||||||||
Sbjct  158  CAATAAATAACAATACCGGGCTCTCAGAG-TCTGGTAATTGGAATGAGTACAATCTAAAT  216

Query  190  CCCCTAACCAAGAACCATTGGAAGGGAAGTCCGGGGGCCGCCACCCCCGTAATTCCCGCC  249
            ||| |||| | || |||||||| || ||||| || | | ||  || | |||||||| ||
Sbjct  217  CCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCT  276

Query  250  CCCATAACCTAAATTTAAATTGGTGGCATTTAAAAGCCCCGAATTGGACTTT-GGGGTTG  308
            || ||| | || |||||| ||| || || ||||||  | || | | || ||| ||||||
Sbjct  277  CCAATAGCGTATATTTAAGTTGTTG-CAGTTAAAAAGCTCGTAGTTGAATTTCGGGGTTA  335

Query  309  GTCGGGTGGGCCGCGCCTGAGGAATGCCC-GG-CTCCCTGTGACTCCTCCTTCCTTAACA  366
            || || ||| |   ||||  || |||  | || ||| |  ||| ||||||||||||  |
Sbjct  336  GTAGGTTGGTCAT-GCCTCTGGTATGTACTGGTCTCAC--TGATTCCTCCTTCCTTATGA  392

Query  367  ACCCTGG-GCCATTAATTTGGTGTTGGCCGGAATTTGGACTGCTACTTTGAAAAA-T-AG  423
            ||| |   |||||||||||||||||   |||||||||||| |||||||||||| | ||
Sbjct  393  ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  452

Query  424  TGCTGTTAGCAGCCCGACACCGCTTGTATAT-TTA--ATGGGAAAAATGACATAGGATCT  480
             | ||||   |||  |  | |||||| |||  |||  || |||| ||||| ||||||  |
Sbjct  453  AG-TGTTTAAAGCAAGCTAACGCTTGAATACATTAGCAT-GGAATAATGAAATAGGACGT  510

Query  481  GCGATATTGTGTTGTC-GTCTCGAGCATAGACGTGATGATCA---GGGAGA-TCGTGGGC  535
             |||| || | ||||  || || || || ||||| ||||| |   |||| | | | ||||
```

```
Sbjct  511  TCGATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGC  570

Query  536  ACTTTATTATACAGTCGT-AGAGGTGATCTTCTAGTA-TTATAGACGACTAACTGCTGCA  593
            |  ||| ||| || | || ||||||||  |||| | | |||| || |||||||| ||||
Sbjct  571  A--TTAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCG  628

Query  594  GCAGC-TGTGACGATGATGTCATCATC-ATCAACACCGATAGTGAGGTGATCGACGACGA  651
              ||| | || | | |||||  ||||  ||||| | ||| ||| ||| |||||| |||||
Sbjct  629  AAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGA  688

Query  652  TCTACTG-CGTCGTATTATCATC-ATC-ACGACGACGACGAGG-ATCG-ATGATGTACTT  706
            ||   |  ||||||| | | | | ||  || | | |||| ||| |||| |||||||  ||
Sbjct  689  TCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTATT  748

Query  707  ATGACTGACTCAT-CGGC  723
             | | |||||||| ||||
Sbjct  749  TTTAATGACTCATTCGGC  766
```

Bark AM1

Rhizophagus irregularis partial 18S rRNA gene, strain DAOM229456, isolate spore 3, clone 03S2_16_10

```
Query  8     TGGTAGGT-ACGGGTGACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACG  66
             |||| ||| |||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  311   TGGT-GGTAACGGGTAACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACG  369

Query  67    GCTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGT  126
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  370   GCTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGT  429

Query  127   AGTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATT  186
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  430   AGTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATT  489

Query  187   TAAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTC  246
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  490  TAAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTC  549

Query  247  CAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGG  306
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  550  CAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGG  609

Query  307  GTTAGTAGGTTGGTCATGCCTCTGGTATGTACTGATCTCACTGATTCCTCCTTCCTTATG  366
            |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  610  GTTAGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCCTTATG  669

Query  367  AACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTA  426
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  670  AACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTA  729

Query  427  GAGTGTTTAAAGCAGGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTT  486
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  730  GAGTGTTTAAAGCAGGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTT  789

Query  487  CGATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCA  546
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  790  CGATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCA  849

Query  547  TTAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAA  606
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  850  TTAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAA  909

Query  607  GCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCA  666
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  910  GCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCA  969

Query  667  GATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTT  726
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  970  GATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTT  1029

Query  727  TTAATGACTCATTCGGCGCCTTACGGGAAA-CAAAGTGTTTGGGGTTC  773
            |||||||||||||||||||||||||||||| |||||||||||| |||
```

```
Sbjct  1030  TTAATGACTCATTCGGCGCCTTACGGGAAACCAAAGTGTTTGGG-TTC  1076
```

WF AM1

Rhizophagus irregularis partial 18S rRNA gene, strain DAOM181602, isolate spore 2, clone ES2_5_4

```
Query  7     TGGTGGT-ACGGGT-ACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGG  64
             ||||||| |||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  311   TGGTGGTAACGGGTAACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGG  370

Query  65    CTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTA  124
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  371   CTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTA  430

Query  125   GTGACAATAAATAACAATACGGGGTTCTTTAGGATCTCGTAATTGGAATGAGTACAATTT  184
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  431   GTGACAATAAATAACAATACGGGGTTCTTTAGGATCTCGTAATTGGAATGAGTACAATTT  490

Query  185   AAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC  244
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  491   AAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC  550

Query  245   AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGG  304
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  551   AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGG  610

Query  305   TTAGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCCTTATGA  364
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  611   TTAGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCCTTATGA  670

Query  365   ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  424
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  671   ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  730

Query  425   AGTGTTTAAAGCAAGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTTC  484
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  731  AGTGTTTAAAGCAAGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTTC  790

Query  485  GATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCAT  544
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  791  GATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCAT  850

Query  545  TAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAG  604
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  851  TAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAG  910

Query  605  CATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAG  664
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  911  CATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAG  970

Query  665  ATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTTT  724
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  971  ATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTTT  1030

Query  725  TAATGACTCATTCGGCGCCTTACGGGAA  752
            ||||||||||||||||||||||||||||
Sbjct  1031 TAATGACTCATTCGGCGCCTTACGGGAA  1058
```

WF AM1

Uncultured Claroideoglomus clone OTU150 18S ribosomal RNA gene, partial sequence

```
Query  15   GACGGGTGACGGAG-ATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCAC  73
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  62   GACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCAC  121

Query  74   ATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAA  133
            || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  122  ATTCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAA  181
```

```
Query  134  TAAATAACAATACCGGGCTCTTTCAGATCTCGTAATTGAAAGGAGTACAATTTAAATCCC  193
            |||||||||||| |||||||||| | |||||||||| || ||||||||||||||||||
Sbjct  182  TAAATAACAATACGGGGCTCTTTCGGGTCTCGTAATTGGAATGAGTACAATTTAAATCCC  241

Query  194  TTAACAAGGAACAATTGGAGGGCAAGTCGGGGGCCACCACCCGCGGTAATTCCAGCTCCA  253
            ||||| |||||||||||||||||||| || |||| || ||||||||||||||||||||
Sbjct  242  TTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCA  301

Query  254  ATAGCGAATATTAAAGTTGTTGCATTTAAAAAGCTCGAATTTGAATTTCGGGATTGACAC  313
            |||||| |||||||||||||||| |||||||||||| | ||||||||||||||||||||
Sbjct  302  ATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGATTGACAC  361

Query  314  GTGGGCCGTGCCTCAGGGGGGAAGAGCTGGCGTAGACTCTTCCTC-CCTTTCGGAACAAA  372
             | || ||||||| | |||| | || |||| |||| |  || ||| ||||  |||  ||
Sbjct  362  ATCGGTCGTGCCTTAAGGGGTATGAACTGGTGTAGTCAATTTCTCACCTTCTGGAG-AAC  420

Query  373  GGGCATTCCCTTAGGTGGGGGGCGCGGGTAGCCCGGACCTTTAGaaaaaaaaaaTTAGAT  432
            ||  || ||||||  |||| | |||||| | || |||||||||     ||||| |||||
Sbjct  421  GGCGATGCCCTTAATTGGGTGTCGCGGGGAACCAGGACCTTTACTTTGAAAAAGTTAGAG  480

Query  433  TGTTTAAAGCTAGCATTTGGCTTGATTACATGGGGATG-ATGAAATAAAATAGGACGCTC  491
            ||||||||||  |||||| |||||| |||||  | ||| |   ||||||||||||||
Sbjct  481  TGTTTAAAGCAGGCATTTTGCTTGAATACATTAGCATGGAAT-AATAAAATAGGACGGCA  539

Query  492  TTATTTCTGTTGTTGTTGGTTTCTAGGATCACCGAAATGATAAAAAGGGAGGGGTGGGGG  551
            | ||| || || |||||||||||||||||||||| |||||| || |||||  | ||||||
Sbjct  540  TGATT-CTATT-TTGTTGGTTTCTAGGATCACCGTAATGATTAATAGGGATAGTTGGGGG  597

Query  552  CAT  554
            |||
Sbjct  598  CAT  600
```

Peat AM3
Uncultured Rhizophagus partial 18S rRNA gene, clone SSA-8

```
Query  10   GGTGTGT-ACGGGTGACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGG  68
            ||||  ||  ||||||  ||||||||||||||||||||||||||||||||||||||||||
Sbjct  32   GGTG-GTAACGGGTAACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGG  90

Query  69   CTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTA  128
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  91   CTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTA  150

Query  129  GTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATTT  188
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  151  GTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATTT  210

Query  189  AAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCGGGGGCCAGCACCCGCGGTAATTCC  248
            |||||||||||||||||||||||||||||||||  ||  |||||||  ||||||||||||
Sbjct  211  AAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC  270

Query  249  AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGAATTTGAATTTCGGGG  308
            |||||||||||||||||||||||||||||||||||||||||||  |  ||||||||||||
Sbjct  271  AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGG  330

Query  309  TTAGTAGGTTGGTCATGCCTCTGGTATGTACGGGTCTCACTGATTCCTCCTTCCTGATGA  368
            ||||||||||||||||||||||||||||||  ||||||||||||||||||||||||||||
Sbjct  331  TTAGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCCTGATGA  390

Query  369  ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  428
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  391  ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  450

Query  429  AGTGTTTAAAGCAAGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTTC  488
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  451  AGTGTTTAAAGCAAGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTTC  510

Query  489  GATCCTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCAT  548
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  511  GATCCTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCAT  570

Query  549  TATTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAG  608
            || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  571  TAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAG  630

Query  609  CATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAG  668
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  631  CATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAG  690

Query  669  ATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTTT  728
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  691  ATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTTT  750

Query  729  TAATGACTCATTCGGCGCCTTACGGGAA  756
            ||||||||||||||||||||||||||||
```

Peat AM3

Uncultured Rhizophagus clone 2 18S ribosomal RNA gene, partial sequence

```
Query  7    GGTGG--ACGGGTAGACGGAGTAGTAGGGTCTCGACTCCGGAGAGGGAGCCTGAGAAACG  64
            |||||  ||||||| |||| ||  ||||| | |||| ||||||||||||||||||||||
Sbjct  37   GGTGGTAACGGGTA-ACGGGGTGTTAGGG-CACGACACCGGAGAGGGAGCCTGAGAAACG  94

Query  65   GCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGT  124
            |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  95   GCTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGT  154

Query  125  AGTGACAATAAATAACAATACCGGGTTCTTTCAGATCGCGAAATTGAAAGGATAACAATT  184
            |||||||||||||||||||| |||||||||| |||| || ||||| || ||  ||||||
Sbjct  155  AGTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATT  214

Query  185  TAAATCTCTTACCAAGAAACAATTGAAGGGCAAGTCGGGGGCCACCACCCGCGGTAATTC  244
            |||||||||||  | || |||||||| |||||||||| || |||| || ||||||||||||
```

```
Sbjct  215  TAAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTC  274

Query  245  CACCTCCAAAAGCGAAAATTAAATTTGTTGCATTTAAAAACCTCGAATTTGAATTTCGGG  304
            || ||||||| |||| | ||||||| |||||||| ||||||| |||| | ||||||||||||
Sbjct  275  CAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGG  334

Query  305  GTTAGTAGGTTGGTCATGCCTCTGGAATGTACGGGTCCCACTGATTCCTCCTTCCTGATG  364
            ||||||||||||||||||||||||| ||||||| |||| ||||||||||||||||||||||
Sbjct  335  GTTAGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCCTGATG  394

Query  365  AACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTA  424
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  395  AACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTA  454

Query  425  GAGTGTTTAAAGCAAGCTAACGCTTGAA-ACATTAGCATGGAATAATGAAATAGGACGTT  483
            |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  455  GAGTGTTTAAAGCAAGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTT  514

Query  484  CGATCCTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCA  543
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  515  CGATCCTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCA  574

Query  544  TTATTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAA  603
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  575  TTAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAA  634

Query  604  GCATTTGCCAAGGATGTTTTCATTAATCAACAACG-AAGTTAGGGGATCGAAGACAATCA  662
            |||||||||||||||||||||||||||| |||| |||||||||||||||||| ||||
Sbjct  635  GCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCA  694

Query  663  -ATACCGTCGTAGTCTTA-CCATAA-CTATGCCGAC-AGGGATCGGATGATGTTA-TTTT  717
             ||||||||||||||||| |||||| |||||||||| |||||||||||||||||| ||||
Sbjct  695  GATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTT  754

Query  718  T-AATGACTCAT-CGG-GCCT-ACGG-AAACCAA-GTGTT-GGGT  755
            | |||||||||| ||| |||| |||| ||||||| ||||| ||||
Sbjct  755  TTAATGACTCATTCGGCGCCTTACGGGAAACCAAAGTGTTTGGGT  799
```

Bark AM3
Paraglomus occultum isolate WDG40 18S ribosomal RNA gene, partial sequence

```
Query  31   TAGGGTCTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCACATCCAAGGAAGGCAGC  90
            |||||| |||||||||||||||||||||||| |||||||||||| ||||||||||
Sbjct  43   TAGGGT-TCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCC-AGGAAGGCAGC  100

Query  91   AGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACAATAAATAACAATATCGG  150
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  101  AGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACAATAAATAACAATACCGG  160

Query  151  GCTCTTATAGTTTGGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGATCCATTG  210
            |||| |  ||| |||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  161  GCTCCTCGAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGATCC-TTG  219

Query  211  GAGGGCAAGTCTGGTGCCCGCAGCCGCGGTAATTCCCGCTCCCATAGCGTATATTTAAGT  270
            |||||||||||||||||| |||||||||||||||| ||| |||||||||||| ||||||
Sbjct  220  GAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC-GCT-CCATAGCGTATA-TTAAGT  276

Query  271  TGTTGCAGTTAAAAAGCTCGTAATTGGACTTTGGGTTTGGTCGTCCGGTCCGCCTTT-GA  329
            |||||||||||||||||||||| |||||| ||||| | || || ||||||||||    |
Sbjct  277  TGTTGCAGTTAAAAAGCTCGTAGTTGGACCTTGGGCTGGGCCGGCCGGTCCGCCCCACGG  336

Query  330  CGAGCACCTGACGTCCTGTCCCTTTTGCCTACGTTACGAATCTGGCCTTATTTGGTTGGG  389
             | ||||| | | ||| ||||||| ||||  || | ||   ||| |||||  |||  |||
Sbjct  337  TGTGCACCGGCCTTCCCGTCCCTTCTGCCGGCGATGCGCTCCTGTCCTTAACTGGACGGG  396

Query  390  TCGTGCCTTCGGCGTTGTTACTTTGAAGAAATTATAGTGCTCTTAGACAAGCCTACGCTT  449
            |||||||| |||||  |||||||||||||||||| |||||||  || |||||| |||||
Sbjct  397  TCGTGCCTCCGGCGCCGTTACTTTGAAGAAATTAGAGTGCTCAAAG-CAAGCCCACGCTC  455

Query  450  TGTATACGTTAGCATGGGATAACATCACAGGATTTCGGTCCTATTGTGTTGGCCTTCGGG  509
            || |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  456  TGGATACATTAGCATGGGATAACATCACAGGATTTCGGTCCTATTGTGTTGGCCTTCGGG  515

Query  510  ATCA-GAGTAATGATTATCAGGGACAATCGGGGGCATTCTTATTTCACAGTCAGAGGAGA  568
            |||  ||||||||||||  |||||||  |||||||||| ||||||| |||||||| ||
```

```
Sbjct  516  ATCGGGAGTAATGATTAAGAGGGACAGCCGGGGGCATTCGTATTTCATAGTCAGAGGTGA  575

Query  569  AAATCTT-GGAGTTATGAAAGACGAACAACTGCGAAAACA-TTTGTCG-GGA-TGTTTT-  623
            || |||| ||| ||||||||||||||| ||||||||| || |||| |  ||| ||||||
Sbjct  576  AATTCTTGGGATTTATGAAAGACGAACCACTGCGAAAGCAGTTTGCCAAGGAATGTTTTT  635

Query  624  C-TT-AATCAAGAACGAAAG-TTGGGGG-C-TCGAA-GAC  657
            | || ||||| ||||||||| ||||||| | ||||| |||
Sbjct  636  CATTAAATCAGGAACGAAAGGTTGGGGGGCCTCGAAAGAC  675
```

Bark AM3

Funneliformis mosseae partial 18S rRNA gene, isolate Att109-25, clone pWD319-2-2

```
Query  12    GTGACGGGTGACGGAGTGTT-GGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACC  70
             || ||||||| |||| ||||| |||  |||  |||||||||||||||||||||| |||||||
Sbjct  338   GTAACGGGTAACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGGCTACC  397

Query  71    ACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGAC  130
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  398   ACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGAC  457

Query  131   AATAAATAACAATATCGGGCTCTTTTGGATCGGG-AATTGGAATGGATACCATCTTAATC  189
             |||||||||||||  ||| ||||||||||  | ||||||||||  ||| || | ||||
Sbjct  458   AATAAATAACAATACAGGGTTCTTTTGGATCTTGTAATTGGAATGAGTACAATTTAAATC  517

Query  190   CCCTAACCAGGAACCATTGGAGGGGAAGTCCGGGGGCCACCACCC-CGGTAATTCCCGCC  248
              | |||| |||||| ||||||||| ||||| || | ||| || || |||||||||| ||
Sbjct  518   TCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTG-CCAGCAGCCGCGGTAATTCCAGCT  576

Query  249   CCCATAACCTAAATTTAAATTGGTGGCA-TTAAAAAGCCCCGAATTGGACTTTGGGGATC  307
             || ||| | || ||| || ||| || || ||||||||| |  | ||| | ||| ||||||
Sbjct  577   CCAATAGCGTATATTAAAGTTGTTG-CAGTTAAAAAGCTCGTAGTTGAA-TTTCGGGATC  634

Query  308   AATATTTCGGCCATGCCGTTGGAATGC-CTGGTTACCTTTGATTTCTGCCCTTCTAACGA  366
             |||||||||| |||||||||| |||| || |||| | |||||||||  |||||||| ||
```

```
Sbjct  635   AATATTTCGGTCATGCCGTTGGTATGCACT-GTTATCATTGATTTCTCACCTTCTAAAGA  693

Query  367   ACC-TGGTGCCATTG-TTTGGTGTTAGGGGGAATTAGGACTGTTACCTTGAAATTAT-A-  422
             ||| |  |||||||  |||||||||| |||||||||||||||||||||||||  || |
Sbjct  694   ACCGTAATGCCATTAATTTGGTGTTACGGGGAATTAGGACTGTTACCTTGAAAAAATTAG  753

Query  423   A-TGCTGTAGCAGCCG-CTCACG-TGTATATACATC-GCGTG-A-TAACATCAAATAG-A  475
             | || | ||  ||| | |||||| |  | ||||||  || || | |||  | |||||| |
Sbjct  754   AGTGTT-TAA-AGCAGGCTCACGCTTGA-ATACATTAGCATGGAATAA--TGAAATAGGA  808

Query  476   CATCCGATT-TGTTT-GTC-GTCTCTATGATCGATGTGATGAT-AGGAG--ATAGTGGGC  529
             ||||||||| | ||| ||  || |||| ||||||||| ||||| |  ||  ||||| ||
Sbjct  809   CATCCGATTCTATTTTGTTGGTTTCTAGGATCGATGTAATGATTAATAGGGATAGTTGGG  868

Query  530   GTC-TTATTACTCA-TCGT-AGAGATGTA-TTGTAGTATT-AT-GACGACTA-CTGCTGC  582
             | | ||| || ||| | || |||| || | || | | ||| || || ||||| || ||||
Sbjct  869   GGCATTAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGC  928

Query  583   G----CATCTGTCACGATGATGTCATCATCA-TCAC-AACGATAG-TAGATGATCGACGA  635
             |    ||| ||||| |  |||||  |||| | |||  ||||| || |||  |||||| ||
Sbjct  929   GAAAGCATTTGTCAAG--GATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGA  986

Query  636   CGATC---TACTGTCGTACGTATCTCATCATCA-CGACTGACGACGATGCATCG-ATGAT  690
             |||||   ||| |||||| || | | | ||| | | | || |||| | | |||| |||||
Sbjct  987   CGATCAGATACCGTCGTA-GTCT-TAACCATAAACTA-TGCCGACTAGGGATCGGATGAT  1043

Query  691   GCTA-TTATG-ACTGACTCAT-CGGC  713
             | || || |  | |||||||| ||||
Sbjct  1044  GTTAATTTTTTAATGACTCATTCGGC  1069
```

WF AM3

Funneliformis mosseae isolate H4_P2 18S ribosomal RNA gene, partial sequence

```
Query  15   ACGGGTGACGGAGTGTTGGGTCTACGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCAC  74
            ||||||  ||||  |||||  ||  |  |||||  ||||||||||||||||||||||||||||||
Sbjct  31   ACGGGTAACGGGGTGTTAGGGC-ACGACACCGGAGAGGGAGCCTGAGAAACGGCTACCAC  89

Query  75   ATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAA  134
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  90   ATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAA  149

Query  135  TAAATAACAATACCGGGCTCTTTTAGATCG-GTAATTGGAAAGAATTCCATCTTAATCCC  193
            |||||||||||||  |||  ||||||  ||||  ||||||||||  ||  |  |  ||  |  ||||  |
Sbjct  150  TAAATAACAATACAGGGTTCTTTTGGATCTTGTAATTGGAATGAGTACAATTTAAATCTC  209

Query  194  CTAACCAAGAACCATTGGAAGGGCAGTTCGGGGGCCACCACCCCCGTTATTTCCGCCCCC  253
             ||||  |  ||||  ||||||  |||||  ||  ||  ||||  ||  ||  ||  ||  ||||  |  ||
Sbjct  210  TTAACGAGGAACAATTGGA-GGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCC  268

Query  254  AAAACCTAAAATTAAATTTGTTCCATTTAAAAACCCCTAATTTGAATTTCGGGATGGTTA  313
            ||  |  |   |  ||||||  |||||  ||  |||||||  |  |   |  ||||||||||||||   ||
Sbjct  269  AATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGATCAATA  328

Query  314  TTTCGGTCATGCCTTTGGAATGCCCGGTTTCCATTGATTTCTCCCCTTCTAACAAACC-T  372
            ||||||||||||  ||||  ||||  |  |||  ||||||||||||  ||||||||  ||||  |
Sbjct  329  TTTCGGTCATGCCGTTGGTATGCACTGTTATCATTGATTTCTCACCTTCTAAAGAACCGT  388

Query  373  GGCCCCTTTGTTTGGGGGTTGCGGGAAATTATTATTGTTTC-TAGAAAAA-TTA-A-TGC  428
                ||  ||  ||  ||  |||  ||||  |||||  |  ||||  |  |  ||||||  |||  |  ||
Sbjct  389  AATGCCATTAATTTGGTGTTACGGGGAATTAGGACTGTTACCTTGAAAAAATTAGAGTG-  447

Query  429  TCTTAAAGGCCGAC--ACTGCGTGTATATTTTA-CG-GGAAACATCAAAT-G-AGATCTG  482
            |  ||||||  |  |  |  ||  ||  ||  |||  |||  |  ||||  ||  ||||  |  |  |||
Sbjct  448  T-TTAAAG-CAGGCTCAC-GCTTGAATACATTAGCATGGAATAATGAAATAGGACATCC-  503

Query  483  TGATTCTGTGTTGTCGGTCTCGAGCATAGTAGTGATGA-TAGGAGGGATAGTGGGCGGCA  541
             ||||||  |  ||||  |||  ||  ||  ||  |  ||  ||||  ||  |||||||||  ||  ||||
```

```
Sbjct  504  -GATTCTATTTTGTTGGTTTCTAGGATCGATGTAATGATTAATAGGGATAGTTGGGGGCA  562

Query  542  -TATTATTCAGTCGT-AGAGATGATCTTCGTAGTATTATAGACGACTA-CTACTGCGAAT  598
             || |||||| | || |||| |||  ||| | |  |||| || ||||| ||||||||||
Sbjct  563  TTAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAA  622

Query  599  -CAGTTGCGAT-GATGTCTTCATCA-TCAC-AACGAT-GTGAGAGGATCGACGACGATCT  653
             || |||| |  ||||| ||||| | |||  |||||  || || ||||||| |||||||
Sbjct  623  GCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCA  682

Query  654  GATA-CGTCGTCGTCTCATC-ATC-ACGATGACGACGATG-ATCG-ATGATGT  701
             |||| |||||| |||| | | ||  || ||| |||| | | |||| |||||||
Sbjct  683  GATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGT  735
```

WF AM3

Rhizophagus irregularis partial 18S rRNA gene, strain DAOM181602, isolate spore 2, clone ES2_5_4

```
Query  13    GTGACGGGTGACGGAGTGTTAGGGTCACGACTCCGGAGAGGGAGCCTGAGAAACGGCTAC  72
             || |||||| |||| ||||||||| |||||| ||||||||||||||||||||||||||||
Sbjct  316   GTAACGGGTAACGGGGTGTTAGGG-CACGACACCGGAGAGGGAGCCTGAGAAACGGCTAC  374

Query  73    CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA  132
             |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  375   CACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA  434

Query  133   CAATAAATAACAATACCGGGTTCTTTAAGATCGCG-AATTGGAAAGGATACCAACTT-AA  190
             |||||||||||||||| |||||||||| |||| || |||||||| |  || ||| || ||
Sbjct  435   CAATAAATAACAATACGGGGTTCTTTAGGATCTCGTAATTGGAATGAGTA-CAATTTAAA  493

Query  191   TCCCCTAACCAAGAACCATTGGAAGGGCAGGCCGGGGGCCACCACCCCCGGAATTTCCGC  250
             || | |||| | |||| ||||| |||||| | | || |||| || || ||| | ||||
Sbjct  494   TCTCTTAACGAGGAACAATTGG-AGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAG  552

Query  251   CCCCAAAACCGAAAATTAAATTTGTTGCATTTAAAAACCCCGAATTTGAATTTCGGGTTT  310
```

```
            | |||| | || | |||||| |||||||| ||||||| | || | |||||||||||| ||
Sbjct  553  CTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGGTT  612

Query  311  AGTAGGTGGGCCATGCCTCTGGAATGTCCGGGTCCCACTGATTCCTCCTTCCTTATGAAC  370
            ||||||| || |||||||||||| |||| | |||| ||||||||||||||||||||||||
Sbjct  613  AGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCCTTATGAAC  672

Query  371  CGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTG-AAAAATTAGAG  429
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  673  CGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAGAG  732

Query  430  TGTTTAAAGCACGCTAACGCTTGAATACATTAGCATGAAA-AATGACATAAGACGTTCGA  488
            |||||||||||  |||||||||||||||||||||||||| || ||||| ||| |||||||||
Sbjct  733  TGTTTAAAGCAAGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTTCGA  792

Query  489  TCTTATGTTGTT-GTTTCTAGCATAGACGTAATGATTA-TAGGGATAGT-GGGGGCATTA  545
            ||||||  |||||  ||||||||  || ||||||||||||  |||||||||  ||||||||||
Sbjct  793  TCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCATTA  852

Query  546  -TATTC-ATTGTCAGAGGTGATC-TCGTAGATTTATAGACGACTAACTACTGCGAA-GCA  601
             |||||  |||||||||||||||   || | |||||||  || ||||||||||||||| |||
Sbjct  853  GTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCA  912

Query  602  TT-GCCGA-GATGTCTTCATCA-TCAC-AACGAT-G-TAGGTCATCGACGACGATCTACT  655
            || ||| | ||||| ||||| | |||  |||||  | ||||  ||||| |||||||   |
Sbjct  913  TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGAT  972

Query  656  ACCGTCGTA-TCTCATC-ATC-ACTATGACGACTA-GGATCGGATGATGATAATTATG-A  710
            ||||||||| ||| | | ||  |||||| |||||| |||||||||||| ||||| |  |
Sbjct  973  ACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTTTTA  1032

Query  711  -TGACTCAT-CGGCGC  724
             |||||||| ||||||
Sbjct  1033 ATGACTCATTCGGCGC  1048
```

Peat FS

Uncultured *Glomus* clone RAL71 18S ribosomal RNA gene, partial sequence

```
Query  13   ACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCACA  72
            ||||||  ||||  |  ||||||  |||  ||||||||||||||||||||  |||||||||
Sbjct  42   ACGGGTAACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGGCTACCACG  101

Query  73   TCCAAGGA-AGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACAA  131
            ||||||||  |||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  102  TCCAAGGAGGGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAA  161

Query  132  TAAATAACAATATCGGG--CTCTTAGAGTTTGGTAATTGGAATGAGTACAATCTAAATCC  189
            ||||||||||||  |||  || || || | | ||||||||||||||||||| ||||||
Sbjct  162  TAAATAACAATACGGGGTTCTTTTGGA-TCTCGTAATTGGAATGAGTACAATTTAAATCT  220

Query  190  CTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCC  249
            |||||||||||  | ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  221  CTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCC  280

Query  250  AATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGGTTTGGTC  309
            ||||||||||||||||||||||||||||||||||||||||||||||||||  ||| |
Sbjct  281  AATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGG-ATGGGC  339

Query  310  GTCA--GGTCCGCCT-TTGACGAGCACCTGACG-TCCTGTCCCTTTTGCCGACGTTACGA  365
              |||   ||| ||||   | || | | |||  |  | ||||||| || || || | ||
Sbjct  340  -TCATTTGTCGGCCTCACGGCGTG-AACTGGTGAGCTTGTCCCTTCTGTCGGCGATGCGC  397

Query  366  ATCTGGCCTTAATTGGTTGGGTCGTGCCTTCGGCGTTGTTACTTTGAAGAAATTAGAGTG  425
              ||||||||||  |||  ||||||||||| || || ||||||||||||||||||||||
Sbjct  398  TCCTGGCCTTAACTGGCCGGGTCGTGCCTCCGACGCTGTTACTTTGAAGAAATTAGAGTG  457

Query  426  CTCAAAGCAAGCCTACGCTTTGTATACGTTAGCATGGGATAACATCACAGGATTTCGGTC  485
            |||||||||||||| |||| || |||| |||||||||||||||||| ||||||||||||
Sbjct  458  CTCAAAGCAAGCCTTCGCTCTGAATACATTAGCATGGGATAACATCATAGGATTTCGGTC  517

Query  486  CTATTGTGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTCGT  545
            |||||  ||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  518  CTATTCTGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGGGCATTCGT  577

Query  546  ATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCATT  605
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  578  ATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACAACTGCGAAAGCATT  637

Query  606  TGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATAC  665
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  638  TGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATCAGATAC  697

Query  666  CGTCCTAGTCTCAACCATAAACGATGCCGACCAGGGATCA-ACGGATGTTGCTTTT--AG  722
            |||| |||||| |||||||||| |||||||| ||| |||| ||| |||||  ||||  |
Sbjct  698  CGTCGTAGTCTTAACCATAAACTATGCCGACTAGGAATCAGACG-ATGTTAATTTTTTAA  756

Query  723  -GACTCCGTTGGCACCTTTTGGGAAACCAAAGTGTTTGGGTTC  764
             |||||  ||||||||||  |||||||||||||||||||||||
Sbjct  757  TGACTCGTTTGGCACCTTACGGGAAACCAAAGTGTTTGGGTTC  799
```

Peat FS

Rhizophagus irregularis partial 18S rRNA gene, strain DAOM229456, isolate spore 3, clone 03S2_16_10

```
Query  7     TGGTGGT-ACGGGT-ACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGG  64
             ||||||| |||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  311   TGGTGGTAACGGGTAACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGG  370

Query  65    CTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTA  124
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  371   CTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTA  430

Query  125   GTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATTT  184
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  431   GTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATTT  490

Query  185   AAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC  244
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  491   AAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC  550

Query  245   AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGG  304
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  551   AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGG  610

Query  305   TTAGTAGGTTGGTCATGCCTCTGGTATGTACTGATCTCACTGATTCCTCCTTCCTTATGA  364
              |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  611   TTAGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCCTTATGA  670

Query  365   ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  424
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  671   ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  730

Query  425   AGTGTTTAAAGCAGGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTTC  484
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  731   AGTGTTTAAAGCAGGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTTC  790

Query  485   GATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCAT  544
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  791   GATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCAT  850

Query  545   TAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAG  604
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  851   TAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAG  910

Query  605   CATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAG  664
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  911   CATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAG  970

Query  665   ATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTTT  724
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  971   ATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTTT  1030

Query  725   TAATGACTCATTCGGCGCCTTACGGGAAA-CAAGGTGTTTG  764
              |||||||||||||||||||||||||||| ||| |||||||
Sbjct  1031  TAATGACTCATTCGGCGCCTTACGGGAAACCAAAGTGTTTG  1071
```

WF FS

Uncultured *Archaeospora* partial 18S rRNA gene, clone La-5

```
Query  14   TGACGGGTGACGGAG-ATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCA  72
            ||||||||||||||| ||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  40   TGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCA  99

Query  73   CATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACA  132
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  100  CATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACA  159

Query  133  ATAAATAACAATATCGGGCTC-TTACAGTTTGGGAATTGGAATGAGTACCATCTAAATCC  191
            |||||||||||||| ||||| | |||  || ||| ||||||||||||||| ||||||||||
Sbjct  160  ATAAATAACAATACCGGGCGCATTAGTGTCTGGTAATTGGAATGAGTACAATCTAAATCC  219

Query  192  CTTAACGAGGAACCATTGGAGGGCAAGTCTGGTGCCCGCAGCCGCGGTAATTCCCGCTCC  251
            |||||||||||||| ||||||||||||||||||||| |||||||||||||||| |||||
Sbjct  220  CTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCC  279

Query  252  CATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAATTGAATTTTGGGTTTGGCC  311
             |||||||||||| ||||||||||||||||||||| ||||||||||||||||||
Sbjct  280  AATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTTGGGTTTGGCC  339

Query  312  GAATGGTCCGCTTTTGGAAAGAACACGGGGTCG-TGGGGCCTTTGCCGATGGGGAAACCC  370
            || ||||||| | ||| || ||  || || ||| | ||||||   ||| | ||| ||
Sbjct  340  GATTGGTCCGTTCTTGTAACGAGTACTGG-TCGGTTGGGCCTCA-CCGCTTGGGGAAGGG  397

Query  371  TAGGGTCTTTTGTGGGG-GTTGGGCATTCCCCAGAACTTTTTCCTGGAAAAAA-TAGTGT  428
            ||  ||| ||| | ||| ||||  |    || ||| ||||  ||| ||||||| ||| ||
Sbjct  398  TAATGTCCTTTATTGGGTGTTGCTCGGAGCCAAGACCTTTA-CCTTGAAAAAATTAGAGT  456

Query  429  GGTTAAAACGCGGATATGCCGGAA-ACCTTTTACTGGGAAAAAATCAAA-AGAACCATGA  486
            | ||||| |  | ||||||| ||| || ||    ||| || || |  || || || | ||
Sbjct  457  GTTTAAAGCAGGCATATGCCAGAATACATTAGCATGG-AATAA-TAGAATAGGACAACGA  514

Query  487  Ctttttttttttt GGTTTCTAGGAACGGTGTAATGAATAACAAGGAATGTTGGGGGCATTC  546
              | | |||| |||||||||||||| || |||||||| ||| | |||  ||||||||||||
```

```
Sbjct  515  TTCTATTTTGTTGGTTTCTAGGACCGTTGTAATGATTAATAGGGATAGTTGGGGGCATTA  574

Query  547  ATATTTCATTTGGCCGAAGGTGGAATTCCCTGGAATTTATGTGAAGAACAACCTACTGCG  606
             ||||  ||  |||  |  ||  ||||  |||||   ||||  ||||   ||||||   |||  |||||||
Sbjct  575  GTATT-CAATTGTCAGA-GGTGAAATTCT-TGGA-TTTAC-TGAAGACTAAC-TACTGCG  628

Query  607  CAAAGCCATTTGGCCAGGGAAGGTTTTCTCTTTAATCTCAGAAACGAAAGGTGAGGGGCA  666
             |||||  |||||  |||  |||  |  |||||   ||  ||||   ||||  ||||||  |  |||||  |
Sbjct  629  -AAAGC-ATTTG-CCAAGGATG-TTTTCA-TT-AATCA-AGAA-CGAAAGTT-AGGGG-A  678

Query  667  TCGGAAAAAACGAATCAAATATACGGTCGTAAGTCCTTACCCAATAAACCTATTGCCGAAC  726
             |||  ||  |  |||  |||  |||   ||  |||||  |||  |||  |||  |||||  |||  |||||  |
Sbjct  679  TCG-AAGA-CGA-TCAGATAC-CG-TCGTA-GTC-TTAACCA-TAAAC-TAT-GCCGA-C  727

Query  727  TAGGGATCCGGGCGAATGGTTGTTCATTGCATCCGCTCCGCCCCTTATGG  776
             ||||||||  ||||||  ||  |   ||||  ||   |  |||||  ||  ||||||||
Sbjct  728  TAGGGATC-GGGCGA-TGTTATTTCAATGACT-CGCTCGGCACCTTATGG  774
```

WF FS

Rhizophagus irregularis partial 18S rRNA gene, strain DAOM229456, isolate spore 3, clone 03S2_16_10

```
Query  10    GTGTGT-ACGGGTGACGGAGTGTTAGGGTCACGACTCCGGAGAGGGAGCCTGAGAAACGG  68
             |||  ||  ||||||  ||||  |||||||||  ||||||  ||||||||||||||||||||||||
Sbjct  313   GTG-GTAACGGGTAACGGGGTGTTAGGG-CACGACACCGGAGAGGGAGCCTGAGAAACGG  370

Query  69    CTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTA  128
             ||||||||||||||||  ||||||||||||||||||||||||||||  ||||||||||||||
Sbjct  371   CTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTA  430

Query  129   GTGACAATAAATAACAATACCGGGCTCTTAAAGATCGGGAAATTGAAAGGAGTACAATTT  188
             ||||||||||||||||||  |||  ||||   ||||  |  |||||  ||  ||||||||||||
Sbjct  431   GTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATTT  490
```

```
Query  189   AAATCCCTTAACAAGAACCATTTGGAGGGCAAGTCGGGGGCCACCACCCGCGGTAATTCC  248
             |||||  ||||||  ||  |  ||  ||||||||||||||  ||  ||||  ||  ||||||||||||||
Sbjct  491   AAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC  550

Query  249   ACCTCCAATAGCGAATATTAAAGTTGTTGCATTTAAAAAGCTCGAATTTGAATTTCGGGT  308
             |  ||||||||||| ||||||||||||||||| ||||||||||| | ||||||||||||
Sbjct  551   AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGG  610

Query  309   TGAGTAGGTGGGCCATGCCTCTGGAATGCACGGGTCTCACTGATTCCTCCTTCCTTATGA  368
             |  |||||||  ||  ||||||||||  |||  ||  ||||||||||||||||||||||||||||||
Sbjct  611   TTAGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCCTTATGA  670

Query  369   ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAA-TTAG  427
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  671   ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  730

Query  428   AGGTGTTTAAAGCAGGCTACCGCTTGTATACATTAGCATGAAATAATG-AATAGGACGTT  486
             |  ||||||||||||||||  ||||||  ||||||||||||  |||||||  ||||||||||
Sbjct  731   A-GTGTTTAAAGCAGGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTT  789

Query  487   CGATCTTATGTTGTTGGTTTCTAG-AT-GACGTAATGA-TAATAGGGATAGT-GGGG-CA  541
             |||||||||  |||||||||||||  ||  ||||||||||  ||||||||||||  ||||  ||
Sbjct  790   CGATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCA  849

Query  542   TTA-TATTCA-TTG-CAGAG-TGAA-TTCGTGGATT-AT-G-AGACTAACTACTGC-AAA  592
             |||  ||||||  |||  |||||  ||||  |||  ||||||  ||  |  |||||||||||||  |||
Sbjct  850   TTAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAA  909

Query  593   GCATT-GC-A-G-ATGTCTCTAT-AATCAAGA-CGAT-GT-AGGG-A-CGA-GAC-ATCA  640
             |||||  ||  |  |  ||||  |  ||  ||||||||  |||  ||  ||||  |  |||  |||  ||||
Sbjct  910   GCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCA  969

Query  641   -ATAC-G-CGTAG-CT-A-C-ATCA-CTATGC--ACTAGG-ATCG-ATGATGT-AATTT  686
              ||||  |  |||||  ||  |  |  ||  |  ||||||  ||||||  ||||  |||||||  |||||
Sbjct  970   GATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTT  1028
```

Marigold Samples

Peat AM1

Claroideoglomus claroideum strain CB15045 18S ribosomal RNA gene, partial sequence

```
Query  16    ACGGGTGACGGGG-TGTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACA  74
             ||||||  ||||||   ||||||||||||||||||||||||||||||||||||||||||
Sbjct  341   ACGGGTAACGGGGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACA  400

Query  75    TCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAAT  134
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  401   TCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACAAT  460

Query  135   AAATAACAATACGGGGCTCTTTCAGGTCGCGTAATTGGAATGATTACAATTTAAATCCCT  194
             |||||||||||||||||||| ||||  |||||||||||||  ||||||||||||||||
Sbjct  461   AAATAACAATACGGGGCTCTTTCGGGTCTCGTAATTGGAATGAGTACAATTTAAATCCCT  520

Query  195   TAACAAGGAACAATTGGAGGGCAAGTCGGGTGCCAGCACCCGCGGTAATTCCAGCTCCAA  254
             ||||  ||||||||||||||||||||  ||||||||||  ||||||||||||||||||||
Sbjct  521   TAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAA  580

Query  255   TAGCGTATATTAAATTTGTTGCATTTAAAAAGCTCGTATTTGAATTTCGGGATTGACACG  314
             ||||||||||||| ||||||||  |||||||||||||  ||||||||||||||||||||
Sbjct  581   TAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGATTGACACA  640

Query  315   TCGGTCGTGCCTTAGGGGGGAAGAGCTGGCGTAGTCTCCTCCTCCCCTTCGGAAGAAAGG  374
             |||||||||||||| ||||  |  ||  ||||  ||||||   |  |  |  |||||  |  ||||  |
Sbjct  641   TCGGTCGTGCCTTAAGGGGTATGAACTGGTGTAGTCAATTTCCCACCTTCTGGAGAACCG  700

Query  375   GCATTCCCTTAGTTgggggggggggggTAACCCGGACCTTTTCTTTaaaaaaaaTAGAGTG  434
               ||  ||||||  |||||  |   |  |||  ||||  ||||||||  ||||  ||||||  |||||||
Sbjct  701   CGATGCCCTTAATTGGGTGTCGCGGGGAACCAGGACCTTTACTTTGAAAAAATTAGAGTG  760

Query  435   TTTAAAGCAGGCATTTTGCTTGATTACATTAG-ATGGAATAATAAAATAGGACGGCATGA  493
             ||||||||||||||||||||||  ||||||||  |||||||||||||||||||||||||||
Sbjct  761   TTTAAAGCAGGCATTTTGCTTGAATACATTAGCATGGAATAATAAAATAGGACGGCATGA  820

Query  494   TTCTATTTTGTTGGTTTCTAGGATCACCGAAATGATTAAAAGGGAGGGTTGGGGGCATTA  553
```

```
            |||||||||||||||||||||||||||| ||||||||| |||||  ||||||||||||
Sbjct  821  TTCTATTTTGTTGGTTTCTAGGATCACCGTAATGATTAATAGGGATAGTTGGGGGCATTA  880

Query  554  CTATTCAATTGTCTGATGTGAAATTCTTGAATTAAAAGAAAACAAACTACTGCTGTTCC-  612
             ||||||||||||  || ||||||||||| ||| |  ||| || |||||||||     |
Sbjct  881  GTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAGCA  940

Query  613  TTCGCCCCCGATGTTTCCACTAAACAAAAAGTAAAGTTAGCGAACCGAT-ACGATCATAT  671
             || |||   ||||||| || ||| ||| ||  |||||||| | | |||  ||||||| ||
Sbjct  941  TTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGAT  1000

Query  672  ACTGTCCTACTCTTACCC-TAGCACCATGCCCACGACGAATCACACTATTTTAATTTTTT  730
             || ||| || ||||| || ||  || ||||| || | |||||| || || ||||||||||
Sbjct  1001 ACCGTCGTAGTCTTAACCATAA-ACTATGCCGACTAGGAATCAGACGATGTTAATTTTTT  1059

Query  731  AATGACTCGT  740
             ||||||||||
Sbjct  1060 AATGACTCGT  1069
```

WF AM1

Paraglomus occultum isolate WDG40 18S ribosomal RNA gene, partial sequence

```
Query  29   GATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCA  88
            |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct  40   GATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCC-AGGAAGGCA  98

Query  89   GCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACAATAAATAACAATACC  148
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  99   GCAGGCGCGCAAATTACCCAATCCTGACACGGGGAGGTAGTGACAATAAATAACAATACC  158

Query  149  GGGCTCAAACGAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGATCCA  208
            ||||||   ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  159  GGGCTCCT-CGAGTCTGGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAGGATCC-  216
```

```
Query  209  TTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTTA  268
            ||||||||||||||||||||||||||||||||||||||| |||||| ||||||||| |||
Sbjct  217  TTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC-GCTCCA-TAGCGTATA-TTA  273

Query  269  AGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTTGGGTTGGGTCGGCCGGTCCGCCTTT  328
            |||||||||||||||||||||||||||||| ||||| |||| ||||||||||||
Sbjct  274  AGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACCTTGGGCTGGGCCGGCCGGTCCGCCCCA  333

Query  329  AGGTGTGCACCGGTTTACTCGTCCCTTCTGTCGGCGATGCGCTCCTGGCCTTAATTGGCC  388
             ||||||||||||  | | |||||||||| |||||||||||||||| |||||| ||| |
Sbjct  334  CGGTGTGCACCGGCCTTCCCGTCCCTTCTGCCGGCGATGCGCTCCTGTCCTTAACTGGAC  393

Query  389  GGGTCGTGCCTCCGGCGTTGTTACTTTGAAGAAATTAGAGTGCTCAAAGCAAGCCTACGC  448
            ||||||||||||||||  |||||||||||||||||||||||||||||||||| ||||
Sbjct  394  GGGTCGTGCCTCCGGCGCCGTTACTTTGAAGAAATTAGAGTGCTCAAAGCAAGCCCACGC  453

Query  449  TCTGTATACATTAGCATGGGATAACATCATAGGATTTCGGTCCTATTACGTTGGCCTTCG  508
            |||| ||||||||||||||||||||| ||||||||||||||||  ||||||||||
Sbjct  454  TCTGGATACATTAGCATGGGATAACATCACAGGATTTCGGTCCTATTGTGTTGGCCTTCG  513

Query  509  GGATC-GGAGTAATGATTAACAGGGACAGTCGGGGGCATTCGTATTTCATAGTCAGAGGT  567
            ||||| |||||||||||||| |||||||| |||||||||||||||||||||||||||||
Sbjct  514  GGATCGGGAGTAATGATTAAGAGGGACAGCCGGGGGCATTCGTATTTCATAGTCAGAGGT  573

Query  568  GAAATTCTT-GGATTTATGAAAGACGAACAACTGCGAAAGCA-TTTGCCAAGGA-TGTTT  624
            ||||||||| |||||||||||||||||| |||||||||||| |||||||||| |||||
Sbjct  574  GAAATTCTTGGGATTTATGAAAGACGAACCACTGCGAAAGCAGTTTGCCAAGGAATGTTT  633

Query  625  T-CATT-AATCAAGAACGAAAG-TTGGGGG-C-TCGAA-GAC  660
            | |||| ||||| ||||||||| ||||||| | ||||| |||
Sbjct  634  TTCATTAAATCAGGAACGAAAGGTTGGGGGGCCTCGAAAGAC  675
```

WF AM1

Rhizophagus irregularis partial 18S rRNA gene, strain DAOM229456, isolate spore 3, clone 03S2_16_10

```
Query  6     TGATGGT-ACGGGT-ACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGG  63
             || |||| |||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  311   TGGTGGTAACGGGTAACGGGGTGTTAGGGCACGACACCGGAGAGGGAGCCTGAGAAACGG  370

Query  64    CTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTA  123
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  371   CTACCACATCCAAGGATGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTA  430

Query  124   GTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATTT  183
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  431   GTGACAATAAATAACAATACGGGGTTCTTTCGGATCTCGTAATTGGAATGAGTACAATTT  490

Query  184   AAATCTCTTAACGAGG-ACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC  242
             |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  491   AAATCTCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC  550

Query  243   AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGG  302
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  551   AGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAATTTCGGGG  610

Query  303   TTAGTAGGTTGGTCATGCCTCTGGTATGTACTGATCTCACTGATTCCTCCTTCCTTATGA  362
             |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  611   TTAGTAGGTTGGTCATGCCTCTGGTATGTACTGGTCTCACTGATTCCTCCTTCCTTATGA  670

Query  363   ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  422
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  671   ACCGTAATGCCATTAATTTGGTGTTGCGGGGAATTTGGACTGTTACTTTGAAAAAATTAG  730

Query  423   AGTGTTTAAAGCAGGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTTC  482
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  731   AGTGTTTAAAGCAGGCTAACGCTTGAATACATTAGCATGGAATAATGAAATAGGACGTTC  790

Query  483   GATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCAT  542
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  791   GATCTTATTTTGTTGGTTTCTAGGATTGACGTAATGATTAATAGGGATAGTTGGGGGCAT  850

Query  543   TAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAG  602
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  851   TAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTGCGAAAG  910

Query  603   CATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAG  662
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  911   CATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAG  970

Query  663   ATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTTT  722
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  971   ATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAGGGATCGGATGATGTTAATTTTT  1030

Query  723   TAATGACTCATTCGGCGCCTTACGGGAAACCAATGTTGTTTTGGGT  768
              |||||||||||||||||||||||||||||| || |||| ||||
Sbjct  1031  TAATGACTCATTCGGCGCCTTACGGGAAACCAAAGT-GTTT-GGGT  1074
```