Linking Above and Below-Ground Interactions in Agro-Ecosystems: An Ecological Network Approach

Bombus terrestris Audax visiting a strawberry flower

Peter Orrell

BSc (Hons) Environmental Science, Manchester Metropolitan University

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Abstract

Belowground microbial communities, such as arbuscular mycorrhizal fungi (AMF), may modify plant reproductive traits, although little is known about how this might then influence pollinator behaviour. This is important as pollinators provide an ecosystem service by contributing towards agricultural production. AMF also provide an ecosystem service by assisting plants with increased access to nutrients and water resources, thereby influencing yields. However, few studies have examined the combined effects of how AMF interact with crop cultivars to alter plant reproductive traits, pollination processes, and ultimately crop yield. Furthermore, the importance of both AMF and pollinators for human perceived crop quality has not been investigated.

In this thesis, I examine the influence of manipulating AMF communities on plant-pollinator interactions, and the role of crop cultivars in mediating these effects, by growing three strawberry (*Fragaria × ananassa*) cultivars inoculated with four AMF communities, and measuring strawberry yield and quality (determined through human taste tests) in two 2-year experiments.

The first experiment was conducted under greenhouse conditions and I found that pollen foraging visits by bumblebees (*Bombus terrestris* Audax) were influenced by both AMF community and strawberry cultivar, whereas nectar foraging visits were only influenced by AMF community. AMF community influenced strawberry yield, without any changes in fruit quality, and effects were consistent across each strawberry cultivar, while AMF community and strawberry cultivar interacted to influence strawberry appearance.

The second experiment was similar to the greenhouse experiment but repeated under field conditions to examine the effects on the naturally occurring pollinator community. Here, I found that while AMF community may influence the visitation of some pollinator taxa, the wild pollinator community provided a high degree of functional redundancy, and strawberry yield was influenced in the same manner as in the greenhouse experiment when plants were exposed to the highly efficient pollinators used in commercial production.

The potential to utilise the above and below-ground interaction data to improve yields relies on the opinions of end users. I conducted a socio-economic analysis of growers' and scientists' perceptions, which showed that key stakeholders believe that interactions between above- and below-ground organisms should be harnessed to improve crop production.

These results show that manipulating a below-ground mutualistic community has effects that cascade through the network to influence plant-pollinator interactions, and alters strawberry yield without loss in quality, with largely predictable outcomes across multiple strawberry cultivars. The interdisciplinary nature of this research revealed that stakeholders believe AMF should be used to improve strawberry production. Understanding the dynamics of these interactions may form part of a toolset for sustainable increases in food security, as well as helping to gain a deeper understanding of the underlying biology that influences ecological networks.

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‘Fish bowl’ in V Block who have been great office mates, have been wonderful to work with throughout my PhD, and were always there to help when advice was needed, as well as sharing interesting ideas and techniques from each other’s projects.

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Declaration of Authorship

I declare that the work contained within this thesis is intellectually my own. Contributions were made to each chapter and the project overall by my supervisors (Dr Alison Bennett, Dr Darren Evans, and Prof Maria Nijnik), who co-designed the project plan and experimental design, as well as reviewing and providing comments on drafts of chapters in the thesis. The greenhouse and field experiment (Chapters 2, 3, and 4) were co-designed with Alison Bennett and Darren Evans, and the social analysis (Chapter 5) was co-designed with Maria Nijnik. Comments on drafts of all chapters were provided by Alison Bennett and Darren Evans. Maria Nijnik provided comments on Chapter 5. Statistical analysis, first drafts, and subsequent edits for all were completed by myself. Philip Smith proofread the thesis, and provided editorial comments on the final draft.

In Chapter 2, during the first year of the greenhouse experiment I worked with Lorna Blackmore during her MSc project, where as one part of the experiment we collaborated to assess the pollen and nectar produced by plants. I designed and lead the experiment, and Lorna refined the methods for pollen sampling, and collected pollen / nectar samples. I completed the analysis, interpreted the results, as well as writing and redrafting the chapter. Lorna also measured pollinator visitation, but utilised timed observations rather than the transects I employed (Lorna’s visitation data is not included in this thesis).

For the assessment of strawberry yield in the greenhouse and field experiments (Chapters 3 and 4), Sandra Caul and undergraduate students in Alison Bennett’s lab group at The James Hutton Institute assisted with the picking and weighing of strawberries, as well as assisting in the final experimental harvest (harvesting plants for assessments of plant biomass), and making slides of roots. Sandra Caul completed the microscopy to measure AMF colonisation rates in Chapter 3. I completed the analysis, interpreted results, wrote and redrafted all results where others assisted in data collection.

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Chapter 1: General Introduction

1.1 Linking above- and belowground interactions in agro-ecosystems

Ecological communities are formed by the species that are present within a habitat. Plants form a range of positive and negative relationships with above- and below-ground communities, which interact via the host plant. For example, belowground mutualists are able to modulate plant traits (Koide, 2000; van Dam and Heil, 2011), which can influence interactions between plants and aboveground insects (Pineda *et al.*, 2010). However, the majority of studies to date have examined belowground (microbe-plant, and root herbivore-plant) and aboveground (plant-pollinator, plant-herbivore, and predator-prey) interactions in isolation. Studies in plant-mediated interactions between above- and belowground communities have largely focused on aboveground antagonists, and the influence of above-belowground interactions on plant mutualists is poorly understood.

The outcome of these interactions can influence the provision of ecosystem services (Wardle *et al.*, 2004), and controlling interactions between above and belowground species has been proposed as one method of increasing our toolset to improve agricultural production systems (Orrell and Bennett, 2013). With a rapidly rising population (Cohen, 2003; Gerland et al., 2014), and concerns over peak phosphorus (Cordell and White, 2011), being able to meet the needs of a growing population and provide food security with sustainable production systems is a growing concern (Godfray *et al.*, 2010). However, to date studies have not examined functional consequences of above-below-ground interactions on crop yields.

Arbuscular mycorrhizal fungi (AMF) are soil dwelling fungi that act as a secondary root system for 80% of plant species (92% of families), providing increased uptake of nutrients and water (Smith and Read, 2008). However, as well as influencing overall plant growth through the improved provision of nutrients and water, AMF are able to influence a range of other factors, such as plant architecture (Koide *et al.*, 1994), pest and pathogen defence (Gehring and Bennett, 2009), drought tolerance (Al-Karaki *et al.*, 2004), gene expression (Hause *et al.*, 2002), resistance to contaminated soils (Diaz *et al.*, 1996), and can influence ecosystem processes (Wardle *et al.*, 2004).

By altering plant traits, AMF can influence interactions between plants and insects. For example, AMF induced changes in plant defensive compounds may increase defence against generalist chewing herbivores (Gehring and Bennett, 2009; Koricheva *et al.*, 2009) and reduce the negative effects of pathogens (Borowicz, 2001), while changes in the release of volatiles may attract enemies of specialist sucking herbivores (Rasmann *et al.*, 2017; Gange *et al.*, 2003). In addition, alterations to plant reproductive traits can influence the frequency of plant-pollinator interactions (Barber and Soper Gorden, 2014). However, there are gaps in our knowledge, for example, we do not know if AMF can influence the foraging behaviour of pollinators, how different entire AMF communities vary in influence, or if AMF induced changes in plant-pollinator have functional consequences for crop yields.

1.2 The importance of pollinators on crop production

Pollinators provide an important ecosystem service by contributing to crop yields and seed set in wild plants. 87.5% of angiosperms, including 87 of the 124 world's most important crop species are either completely or partially dependent on pollination by animals, which accounts for approximately 35% of global crop production (Klein *et al.*, 2007; Ollerton *et al.*, 2011). Without pollination, crop yields would fall below current levels of consumption (Gallai *et al.*, 2009). In addition, insect pollination can improve the quality of crop yields by reducing malformations, improving fruit traits such as colour and firmness (Klatt *et al.*, 2014). By improving crop yields, pollination is worth between £430-510 million as an ecosystem service for commercial agriculture in the UK, which accounts for approximately 8% of the total value of crops produced (NEA, 2011; Breeze *et al.*, 2012). Furthermore, 20% of land dedicated to commercial crop production in the UK is comprised of pollinator dependent crops, which has increased by 38% since 1989 (NEA, 2011).Changes in plant reproductive traits can influence plant-pollinator interactions (Buchmann and Cane, 1989; Harder, 1990; Real and Rathcke, 1991; Poulton *et al.*, 2001a; Buide, 2006; Cahill *et al.*, 2008; Soto *et al.*, 2013), and by influencing these traits, belowground organisms can influence how plants interact with pollinators (Barber and Soper Gorden, 2014). As such, belowground organisms may play an indirectly influence an important aboveground ecosystem service.

The ability of wild pollinators to fulfil crop pollination requirements has been shown to be reduced in intensive production systems (Kremen *et al.*, 2002), and with recent declines in wild and domesticated pollinators (Biesmeijer *et al.*, 2006; Colla and Packer, 2008), understanding how above- and belowground organisms interact is necessary in order to exploit the ecosystem services provided by them and improve crop yields.

1.3 The influence of AMF on plant reproductive traits

AMF can influence plant reproductive traits, such as floral display (Bryla and Koide, 1990; Stanley *et al.*, 1993; Lu and Koide, 1994; Koide, 2000; Pendleton, 2000; Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Poulton *et al.*, 2002; Scagel, 2004; Gange and Smith, 2005; Wolfe *et al.*, 2005; Perner *et al.*, 2007; Varga and Kytöviita, 2010b; Aguilar-Chama and Guevara, 2012), as well as specific male (pollen) (Lau *et al.*, 1995; Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Poulton *et al.*, 2002; Kiers *et al.*, 2010), and female (nectar) (Gange and Smith, 2005; Kiers *et al.*, 2010; Barber *et al.*, 2013b) traits. Below we review the current evidence of how AMF can influence each of these factors.

1.3.1 The influence of AMF on floral display

Floral display plays an important role in pollinator attraction, and while the majority of studies illustrate that AMF can increase the number of flowers a plant produces (Bryla and Koide, 1990; Stanley *et al.*, 1993; Lu and Koide, 1994; Koide, 2000; Pendleton, 2000; Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Poulton *et al.*, 2002; Scagel, 2004; Gange and Smith, 2005; Wolfe *et al.*, 2005; Perner *et al.*, 2007; Sudova, 2009; Varga and Kytöviita, 2010b; Becklin *et al.*, 2011), two studies have shown that AMF can reduce flower size (Ganade and Brown, 1997; Cahill *et al.*, 2008). However, both studies that showed a reduction in flower size utilised fungicides to supress the AMF community, which influence the entire fungal community. As such, 'AMF treatments' in these studies also include pathogenic fungi, which may have been responsible for the reduction in flower number. These influences also depend on the plant species, for example, Gange & Smith (2005) found that AMF differentially influenced both the number and size of flowers in three plant species: *Centaurea cyanus* (Cornflower), *Tagetes erecta* (Mexican marigold), and *Tagetes patula*(French marigold). AMF inoculation increased the number of flowers in *C. cyanus* and *T. patula*, but not in *T. erecta*. Conversely, AMF did not influence the flower size of *C. cyanus*, but increased flower size in *T. patula* and *T. erecta*. Thus AMF can influence the number and size of flowers plants produce, but this varies with plant species.

1.3.2 The influence of AMF on male reproductive traits

Studies have shown that AMF can influence a range of male reproductive factors, such as the quantity of pollen produced per flower, the size of pollen grains, and pollen nutritional content. For example, Lau et al. (1995) found that in *Cucurbita pepo* (Courgette), AMF significantly increased the size of pollen grains. Two studies by Poulton et al. (2001b; 2002), found that in *Lycopersicon esculentum* (Tomato), AMF and P fertilisation similarly increased pollen production, indicating that AMF may be able to improve male reproductive traits through increased plant nutrition. Furthermore, in an experiment where Varga & Kytöviita (2010) manipulated *Geranium sylvaticum* (Wood cranesbill), AMF produced significantly more functional stamens, increasing the quantity of pollen per flower. However, AMF does not always increase pollen production, for example, Kiers et al., (2010), found a significant reduction in pollen production in *Cucumis sativus* (Cucumber) in mycorrhizal plants. These results suggest that AMF may increase pollen production through the facilitation of P uptake, however, the traits of the individual plant species can influence the direction of the interaction.

1.3.3 The influence of AMF on female reproductive traits

We expect AMF induced changes in nectar production to result from the influence of AMF on water and carbon within the plant. For example, AMF can influence water uptake (Smith and Read, 2008), and water use efficiency (Kaya *et al.*, 2003), which may lead to an increase in the amount of water a plant has available for nectar production (nectar quantity). However, AMF use up to 20% of plant photosynthates (Jakobsen and Rosendahl, 1990), which may reduce the amount of carbohydrates available for nectar sugar content (nectar quality), but can also increase the photosynthetic rate of the plant, potentially increasing available carbohydrates. Gange & Smith (2005) found that AMF increased the quantity of nectar secreted in *T. patula* and *T. erecta*, but not in *C. cyanus*. and increased the sugar content in *T. erecta*. In addition, Kiers et al. (2010) found that in *Cucumis sativus* AMF increased the quantity of nectar produced, but this depended on P fertilisation, with AMF providing the greatest benefit to plants with supplemental P. As such, AMF has the potential to influence female reproductive traits, but these effects depend on the plant species and its growing conditions, such as light and temperature levels, as well as water and nutrient availability.

1.4 The response of pollinators to changes in plant reproductive traits

Changes in plant reproductive traits have an important role in determining plant-pollinator interactions. The frequency and efficiency of pollination services has been shown to be influenced by changes in floral rewards, such as the quantity and nutritional quality (amino acid content) of pollen and the quantity and nutritional quality (sugar content) of nectar, the number of flowers, and floral display (Buchmann and Cane, 1989; Harder, 1990; Real and Rathcke, 1991; Ashman *et al.*, 2000; Poulton *et al.*, 2001a; Buide, 2006; Cahill *et al.*, 2008; Willmer, 2011; Soto *et al.*, 2013).

Pollinator visitation rates do not only depend on the number of flowers available to pollinators as other plant reproductive traits play important roles. For example, while some studies have found that plants with a higher number of open flowers have significantly more visits from pollinators (Buide, 2006), other studies have found that pollinator visitation can be determined not by the number of flowers a plant has but rather by the quantity of nectar per flower (Real and Rathcke, 1991). Bees are thought to be able to detect nectar resources remotely and anthers may act as visual cues for male rewards, both influencing attraction (Ashman *et al.*, 2000). If AMF can influence the level of these rewards, pollinator visitation, and in turn crop yield, could be increased.

However, the promotion of a single floral resource may not increase pollinator visitation, and in gynodioecious plants, such as *Fragaria virginiana* (Virginia strawberry), pollinators preferentially visit flowers that offer adequate male and female rewards (Ashman *et al.*, 2000; Asikainen and Mutikainen, 2005). For example, Cresswell and Robertson (1994) found that bumblebees prefer *Campanula rotundifolia* (Harebell) flowers displaying both pollen and nectar when overall pollen availability in floral patches was high, but selectively visited flowers displaying relatively high levels of pollen and a lack of nectar (male phase) when pollen availability was lower. As such, in crop systems dominated by a single plant species, the influences of AMF or the inherent traits of crop cultivars on floral resources could play important roles in determining which plants are most frequently visited by pollinators.

1.5 The influence of AMF on plant-pollinator interactions

As AMF can influence plant reproductive traits, and pollinators respond to changes in these rewards, AMF can indirectly influence plant-pollinator interactions, although there are a limited number of studies to date that examine AMF-plant-pollinator interactions. For example, Wolfe et al. (2005) found that pollinator visitation increased with AMF inoculation in *Chamerion angustifolium* (Fireweed) in the field, however only visits by bumblebees and honeybees were recorded. Cahill et al. (2008) support this result by proposing that AMF suppression reduces pollinator visitation, and alters the visiting community to be dominated by small bees and Diptera over larger bees in six grassland plant species (*Achillea millefolium* (Yarrow), *Aster laevis* (Smooth blue aster), *Campanula rotundifolia* (Harebell), *Cerastium arvense* (Field mouse-ear), *Erigeron philadelphicus* (Common fleabane), and *Solidago missouriensis* (Missouri goldenrod). However, this study utilised a fungicide to inhibit AMF, and these influences may have resulted from suppressing other soil organisms, such as pathogenic fungi or nematodes. In addition, these influences may affect pollinator taxa differently. For example, Gange and Smith (2005) found that AMF-plant-pollinator interactions depend upon the plant species, and that pollinator taxa responded differently to plants associated with AMF, increasing Hymenoptera vistis to *C. cyanus* and *T.erecta*, but only increasing Diptera visits in *C. cyanus*.

AMF species can also influence plant-pollinator interactions. For example, Varga and Kytöviita (2010) found that in *Geranium sylvaticum* (Wood cranesbill) *Glomus hoi* reduced visits from Hymenoptera, but *Glomus claroideum* did not. Furthermore, Barber et al., (2013a) examined the influence of several AMF inocula on pollinator visitation in *Cucumis sativus* (Cucumber). Although there was no overall difference in total visitation rates, honeybees preferred plants without AMF, bumblebee visits were higher on plants with *Rhizophagus irregularis*, and Lepidoptera preferentially visited plants with either *Glomus clarum* or a mix of three AMF species. However, previous studies that have examined AMF-plant-pollinator interactions have not measured the influence of AMF on pollinator foraging behaviour, if these influences are mediated by the traits of crop cultivars, or the influences of whole AMF communities that plants associate with in the field.

Determining the influence of AMF on both domesticated and wild pollinators is particularly important, as changes in these visits can have important consequences for crop yields. For example, the foraging behaviour of different pollinators can influence the quantity and quality of crop yields (Chagnon *et al.*, 1993), however, to date it remains untested if AMF can influence pollinator behaviour. In field grown crops that are exposed to wild pollinators, visiting pollinators have varying levels of efficiency, with Hymenoptera the most efficient (Schemske and Horvitz, 1984; Fishbein and Venable, 1996; Bingham and Orthner, 1998; Ivey *et al.*, 2003). As such, increasing these visits could potentially improve yields, however, other studies have shown that there is a high degree of functional redundancy in wild pollinator communities (Garibaldi *et al.*, 2013), and *Syrphidae* and other Diptera may be able to fulfil crop pollination requirements (Orford *et al.*, 2015). Although AMF have been shown to differentially influence the visitation of pollinator taxa, it remains to be tested if these changes have functional consequences for crop yields.

1.5.1 The potential for crop cultivars to mediate plant-pollinator interactions

While the influence of AMF on plant-pollinator visitation depends upon the species present in both the above- and belowground communities, these interactions may also be mediated by crop cultivar. Although there are few studies that measure the influences of multiple crop cultivars on AMF induced changes in plant reproductive traits and pollinator visitation, Lau *et al.* (1995) found that cultivars of *Cucurbita pepo* (courgette) differed in pollen production and pollen grain size, and interacted with AMF to influence flower production differently for each cultivar. In addition, Poulton *et al.* (2001b) found that AMF influenced pollen production in one cultivar of *Lycopersicon esculentum* (tomato), but not in another, and in a later study found that AMF influenced pollinator visitation in only one tomato phenotype (Poulton *et al.*, 2001a). However, as AMF-plant-pollinator interactions is an emerging field with relatively few (six) studies, to date it remains to be tested if crop cultivars can mediate the influences of AMF on pollinator visitation.

1.5.2 The potential for AMF to influence ecological networks

Ecological networks (Memmott, 1999) are increasingly being used to quantitatively describe interactions between communities of species (Bascompte and Jordano, 2013). The study of ecological networks has been used to answer fundamental questions about how changes in mutualistic interactions between communities can influence biodiversity, co-evolution, and ecosystem function, by examining the impacts of habitat loss, species extinctions, invasive species, climate change (Bascompte and Jordano, 2013). However, these networks tend to focus on bipartite interactions between species such as plants and pollinators, and it is not understood how factors such as the concurrent interaction of plants with other organisms (such as AMF) or the inherent traits of crop cultivars can influence interactions within these networks. Including these aspects in studies of species interactions may offer the opportunity to increase the resolution of ecological networks and the dynamics that influence pollinator interactions within a plant species, helping us to further understand factors that influence seed set in wild species and crop production in agriculture.

1.6 Utilising strawberry as a model system to test plant-pollinator interactions

Strawberry (*Fragaria x ananassa*) provides an ideal model system to study AMF-plant-pollinator interactions for several reasons. In order to produce perfectly shaped strawberries and reach the highest commercial value, strawberries require all stigmas to be pollinated (Free, 1993). Stigmas that are not pollinated will not develop, impairing the production of the strawberry, and resulting in misshapen fruit. Additionally, Klatt *et al.* (2014) found bee pollination increased yield, fruit shape, redness, firmness, sugar-acid ratios, shelf life, and commercial grades over wind pollination and selfing. However, strawberry pollination does not rely solely on attaining a sufficient number of pollinator visits, and Chagnon *et al.* (1993) found that when strawberries are visited by both wild pollinators and honeybees, perfectly shaped fruits were more likely to develop. As such, insect pollination is a vital component of determining the quantity and quality of strawberry yields, and insufficient pollination results in a loss in yield. Furthermore, wild pollinators have an important impact on strawberry yields, and if the wild pollination community cannot fulfil functional complementarity in the absence of hives of Hymenoptera, yields are reduced.

In addition, examining factors that influence the quantity and quality of strawberry yields is important, as strawberry is a commercially important crop globally (FAOSTAT, 2017), makes an important contribution to human nutrition (Giampieri *et al.*, 2012), and production is rapidly increasing (DEFRA, 2008; DEFRA, 2011; DEFRA, 2016). As well as the amount of fruit produced, the quality of yields has an important role in determining their market value (Klatt *et al.*, 2014).

1.6.1 Commercial strawberry pollination

There are two main methods of commercial strawberry production; growing in substrate on table top systems under cover such as polytunnels, and direct planting into field soils (DEFRA, 2016) (Figure 1.1). Growth within substrate in table top systems under the cover of polytunnels has increased in recent years, due to the potential for increases in yield, shorter maturation time, and the ability to evenly control a short window for harvest, and now forms the majority of large scale strawberry production (DEFRA, 2016).

Figure 1.1: Comparison of strawberry growing methods; tabletop substrate system (left) (Anstiss, 2010), and plasticulture (right) (Miller, 2013).

Polytunnels are typically supplemented with hives of bumblebees (Figure 1.2) purchased annually by growers (Velthuis and Van Doorn, 2006), typically containing *Bombus terrestris,* whereas field grown strawberries are sometimes pollinated by hives of honeybees. However, because both systems are exposed to the open environment, they are also visited by a range of wild pollinators (Nye and Anderson, 1974).

Figure 1.2: *Bombus terrestris* Audax visiting a strawberry flower

1.7 The influence of AMF on strawberry

Although the influences of AMF on plant growth have been widely studied (Smith and Read, 2008), these studies have typically focused on either model or wild plant species, as AMF communities are often reduced in intensive agricultural systems. As such, there are far fewer studies that have examined the impacts of AMF on crop yields. Despite this, there is evidence that AMF may have the potential to improve crop production. For example, Ceballos *et al.* (2013) showed that AMF can improve yields of *Manihot esculenta* (cassava) under reduced phosphorus fertilisation, and Baslam *et al.* (2011a) found that AMF improve *Lactuca sativa* (lettuce) yields (although this is a direct result of increased plant growth, and does not rely on the production of other plant organs). However, to date there are no studies that test how crops respond to multiple natural AMF communities, if the effects of these communities vary across crop cultivars, or if human perceived crop quality is influenced.

Previous studies have examined multiple ways in which AMF can influence strawberry. For example, studies have shown that AMF can increase strawberry biomass (Vestberg, 1992; Sharma and Adholeya, 2004; Fan *et al.*, 2011; Matsubara, 2011; Robinson-Boyer *et al.*, 2016), fruit yield (Sharma and Adholeya, 2004; Robinson-Boyer *et al.*, 2016), runner production (Vestberg, 1992; Sharma and Adholeya, 2004), tolerance to pathogens such as *Fusarium oxysporum* (Fusarium wilt) (Matsubara, 2011) and Phytophthora spp. (Norman and Hooker, 2000; Vestberg *et al.*, 2004), as well as resistance to stresses such as salinity (Fan et al., 2011; Sinclair *et al.*, 2014). However, conversely, other studies have shown either no increase (Camprubí *et al.*, 2007), or a decrease (Sinclair *et al.*, 2014) in strawberry yield with AMF inoculation.

One potential mechanism for these differences in responses is variation in effects due to biotic and abiotic environmental factors. Vestberg *et al.* (2004) examined the influence of *Glomus mosseae* on strawberry growth in seven experiments conducted in three locations either in the summer or winter, and in the presence or absence of *Phytophthora cactorum* (strawberry crown rot). *G. mosseae* only improved strawberry growth when plants were infected by *P. cactorum*, and only in the autumn in less favourable growth conditions. In addition, AMF reduced growth in sterilised peat, but had no effect in unsterile peat. Furthermore, Sharma and Adholeya (2004) found that AMF induced improvements in strawberry yield, runner production, biomass, and shoot P content, but the effects were no longer present under high P fertilisation.

Another mechanism through which differences in responses arise results from the specific strawberry cultivars or AMF species present. For example, Vestberg (1992) examined the influence of six AMF strains on ten strawberry cultivars, and found that in one cultivar, all strains improved growth, whereas in two other cultivars, only two strains improved growth. Furthermore, Sinclair *et al.* (2014) found that while AMF were able improve plant growth and fruit quality under salt stress, the effects were dependent on the three strawberry cultivars and three AMF species tested, with AMF species and strawberry cultivar interacting to influence the level of AMF colonisation, brix, and fruit acidity. Conversely, Fan *et al.* (2011) found that strawberry cultivar did not influence increases in biomass under salt stress, indicating that some cultivars are able to mediate responses to a greater degree than others. In addition, this study only utilised a single AMF species, and AMF-cultivar interactions may be more pronounced in some AMF species compared to others. As such, the ability of strawberry cultivars to mediate responses to AMF colonisation may depend on the specific strawberry cultivars and AMF species present.

Despite the potential for cultivars to respond differently to an AMF species, Santos-González *et al.* (2011)*,* found that the assemblage of AMF communities strawberry plants associate with were determined not by cultivar, but rather by soil. As such, strawberry plants associate with the same AMF species regardless of cultivar, though each cultivar will respond differently to the AMF species present. However, previous evidence has been limited to the examination of the influence of individual AMF species, or combinations of several species, and it remains to be tested if the influences of entire natural AMF communities are also mediated by strawberry cultivar. In addition, despite the importance of pollination for fruit production, to date there are no studies that examine if AMF can influence plant-pollinator interactions in strawberry, and if these changes have functional consequences for crop yields.

1.8 Aims of the thesis and overview of data chapters

As well as potentially improving crop yields through increasing nutrient acquisition and plant growth (Smith and Read, 2008), AMF can influence plant-pollinator interactions (Barber and Soper Gorden, 2014), which may also benefit crop yields. However, while AMF have been shown to influence the frequency of pollinator visits, it is unknown if AMF can influence pollinator behaviour (if pollinators forage for pollen or nectar, and the amount of time spent foraging for each resource), and it remains to be tested if these influences on pollinator visitation have functional consequences for crop yields. Additionally, previous studies that examine AMF-plant-pollinator interactions rely on either a single AMF species, or a combination of a limited number of dominant AMF species, and it remains to be tested how plant-pollinator interactions are influenced by entire natural AMF communities that crops interact with in the field. Furthermore, previous studies have examined changes in AMF and crop cultivar in isolation, and it remains to be tested if multiple natural AMF communities interact with several crop cultivars to influence plant-pollinator interactions. Whilst controlling above-below-ground interactions in agricultural systems could maximise the ecosystem services they provide and improve crop yields, the uptake of new management techniques relies on the interest and motivations of the end user, and it is unknown how stakeholders value the ecosystem services provided by AMF and pollinators or their knowledge of above-below-ground interactions. As such, we aim to answer four primary questions to determine how AMF can influence both plant-pollinator interactions, and crop yields:

1. Can AMF influence pollinator foraging behaviour?
2. Can AMF influence strawberry yield quantity and quality?
3. Can AMF communities influence wild pollinator visitation, and does this have consequences for strawberry yield?
4. What are the perceptions of growers on wild pollinators and the introduction of AMF into commercial production?

In order to answer these four questions, we utilised an interdisciplinary approach that started in the greenhouse and ended with the consumer in order to first examine the fundamental biology of above-belowground AMF-plant-pollinator interactions, and then the functional consequences of these interactions both from the perspectives of plants (reproduction) and humans (quality of produce, and value placed on the ecosystem services of AMF and pollinators).

We accomplished this through conducting three experiments: a greenhouse experiment, a field experiment, and a social analysis. For the greenhouse and field experiments, we chose to compare two natural AMF communities extracted from field soils surrounding the location where both experiments took place, a commercial inoculant, and sterile control plants. For the natural AMF communities, we selected two communities that varied in the diversity of morphospecies, as the influence of communities of organisms that have close relationships with plant root systems has been predicted to vary based on their diversity (Wardle *et al.*, 2004). We utilised a commercial inoculant for three reasons: first, to determine if first, commercially available inocula are currently suitable for use in strawberry production; second, if inocula produced in an intensive environment have the same influence as locally sourced AMF communities when tested in the field; and third, if inocula produced with plants that do not rely on pollination are less adapted to promote plant-pollinator interactions. We tested three commonly used commercial cultivars of strawberry: Elsanta and Sonata, which are the two predominant varieties used in the UK, along with Darselect, a variety more commonly used in mainland Europe , and exhibits different growth habits. The same AMF communities and strawberry cultivars were used in both the greenhouse and the field, and both experiments were conducted over two years, as commercial strawberry production typically utilises plants for two cropping seasons. Finally, in the social analysis, we targeted strawberry growers in the UK and USA (FAOSTAT, 2017), as these countries are two of the world's largest producers of strawberries, along with experts (scientists) who study interactions between AMF and crops.

1.8.1 Chapter 2: Belowground mutualists and crop cultivars influence pollinator foraging behaviour

The importance of pollinators in agro-ecosystems has widely been accepted as vital for the production of many crops (Free, 1993), and the loss of all pollinators would reduce global food production to below current levels of consumption (Gallai *et al.*, 2009). However, on a local scale within a single crop species, we do not fully understand all of the dynamics that shape plant-pollinator interactions. Although AMF have been shown to influence plant reproductive traits, and pollinator visitation rates, it is unknown if they can also influence the foraging behaviour of pollinators, or if these influences are mediated by crop cultivar. Strawberry flowers require all pistils to be pollinated to prevent deformed fruit (Free, 1993), and the foraging behaviour of bees has been shown to influence which stigmas within a flower are pollinated (Chagnon *et al.*, 1993). As such, in Chapter 2 we aim to determine if AMF can influence the foraging behaviour of pollinators, and if these influences vary across multiple cultivars.

In Chapter 2, I examine the influences of AMF and strawberry cultivar on *Bombus terrestris* Audax, which is commonly used in commercial strawberry production. I test if either AMF community or strawberry cultivar can influence plant reproductive traits, and in turn the frequency and duration of visits, or foraging behaviour of pollinators in the greenhouse over two years. I predict that AMF communities will influence these traits, but that both of the natural communities will be adapted to promote plant reproduction over the Commercial Inoculant. As AMF influences have been shown to vary between plant species, and there is some evidence that AMF differentially influence the reproductive traits of crop cultivars, I predict that they will also vary between crop cultivar.

1.8.2 Chapter 3: Mycorrhizal fungi influences strawberry yield with no loss in fruit quality across multiple cultivars

Although it is widely understood that AMF can influence plant growth (Smith and Read, 2008), there is limited evidence as to how these changes translate to crop yields (Sharma and Adholeya, 2004; Camprubí *et al.*, 2007; Baslam *et al.*, 2011a; Ceballos *et al.*, 2013; Sinclair *et al.*, 2014; Robinson-Boyer *et al.*, 2016). Furthermore, the influences of AMF can be specific to a plant species, and other studies have explored interactions between a single AMF species or combination of species (Vestberg, 1992; Fan *et al.,* 2011; Sinclair *et al.*, 2014) but to date, there is no evidence of if the influence of an entire natural AMF community on crop yield is consistent across multiple crop cultivars. In addition, although there are some studies that have examined how AMF influence the nutritional content or essential oil quality of yields (Gupta *et al.*, 2002; Kapoor *et al.*, 2002; Kapoor *et al.*, 2004; Baslam *et al.*, 2011a), other measures of yield quality are lacking. As such, in Chapter 3 we aim to determine how AMF influences the yield of multiple strawberry cultivars, and if these changes affect human perceived fruit quality.

In Chapter 3, I continue the experiment set up in Chapter 2 to determine the influences of AMF community and crop cultivar on both the quantity and quality of strawberry yield. I measure the number of strawberries produced, the total yield of each plant, and the average yield per strawberry. In addition, I also conduct human taste tests, in order to determine the functional consequences of these interactions from a human perspective. I determined human preference for strawberries produced by each AMF treatment or strawberry cultivar, by conducting a taste test and asked participants to rate strawberries based on seven commonly used variables in commercial trials, from appearance to flavour, and juiciness to sweetness. I predict that the Commercial Inoculant will provide the greatest benefit, as it is adapted to support plant growth under intensive conditions, whereas natural communities are likely adapted to support plants facing a range of biotic and abiotic stresses in the field that are not present to the same degree in the glasshouse, but that this may depend on crop cultivar.

1.8.3 Chapter 4: The effects of AMF community and strawberry cultivar on interactions with wild pollinators and strawberry yield

Examining wild pollinator communities is important as they can influence crop yields through several dynamics. First, the efficiency of pollinators varies, and in turn, the value of each individual visit depends on pollinator taxa, with Hymenoptera providing the most efficient visits (Schemske and Horvitz, 1984; Fishbein and Venable, 1996; Bingham and Orthner, 1998; Ivey *et al.*, 2003). However, secondly, the diversity of wild pollinator communities can provide a high degree of functional redundancy. For example, some *Syrphidae* species can carry similar pollen loads to bees, and while each visit is less efficient, this can be mitigated by a higher frequency of visits by Diptera (Orford *et al.*, 2015). In addition, supplementing crops with bees has been found to only improve production in 14% of production systems (Garibaldi *et al.*, 2013). Third, improving the diversity of visits to a plant can improve yields (Hoehn *et al.*, 2008), as pollinators exhibit different foraging techniques, and visits from several different pollinators can provide functional complementarity, improving the quantity, quality, and market value of strawberry yields (Chagnon *et al.*, 1993). A limited number of studies have previously found that AMF can influence the visitation of wild pollinators (Gange and Smith, 2005; Varga and Kytöviita, 2010b; Barber *et al.*, 2013a), which has the potential to influence crop yields. However, as described in Section 1.5, it is unclear how whole AMF communities that plants naturally associate with in the field can influence aboveground interactions with wild pollinators, or if these effects are mediated by crop cultivars. Furthermore, it is unclear if changes in pollinator visitation have functional consequences for crop yields in the field. As such we aim to determine if AMF communities can influence the visitation of wild pollinators, if these influences vary between strawberry cultivars, and if changes in visitation lead to differences in crop yields.

In Chapter 4, I use the same AMF communities and strawberry cultivars as in the greenhouse, but measure their influences on the wild pollinator community. I measure the frequency of visits and species richness of wild pollinators overall, along with sub-taxa of pollinators. To determine if changes in pollinator visitation has a functional consequence for crop yields, or if redundancy is provided by other pollinators in the community, I measure the number of strawberries produced, total yield, and average weight of each strawberry. I predict that the natural AMF communities will be better adapted to support plant reproduction, and will have the greatest benefit to pollinator visitation. In addition, as they are adapted to the local environmental conditions, I predict that they will also provide the greatest yields.

1.8.4 Chapter 5: Perceptions on the introduction of AMF as a novel biotechnology in the production of soft fruit: an analysis using Q-methodology

Concerns over global food security are increasing (Godfray et al., 2010) with a rapidly rising population (Cohen, 2003; Gerland et al., 2014). Fertiliser prices are increasing and becoming increasingly volatile with worries over peak phosphorus (Cordell and White, 2011), however biological amendments that maximise the ecosystem services of AMF and pollinators could provide one part of a toolset to increase sustainable intensification and improve food security. There is a rise in interest in utilising other biological amendments such as pest and pathogen control (van Lenteren, 2000; Marrone, 2009; Bhattacharyya and Jha, 2012), and 'Integrated Nutrient Management' (Gruhn et al., 2000; Wu and Ma, 2015). Although the use of rhizobia to fix nitrogen in legumes has been established since the 1900s (Catroux et al., 2001), and AMF have been proposed as a method to reduce reliance on phosphorus fertilisation (Roy-Bolduc and Hijri, 2011; Orrell and Bennett, 2013), they have yet to be implemented in commercial agricultural production, due to a range of reasons, including a lack of proven cost-effective inocula that meets the needs of growers, high fertilisation levels, and the use of fungicides in many production systems. In order for the successful uptake of a new technology it is vital to understand the perceptions of the end users and other stakeholders in the industry in order to develop products that meet their needs. In addition, although AMF and wild pollinators are present in crop production systems, and may improve crop production, there is currently no evidence in academic literature as to how much value growers place on the ecosystem services they provide. Ultimately, the uptake of controlling these interactions to maximise the ecosystem services they provide is decided by the end users. As such, we aim to characterise the viewpoints of growers and experts in the soft fruit production industry on the implementation of AMF in commercial production systems, as well as how much they value wild pollinators.

In Chapter 5, I move from understanding the biology of these interactions to determining the perceptions of those who could exploit them by using an innovate method (Q-methodology) that has been utilised in social science to examine sustainability efforts, but has not explored the introduction of a novel biotechnology (such as AMF), in order to survey the viewpoints of growers and experts in strawberry production, and determine which predominant sets of viewpoints are present in the industry. I measure perceptions on the introduction of AMF in commercial strawberry production as a novel biotechnology to improve yields, and examine if opinions are motivated by intrinsic or extrinsic factors. In addition, I measure the value that stakeholders place on wild pollinators. Finally, I determine if experts (scientists) share the same viewpoints as growers, or if they have their own set of opinions. Based on previous Q-methodology studies in sustainability, I predict that growers will fall into two predominant viewpoints – those with an intrinsic care for sustainability, and those who are focused on profits and production. Due to the regular use of commercial hives of pollinators in their production systems, I predict that growers will not place a high value on the wild pollinators visiting their crops.

Chapter 2: Belowground Mutualists and Crop Cultivars Influence Pollinator Foraging Behaviour

Abstract

Previous studies have explored interactions between plants and the organisms they interact with extensively, however, multi-trophic interactions between above- and below-ground mutualisms are poorly understood. For example, whilst belowground organisms have been shown to influence plant reproductive traits, and pollinators respond to changes in floral rewards, these interactions are typically studied in isolation. Arbuscular mycorrhizal fungi (AMF) have been shown to influence the frequency of pollinator visits, but it is unknown if these changes also influence the foraging behaviour of pollinators, if these effects are mediated by crop cultivars, or how entire natural AMF communities effect these interactions. In this chapter, I examine AMF-plant-pollinator interactions in the controlled environment of a greenhouse, utilising two natural AMF communities, a commercial inoculant, and a sterile control with three commercial strawberry cultivars (Elsanta, Sonata, and Darselect).I measured if AMF community can influence plant reproductive traits, and the frequency, duration, and foraging behaviour of *Bombus terrestris* Audax within several strawberry cultivars. I find that AMF communities can influence floral display and nectar production, with one of the natural communities (Community 1) improving these traits over the commercial inocula. AMF community also influenced the frequency, and duration of total pollinator visits, as well as for each foraging behaviour. Community 1 had fewer visits than plants with the commercial inocula, and these visits were shorter than control plants. AMF community also influenced the foraging behaviour of pollinators, and Community 1 had the fewest and shortest pollen foraging visits. Community 1 also had the fewest nectar foraging visits, but of the longest duration. While AMF-plant-pollinator interactions have previously been shown to vary between plant species, I find that AMF communities influence pollinator visits similarly across multiple crop cultivars, and found no interaction between AMF and strawberry cultivar. As such, AMF can influence the foraging behaviour of pollinators, which may have important consequences for crop yields, could add additional resolution to the study of ecological networks.

2.1 Introduction

Pollinators interact with 75% of all crop species worldwide, and are responsible for improving the yield of 35% of crops globally (Klein *et al.*, 2007; Ollerton *et al.*, 2011). The nature of the pollination services provided play an important role in determining fruit set, and crop yield quantity and quality (Chagnon *et al.*, 1993; Mayfield *et al.*, 2001; Ivey *et al.*, 2003). In crop production systems dominated by a single plant species, there may be localised influences that play important roles in determining the nature and efficiency of pollinator visits, and in turn crop yields. For example, pollinators preferentially visit some crop cultivars over others, and belowground organisms can alter the expression of plant reproductive traits, both of which can change the frequency of pollinator visits or their foraging behaviour. By increasing the resolution at which interactions between pollinators and crops are studied, we are able to gain a deeper understanding of the functional consequences for crop yields, furthering our understanding of plant-pollinator interactions and providing important information for growers.

2.1.1 The influence of AMF on plant reproductive traits

Studies have shown that pollinators respond to changes in the floral rewards offered by a plant (Buchmann and Cane, 1989; Harder, 1990; Real and Rathcke, 1991; Poulton *et al.*, 2001a; Soto *et al.*, 2013), and its floral display (Buide, 2006; Cahill *et al.*, 2008), influencing the frequency and duration of visits. However, these traits can be influenced by factors that are commonly overlooked.

For example, belowground organisms can influence a range of plant traits, such as defensive compounds (van Dam and Heil, 2011), and reproductive traits (Koide, 2000), affecting the interaction between plants and aboveground insects (Pineda *et al.*, 2010). All terrestrial ecosystems contain above- and below-ground species, and by influencing plant reproductive traits, interactions between above- and below-ground organisms can play an important role in the provision of ecosystem services, such as pollination (Wardle *et al.*, 2004). Arbuscular mycorrhizal fungi (AMF) are plant mutualists living in the rhizosphere, acting as a secondary root system, increasing nutrient and water uptake, in return for carbon from the plant (Smith and Read, 2008). However, as well as influencing plant growth through the provision of nutrients, AMF can affect plant reproduction through influencing a range of plant reproductive traits, and in some studies AMF have been shown to have a greater impact on the reproductive traits of a plant than vegetative traits relating to plant growth (Poulton *et al.*, 2002).

AMF can improve floral display by increasing the number or diameter of flowers (Bryla and Koide, 1990; Stanley *et al.*, 1993; Lu and Koide, 1994; Koide, 2000; Pendleton, 2000; Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Poulton *et al.*, 2002; Scagel, 2004; Gange and Smith, 2005; Wolfe *et al.*, 2005; Perner *et al.*, 2007; Varga and Kytöviita, 2010b; Aguilar-Chama and Guevara, 2012). However, there are conflicting explanations as to how AMF can influence male and female reproductive traits. AMF have been shown to improve male reproductive traits through improving pollen production, the size of pollen grains, pollen tube growth, pollen phosphate content, and the success of pollen siring seeds (Lau *et al.*, 1995; Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Poulton *et al.*, 2002), but have a negative influence on pollen production in other studies (Kiers *et al.*, 2010). Similarly, nectar production and sugar content increase with AMF inoculation in some plant species, but not others (Gange and Smith, 2005; Kiers *et al.*, 2010; Barber *et al.*, 2013b).

As such, the influence of AMF on plant reproductive traits depends on the plant species, and the specific trait measured, and AMF do not always improve reproductive traits (Varga and Kytöviita, 2010b). For example, Gange and Smith (2005) found that AMF increased the number of flowers in *Centaurea cyanus* and *Tagetes patula* but not in *Tagetes erecta*, increased flower size in only *T. patula* and *T. erecta* but not *C. cyanus*, and only increased nectar sugar content in *T. erecta*. There may also be trade-offs between male and female reproductive traits, and Kiers *et al.* (2010) found that AMF increased flower diameter and nectar production, but decreased pollen production in *Cucumis sativus* .

Changes in plant reproductive traits may also depend on AMF species associated with the plant. For example, *Glomus claroideum* increased the flowering shoot mass and in turn proportion of flowering plants in *Geranium sylvaticum*, whereas *Glomus hoi* increased root mass, but not the proportion of flowering plants (Varga and Kytöviita, 2010a).

2.1.2 AMF influences on pollinator visitation

Changes in plant reproductive traits play important roles in determining plant-pollinator interactions. Plants inoculated with AMF have been shown to receive more pollinator visits than non-mycorrhizal plants in some plant species (Poulton *et al.*, 2001a; Gange and Smith, 2005; Wolfe *et al.*, 2005; Cahill *et al.*, 2008), however AMF induced changes in pollinator visitation rate are dependent on the plant species, and do not always improve insect visitation (Cahill *et al.*, 2008; Varga and Kytöviita, 2010b).

Previous studies that have explored AMF-plant-pollinator interactions have manipulated treatments with a single AMF species, however most plants (including crops) are colonised by communities of AMF in the field, and as such it is important to understand the influences of natural AMF communities on plant-pollinator interactions. Only a single study to date has examined the effects of inoculating plants with multiple AMF species on pollinator visitation. Barber *et al.* (2013a) used four single species inoculants, along with an inoculant consisting of three AMF species, and found that honey bees, bumble bees, and Lepidoptera responded differently to each AMF treatment. However, this study used one mixed species treatment of three species, and did not compare multiple mixed AMF communities, or naturally occurring AMF communities that crops would associate with in the field.

In addition, previous studies have mainly focused on insect visitation rates, but have not explored changes in the foraging behaviour of pollinators. This is important as the nature of the interactions that take place can play an important role in crop yields. For example, the behaviour of pollinators while visiting a flower has been shown to play an important role in determining crop yields (Chagnon *et al.*, 1993), and some visits are more beneficial to plant reproduction than others. Bees that visit to only forage for nectar are more likely to only cause incidental pollen deposition, as they do not always carry significant pollen loads (Free, 1968).

Bumblebees utilise multiple foraging techniques, and can forage for pollen, nectar, or both (Free, 1968). Pollen foraging bees either travel in a circle around flowers collecting pollen from each anther, coming into contact with stigmas, or stand directly on the central stigmas, pivoting to reach each anther and as such depositing pollen on stigmas (Free, 1968). Pollen foraging bees can also exhibit ‘buzz’ pollination, in which they vibrate their flight muscles without moving their wings, sonicating the flower, causing pollen to be ejected from anthers, and distributing some of the pollen load from their bodies (De Luca and Vallejo-Marín, 2013), which can improve the chances of successful pollination (Kawai and Kudo, 2009), and occurs in many agricultural crops (Buchmann *et al.*, 1983; Vallejo-Marín *et al.*, 2010).

To date, it is unclear as to whether entire AMF communities (as opposed to single AMF species) can influence pollinator visitation rates across crop cultivars, or the effects on pollinator foraging behaviour. Although these influences have been shown to depend on AMF-plant interactions, AMF-plant-pollinator interactions have not previously been studied in soft fruit.

2.2 Methods

2.2.1 Study System

Strawberries are an ideal model crop to test these interactions, due to their pollinator dependence and high economic value (FAOSTAT, 2017). A crop's reliance on pollinators is measured in dependency ratios, which calculate losses in production in the absence of pollinators (Gallai *et al.*, 2009). Strawberries are 10 to 40% dependent on pollination (Gallai *et al.*, 2009), and poor pollination results in a significant loss for growers, influencing fruit quantity, quality, and market value (Roselino *et al.*, 2009; Klatt *et al.*, 2014). Strawberry cultivars produce varying floral rewards, including male traits such as pollen protein content, amino acid composition (Grunfeld *et al.*, 1989), and female traits such as nectar production, sugar concentration, and sugar production per flower (Abrol, 1992), and bees can discriminate between these floral rewards (Buchmann and Cane, 1989; Harder, 1990), preferentially visiting plants with greater rewards (Harder, 1990; Abrol, 1992). Global strawberry production was worth $18 billion USD in 2014, an increase of over 133% in the previous decade (FAOSTAT, 2017), and controlling AMF-plant-pollinator interactions offers growers the opportunity to improve the quantity and quality of their yields. AMF are commonly present in commercial strawberry crops due to the use of unsterilised substrates in plant propagation, and understanding the influences of AMF communities on strawberry pollination could offer the opportunity to control these interactions, improving pollination and increasing strawberry yields. Here we test if strawberry-pollinator interactions are influenced by AMF community, and if these influences are mediated by strawberry cultivar in a controlled environment using four AMF treatments (two natural communities, a commercial inoculant, and a sterile control), and three commercial cultivars of strawberry (*Fragaria* × *ananassa*). We aim to determine if:

1. AMF community influences the reproductive traits of strawberry
2. AMF community influences the frequency of pollinator visits
3. AMF community can influence the foraging behaviour of pollinators
4. Effects are consistent across multiple strawberry cultivars

As studies have previously shown that individual AMF species can influence plant reproductive traits and pollinator visitation rates, we predict that whole AMF communities will also influence both of these factors. Commercially produced inocula are typically produced with plants that do not rely on insect pollination, and as such we predict that the natural communities will be adapted to promote reproductive traits and in turn improve pollinator visitation. Although the influence of AMF on pollinator behaviour is untested, we predict that AMF induced changes in male and female reproductive traits will influence the foraging behaviour of pollinators. Reproductive traits within a crop species vary across cultivars, and as the influence of AMF on plant traits can depend on plant genotype / crop cultivar, we predict that AMF community and strawberry cultivar will interact, and the influence of an AMF community will depend on the strawberry cultivar it is associated with.

In order to test the impact of AMF on pollinator visitation and plant reproductive traits, we conducted a 4 x 3 (four AMF treatments, and three strawberry cultivars) factorial complete block randomised experiment, and selected three AMF communities: a commercial inoculant, and two natural communities, varying in diversity, along with a sterile control. The commercial inoculant was RootGrow Professional (PlantWorks, Sittingbourne, UK), hereafter named ‘Commercial Inoculant’ (Robinson-Boyer *et al.*, 2016), and the two natural communities were derived from sites surrounding The James Hutton Institute, Scotland, UK. The first natural AMF community (hereafter named ‘Community 1’) was extracted from a plant species poor barley field margin (56°27'21.5"N 3°04'33.9"W), and contained a low diversity of AMF species. The second natural AMF community (hereafter termed ‘Community 2) was extracted from a plant species rich wildflower meadow (56°27'27.6"N 3°03'57.4"W), and contained a high diversity of AMF species. In addition, control plants, with sterile AMF spores were used to rule out influences from the physical properties or other microbes present in each inoculant. The communities tested were sourced from two sites within The James Hutton Institute rather than strawberry fields, as commercial strawberry production in Scotland is based on table top systems in coir (see Section 1.6.1), and contain little to no spores.

Prior to the main experimental phase, AMF inocula was produced by extracting AMF communities and inoculating strawberry plants, which were allowed to grow for four months, before their substrate was used to inoculate experimental plants, as described below. In June 2013, AMF spores were extracted from all three communities (commercial inoculant and two natural communities), using wet sieving and sucrose centrifugation (Daniels and Skipper, 1982). I extracted 100 ml of field soil / commercial inoculant for every litre of substrate used to pot the plants in the pre-experimental phase (Bennett *et al.*, 2016). Supernatant lacking spores was removed to concentrate the solution, and pots were inoculated with 1 ml of spore solution. A microbial wash was produced from each extract and 1 ml added to each pot, in order to rule out the influences of non-AMF microbes (Bennett *et al.*, 2016). To produce the microbial wash, supernatant fluid from the spore solution was removed and vacuum filtered through filter paper with 11 µm pore size (No. 1, 125mm, Whatman, Buckinghamshire, England). Half of each spore solution and microbial wash was steam sterilised (121°C, 15 psi for 20 minutes), in order to add each solution to each treatment to rule out any influences of physical, chemical, or biological properties of the solutions (Table 2.1), and only test the influence of the AMF communities. Community 1 contained 3.33 ± 0.33 morphospecies, with a density of 26 ± 0.58 spores ml^{-1} and Shannon's diversity Index of H=1.18 ± 0.09, and Community 2 contained an average richness of 6.33 ± 0.33 morphospecies at a density of 30 ± 2.52 ml^{-1} and a diversity of H=1.77 ± 0.05. The commercial inocula contained 4.33 ± 0.33 morphospecies, with a density of 28.66 ± 1.33 ml^{-1} and a diversity of H=1.43 ± 0.07. Morphospecies 1 and 2 were present in all three inocula, and morphospecies 8 was present in both Community 1 and the commercial inocula.

Table 2.1: An overview of AMF communities and microbial wash added to each treatment. In order to produce treatments which examine the effect of a single AMF community whilst excluding influences of the physical, chemical, and biological properties associated with each inoculant, combinations of sterile (orange boxes) and live (blue boxes) AMF spores and microbial wash were added. For example, to produce the ‘Community 1’ treatment, live AMF spores from Community 1 and sterile spores from all other inocula were added, along with sterile microbial wash from Community 1 and live microbial washes from all other communities. As such, the Community 1 treatment then contained spores from all treatments (although only spores from Community 1 were live), as well as the physical / chemical properties, and microbes present in all other treatments. Orange cells indicate the addition of sterile inocula, and blue cells indicate the addition of live inocula.

Inocula	**Experimental Treatment**							
	Sterile		**Community 1**		**Community 2**		**Commercial**	
	AMF Community	*Microbial Wash*	*AMF Community*	*Microbial Wash*	*AMF Community*	*Microbial Wash*	*AMF Community*	*Microbial Wash*
Sterile	Sterile	Live	Sterile	Live	Sterile	Live	Sterile	Live
Community 1	Sterile	Live	Live	Sterile	Sterile	Live	Sterile	Live
Community 2	Sterile	Live	Sterile	Live	Live	Sterile	Sterile	Live
Commercial	Sterile	Live	Sterile	Live	Sterile	Live	Live	Sterile

2.2.2 Inocula production

Prior to the main experimental phase, in order to generate inocula for the experimental plants, three cultivars of cold-stored strawberry plants, ‘Elsanta’, ‘Sonata’, and ‘Sweetheart’ were obtained from a UK based propagator (R W Walpole Ltd, Kent, UK), and the previously described inocula were added to the plants. As AMF have been shown to develop host specificity, multiple strawberry cultivars were used during the production phase to prevent communities from becoming adapted to a single strawberry cultivar. Darselect (used in the main experimental phase) was not used, as this cultivar was not available from this supplier. Roots were removed from all plants to exclude AMF present from the propagation process, before plants were potted in 2 L pots with strawberry mix coir. Plant pots were sterilised in a bleach solution (‘Domestos Thick Bleach Original’ – primary active ingredient NaClO, Unilever, London, UK) before use, and all substrates were twice steam sterilised in an autoclave (121°C, 15 psi for 2 hours). Two weeks later, when new roots were beginning to establish, roots from 5 plants from each variety were sampled and stained with trypan blue (Koske and Gemma, 1989), and assessed with the gridline intersect method (McGonigle *et al.*, 1990) to confirm the absence of pre-existing AMF. Following this, the plants were inoculated with 1 ml of live and sterile AMF spores and microbial wash as indicated in Table 2.1. These plants were grown for four months in order for AMF colonisation to become fully established, and spores to be produced throughout the substrate. At the end of the growth period, substrates and root fragments were collected and pooled by AMF community in order to produce inoculant for the experimental plants.

2.2.3 Experimental Design

In order to test the influences of multiple AMF communities on pollinator visitation and plant reproductive traits across several strawberry cultivars, a four (AMF community) by three (strawberry cultivar) factorial randomised glasshouse experiment was conducted over two years. In September 2013, strawberry plants for the main experimental stage (misted tips that had been rooted into coir) were purchased from Nessen BV, Netherlands (a supplier which was able to provide plants free of previous AMF colonisation). Three cultivars were used - Elsanta and Sonata were selected to represent the two main varieties used in commercial production in the UK, and Darselect in order to provide a more distantly related cultivar grown in the EU, along with having different growth habits, such as flower phenology, and typical yields.

Before planting, roots from three plants from each cultivar were stained using Trypan Blue (Koske and Gemma, 1989) and assessed using the grid-line intersect method to confirm that they were free of AMF from the propagation process. Plants were then potted in 0.5 L sterile pots with strawberry mix coir (Bulrush Horticulture Ltd, Londonderry, UK), containing 100 ml of the appropriate treatment inocula (substrate and root fragments mix) produced in the inocula production phase (described above) (Table 2.1). Sixty plants of each strawberry cultivar were used across each of the four AMF communities, creating a replication level of 15 plants per treatment and 180 plants in total. Plants were grown under cool house conditions, with no supplemental lighting or heating, greenhouse fans permanently on, and a temperature range of 2-5°C for 6 months over winter to establish full AMF colonisation before the flowering period in the following year. In April 2014, at the end of this overwintering period, plants were repotted into sterile 3 L pots with strawberry mix coir containing 1/3 of the recommended application rate of fertiliser for strawberries to create a low P environment (2.7 g/L – Osmocote 14-16M, Geldermalsen, Netherlands). Plants were arranged into three blocks, each containing an equal number of plants from each treatment, and the location of each plant within the block was completely randomised. There were 20 rows of plants within each block, each containing three plants (Figure 2.1). Each pot was separated by 20 cm gap between the adjacent pot. Plants were grown for a total of two years over the course of the study, and during winter months (October to March) each year, environmental conditions in the glasshouse were altered to reflect outdoor conditions as described above. During the summer months, plants had 18 hours of light per day, with supplemental lighting when light levels fell below 150 W/m^2, temperatures maintained at 21°C during the day, 16°C during the night, and an overhead screen closed when light values exceed 450 W/m^2. Additional fertiliser was not applied at any stage, as Osmocote is a slow release fertiliser. Fungicides were not used at any stage, and any evidence of powdery mildew was treated with a mixture containing rapeseed oil (15.6 ml/L), anionic and non-ionic surfactants (7.8 ml/L) (Ecover Zero Washing Up Liquid, Ecover UK Ltd., Richmond, UK), and bicarbinate of soda (15.6 g/L) in sterile distilled water. *Chrysoperla rufilabris* (Green lacewing) larvae were used to control aphids, and *Phytoseiulus persimilis* were used to control *Tetranychus urticae* (Red spider mite) if either pest was sighted in the glasshouse.

At the initiation of flowering, a commercial hive of *Bombus terrestris* Audax (Standard Hive, BioBest, Netherlands), containing approximately 80 workers was placed within the greenhouse in order to provide pollinators for the experiment. Visitation rates and foraging behaviour were determined by releasing 15 bees from the hive and walking a 1-hour transect through the greenhouse. Transects were conducted daily from Monday to Friday during the flowering period, and each transect was started at a randomly allocated location in the greenhouse. An observer would slowly walk the transect along each block of plants, observing the three rows (9 plants) directly adjacent (Figure 2.1). When a bee was observed to land on a flower, a timer was started to measure the duration of the visit, and the foraging behaviour was observed. Foraging behaviour was classed as 1) foraging for pollen, 2) foraging for nectar, 3) foraging for both pollen and nectar, or 4) non-foraging visits (when a bee would land on a flower but collecting neither pollen or nectar). The total number of visits per flower were calculated by combining these measures. Two measures of the duration were used for analysis – the total duration of each visit type observed during all transects a plant received, and the average duration of visits (calculated by dividing the total duration by the number of visits).

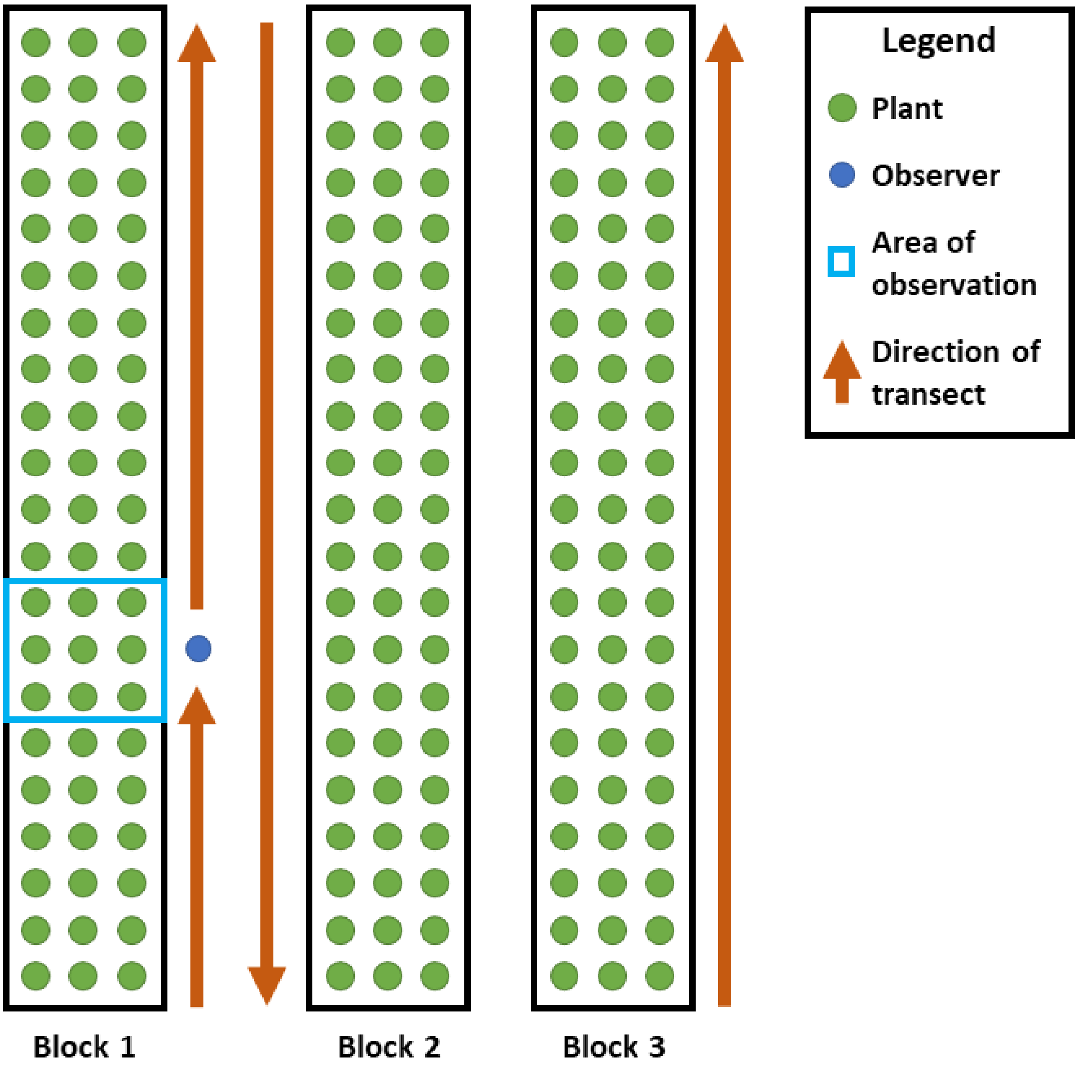


Figure 2.1: Illustration of the layout of plants, block, transect route, and observation window in the greenhouse. Black rectangles show each block, containing 60 plants each (green circles). Transects were started at a random location, and the observer (blue circle) monitored 9 plants from the three rows adjacent as passed (light blue square), walking in the direction indicated in by the orange arrows. The direction walked (in the direction illustrated, or the reverse) was determined randomly using a random number generator.

To determine male and female reproductive capacity, individual flowers were enclosed in Enviromesh (Ultrafine, Agralan Ltd, UK), until petals had opened. Nectar quantity was then measured by probing the nectaries with a 0.1 µl capillary tube, and measuring the quantity of nectar extracted with digital callipers. Nectar was harvested at two points in the day, during the morning (10-11 am), and the afternoon (2-3 pm), as nectar is secreted throughout the day, and the timing of the secretion may depend on AMF influences or strawberry cultivar. Anthers were then harvested from the same flowers as a proxy metric to determine male reproductive capacity, before being counted, freeze-dried, and weighed on a micro-balance.

A subsample of plant roots were stained with Trypan Blue (Koske and Gemma, 1989) at the end of the experiment, and assessed using the grid-line intersect method (McGonigle *et al.*, 1990). Results can be found in Section 3.3.3.

2.2.4 Statistical Analysis

All statistical analyses were conducted in R version 3.2.4 (R Development Core Team, 2016). Pollinator visitation variables were analysed using a three-way repeated measures general linear mixed effect model, using AMF community, cultivar, and year as fixed factors, with random factors of plant identification number to account for repeated measures and block number to account for position in the greenhouse, using the 'nlme' package (Pinheiro *et al.*, 2016). Plant size varied across AMF treatments (see Chapter 3), and as such plants from different treatments had different numbers of flowers. We aimed, however, to determine how AMF can influence pollinator visitation through changes in plant reproductive traits, rather than if changes were due to an increase in plant size and number of flowers (which could lead to more visits). As such, to control for these differences and to only look at the influences on visitation as a result of changes in plant reproductive traits, we divided each of the visitation variables by the number of flowers a plant had, a measure commonly used in previous studies (Poulton *et al.*, 2001a; Gange and Smith, 2005; Varga and Kytöviita, 2010b; Barber *et al.*, 2013a). Where main effects were significant, differences between AMF communities and strawberry cultivars were compared using Tukey's honestly significant difference (HSD), using the 'lsmeans' R package (Lenth, 2016).

Due to the potential for spatial auto-correlation in the pollinator visitation variables (i.e. the floral display or volatiles released by a plant may influence the visits of pollinators to plants surrounding it), Moran's I test was used to test for spatial auto-correlation. The average duration of combined visits (p = 0.002), and the duration of nectar visits (p = 0.049) were significantly spatially auto-correlated, and as such the 'corExp' correlation correction was added to the models, using the plant's row and column as plant location.

Plant reproductive traits were analysed using a similar model, however as this was only assessed during the first year, year was excluded as a fixed factor. Model assumptions were checked, and variables that did not satisfy a normal distribution of the residuals were transformed using nine different data transformations, and the best fit used for final results by comparing model residuals. All plant reproductive traits were square root transformed, except for the number of anthers, which was transformed to the power of three, and average anther weight, which was not transformed. The number and duration of combined visits were square root transformed and the average duration was not transformed. All pollen visits were square root transformed, and all nectar visits were not transformed.

2.3 Results

2.3.1 Plant Reproductive Traits

AMF community influenced the number of flowers, with Community 1 producing significantly more flowers than the commercial inoculant (Figures 2.2a & S2.1a, Table 2.2). AMF also influenced the volume of nectar that flowers produced in the afternoon, but not in the morning, and had no impact on the number and mass of anthers (Figures 2.2b & S2.1b, Table 2.2).

There was a significant effect of strawberry cultivar on all plant reproductive traits, with Elsanta producing more flowers but less nectar than Sonata. Both Elsanta and Sonata had more flowers and nectar than Darselect, but fewer anthers, which weighed less (Figures 2.2c & S2.1c, Table 2.2). AMF community and strawberry cultivar only interacted to influence the number of anthers.

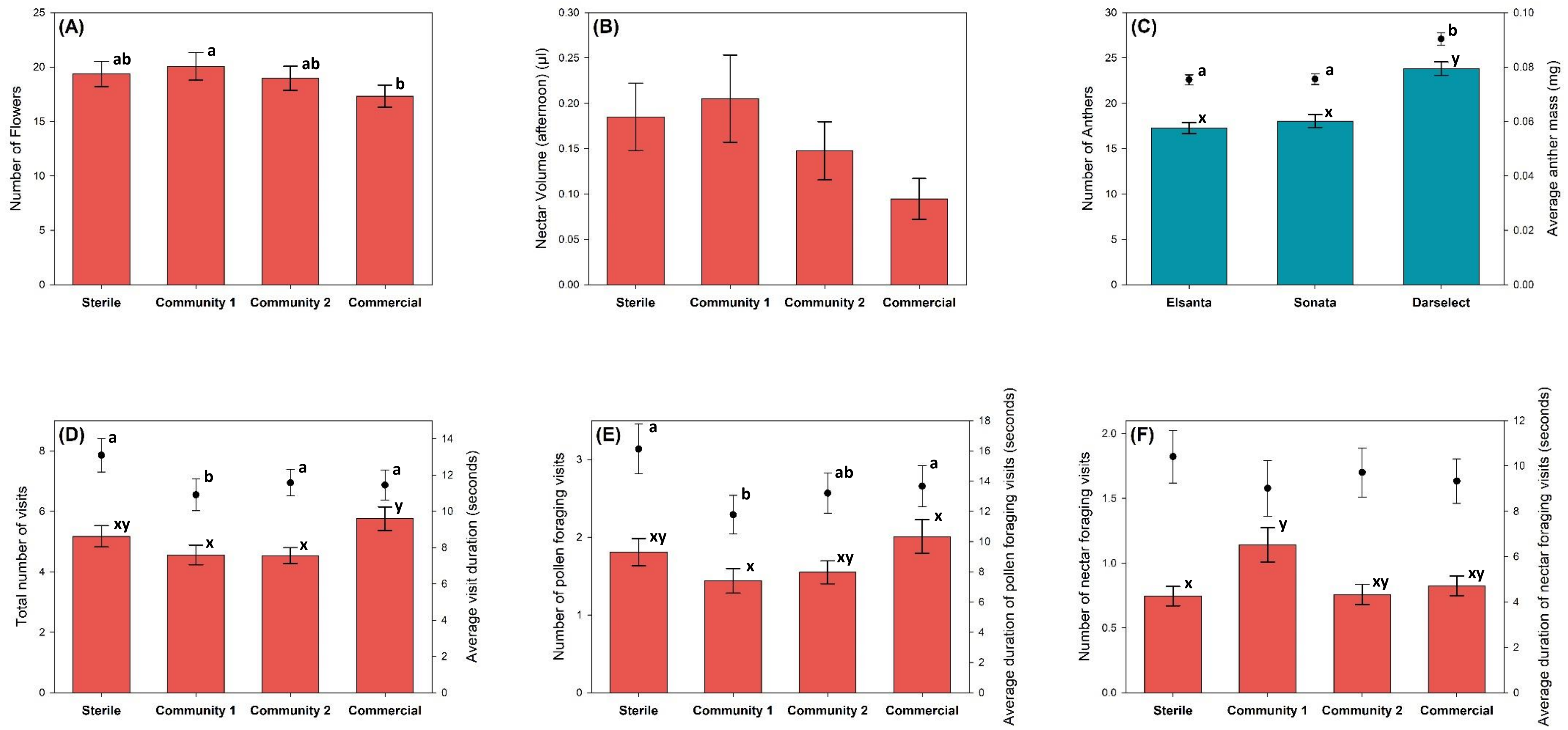


Figure 2.2: Plant reproductive traits and pollinator visitation: (a) Number of flowers by AMF community, (b) amount of nectar produced in the afternoon (µl) by AMF community, (c) number (dots) and average mass (mg) (bars) of anthers by strawberry cultivar, (d) total number (dots) and average duration (seconds) (bars) of bumblebee visits, (e) number (dots) and average duration (seconds) (bars) of pollen foraging visits, and (f) number (dots) and average duration (seconds) (bars) of nectar foraging visits. Values represent least squares means (±SE). Lower case letters adjacent to upper error bars represent post-hoc contrasts between treatments. Treatments that share letters had indicate no significant difference, whereas different letters indicate a significant difference between treatments. Where two variables are shown on the same graph, letters 'a', 'b', and 'c' are used for the left x-axis, and 'x', 'y', and 'z' for the right y-axis. Where post-hoc contrasts did not reveal significant differences between individual treatments, letters are not shown. Figures showing data for both AMF community and strawberry cultivar can be found in Figure S2.1 in Section 2.6.

Table 2.2: The influences of AMF community and strawberry cultivar on plant reproductive traits. Tukey's HSD contrasts between AMF communities and strawberry cultivars are shown below each man effect when the main effect was significant. The statistic column shows F values for main effects, and estimates of differences for contrasts. Error degrees of freedom are listed at the bottom of each column. Significant p-values are in bold, and trends are italicised.

	Plant Reproductive Traits																				
	Flower Number			Nectar Volume (Morning)			Nectar Volume (Afternoon)			Total Nectar Volume			Number of Anthers			Total Anther Mass			Average Anther Mass		
	df	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*
AMF Community	3	2.65	**0.050**	3	0.38	0.767	3	3.13	**0.030**	3	0.43	0.735	3	1.83	0.147	3	1.65	0.185	3	2.13	0.103
Sterile vs. Community 1	3	-0.70	0.947				3	-0.02	0.967												
Sterile vs. Community 2	3	0.40	0.999				3	0.04	0.851												
Sterile vs. Commercial	3	2.04	0.161				3	0.09	0.221												
Community 1 vs. Community 2	3	1.10	0.900				3	0.06	0.613												
Community 1 vs. Commercial	3	2.74	**0.045**				3	0.11	0.100												
Community 2 vs. Commercial	3	1.64	0.213				3	0.05	0.715												
Cultivar	2	36.39	**<0.001**	2	22.09	**<0.001**	2	24.85	**<0.001**	2	35.05	**<0.001**	2	24.95	**<0.001**	2	41.97	**<0.001**	2	25.04	**<0.001**
Elsanta vs. Sonata	2	4.12	**<0.001**	2	-0.10	*0.060*	2	-0.10	**0.017**	2	-0.20	**0.009**	2	-0.08	0.999	2	-0.06	0.765	2	< -0.01	0.688
Elsanta vs. Darselect	2	6.99	**<0.001**	2	0.19	**<0.001**	2	0.14	**<0.001**	2	0.33	**<0.001**	2	-4.49	**<0.001**	2	-0.87	**<0.001**	2	-0.02	**<0.001**
Sonata vs. Darselect	2	2.88	**<0.001**	2	0.29	**<0.001**	2	0.24	**<0.001**	2	0.53	**<0.001**	2	-4.41	**<0.001**	2	-0.81	**<0.001**	2	-0.02	**<0.001**
Year	1	501.47	**<0.001**																		
Community*Cultivar	6	0.86	0.522	6	0.44	0.853	6	1.60	0.157	6	1.26	0.282	6	2.72	**0.019**	6	1.31	0.261	6	0.70	0.652
Community*Year	3	1.75	0.159																		
Cultivar*Year	2	2.43	0.091																		
Community*Cultivar*Year	6	0.20	0.975																		
Error	168			91			91			91			80			80			80		

2.3.2 Pollinator Visitation

AMF community significantly affected the total number of bumblebee visits, and the duration of these visits. Community 1 had the least number of visits compared to any other treatment, and the average duration of visits to plants with Communities 1 and 2 was shorter than plants with the Commercial Inoculant (Figures 2.2d & S2.1d, Table 2.3).

Strawberry cultivar influenced the number and duration of visits, with Elsanta having a higher number but shorter duration of visits than Darselect. The average duration of visits to Sonata plants was higher than Elsanta, but lower than Darselect. AMF community and strawberry cultivar did not interact to influence any visitation variables. We found a significant effect of year as plants were larger and more vigorous in year 2, and larger plants have improved reproductive traits, producing more pollen and nectar in each flower, influencing pollinator visitation (Table 2.3).

Table 2.3: The influence of AMF community and strawberry cultivar on total bumblebee visits per flower. Tukey's HSD contrasts between AMF communities and strawberry cultivars are shown below each main effect when the main effect was significant. The statistic column shows *F* values for main effects, and estimates of differences for contrasts. Error degrees of freedom are listed at the bottom of each column. Significant *p*-values are in bold, and trends are italicised.

	Total Flower Visits per Flower								
	Visit Frequency			Visit Duration			Average Visit Duration		
	df	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*
AMF Community	3	5.84	**<0.001**	3	3.28	**0.022**	3	4.02	**0.009**
Sterile vs. Community 1	3	1.31	**0.003**	3	9.58	0.184	3	1.02	0.193
Sterile vs. Community 2	3	0.91	0.833	3	15.53	0.644	3	1.06	0.436
Sterile vs. Commercial	3	0.99	1.000	3	1.30	0.813	3	-0.98	0.689
Community 1 vs. Community 2	3	-0.40	**0.036**	3	5.95	0.831	3	0.04	0.961
Community 1 vs. Commercial	3	-0.32	**0.002**	3	-8.28	**0.021**	3	-2.00	**0.012**
Community 2 vs. Commercial	3	0.08	0.791	3	-14.24	0.172	3	-2.04	**0.048**
Cultivar	2	7.50	**<0.001**	2	16.29	**<0.001**	2	23.35	**<0.001**
Elsanta vs. Sonata	2	0.34	0.096	2	-13.80	**0.005**	2	-1.47	**0.045**
Elsanta vs. Darselect	2	1.05	**0.001**	2	-13.14	**<0.001**	2	-3.57	**<0.001**
Sonata vs. Darselect	2	0.71	0.179	2	0.66	**0.032**	2	-2.11	**<0.001**
Year	1	9.41	**0.003**	1	10.75	**0.001**	1	36.29	**<0.001**
Community*Cultivar	6	0.93	0.472	6	0.86	0.523	6	0.78	0.588
Community*Year	3	3.86	**0.011**	3	1.88	0.135	3	1.30	0.276
Cultivar*Year	2	6.32	**0.002**	2	8.58	**<0.001**	2	3.09	**0.048**
Community*Cultivar*Year	6	1.38	0.224	6	0.49	0.813	6	0.83	0.547
Error	168			168			168		

2.3.3 Pollen and Nectar Foraging Visits

AMF community influenced both the number and duration of visits when the bumblebees were foraging for pollen. Community 1 had fewer pollen foraging visits than the Commercial Inoculant or sterile control plants, and these visits were shorter than visits to plants with the commercial inoculant (Figures 2.2e & S2.1e, Table 2.4). Strawberry cultivar also influenced the number and duration of pollen foraging visits, with Darselect having more and longer duration visits than either Elsanta and Sonata, and Elsanta had the shortest (Table 2.4).

Nectar foraging visits were only influenced by AMF community, and not plant genotype. There was a trend for Community 1 to have fewer visits than the commercial inocula, as well as having a longer average visit duration than sterile plants (Figures 2.2f & S2.1f, Table 2.4). AMF community and strawberry cultivar did not interact to influence any visitation variables (Table 2.4).

Table 2.4: The influence of AMF community and strawberry cultivar on bumble be visits per flower by foraging behaviour. Tukey's HSD contrasts between AMF communities and strawberry cultivars are shown below each man effect when the main effect was significant. The statistic column shows F values for main effects, and estimates of differences for contrasts. Error degrees of freedom are listed at the bottom of each column. Significant p-values are in bold, and trends are italicised.

	Flower Visits by Foraging Type per Flower																	
	Pollen Visits Frequency			Pollen Visits Duration			Pollen Visits Average Duration			Nectar Visits Frequency			Nectar Visits Duration			Nectar Visits Average Duration		
	df	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*
AMF Community	3	3.26	**0.023**	3	3.20	**0.025**	3	4.05	**0.008**	3	2.71	**0.047**	3	0.15	0.932	3	3.07	**0.029**
Sterile vs. Community 1	3	0.84	**0.042**	3	12.65	0.107	3	1.90	0.148	3	0.24	0.112	3			3	-2.26	**0.034**
Sterile vs. Community 2	3	0.57	0.849	3	11.80	0.659	3	1.34	0.910	3	0.12	1.000	3			3	-0.07	0.982
Sterile vs. Commercial	3	0.48	1.000	3	2.31	0.941	3	-1.03	0.571	3	0.19	0.997	3			3	-0.46	0.956
Community 1 vs. Community 2	3	-0.28	0.253	3	-0.85	0.673	3	-0.56	0.460	3	-0.12	0.118	3			3	2.19	0.089
Community 1 vs. Commercial	3	-0.37	**0.033**	3	-10.33	**0.025**	3	-2.93	**0.004**	3	-0.06	*0.071*	3			3	1.80	0.122
Community 2 vs. Commercial	3	-0.09	0.806	3	-9.48	0.316	3	-2.37	0.210	3	0.07	0.996	3			3	0.38	0.999
Cultivar	2	12.63	**<0.001**	2	15.95	**<0.001**	2	17.91	**<0.001**	2	0.56	0.572	2	0.59	0.553	2	2.18	0.116
Elsanta vs. Sonata		-0.02	0.099		-11.53	**0.009**		-2.03	**0.014**									
Elsanta vs. Darselect		-0.44	**<0.001**		-17.16	**<0.001**		-4.02	**<0.001**									
Sonata vs. Darselect		-0.43	**0.011**		-5.63	**0.023**		-1.99	**0.006**									
Year	1	2.29	0.132	1	8.71	**0.004**	1	42.18	**<0.001**	1	0.33	0.566	1	0.41	0.521	1	24.22	**<0.001**
Community*Cultivar	6	0.57	0.755	6	1.13	0.345	6	1.73	0.117	6	1.25	0.283	6	0.87	0.519	6	0.34	0.915
Community*Year	3	1.28	0.282	3	1.22	0.305	3	1.88	0.135	3	4.56	**0.004**	3	0.19	0.906	3	3.27	**0.023**
Cultivar*Year	2	19.94	**<0.001**	2	16.22	**<0.001**	2	16.72	**<0.001**	2	1.43	0.242	2	2.27	0.107	2	1.68	0.189
Community*Cultivar*Year	6	0.91	0.486	6	0.51	0.804	6	1.47	0.190	6	0.97	0.447	6	0.72	0.634	6	0.18	0.981
Error	168			168			168			168			168			168		

2.4 Discussion

Both AMF community and strawberry cultivars affected plant reproductive traits and pollinator visitation frequency, the duration of visits, and foraging behaviour. Strawberry cultivar influenced all plant reproductive traits, whereas AMF community only affected the number of flowers and the amount of nectar produced in the afternoon. Strawberry cultivar and AMF community only interacted to influence the number of anthers, indicating that the influences of entire natural AMF communities are largely consistent across the strawberry cultivars we tested. The specific AMF community strawberry plants associate with had an important role in determining pollinator visitation, also determining pollinator foraging behaviour. While plants with AMF Community 1 had the greatest number of flowers, the number and duration of total and pollen foraging visits by *Bombus terrestris* Audax were lower per flower. However, the number of nectar foraging visits was increased, while the duration of these visits increased. Reducing the number of nectar foraging visits could improve yields (see Chapter 3 for strawberry yield), by reducing scent markers left by bees after inefficient nectar foraging visits that discourage subsequent highly efficient pollen foraging visits. The number of spores was similar in each of the AMF treatments (see Section 2.2.1), and as such, the effects observed were not the result of the number of propagules within each inocula, but rather were determined by the species present in each community.

2.4.1 AMF influences strawberry flower number and nectar production

Most studies have found that the presence of AMF increases flower number (Bryla and Koide, 1990; Stanley *et al.*, 1993; Lu and Koide, 1994; Koide, 2000; Pendleton, 2000; Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Poulton *et al.*, 2002; Scagel, 2004; Wolfe *et al.*, 2005; Perner *et al.*, 2007; Varga and Kytöviita, 2010b; Aguilar-Chama and Guevara, 2012), but we found that AMF community differentially influenced flower number, with Community 1 producing significantly more flowers than the Commercial Inoculant. Previous research utilised either single AMF species, or a blend of two to three AMF species, whereas we tested natural AMF communities that crops would associate with in the field. Previous studies have shown that the number of flowers a plant produces depends on the presence or absence of AMF (Gange and Smith, 2005), and here we have shown that flower number also depends on which AMF community plants associate with.

In addition to flower number, AMF may benefit plants through improving other reproductive traits, influencing male and female reproductive traits (Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Poulton *et al.*, 2002; Gange and Smith, 2005; Kiers *et al.*, 2010; Barber *et al.*, 2013b), and we found that the AMF communities we tested only influenced nectar production, although only in the afternoon. In *Lycopersicon esculentum* (tomato), AMF induced changes in male reproductive traits due to improved phosphorus acquisition (Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Poulton *et al.*, 2002). However, we found no change in male reproductive traits, suggesting strawberry does not require high P inputs to fulfil male reproductive functions, and that changes in the number of pollen foraging visits may be due to the attraction of pollinators to flowers, and the changes in the duration of these visits could result from differences in the nutritional quality of the pollen produced.

Pollinator visitation rates and behaviour are influenced by the floral rewards available (Buchmann and Cane, 1989; Harder, 1990; Real and Rathcke, 1991; Poulton *et al.*, 2001a; Soto *et al.*, 2013), and differences in floral rewards determined by the inherent traits of crop cultivars can play important roles in shaping plant-pollinator interactions. Strawberry cultivar had a strong effect on plant reproductive traits, significantly influencing all male and female measures. Although there has been some analysis characterising the reproductive traits of strawberry cultivars previously (Grunfeld *et al.*, 1989; Abrol, 1992; Żebrowska, 1998), these studies used cultivars of strawberry that are no longer used in commercial production. By utilising currently used commercial cultivars we can inform growers of the selection of cultivars that promote pollination. For example, planting cultivars with low levels of floral rewards adjacent to other cultivars or crops with a higher level of rewards will deter pollinator visits to the target crop, and in turn reduce the quality and market value of the yield (Kakutani *et al.*, 1993; Żebrowska, 1998; Roselino *et al.*, 2009; Klatt *et al.*, 2014), and management could be improved by planting cultivars with similar levels of floral rewards in close proximity. In addition, strawberry breeding programmes could be improved by informing breeders of which cultivars support pollination, in order to produce highly efficient cultivars. The cultivars we tested exhibit a trade-off between the number of flowers and foraging resources, and while Elsanta produced the most flowers, it had less foraging resources than Sonata or Darselect.

Previous studies have found the potential for interactions between crop cultivars and AMF species (Vestberg, 1992; Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Fan *et al.*, 2011; Sinclair *et al.*, 2014), however, our results show that AMF community and crop cultivar only interact to influence the number of anthers produced in strawberry. AMF have been shown to influence pollen production in some tomato cultivars, but not others (Poulton *et al.*, 2001b), and also interact to influence other traits, such as the number of flowers produced (Poulton *et al.*, 2001b), pollen siring success (Poulton *et al.*, 2001a), and pollinator visitation rates (Poulton *et al.*, 2001a). However, other traits, such as the number of flowers, or pollen germination and tube growth, did not interact (Lau *et al.*, 1995; Poulton *et al.*, 2001a; Poulton *et al.*, 2001b). Thus, although the influences of AMF on plant reproductive traits can be mediated by different crop cultivars, we find that the influences of an entire AMF community to be largely consistent across the strawberry cultivars we tested. Because of this overall lack of interactions, beneficial AMF communities that improve strawberry pollination could be utilised to improve multiple strawberry cultivars.

2.4.2 AMF influences pollinator visitation and foraging behaviour

Efficient pollination improves both the quantity and quality (colour, firmness, shelf life, commercial grade) of strawberry yields, and in turn market value (Roselino *et al.*, 2009; Klatt *et al.*, 2014). Poor pollination results in misshapen fruits (Kakutani *et al.*, 1993; Żebrowska, 1998; Roselino *et al.*, 2009; Klatt *et al.*, 2014), as strawberries require all pistils within a flower to be pollinated for a perfectly shaped fruit to develop (and in turn a high commercial grade) (McGregor, 1976). Increasing the number and duration of bumblebee visits improves the likelihood of effective pollination, and in turn improves crop yields (Chagnon *et al.*, 1989). We found that both AMF community and strawberry cultivar play important roles in determining the frequency and duration of pollinator visits, and thus controlling AMF communities could lead to an improvement in strawberry yields in commercial production.

Examining the foraging behaviour of pollinators allows us to understand the dynamics of plant-pollinator interactions that play important roles in determining yield quantity and quality in commercial crops. Whilst the frequency (Cnaani *et al.*, 2006) and duration (Hodges, 1985) of nectar foraging visits by bumblebees has been shown to be related to the quantity of nectar within a flower, cultivar mediated changes in nectar volumes did not influence nectar foraging visits. However, AMF community mediated changes in nectar volume influenced the duration of nectar foraging visits.

Although Free (1968) found that honeybees spent similar amounts of time foraging for either nectar or pollen (9.8 to 10.7 seconds respectively), we found that bumblebees spent less time on each flower when visiting for nectar (4.9 seconds) compared to pollen (8.7 seconds). Controlling nectar foraging visits is particularly useful for growers, as during these short visits, bees do not always carry pollen loads and only deposit pollen incidentally (Free, 1968). In addition, bumblebees leave scent markers when they visit a flower, and bees will then avoid these flowers, reducing the likelihood of a subsequent visit (Goulson *et al.*, 1998). The combination of short visits with poor pollen deposition and the discouragement of an additional visit increases the chance of incomplete pollination and in turn the development of a poor quality fruit (Kakutani *et al.*, 1993; Żebrowska, 1998; Roselino *et al.*, 2009; Klatt *et al.*, 2014). We found that Community 1 showed a trend to reduce the number of these visits over the commercial inoculant, while significantly increasing the duration of nectar foraging visits over sterile plants. As such, controlling the belowground AMF community may lead to improvements in yield quality through a reduction in the number of nectar foraging visits, while increasing their duration and in turn incidental pollen deposition.

One method of understanding wider plant-pollinator interactions in agro-ecosystems is through the use of ecological interaction networks, by measuring the frequency of interactions between species. These networks, however, are typically captured as a snapshot of the species present and the frequency of their interactions, and there may be important dynamics that are not typically described in these studies that shape these networks and their functions. The inherent traits of plant genotypes, the potential for indirect influences from other organisms, and the nature of the interactions that occur may have important roles in determining the functional outcome of the network. We propose that including plant genotypes or crop cultivars within species interaction networks offers the opportunity to increase the level of resolution of plant-pollinator interaction networks. In addition, incorporating the indirect influences of symbionts such as AMF or herbivores that can have indirect influences on plant-pollinator interactions (Pineda *et al.*, 2010; Barber and Soper Gorden, 2014) into interaction networks allows the study of these systems in settings that more closely mimic a natural environment (see Chapter 4).

Crop production systems may be improved by selecting crop cultivars and AMF communities that promote efficient pollination services to improve yield quantity and quality for growers.

Maintaining efficient plant-pollinator interactions is not only vital in pollinator dependent crops such as strawberry, but is important in the provision of ecosystem services in the wider agro-ecosystem. Future studies that examine the roles of plant genotypes and AMF influences on pollination could provide benefits to crop production, and in other areas such as habitat restoration projects and in the creation of wildflower margins that are designed to benefit pollinators, through the selection of crop cultivars / plant genotypes and AMF communities that maximise pollinator efficiency.

2.5 Conclusion

Here we have shown that altering a belowground AMF-plant network has consequences that cascade through the network to influence aboveground plant-pollinator interactions. As well as influencing plant reproductive traits and overall visitation rates, both AMF communities and strawberry cultivars have roles in determining not only overall visitation rates, but also the foraging behaviour of *Bombus terrestris* Audax. However, these changes are not just related to the presence or absence of AMF, and the specific AMF community a plant associates with influences these interactions. Maintaining beneficial plant-pollinator interactions is vital in pollinator dependent crops such as strawberry to maximise yields, and controlling these associations to promote improved pollination services could provide an important tool to improve yields and increase food security.

2.6 Supplementary Information

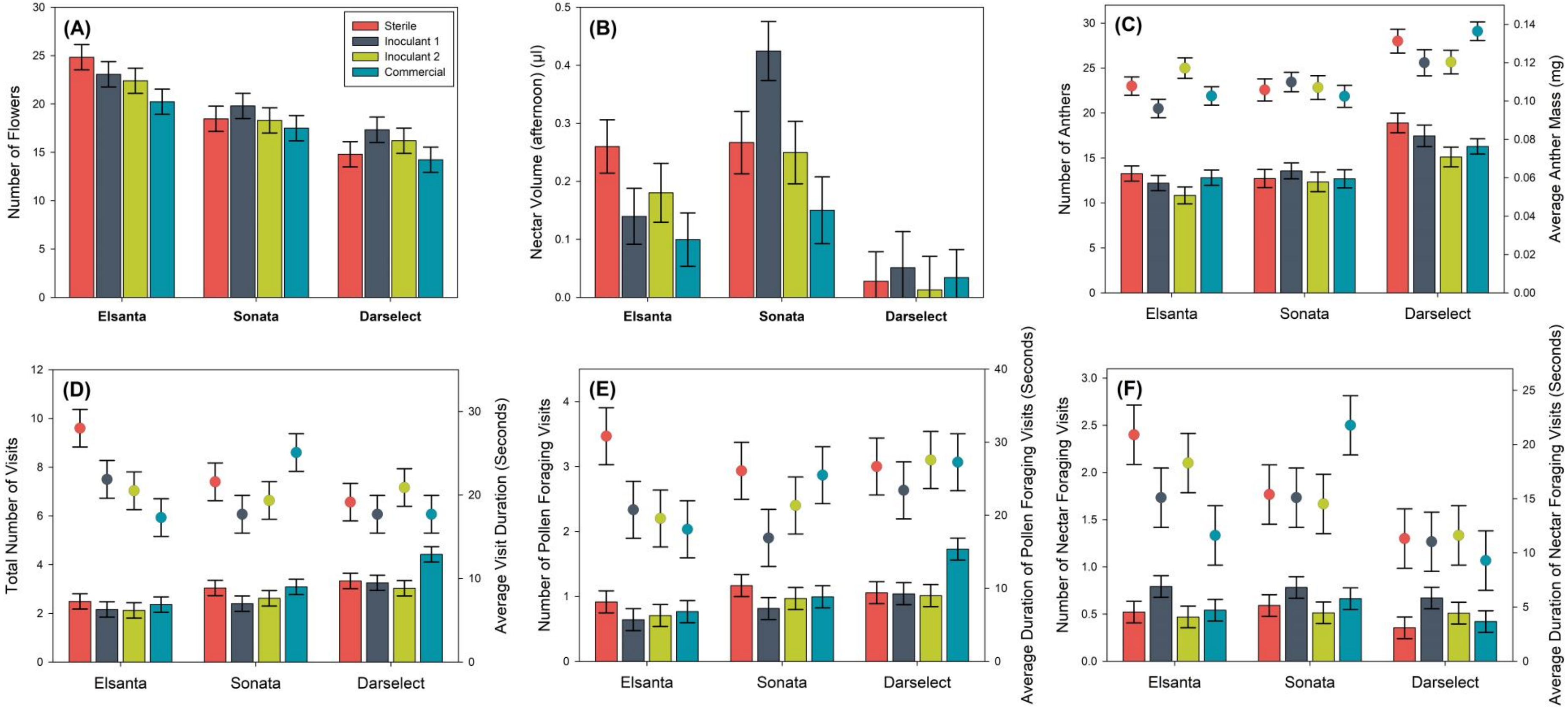


Figure S2.1: Plant reproductive traits and pollinator visitation: (a) Number of flowers by AMF community and strawberry cultivar, (b) amount of nectar produced in the afternoon (µl) by AMF community and strawberry cultivar, (c) number (dots) and average mass (mg) (bars) of anthers by strawberry cultivar and strawberry cultivar, (d) total number (dots) and average duration (seconds) (bars) of bumblebee visits by strawberry cultivar and strawberry cultivar, (e) number (dots) and average duration (seconds) (bars) of pollen foraging visits by strawberry cultivar and strawberry cultivar, and (f) number (dots) and average duration (seconds) (bars) of nectar foraging visits by strawberry cultivar and strawberry cultivar. Values represent least squares means (±SE). The legend for all figures can be found in Figure A.

Chapter 3: Mycorrhizal Fungi Influence Strawberry Yield with no Loss in Fruit Quality Across Multiple Cultivars

Abstract

Global food security is currently facing multiple pressures, and concerns over peak phosphorus are driving volatile fertiliser prices that are rapidly increasing. Arbuscular mycorrhizal fungi (AMF) are able to improve plant acquisition of phosphorus, and potentially improve yields but, despite the availability of several commercial inoculants, have yet to be widely implemented in mainstream agriculture. In addition, the potential use of AMF in agriculture relies on selecting the most beneficial inoculant, as AMF may have either positive or negative effects on plant growth, and the direction of these effects can depend on the specific combination of AMF community and crop species. Commercial strawberry crops are often colonised by AMF at the propagation stage, and as such represent an ideal study-system for examining yield responses to multiple AMF communities. Here I tested four AMF communities, including a commercial inoculant, on three strawberry genotypes in a two-year greenhouse study, and found that AMF are able to significantly influence strawberry yield, with no reduction in fruit quality. I found that in several measures, a natural AMF community extracted from a typical agricultural field margin significantly outperformed the Commercial Inoculant. Overall, there were no significant interactions between AMF community and strawberry cultivar on the response variables studied (except for human perceived strawberry appearance), and as such AMF influenced all strawberry cultivars similarly. As such, AMF hold the potential to improve strawberry production, however selecting an optimal AMF community is key to maximising the potential benefits. As such, AMF have important influences in strawberry yields, and controlling these interactions may form part of a toolset to improve the sustainable intensification of production systems.

3.1 Introduction

With a rapidly growing population (Cohen, 2003; Gerland *et al.*, 2014), there are increasing concerns over global food security (Godfray *et al.*, 2010). The quality and availability of nutrients vital to crop growth, such as phosphorus, are decreasing as concerns over peak phosphorus (a decline in phosphorus availability with the depletion of phosphate rock reserves) rise, and fertiliser prices become increasingly volatile (Cordell and White, 2011). To mitigate these pressures, alternatives to high input chemical fertilisation, such as the use of biological amendments, are required to improve food security. There has been a rise in interest in the use of biological amendments in agriculture, such as 'Integrated Nutrient Management' (Gruhn et al., 2000; Wu and Ma, 2015). The market for biological control of agricultural pests is increasing rapidly (Marrone, 2009), and is standard practice in some production systems in Europe, while growing in others (van Lenteren, 2000; Marrone, 2009), and for pathogens in the USA and China (Bhattacharyya and Jha, 2012). The proposed use of beneficial microbes to reduce reliance on chemical fertiliser inputs (Roy-Bolduc and Hijri, 2011; Orrell and Bennett, 2013) may form an important part of a toolset to meet increasing demands, improve food security, and achieve sustainable intensification. For example, the inoculation of legumes with rhizobia in order to provide nitrogen fixation has been established in agriculture since the 1900s, and inoculated soybeans were estimated to cover 10-20 million hectares per year at the beginning of this century (Catroux *et al.*, 2001). Despite this, improvements have not been made in utilising microbial amendments for non-legume crop species, or in inoculants that contribute to phosphorus fertilisation. There is some evidence that these microbes can reduce the level of phosphorus inputs required (Sharma and Adholeya, 2004; Ceballos *et al.*, 2013), however, although gaining traction in developing countries where the cost of chemical fertilisers can be prohibitively expensive, beneficial organisms that could be utilised to improve fertilisation and reduce reliance on nutrient inputs are not widely used in agriculture in developed countries (Vessey, 2003; Roy-Bolduc and Hijri, 2011).

An example of these beneficial organisms are arbuscular mycorrhizal fungi (AMF). AMF are soil-dwelling fungi that associate with 92% of plant families, including many crop species (Wang and Qiu, 2006), forming mutualistic relationships in which the fungi act as a secondary root system for the plant, providing increased nutrient and water uptake, in exchange for carbon provided by the plant (Smith and Read, 2008). AMF have been shown to improve crop yields in some tropical or arid agricultural systems (Sharma and Adholeya, 2004; Ceballos *et al.*, 2013), however, their effectiveness in a wider range of crops, such as soft fruits, or in temperate regions, remains poorly understood.

AMF, however, do not always provide positive effects. The specific AMF species present can determine the efficiency of phosphorus uptake (Klironomos, 2000), and the specific combination of AMF and plant species can dictate crop yields (Johnson *et al.*, 1992). In addition, AMF have been shown to produce varying effects depending on the crop cultivar, in crop growth (Baon *et al.*, 1993), and yield quality (Baslam *et al.*, 2011a; Baslam *et al.*, 2011b). As such, the combination of AMF species and crop cultivar can determine if interactions are positive or negative, however, the influence of multiple entire natural AMF communities on yield quantity and quality across several crop cultivars remains untested.

Strawberry crops play an important role in the world economy, with an estimated 12.4 million tonnes of strawberries produced in 2014, an increase of 52.4% in the previous decade (FAOSTAT, 2017). The costs of fertilisation are rising (Cordell and White, 2011), with current costs for strawberry at approximately $2,000 USD per hectare under US field conditions (Klonsky, 2012). In order to maintain cost effectiveness and mitigate the rising costs of fertiliser inputs, beneficial microbes such as AMF could form part of an important toolset to mitigate these cost increases, whilst simultaneously improving the sustainability of production systems. AMF are a natural component of the strawberry production system, often forming associations with plants during the propagation stage (personal observation), and hold the potential to improve crop yields. However, as the AMF species present are not currently controlled within production systems, these associations may produce positive or negative effects on strawberry yields. AMF have previously been shown to improve crop yields under a reduced fertiliser regime in cassava (Ceballos *et al.*, 2013). Similar work on strawberry crops showed that AMF improved fruit production at all but the highest P application level, in P limited soil in India (Sharma and Adholeya, 2004). However, both of these studies were in tropical or arid systems, and while numerous studies have shown that AMF have the potential to influence strawberry growth, and a range of other plant traits, studies of the impact of AMF on fruit yield and quality are far fewer (see Section 1.7).

Strawberry cultivar has been shown to have a dominant influence on yields over abiotic environmental influences (Capocasa *et al.*, 2008; Crespo *et al.*, 2010; Gündüz and Özdemir, 2014), and the responses of crops to AMF colonisation have been shown to vary across multiple cultivars in several crop species (Menge and Johnson, 1978; Jun and Allen, 1991; Hetrick *et al.*, 1996; Al-Karaki *et al.*, 2001). Strawberry cultivar also has the potential to interact with and mediate the influence of an AMF species or blend of several species (Vestberg, 1992; Fan *et al.*, 2011; Sinclair *et al.*, 2014), however it is unknown if strawberry cultivars interact with entire AMF communities to influence yield quantity and quality.

In order to harness the potential of AMF to improve sustainable intensification, and improve food security, it is important to understand the effects of AMF on strawberry yield quantity and quality, within a range of strawberry cultivars. Measuring human perceived strawberry quality is important, as factors such as the colour of fruits are used by consumers as indicators of fruit quality, and influence purchasing decisions (Caner *et al.*, 2008). Given that different AMF species have varying effects on plant hosts, and the influence of AMF can depend on crop cultivar, it is important to test combinations of multiple entire AMF communities and crop cultivars. Here we test yield quantity and fruit quality in a fully replicated experimental design, utilising three common commercial cultivars of strawberry, grown with four AMF communities. The objectives of this study are to determine if:

1. AMF communities can influence strawberry yield
2. AMF can influence human perceived fruit quality
3. If AMF influences are mediated by strawberry cultivar

By examining the relationship between AMF and strawberries, it is possible to determine whether AMF can be used in the sustainable intensification of strawberry production. We predict that AMF communities will influence strawberry yield and quality, and that the commercial inoculant will provide the greatest benefit, as it is adapted to a plant growth under controlled conditions. As Community 1 was found to influence the frequency and duration of nectar foraging visits in Chapter 2, we predict that it will also promote strawberry yield. As the influences of AMF have previously been shown to vary between plant genotypes, we predict that AMF influences on strawberry yield and quality will depend on crop cultivar.

3.2 Methods

3.2.1 Study System

In order to test the influence of multiple AMF communities on the yield of several strawberry cultivars, after the flowering period we continued the experiment described in Chapter 2 in order to determine strawberry yield quantity and quality. Following the analysis of pollinator visitation, the same plants used in Chapter 2 were used to assess strawberry yield. Briefly, we conducted a 3 x 4 complete randomised block experiment with three commercial strawberry cultivars (Elsanta, Sonata, and Darselect), and four AMF communities (two natural communities – ‘Community 1’ and ‘Community 2’, a commercial inoculant, and sterile control plants) across two years. The two natural AMF communities were extracted from field soils surrounding The James Hutton Institute, and the Commercial Inocula, RootGrow Professional was purchased from PlantWorks (Sittingbourne, Kent, UK). Plants were maintained in the same conditions in the glasshouse as described in Chapter 2 through the fruiting stage.

3.2.3 Experimental Design

In order to determine crop yield, fruits were harvested daily upon ripening, and fresh weights of each individual strawberry recorded, before being stored in a freezer and subsequently freeze-dried. Six strawberries from each plant were harvested at random intervals during the fruiting period, and three of these were crushed and the resulting juice used to measure Brix (a commonly used measure of sugar content) using a handheld Brix meter (Ref113/114, Index Instruments, Huntingdon, England), and the remaining three used for taste testing. Brix meters are a commonly used measure of fruit sugar content, by determining the refractive index of fruit juices, providing a measure of fruit quality. In August 2015, two years after the initial planting, plants were harvested by cutting off the above ground material (separated into leaves and crowns), and washing the substrate from the roots, before each constituent part of the plant was individually bagged, and aboveground parts dried at 70°C for one week and roots freeze-dried for 48 hours before being weighed to determine plant biomass. AMF colonisation was assessed by staining a subsample of roots using Trypan Blue (Koske and Gemma, 1989) and assessed using the grid-line intersect method (McGonigle *et al.*, 1990).

3.2.4 Taste Testing

Taste testing was conducted by presenting volunteer human testers a set of strawberries and asking them to score the strawberries on a range of characteristics. Taste testing sessions were conducted twice weekly during the fruiting period, and depending on how many fruits were ripe at each session, between four to eight human testers were used for each session, and each presented with six randomly selected strawberries. Testers were asked to score each strawberry on a scale of one to five, with one being the poorest score, and five being the best score. Testers were advised to rate the strawberries individually, comparing them to strawberry quality overall, rather than drawing direct comparisons to the selection of the other test strawberries with which they were presented. Each strawberry was presented in an individual polythene bag coded so that neither the subject nor the tester knew which treatment they came from. Testers were asked to give scores for appearance (how unattractive (score of 1) or attractive (score of 5) the strawberry appeared overall), firmness (how soft (score of 1) or firm (score of 5)), colour (how orange (score of 1) or dark red (score of 5)), brightness (how pale (score of 1) or deep (score of 5) the colour was), flavour (overall poor (score of 1) or good (score of 5) strawberry flavour), and sweetness (if the strawberry had little (score of 1) or a lot (score of 5) of sweetness) (Figure S3.1). Data collection sheets contained two gradient scales indicating a standard range of colours to be used to assess colour and brightness (See Figure S3.1). Participants were encouraged to ensure that scores were provided for each category, however at times testers made mistakes and did not provide scores for all categories.

3.2.5 Statistical Analysis

All statistical analyses were conducted in R version 3.2.4 (R Development Core Team, 2016). Fruit yield and taste test variables were analysed using a three-way repeated measures general linear mixed effect model, using AMF community, cultivar, and year as fixed factors, with random factors of plant identification number to account for repeated measures and block number to account for position in the greenhouse, using the ‘nlme’ package (Pinheiro *et al.*, 2016). Plant biomass and AMF colonisation were analysed using a similar model, however as this was only assessed at the end of the study, year was excluded as a fixed factor. Model assumptions were checked, and variables that did not satisfy a normal distribution of model residuals were transformed, and the best fit used for final results. Number of fruits, plant aboveground biomass, belowground biomass, number of leaves, leaf biomass, number of crowns, and AMF colonisation and brix were square root transformed. Crown biomass was log transformed. All other variables were not transformed. Where main effects were significant, differences between AMF communities and strawberry cultivars were compared using Tukey’s honestly significant difference (HSD), using the ‘lsmeans’ R package (Lenth, 2016).

3.3 Results

3.3.1 Strawberry yield

Fruit yield was measured as the total weight of all strawberries produced per year (total yield), the number of strawberries produced each year (number of fruits), and the average weight of strawberries in each year (average fruit weight). AMF community influenced the total yield, and number of fruits, but not the average fruit weight. Community 1 produced a significantly higher number of strawberries and total yield than the commercial inoculant (Figure 3.1a & S3.2a, Tables 3.1 & S3.1).

Strawberry cultivar influenced all yield variables (total yield, number of strawberries, and average strawberry weight). Elsanta had significantly higher yields, and number of strawberries, but a lower average strawberry weight than either Sonata on Darselect. Cultivar and year interacted to influence total yield and average fruit weight, with a trend for AMF community and strawberry cultivar interacting to influence average fruit weight (Tables 3.1 & S3.1).

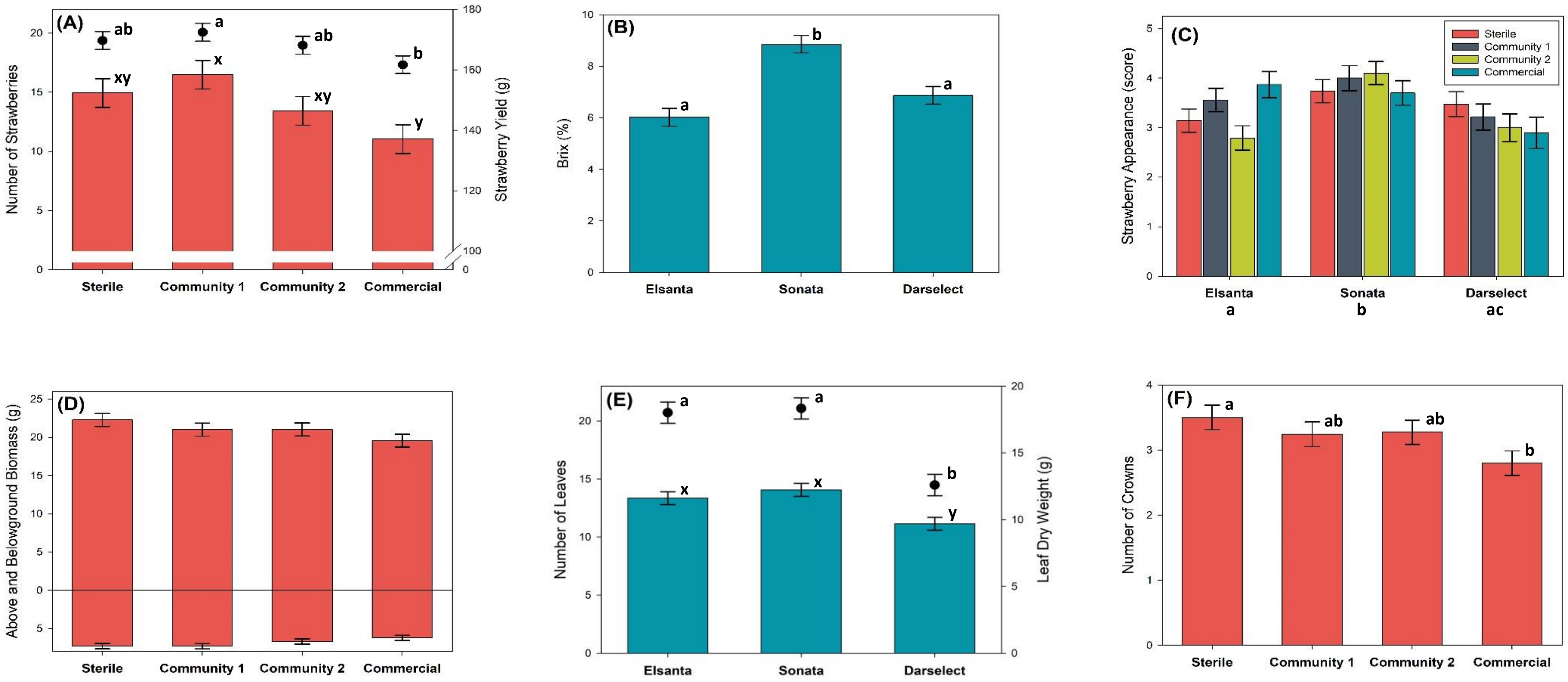


Figure 3.1: Strawberry yield, fruit quality, and plant growth. A) Number (dots) and yield (bars) of strawberries by AMF community, B) sugar content (brix) by strawberry cultivar, C) human perceived strawberry appearance by both AMF community and strawberry cultivar (bars are grouped by strawberry cultivar (x-axis), and see legend for colours of AMF communities, D) aboveground (upper bars) and belowground (lower bars) plant biomass by AMF community, with the line at zero on the y-axis separating above and belowground biomass, E) number (dots) and dry weight (bars) of leaves by strawberry cultivar, and F) number of crowns by AMF community. Values represent least squares means (±SE). Lower case letters adjacent to upper error bars represent post-hoc contrasts between treatments. Treatments that share letters had indicate no significant difference, whereas different letters indicate a significant difference between treatments. Where two variables are shown on the same graph, letters 'a', 'b', and 'c' are used for the left x-axis, and 'x', 'y', and 'z' for the right y-axis. In graph C, letters are located below cultivar names on the x-axis. Where post-hoc contrasts did not reveal significant differences between individual treatments, letters are not shown. Figures showing data for both AMF community and strawberry cultivar can be found in Figure S2.1 in Section 3.6.

Table 3.1: The influence of AMF community and strawberry cultivar on strawberry yield in the greenhouse. Tukey's HSD **c**ontrasts between individual strawberry cultivars and AMF inoculants are shown below each main effect when a main effect was significant. Statistics show *F* values for main effects, and estimates for contrasts. Error degrees of freedom are listed at the bottom of each column. Significant *P* vales are in bold, and trends are italicised.

	Strawberry Yield								
	Total Yield			Number of Fruits			Avergare Fruit Weight		
	df	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*
AMF Community	3	3.66	**0.014**	3	2.65	**0.050**	3	0.33	0.806
Sterile vs. Community 1	3	-6.06	0.804	3	-0.70	0.947			
Sterile vs. Community 2	3	5.92	0.815	3	0.40	0.999			
Sterile vs. Commercial	3	15.26	0.109	3	2.04	0.161			
Community 1 vs. Community 2	3	11.98	0.285	3	1.10	0.900			
Community 1 vs. Commercial	3	21.32	**0.010**	3	2.74	**0.045**			
Community 2 vs. Commercial	3	9.35	0.507	3	1.64	0.213			
Strawberry Cultivar	2	6.34	**0.002**	2	36.39	**<0.001**	2	27.62	**<0.001**
Elsanta vs. Sonata	2	14.72	**0.033**	2	4.12	**<0.001**	2	-0.54	**0.046**
Elsanta vs. Darselect	2	19.99	**0.002**	2	6.99	**<0.001**	2	-1.64	**<0.001**
Sonata vs. Darselect	2	5.27	0.638	2	2.88	**<0.001**	2	-1.10	**<0.001**
Year	1	564.60	**<0.001**	1	501.47	**<0.001**	1	0.03	0.857
Community*Cultivar	6	0.97	0.444	6	0.86	0.522	6	2.14	*0.051*
Community*Year	3	2.26	0.084	3	1.75	0.159	3	1.89	0.134
Cultivar*Year	2	5.60	**0.004**	2	2.43	0.091	2	7.64	**0.001**
Community*Cultivar*Year	6	0.31	0.931	6	0.20	0.975	6	0.68	0.664
Error	168			168			168		

3.3.2 Taste test / Fruit Quality

AMF community did not influence any strawberry taste test variable, except for strawberry appearnce, where AMF also interacted with strawberry cultivar to influence fruit appearance (Figure 3.1c). Each strawberry cultivar responded differently to an AMF community, and the commercial inoculant improved strawberry appearance in Elsanta, had no effect in Sonata, and impaired appearance in Darselect. Strawberry cultivar influenced all taste test variables, and year only influenced strawberry colour, with cultivar and year interacting to influence appearance, firmness, flavour, and sweetness. Elsanta produced fruits that had better colour, but a worse overall appearance, brightness, and sweetness compared to Sonata, and less firm fruit than Darselect. Fruit from Sonata plants had significantly better appearance, sweetness and juiciness, but were less firm than Darselect fruit. Both human taste testing and Brix measurements showed that fruit sugar content was influenced only by strawberry cultivar, with Sonata producing sweeter strawberries than either Elsanta or Darselect (Figure 3.1b & S3.2b, Table 3.2 & S3.2).

Table 3.2: The influence of AMF community and strawberry cultivar on yield quality. Tukey's HSD **c**ontrasts between individual strawberry cultivars and AMF inoculants are shown below each main effect when a main effect was significant. Statistics show *F* values for main effects, and estimates for contrasts. Error degrees of freedom are listed at the bottom of each column, and vary when testers did not record a value for all questions asked. Significant *P* vales are in bold, and trends are italicised.

Strawberry Quality (Taste Test)

	Appearance			Firmness			Colour			Brightness		
	df	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*
AMF Community	3	0.62	0.606	3	0.32	0.812	3	0.02	0.996	3	0.78	0.507
Strawberry Cultivar	2	14.11	**<0.001**	2	16.29	**<0.001**	2	4.45	**0.013**	2	4.55	**0.012**
Elsanta vs. Sonata	2	-0.54	**0.007**	2	0.32	*0.069*	2	0.37	**0.010**	2	-0.46	**0.012**
Elsanta vs. Darselect	2	0.19	0.550	2	-0.53	**0.002**	2	0.27	0.107	2	-0.35	0.100
Sonata vs. Darselect	2	0.74	**0.001**	2	-0.85	**<0.001**	2	-0.10	0.729	2	0.11	0.778
Community*Cultivar	6	2.33	**0.035**	6	1.03	0.409	6	1.67	0.132	6	0.49	0.816
Error	159			165			165			165		
Year	1	1.35	0.250	1	1.95	0.166	1	19.62	**<0.001**	1	0.25	0.616
Community*Year	3	0.22	0.879	3	0.49	0.691	3	0.50	0.686	3	1.02	0.386
Cultivar*Year	2	3.44	**0.038**	2	3.27	**0.042**	2	2.90	*0.059*	2	1.22	0.298
Community*Cultivar*Year	6	2.24	**0.050**	6	0.98	0.442	6	1.29	0.267	6	0.64	0.702
Error	64			110			110			110		

	Flavour			Sweetness			Juciness			Brix		
	df	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*
AMF Community	3	0.84	0.474	3	0.07	0.975	3	0.93	0.429	3	0.19	0.903
Strawberry Cultivar	2	8.08	**<0.001**	2	8.38	**<0.001**	2	4.32	**0.015**	2	18.95	**<0.001**
Elsanta vs. Sonata	2	-0.53	**0.007**	2	-0.59	**0.003**	2	-0.23	0.296	2	-2.84	**<0.001**
Elsanta vs. Darselect	2	0.02	0.996	2	-0.06	0.941	2	0.26	0.272	2	-0.85	0.281
Sonata vs. Darselect	2	0.55	**0.009**	2	0.53	**0.015**	2	0.49	**0.011**	2	1.98	**<0.001**
Community*Cultivar	6	1.16	0.331	6	0.87	0.519	6	1.65	0.137	6	1.23	0.294
Error	165			165			165			161		
Year	1	1.26	0.265	1	2.95	0.089	1	0.98	0.324			
Community*Year	3	0.88	0.455	3	1.34	0.265	3	1.98	0.122			
Cultivar*Year	2	9.08	**<0.001**	2	3.61	**0.030**	2	1.56	0.215			
Community*Cultivar*Year	6	1.35	0.241	6	0.61	0.725	6	0.31	0.932			
Error	110			110			101					

3.3.3 Plant biomass

Plant biomass was measured as total aboveground biomass and total belowground biomass, as well as the number and dry mass of the constituent aboveground plant organs (leaves and crowns). AMF community reduced belowground plant biomass, and the number of crowns produced, with a trend to reduce aboveground biomass (Figures 3.1 & S3.2, Tables 3.3 & S3.3). Plants with the Commercial Inoculant produced significantly fewer crowns and a trend to lower belowground biomass than sterile control plants.

Strawberry cultivar influenced all measures of plant biomass (above and belowground biomass, number and biomass of leaves, and number and biomass of crowns) (Figures 3.1 & S3.2, Tables 3.3 & S3.3). Elsanta plants had significantly more aboveground biomass than Sonata plants, driven by a significant increase in crown biomass. Both Elsanta and Sonata were significantly bigger than Darselect plants for all measures.

Table 3.3: The influence of AMF community and strawberry cultivar on plant biomass. Tukey's HSD **c**ontrasts between individual strawberry cultivars and AMF inoculants are shown below each main effect when a main effect was significant. Statistics show *F* values for main effects, and estimates for contrasts. Error degrees of freedom are listed at the bottom of each column, and vary when testers did not record a value for all questions asked. Significant *P* vales are in bold, and trends are italicised.

	Plant Growth																	
	Aboveground Biomass			Belowground Biomass			Number of Leaves			Leaf Biomass			Number of Crowns			Crown Biomass		
	df	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*
AMF Community	3	2.54	*0.059*	3	2.79	**0.042**	3	1.74	0.162	3	2.17	0.094	3	3.20	**0.025**	3	2.03	0.112
Sterile vs. Community 1				2	-0.02	0.998							2	0.26	0.666			
Sterile vs. Community 2				2	0.61	0.452							2	0.23	0.677			
Sterile vs. Commercial				2	1.07	*0.062*							2	0.70	**0.015**			
Community 1 vs. Community 2				2	0.62	0.558							2	-0.03	1.000			
Community 1 vs. Commercial				2	1.08	0.093							2	0.45	0.224			
Community 2 vs. Commercial				2	0.46	0.730							2	0.47	0.222			
Strawberry Cultivar	2	26.68	**<0.001**	2	55.26	**<0.001**	2	21.00	**<0.001**	2	9.84	**<0.001**	2	16.16	**<0.001**	2	56.68	**<0.001**
Elsanta vs. Sonata	2	2.38	**0.023**	2	0.87	0.085	2	-0.37	0.984	2	-0.63	0.678	2	-0.09	0.782	2	2.94	**<0.001**
Elsanta vs. Darselect	2	6.41	**<0.001**	2	3.49	**<0.001**	2	6.23	**<0.001**	2	1.91	**0.003**	2	1.07	**<0.001**	2	4.53	**<0.001**
Sonata vs. Darselect	2	4.04	**<0.001**	2	2.62	**<0.001**	2	6.60	**<0.001**	2	2.54	**<0.001**	2	1.15	**<0.001**	2	1.58	**<0.001**
Community*Cultivar	6	1.15	0.337	6	0.83	0.547	6	1.37	0.229	6	1.45	0.200	6	0.67	0.672	6	0.71	0.644
Error	166			166			166			166						166		

AMF colonisation rates were significantly influenced by AMF community ($F_{3,148}$ =50.17, p<0.001), with a trend for strawberry cultivar ($F_{2,148}$ =2.98, p=0.054), and a significant interaction between AMF community and strawberry cultivar ($F_{6,148}$ =2.356, p=0.033) (Figure 3.2). AMF colonisation was higher in the inoculated treatments (Community 1, Community 2, and the Commercial Inocula) than sterile control plants (p<0.001), but colonisation rates did not differ between inoculated treatments (p>0.05).

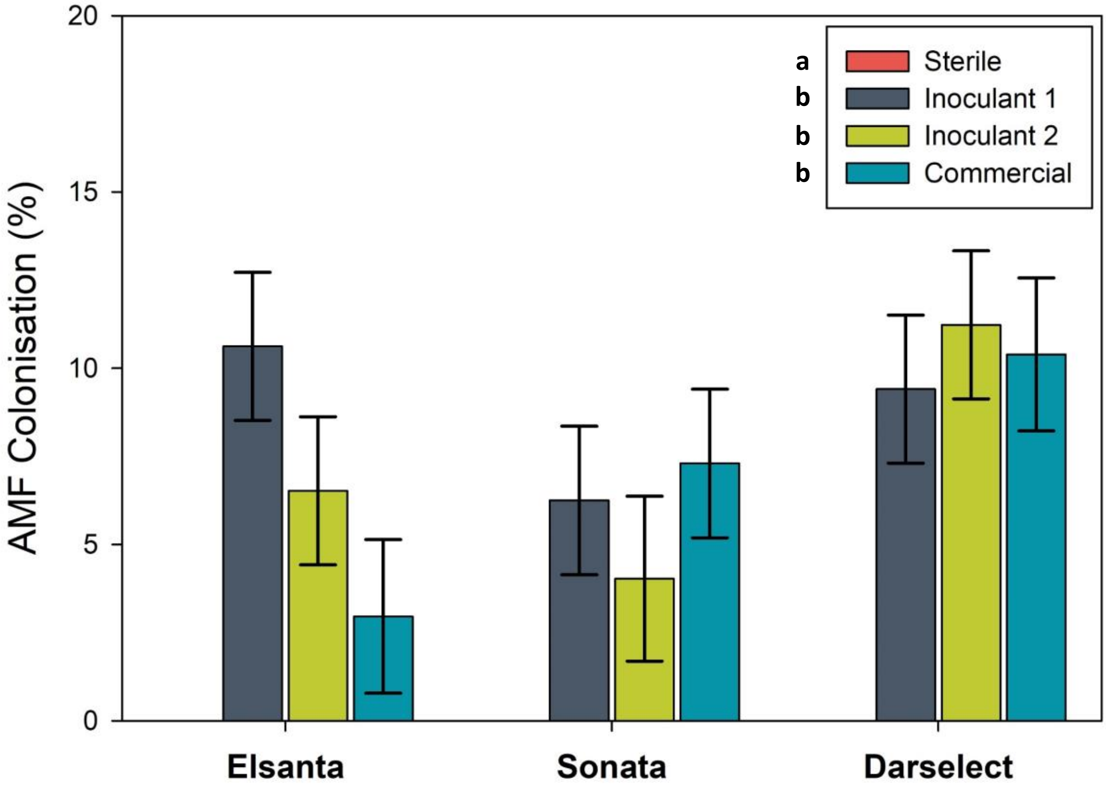


Figure 3.2: Percentage of roots colonised by each AMF community across strawberry cultivars. Bars are grouped by strawberry cultivar (x-axis), and see legend for colours of AMF communities. Sterile (red) bars are not visible, as sterile plants were not colonised. Values represent least squares means (±SE). Lower case letters adjacent to the figure legend represent post-hoc contrasts between treatments. Treatments that share letters had indicate no significant difference, whereas different letters indicate a significant difference between treatments.

3.4 Discussion

We predicted that the commercial inoculant would provide the greatest benefit to fruit yield and quality due to being adapted to high-intensity growth environments, and that the natural AMF would provide less benefit. However, we found that the greatest benefits were provided by the lower diversity natural AMF Community 1, over either the higher diversity 'Community 2' or the commercial inoculant, indicating that whole AMF communities may promote strawberry yield over commercial inocula.

3.4.1 Strawberry Yield

Here we have shown that AMF are able to influence the quantity of strawberry yield, without altering yield quality (average fruit weight, sugar content, human perceived taste test). As strawberry plants are naturally colonised by AMF at the propagation stage, controlling these interactions could improve yields and prevent losses from negative interactions. The influence of AMF on yield was dependent on the specific AMF community, with Community 1 producing higher yields than the commercial inoculant. We found a 14.44% difference in yields between the best (Community 1) and worst (Commercial inoculant) AMF communities, and as strawberry plants naturally associate with AMF in production systems, growers may be able to improve yields if these interactions are controlled. Based on our results and global production figures (FAOSTAT, 2017), utilising a beneficial AMF inoculant could increase yields global yields by 1.8 million tons. In addition, AMF community only interacted with strawberry cultivar for fruit appearance, indicating that these influences are predictable across a range of strawberry cultivars.

AMF have previously been shown to have the potential to positively or negatively impact strawberry yield (Sharma and Adholeya, 2004; Camprubí *et al.*, 2007; Sinclair *et al.*, 2014; Robinson-Boyer *et al.*, 2016), which may be explained through an increase in plant nutrition. AMF can influence strawberry growth through increased plant nutrient content (Marschner and Dell, 1994). Increased plant nutrition has been shown improve strawberry yields (May and Pritts, 1993), however AMF species (Raju *et al.*, 1990; Munkvold *et al.*, 2004) and strains (Munkvold *et al.*, 2004) have different capacities for nutrient uptake. Thus, variation in nutrient provision between AMF communities could explain variation in strawberry yield.

AMF may improve strawberry production through other means. For example, Niemi and Vestberg (1992) found that inoculation with several different AMF strains could improve runner production by 76%, with Sharma and Adholeya (2004) finding similar results in a range of P fertilisation levels, except for at the highest application rate. Strawberry runners provide two benefits by 1) producing fruits and increasing yield, and 2) providing stock for plant propagators. As such, AMF hold the potential to not only improve strawberry yield, but additional factors of strawberry cultivation, such as propagation, improving commercial strawberry production in several ways.

AMF do not always form mutualistic relationships, as AMF that promotes plant growth in one plant species can be parasitic on another (Klironomos, 2003), and species-specific interactions could reduce yields. Even cultivars of other crop species can have varying responses to AMF (Menge and Johnson, 1978; Jun and Allen, 1991; Hetrick *et al.*, 1996). However, we found although changes in yield differed by AMF community, the influences of an AMF community were consistent across multiple strawberry cultivars. Our results show that in strawberry, that apart from strawberry appearance, AMF community did not interact with strawberry cultivar, and as such, the influence of our AMF communities on a strawberry cultivar had predictable influences on other strawberry cultivars.

However, AMF interactions are context dependent, depending on the conditions of the growing system (Barber *et al.*, 2013; Johnson *et al.*, 2015), and as such the influences of AMF on strawberry yield must be tested in the field, in order to determine how these communities influence strawberry yield when exposed to natural environmental conditions (see Chapter 4).

3.4.2 Taste Test

Examining human perceived measures of strawberry quality is vital, as consumers use aspect such as the colour and firmness of fruit as indicators of quality, and influence purchasing decisions (Caner et al., 2008). Strawberry cultivar had the most consistent and strongest influence over all taste test results, and previous research has shown that strawberry variety has the largest impact on factors that influence taste and nutritional content over abiotic factors such as environmental conditions (Capocasa *et al.*, 2008; Crespo *et al.*, 2010; Gündüz and Özdemir, 2014). Cultivars influence fruit quality through changes in the composition of chemical compounds within the fruit. Studies have shown that strawberry cultivars show different levels of individual sugars, organic acids, and vitamin C, which are responsible for strawberry taste, and anthocyanins which give strawberries their colour (Crespo *et al.*, 2010).

While AMF influence strawberry yield, except for appearance, human perceived fruit quality was not affected by AMF, and thus improvements in yield did not result in a loss of quality. Although no difference was found in human perceived taste of the strawberries, AMF community interacted with strawberry cultivar to affect strawberry appearance, with each strawberry cultivar responding differently to each AMF community. Strawberry appearance has a large role in determining customer choice of products, and in turn the price received by growers. Due to recent focus on the nutritional advantages of eating strawberries, research is currently looking to improve strawberry cultivars through molecular marker based breeding programmes (Urrutia *et al.*, 2015), and incorporating a focus on producing cultivars that are able to exploit AMF induced phosphorus uptake holds the potential to further improve strawberry yield whilst maintaining fruit quality, and a reduction in the level of nutrient inputs required (Bucher, 2007).

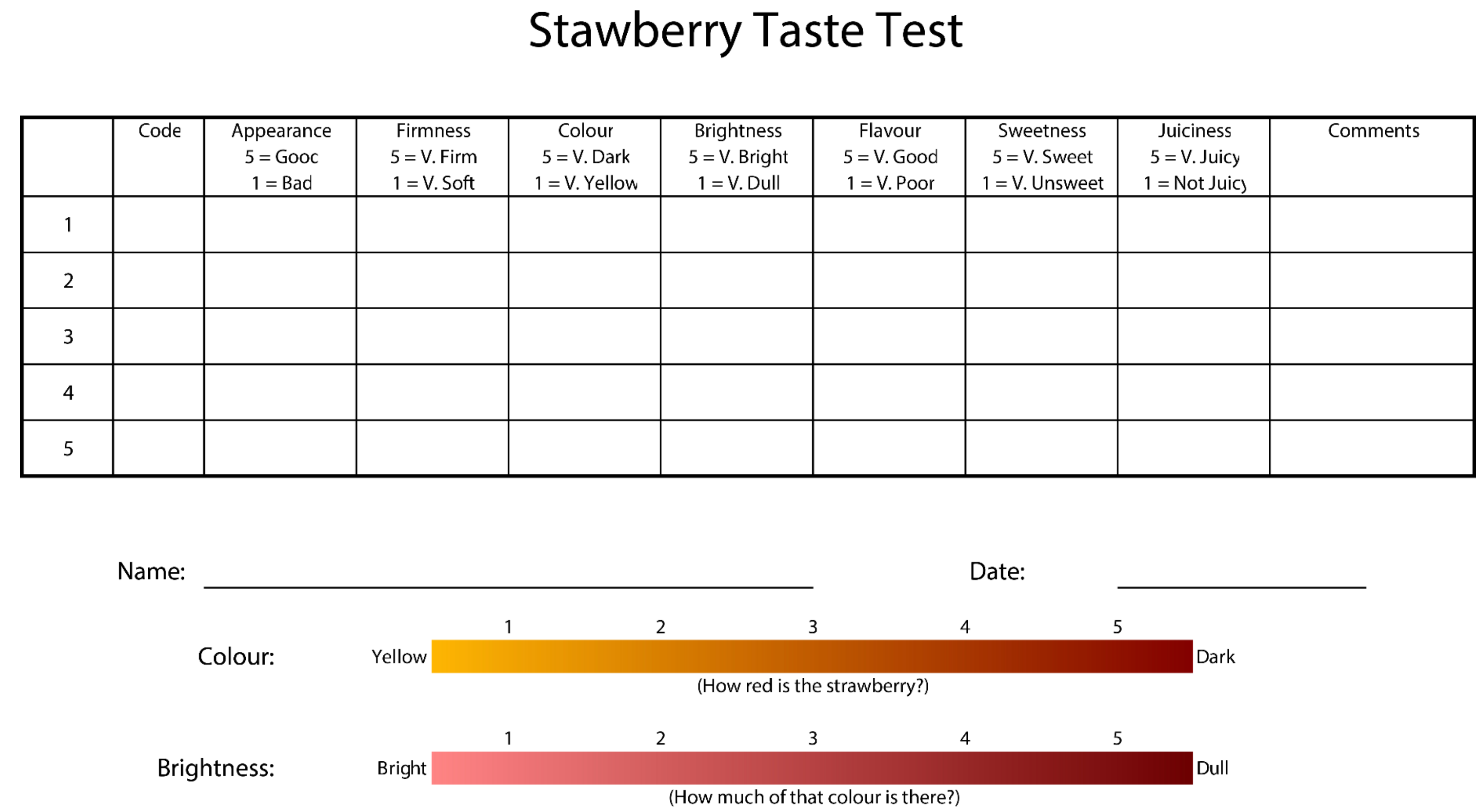
By studying the influence of AMF communities on commercially produced strawberry cultivars, we are able to not only further our understanding of AMF-crop interactions, but also inform growers of techniques that may be able to improve the production of commercially important crop species. By controlling which AMF community strawberry plants associate with, we may be able to increase crop yields with no loss in quality.

Understanding interactions between AMF communities and strawberry cultivars in commercially important crops species, such as strawberry, presents an opportunity to improve production systems, and controlling these interactions will mitigate crop losses resulting from negative AMF interactions, and play an important role in food security and sustainable intensification. Research in this area may form an important tool in sustainably improving the quantity and quality of yields in commercial strawberry production and other soft fruits, helping to further safeguard food security in times of rapid population growth, and pressures such as increases in costs and a reduction in the availability of agro-chemicals.

3.5 Conclusion

While the influence of AMF on plant growth has been extensively studied, there is little evidence of their influence on crop yields, if these impacts are mitigated by the traits of crop cultivars, or if changes in yield can affect crop quality. Here we have shown that AMF communities that plants associate with in the field have the potential to improve strawberry yield quantity by 14%, if the community a plant associates with is controlled. We measured the functional consequences of altering the ecosystem service provided by AMF from a human perspective by conducting a taste test, and found that these improvements in yield do not result in any loss in human perceived fruit quality. AMF only interacted with strawberry cultivar to influence human perceived strawberry appearance, indicating that AMF communities largely have the same influence on multiple strawberry cultivars. As strawberry plants are naturally colonised by AMF, controlling these interactions represents an opportunity to improve yields and strawberry production. However, AMF influences on strawberry yield must be tested in the field, where plants are exposed to similar conditions to commercial production systems. With a rapidly growing population, increasing pressures on food security, and concerns over peak phosphorus, understanding and utilising interactions with potentially beneficial microbes presents an opportunity to improve sustainable intensification and increase food security.

3.6 Supplementary Information



Stawberry Taste Test

	Code	Appearance 5 = Gooc 1 = Bad	Firmness 5 = V. Firm 1 = V. Soft	Colour 5 = V. Dark 1 = V. Yellow	Brightness 5 = V. Bright 1 = V. Dull	Flavour 5 = V. Good 1 = V. Poor	Sweetness 5 = V. Sweet 1 = V. Unsweet	Juiciness 5 = V. Juicy 1 = Not Juicy	Comments
1									
2									
3									
4									
5									

Name: ______________________ Date: ____________

Colour: Yellow 1 2 3 4 5 Dark

(How red is the strawberry?)

Brightness: Bright 1 2 3 4 5 Dull

(How much of that colour is there?)

Figure S3.1: Taste test data entry sheet provided to human tasters. Each taster was asked to score the appearance, firmness, colour, brightness, flavour, sweetness, and juiciness of each strawberry on a scale of 1 to 5. Gradient scales were provided to give a standardised range for colour and brightness and to show the difference between the two variables.

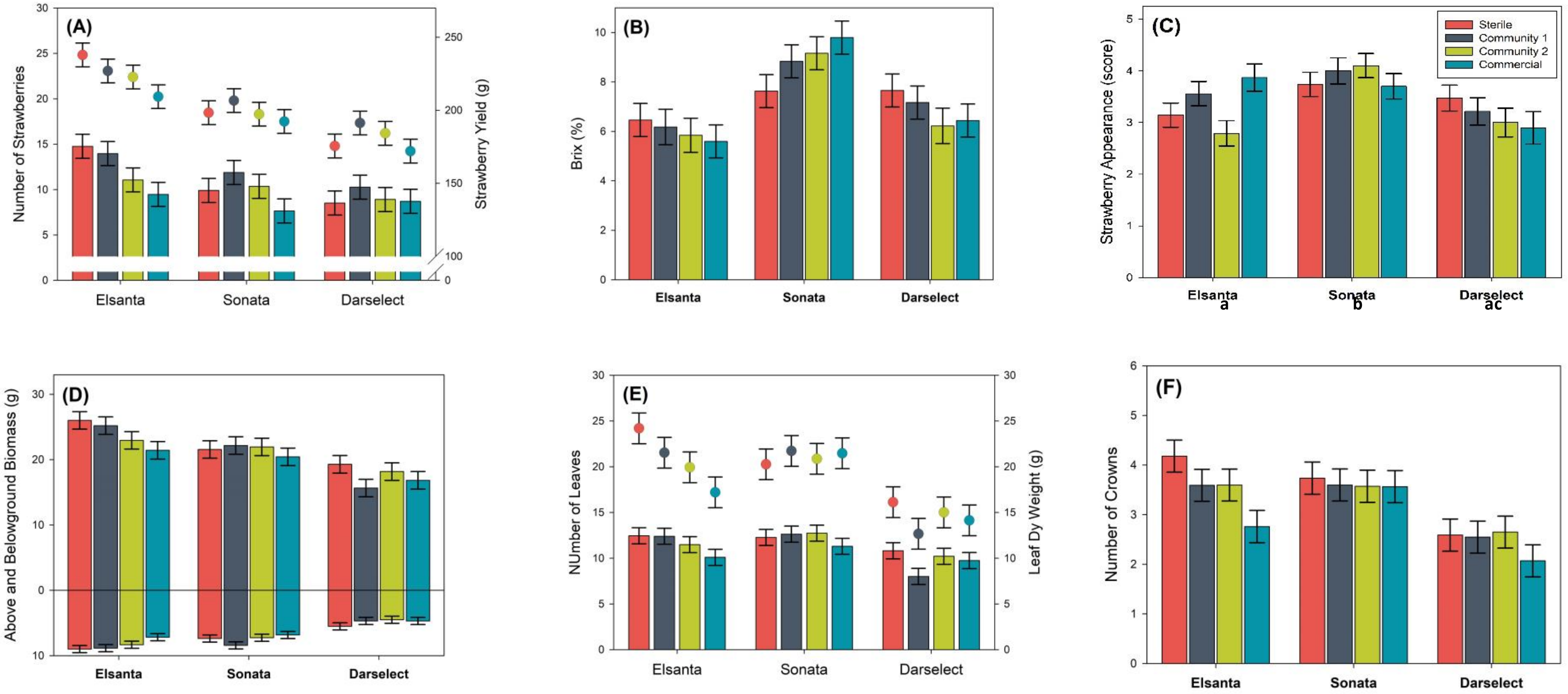


Figure S3.2: Strawberry yield, fruit quality, and plant growth. A) Number (dots) and yield (bars) of strawberries by AMF community and strawberry cultivar, B) sugar content (brix) by AMF community and strawberry cultivar, C) human perceived strawberry appearance by AMF community and strawberry cultivar (bars are grouped by strawberry cultivar (x-axis), and see legend for colours of AMF communities, D) aboveground (upper bars) and belowground (lower bars) plant biomass by AMF community and strawberry cultivar, with the line at zero on the y-axis separating above and belowground biomass, E) number (dots) and dry weight (bars) of leaves by AMF community and strawberry cultivar, and F) number of crowns by AMF community and strawberry cultivar. Values represent least squares means (±SE). The legend for all figures can be found in Figure C.

Table S3.1: Summary of least squares means and standard errors for yield variables. AMF communities or cultivars with different letters are significantly different from each other, and shared letters indicate no significant difference (see Table 3.1). Contrasts are only shown where a main effect was significant.

	Strawberry Yield								
	Total Yield			Number of Fruits			Avergare Fruit Weight		
	Mean	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*
AMF Community									
Sterile	152.32	4.75	ab	19.37	0.75	ab	152.32	4.75	
Community 1	158.38	4.75	a	20.07	0.75	a	158.38	4.75	
Community 2	146.40	4.75	ab	18.97	0.75	ab	146.40	4.75	
Commercial	137.06	4.75	b	17.32	0.75	b	137.06	4.75	
Strawberry Cultivar									
Elsanta	160.11	4.11	a	22.63	0.65	a	160.11	4.11	a
Sonata	145.39	4.11	b	18.52	0.65	b	145.39	4.11	b
Darselect	140.12	4.11	b	15.64	0.65	c	140.12	4.11	c

Table S3.2: Summary of least squares means and standard errors for strawberry quality variables. AMF communities or cultivars with different letters are significantly different from each other, and shared letters indicate no significant difference (see Table 3.2). Contrasts are only shown where a main effect was significant.

Strawberry Quality (Taste Test)												
	Appearance			Firmness			Colour			Brightness		
	Mean	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*
AMF Community												
Sterile	3.45	0.14		3.41	0.12		3.56	0.10		3.27	0.13	
Community 1	3.59	0.14		3.52	0.12		3.56	0.11		3.02	0.14	
Community 2	3.30	0.15		3.59	0.12		3.53	0.11		3.22	0.14	
Commercial	3.49	0.16		3.45	0.13		3.53	0.11		3.10	0.14	
Strawberry Cultivar												
Elsanta	3.34	0.12	a	3.42	0.10	a	3.76	0.09	a	2.88	0.11	a
Sonata	3.89	0.12	b	3.10	0.10	a	3.39	0.09	b	3.34	0.11	b
Darselect	3.15	0.14	a c	3.95	0.11	b	3.49	0.10	ab	3.23	0.13	ab

	Flavour			Sweetness			Juciness			Brix		
	Mean	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*
AMF Community												
Sterile	3.15	0.14		2.96	0.14		3.27	0.13		7.25	0.39	
Community 1	3.03	0.15		2.92	0.15		3.45	0.13		7.39	0.40	
Community 2	3.40	0.15		3.07	0.15		3.25	0.13		7.08	0.40	
Commercial	3.18	0.15		3.01	0.15		3.51	0.14		7.28	0.39	
Strawberry Cultivar												
Elsanta	3.02	0.12	a	2.77	0.12	a	3.38	0.11	ab	6.02	0.34	a
Sonata	3.55	0.12	b	3.36	0.12	b	3.61	0.11	a	8.85	0.33	b
Darselect	3.00	0.13	a	2.84	0.14	a	3.12	0.12	b	6.87	0.34	a

Table S3.3: Summary of leas squares means and standard errors for growth variables. AMF communities or cultivars with different letters are significantly different from each other, and shared letters indicate no significant difference (see Table 3.3). Contrasts are only shown where a main effect was significant.

	Plant Growth																	
	Aboveground Biomass			Belowground Biomass			Number of Leaves			Leaf Biomass			Number of Crowns			Crown Biomass		
	Mean	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*	*Mean*	*St. Error*	*Group*
AMF Community																		
Sterile	22.28	0.85		7.31	0.35		20.20	1.03		11.85	0.54		3.50	0.19	a	10.73	0.39	
Community 1	21.01	0.85		7.32	0.35		18.64	1.03		11.01	0.54		3.24	0.19	ab	10.31	0.39	
Community 2	21.03	0.85		6.70	0.35		18.60	1.03		11.48	0.54		3.27	0.19	ab	9.86	0.39	
Commercial	19.56	0.85		6.24	0.35		17.60	1.03		10.38	0.54		2.80	0.19	b	9.61	0.40	
Strawberry Cultivar																		
Elsanta	23.90	0.77	a	8.35	0.31	a	20.72	0.91	a	11.61	0.48	a	3.53	0.16	a	12.61	0.35	a
Sonata	21.52	0.77	b	7.48	0.31	a	21.08	0.91	a	12.23	0.48	a	3.62	0.16	a	9.67	0.36	b
Darselect	17.49	0.77	c	4.86	0.31	b	14.48	0.91	b	9.69	0.48	b	2.46	0.16	b	8.09	0.35	c

Chapter 4: The Effects of AMF Community and Strawberry Cultivar on Interactions with Wild Pollinators and Strawberry Yield

Abstract

Ecological networks examine bi-partite interactions between communities, however do not take into account aspects such as AMF-plant interactions, or the traits of crop cultivars, which may influence plant-pollinator interactions. In addition, it is poorly understood if altered network structures have functional consequences. While previous studies have shown that AMF can influence interactions between plants and wild pollinators it remains unknown if these changes have consequences for crop yields. The visitation of wild pollinators can influence yields through the efficiency of individual visits, or by functional redundancy provided by an increase in visit frequency by highly abundant species, and complementary foraging techniques resulting from visits by a diverse community of pollinators. In this chapter, I measure the frequency of visits by wild pollinators overall, along with sub-taxa of pollinators, and determine the functional consequences on crop yields compared to visits by bumblebees in the greenhouse (Chapters 2 and 3). I found that AMF community did not influence the frequency of wild pollinator visitation overall, but did influence the frequency of visits from Hymenoptera (although Hymenoptera visits were low, and may have been an effect of sampling completeness). I measured the yield of plants, and found the same pattern of yield between AMF communities as in the greenhouse, indicating that the wild pollinator community was able to fulfil crop pollination requirements to the same degree as the highly efficient *Bombus terrestris* Audax in the greenhouse. Both of the natural AMF communities improved strawberry yield to a greater degree than in the greenhouse, indicating that AMF communities may provide the greatest benefit to crop yields in environments they are adapted to. As such, wild pollinators provided a high degree of functional redundancy, and these results support the value of the ecosystem service they provide.

4.2 Introduction

The indirect influences of belowground plant mutualists (Barber and Soper Gorden, 2014), such as Arbuscular mycorrhizal fungi (AMF) (Poulton *et al.*, 2001; Gange and Smith, 2005; Wolfe *et al.*, 2005; Cahill *et al.*, 2008; Varga and Kytöviita, 2010b; Barber *et al.*, 2013), and the inherent traits of crop cultivars (Parker, 1981; Hagler *et al.*, 1990; Schneider *et al.*, 2002; Chambó *et al.*, 2011), have both been shown to influence plant-pollinator interactions. Previously, studies of how these factors affect plant-pollinator interactions have focused on the visitation rates of broad taxonomic groups of pollinators (typically orders), however utilising a community level approach (Gehring and Bennett, 2009) and examining interaction networks (Memmott, 1999) offers the opportunity to study the dynamics of these interaction networks at a higher resolution. As defined by Bennett *et al.* (2017), a community approach explores interactions between multiple species interacting concurrently as communities, rather than interactions between individual species. Furthermore, this approach can be used to understand the functional consequences on the provision of ecosystem services, such as pollination, by altered network structures (Thompson *et al.*, 2012). However, to date it remains untested as to how the influences of either AMF communities or crop cultivars can influence the interactions of wild pollinator communities and if this has functional consequences for crop yields.

Pollination is a key ecosystem service, contributing significantly to crop production, and the loss of pollinators would reduce the yields of commercially important crops below current levels of consumption (Gallai *et al.*, 2009). Insect pollination can improve both the quantity and quality of crop yields (Klatt *et al.*, 2014), however, crop production does not only rely on simply having a sufficient abundance of pollinators. For example, pollinators have different foraging techniques, and visits to flowers by multiple pollinators improves the likelihood of all stigmas receiving pollen (Chagnon *et al.*, 1993). As such, the diversity of pollinator visits has been shown to play an important role in determining not only the quantity, but also the quality and market value of yields (Chagnon *et al.*, 1993).

4.2.1 Improving the resolution of plant-pollinator interactions

One method of analysing interactions between plant and pollinator communities is through the use of ecological networks, which can quantitatively describe species interactions and emergent network structure/topology (Memmott, 1999). To date, studies of ecological networks have tended to focus on bipartite interactions between species (e.g. plants and flower-visiting insects) (Bascompte and Jordano, 2013), but manipulative experimental approaches are relatively rare: tending to focus on the impacts of an altered plant species community on network structure (Lopezaraiza–Mikel *et al.*, 2007), or often relying on *in silico* simulations (Memmott *et al.*, 2004; Memmott *et al.*, 2007; Rezende *et al.*, 2007). Changes in the structure of plant communities have been shown to influence pollinator interactions, affecting factors that are important to crop production, such as pollinator visitation rates, species richness, and network structure, as well as pollen transport networks (Lopezaraiza–Mikel *et al.*, 2007; Bartomeus *et al.*, 2008; Forup *et al.*, 2008; Vilà *et al.*, 2009). However, in crop production systems that are dominated by a single plant species, there may be other factors, such as concurrent interactions between plants and other organisms, or the inherent traits of plant genotypes / crop cultivars that play important roles in determining the structure and functional consequences of plant-pollinator interaction networks. Hence, utilising a community level approach that examines interactions between multiple species interacting concurrently offers the opportunity to understand the dynamics that influence plant-pollinator interactions on a local scale.

Crop production systems are typically dominated by a single plant species, and as such, examining factors that influence the pollination of this dominant plant species, such as concurrent interactions with other mutualists, and how these influences are mediated by the traits of crop cultivars could help us to understand patterns of how pollinator communities are influenced at a localised level, which species are important for pollination, and how altering these aspects might influence crop yields. By examining multiple interacting communities to study the underlying dynamics within interaction networks, we can further our understanding of the structure and assemblage of crop-pollinator interaction networks embedded in wider ecosystems, and inform the management of crops to maximise the efficiency of pollination services.

Plant-pollinator communities are widely thought to be generalised amongst species (Waser *et al.*, 1996), with most plants and pollinators interacting with several species, and Waser *et al.* (1996) suggest that generalisation can be predicted if temporal and spatial variation is taken into account. However, in crop production systems with a single plant species, if pollination services can be influenced by interactions with other mutualists (Barber and Soper Gorden, 2014), interactions may be more locally specialised than presumed, which suggests that the influences of interactions with other plant mutualists, such as AMF, as well as the inherent traits of plant genotypes / crop cultivars may play important roles in determining pollinator interactions within a plant species. We have shown in Chapter 2 that both of these factors play roles in determining the floral rewards offered by plants, and influence plant-pollinator interactions in a controlled environment. In this study, we examine the influence of these factors on wild pollinator interactions in an open field experiment.

4.2.2 Improving crop production by utilising a community level approach

Soft fruits, such as strawberry, are commercially important crops (FAOSTAT, 2017), and strawberry yield is estimated to be between 10 and 40% dependent on pollinators (Gallai *et al.*, 2009). The pollination of commercial strawberry crops is often supplemented by hives of bumblebees, but their flowers are also visited by a diverse range of wild pollinators, and floral visits by pollinators with multiple foraging techniques have been shown to improve the quantity and quality of yields (Chagnon *et al.*, 1993). Being able to manage communities of wild pollinators in crop production systems to maximise pollination services could offer the potential to increase yields.

Previous studies have utilised community level approaches to examine plant-pollinator interactions, and while the structure of complex networks has been shown to influence their function (Strogatz, 2001; Pascual and Dunne, 2006), the functional consequences of altering multi-trophic interactions is poorly understood (Kinzig *et al.*, 2001; Thompson *et al.*, 2012), particularly in agricultural crops. Examining how changes in pollinator interactions affect crop yields is one way of quantifying the functional consequences of altered network structure. In Chapter 3 we found that both AMF community and crop cultivar influenced strawberry yield when pollinated by a single pollinator species, however while manipulations of AMF species has been shown to influence wild pollinators in a limited number of studies (Gange and Smith, 2005; Varga and Kytöviita, 2010b; Barber *et al.*, 2013), it remains to be understood how AMF communities influence the visitation rates of wild pollinator taxa, or the resulting influence on crop yield. If AMF can promote the visitation of pollinators belonging to taxa that exhibit efficient pollination, such as Hymenoptera (Schemske and Horvitz, 1984; Fishbein and Venable, 1996; Bingham and Orthner, 1998; Ivey *et al.*, 2003), crop yields may be improved through pollination by these highly efficient pollinators. However, in wild pollinator communities, the pollination success of a plant is likely to be determined by visits from multiple pollinator species, and a reduction in the pollination services from one taxa of pollinators may be supplemented by an increase in visits by pollinators from another, creating functional redundancy, thus mitigating any changes (e.g. recent species declines and losses (Biesmeijer *et al.*, 2006; Potts *et al.*, 2010; Potts *et al.*, 2015)) from highly efficient pollinators (Garibaldi *et al.*, 2013). For example, *Syrphidae* species can carry equal pollen loads to bees (Orford *et al.*, 2015), and the abundance of Diptera in agro-ecosystems could fulfil the pollination requirements of crops (Orford *et al.*, 2015).

Furthermore, the structure of pollinator communities and diversity of visits a plant receives has an important role in determining crop yields. For example, in pollinator dependent crops such as strawberry (Gallai *et al.*, 2009), diverse plant-pollinator interactions can foster functional complementarity, where different foraging techniques of pollinators supplement each other to improve pollination efficiency, reduce misshapen strawberries, and improve yields (Chagnon *et al.*, 1993). Understanding the influences of multiple interacting communities of species is vital when managing pollinators in agro-ecosystems for crop production (Allen-Wardell *et al.*, 1998), and whilst pollinator communities in commercial strawberry production systems cannot be enhanced by altering the plant species, improvements in yield could be fostered through associating crop cultivars with AMF communities that promote pollinator visitation (Poulton *et al.*, 2001). As strawberry crops naturally associate with AMF communities, and several cultivars of strawberry are typically present in a field, understanding how these factors influence the structure of plant-pollinator communities could offer the opportunity to increase yields through improved plant-pollinator interactions

There are multiple mechanisms through which AMF can improve crop production, and whilst AMF have been shown to influence plant-pollinator interactions, they also play an important role in nutrient and water acquisition, influencing plant vigour (Smith and Read, 2008). As such, realised crop yields may be dependent on: i) AMF influences on the provision of nutrient and water resources to plants; ii) AMF influences on plant-pollinator interactions; or iii) the diversity of the visiting pollinator community and the provision of functional redundancy provided by other pollinator taxa if the visitation rates of highly efficient pollinators are altered.

Unlike typical studies of plant-pollinator interaction networks, which examine changes in plant species communities (Lopezaraiza–Mikel *et al.*, 2007; Bartomeus *et al.*, 2008; Forup *et al.*, 2008; Vilà *et al.*, 2009), for the first time we utilise a single crop species and manipulate below-ground AMF communities and crop cultivars to determine the effects on above-ground pollinator visitation rates, diversity, and wild pollinators in an agro-ecosystem. Combined with this community approach to pollinator visitation, we also examine the functional consequences of strawberry yield. Determining differences in the structure of plant-pollinator communities between AMF communities and strawberry cultivars will help to explain the functional impact of an altered belowground network on an aboveground network, and if these changes are mediated by crop cultivars. We aim to determine if within a single crop species, belowground AMF communities or strawberry cultivar can influence:

1) The overall frequency and diversity of visits from wild pollinators
2) The frequency and diversity of pollinator visits within individual taxa
3) If AMF induced changes in *Bombus terrestris* Audax visits found in Chapter 2 translate to wider Hymenoptera species
4) If the influences of AMF on strawberry yield observed in a controlled environment with a single pollinator species (Chapter 3) occur in the field with a diverse pollinator community

We predict that AMF community and crop cultivar will influence pollinator visitation, but this may depend on pollinator taxa, and expect changes in the visitation rates of Hymenoptera as observed in Chapter 2. Based on our results from Chapter 3, we predict that strawberry yield will be influenced by AMF, with Community 1 (a low diversity natural AMF community extracted from the same field as the experimental plot, which provided the greatest benefit on strawberry yield in the greenhouse) providing the largest improvement.

4.3 Methods

In order to determine the influences of altering belowground AMF communities and crop cultivars on the diversity and frequency of pollinator visitation rates of wild pollinators, and in turn strawberry yield we utilised the same AMF communities and crop cultivars described as in the greenhouse study (see Chapters 2 and 3) in the field. Strawberry plants were prepared and inoculated as described in Chapter 2 (Section 2.2). Briefly, two natural AMF communities were extracted from field soils surrounding The James Hutton Institute, Scotland, UK, (termed 'Community 1' and 'Community 2') along with a commercial inoculant (RootGrow Professional, PlantWorks, Sittingbourne, UK) (termed 'Commercial') and added to pots. Three cultivars (Elsanta, Sonata, and Sweetheart) of strawberry were inoculated and grown for four months to produce inocula for the main experimental phase. The substrate from these plants was then removed and pooled by AMF community to produce bulk inocula for the experimental plants.

4.3.1 Study System

The experimental site was located in 'Quarry Field' at The James Hutton Institute (56°27'13.61"N, 3° 4'44.74"W), and was primarily surrounded by commercial barley (*Hordeum vulgare*) production. The site was located 15 m from a small (0.1 ha) experimental plot of raspberry (*Rubus idaeus*), 20 m from a species poor hedgerow / field margin (containing *Bellis perennis, Crataegus monogyna, Fagus sylvatica, Fragaria vesca, Fumaria officinalis, Heracleum sphondylium, Matricaria discoidea, Ranunculus repens, Rosa canina, Rubus fruticosus, Taraxacum officinale, Trifolium pratense*, and *Trifolium repens*), 150 m from a mixed deciduous woodland (4.2 ha), and 300 m from the river Tay (Figure 4.1).

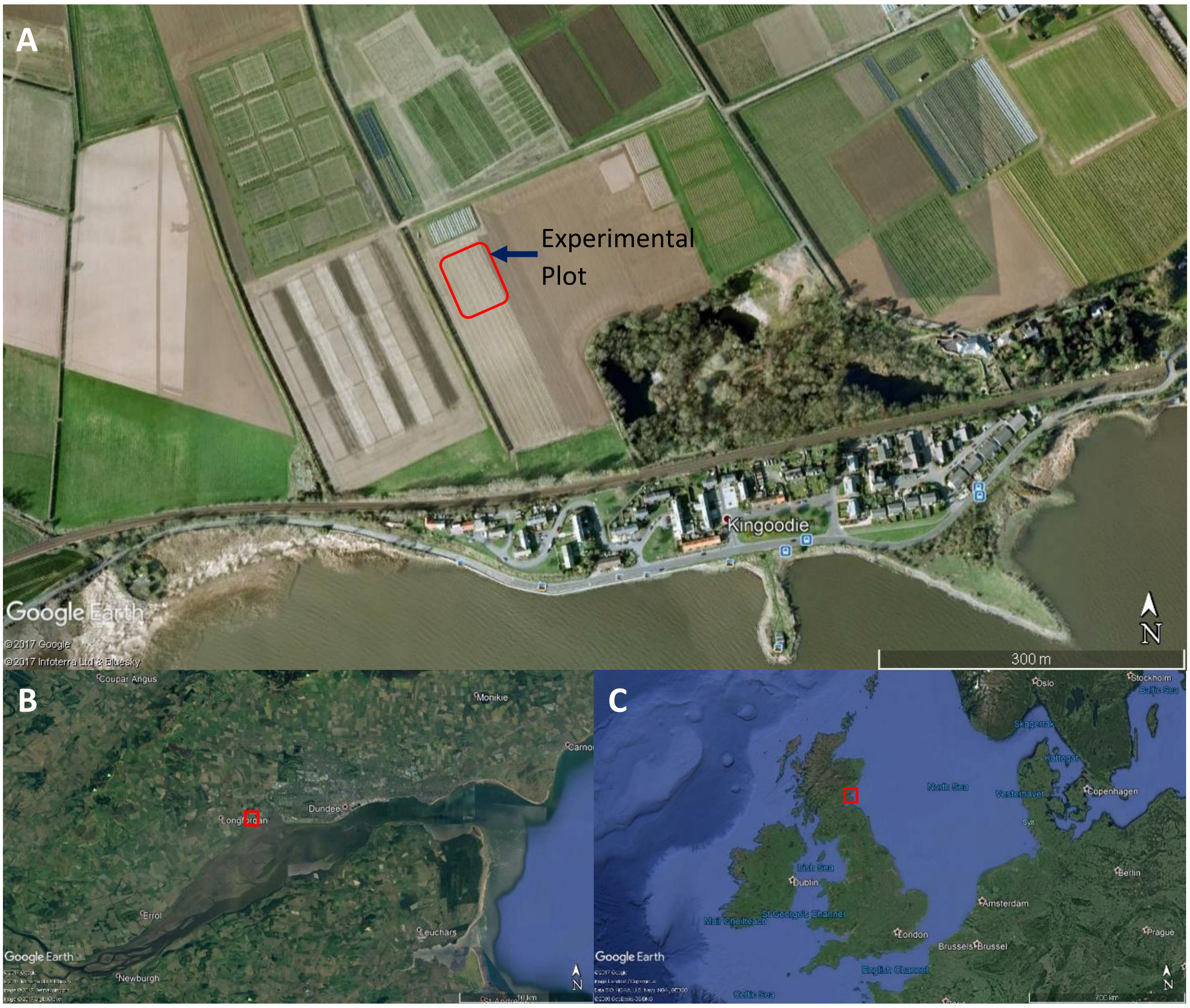


Figure 4.1: Location of the experimental site. Aerial view of A) the site of the experimental plot and surrounding fields, B) the site within the wider region, and C) the location of the region within the UK (Google Earth, 2017a; Google Earth, 2017b; Google Earth, 2017c).

For the main experimental phase, three cultivars of strawberry were utilised (Elsanta, Sonata, and Darselect), and 216 plants of each cultivar were divided among the four AMF treatments (Community 1, Community 2, Commercial, and a sterile control), creating a replication level of 72 plants per treatment and 864 plants in total. These experimental plants were initially grown in 0.5 L pots containing 100 ml of the previously produced bulk inocula, and grown for 6 months in a glasshouse to establish full AMF colonisation, before being repotted in 3 L pots with sterilised coir (see Chapter 2).

After the initial 6-month glasshouse stage to establish AMF colonisation, plants were moved to the field, where we conducted a 4 x 3 factorial replicated complete block experiment in which we manipulated four AMF communities, and three strawberry cultivars. Plants were arranged in a randomised block design with 8 rows of plants (acting as blocks), each containing equal replication of each treatment, for a total of 108 plants per row, and the location of each plant within the block was completely randomised. Each row consisted of a raised bed, upon which wooden blocks were placed to raise plant pots from the surface of the soil to prevent contamination of the pots from AMF in the field soils. An empty plant pot of the same size in which the plant was potted (3 L) was then screwed onto the block to provide a further barrier to contamination, and the wooden blocks secured in place with high tensile wire attached to wooden posts sunken into the raised beds after every 6 pots, with a 25 cm gap between each pot. Irrigation was provided by overhead irrigation lines with a dripper positioned above every pot (Figure 4.2).

Figure 4.2: Physical set up of the experiment in the field showing: A) the organisation of the row based design and setup of irrigation lines and support posts, and B) the setup for an individual plant, consisting of a wooden block and secondary plant pot to provide a barrier to contamination from AMF in the field soil, and an overhead irrigation line with drippers positioned directly above each plant.

4.3.2 Sampling and identification of pollinators

Plants were maintained in the field for two flowering seasons, from May 2014 to August 2015. During the flowering period (May to mid June), one hour transects were conducted daily five times per week for five weeks (25 transects per year). Transects started at a randomly assigned location within the experimental plot, and were completed by slowly walking along each row, observing plants individually within the row. If a pollinator was present on a plant as it was passed during the transect and observed to be feeding on a flower, it would be captured using either a circular 40 cm net (E6741F & E6742, Watkins and Doncaster, Leominster, UK) for larger specimens (larger Hymenoptera and *Syrphidae*), or an aspirator (aka pooter) (E710, Watkins and Doncaster, Leominster, UK) for smaller specimens. Once captured, specimens were individually enclosed in a small hand constructed paper envelope and sealed. Envelopes containing specimens were immediately deposited in a 1 L clip top cylindrical glass jar (0025.491, Kilner, Liverpool, UK), containing 3 cm of shredded paper infused with 10 ml of ethyl acetate in the base to euthanise the specimen. A perforated cardboard barrier separated specimen envelopes from the infused shredded paper in the base.

Once each transect was completed, specimens were immediately returned to the laboratory and specimens stored in a -20°C freezer for 48 hours. Pollinators were then pinned before being taxonomically identified under a stereo microscope using standard keys. *Bombus* spp. were identified using Benton (2006), other Hymenoptera using Collins (2012), Else (2014), and Else and Wright (2006), *Syrphidae* spp. using Stubbs *et al.* (2002), and other Diptera using Unwin (1981).

Strawberry yield was determined by collecting fruits daily when ripe, individually bagged and labelled in the field, and fresh weights of each strawberry were recorded in the lab before being stored at -20 °, and freeze-dried.

4.3.3 Statistical analysis

Yield was calculated as the total fresh weight of all strawberries produced per plant, number of strawberries per plant, and average yield per strawberry (in order to determine if changes in pollinator visitation altered the yield for each strawberry). Yield variables were analysed using a three-way repeated measures general linear mixed effect model, with the ‘nlme’ R package (Pinheiro *et al.*, 2016). AMF community, strawberry cultivar, and year were included in the model as fixed factors, with plant number and row (acting as a block) as random factors to account for repeated measures and row number to account for position in the field, and plant location. Yield was included as a fixed rather than a random factor as plants were larger in the second year. Where main effects were significant, differences between AMF communities and strawberry cultivars were compared using Tukey's honestly significant difference (HSD), using the 'lsmeans' R package (Lenth, 2016).

For pollinator visitation, we utilised rarefaction curves to check for sampling completeness, and to compare the estimated species richness between treatments (Gotelli and Colwell, 2001) to determine if AMF community or strawberry cultivar influenced expected species richness. EstimateS (Colwell, 2005) was used to predict the estimated number of species for each AMF community and strawberry cultivar, and to create sample based rarefaction curves based on the analytical estimated species. 95% confidence intervals, and rarefaction curves were estimated by extrapolating samples by a factor of 3. Sampling completeness for each AMF community and strawberry cultivar was calculated by dividing the observed number of species by the Chao2 predictor (Chao, 1987), also generated by EstimateS. Comparisons of expected species richness were completed in R (R Development Core Team, 2016), by comparing Chao predictors with AMF community, strawberry cultivar, and year as fixed effects and sample as a random effect using a general linear mixed effect model, with the 'nlme' R package (Pinheiro *et al.*, 2016).

We tested for differences between the frequency and observed species richness of pollinator visits between AMF communities and strawberry cultivars for pollinator visits overall in addition to subsets of pollinator taxa (Hymenoptera, Diptera, Diptera excluding *Syrphidae* spp., *Syrphidae* spp., and all other visitors (Hemiptera, Coleoptera, and Neuroptera)) in R using GLMM models. *Syrphidae* were examined separately from other Diptera as *Syrphidae* are typically substantially larger than other Diptera (and may carry a higher pollen load), and have different foraging and behavioural patterns than smaller Diptera. The data was zero inflated (including a high number of single visits to plants), and to account for zero inflation the 'glmmADMB' R package (Skaug *et al.*, 2015) was used to construct generalised linear mixed effect models, utilising AMF community, strawberry cultivar, and year as fixed effects, and plant number and row as random effects, replicated by individual plant. For all variables, models were created with Poisson, negbinom, and negbinom1 families, and AIC values (Akaike Information Criterion, which are used to compare the fit of models between families and link functions) compared to select the model with the best fit. For visit frequency, final models for total pollinators were constructed with the negbinom family, Hymenoptera and 'other orders' with the negbinom1 family, and all others with the Poisson family. For observed species richness, Hymenoptera used negbinom1, 'other orders' used negbinom, and all other used the Poisson family. Interaction networks were created using Food Web Designer (Sint and Traugott, 2016a; Sint and Traugott, 2016b), to visually illustrate interactions in the plant-pollinator network.

4.4 Results

4.4.1 Strawberry Yield

Strawberry yield was influenced by AMF community, strawberry cultivar, and year, with an interaction between strawberry cultivar and year. Both of the natural AMF communities tested had significantly higher total yields and number of fruits per plant than the commercial inocula. Community 1 had increased yields over sterile control plants, with a trend for Community 2 to improve yields. Elsanta had significantly higher yields, and number of fruits than both Sonata and Darselect, but had a lower average fruit weight than Darselect. Sonata did not have higher total yields per plant than Darselect, but rather had a higher number of fruits that were of a smaller weight. AMF community and strawberry cultivar did not interact to influence any yield variables, indicating that the influence of the AMF community was consistent across the strawberry cultivars we tested (Table 4.1, Figures 4.3 & S4.1). We found a similar pattern of influences on yield between AMF communities in the field as in the greenhouse (Chapter 3), with Community 1 providing the greatest benefit. However, the magnitude of differences between AMF communities was greater in the field, and both Community 1 and 2 improved yields over the Commercial Inoculant.

Table 4.1: The influence of AMF community and strawberry cultivar on strawberry yield in the field. Tukey's HSD contrasts between individual strawberry cultivars and AMF communities are shown below each main effect when a main effect was significant. Statistics show F values for main effects, and estimates for contrasts. Error degrees of freedom are listed at the bottom of each column. Significant *P* vales are in bold, and trends are italicised.

	Strawberry Yield								
	Total Yield			Number of Fruits			Averagre Fruit Weight		
	df	*Statistic*	*P*	*df*	*Statistic*	*P*	*df*	*Statistic*	*P*
AMF Community	3	5.88	**<0.001**	3	4.59	**0.003**	3	3.02	**0.029**
Sterile vs. Community 1	3	-9.55	0.448	3	-1.21	0.178	3	-0.35	0.222
Sterile vs. Community 2	3	-9.18	*0.063*	3	-1.12	0.248	3	0.03	0.999
Sterile vs. Commercial	3	3.71	0.743	3	0.71	0.634	3	0.05	0.993
Community 1 vs. Community 2	3	0.36	0.999	3	0.09	0.999	3	0.39	0.165
Community 1 vs. Commercial	3	13.26	**0.002**	3	1.92	**0.007**	3	0.41	0.126
Community 2 vs. Commercial	3	12.90	**0.003**	3	1.84	**0.013**	3	0.02	0.999
Strawberry Cultivar	2	16.72	**<0.001**	2	66.99	**<0.001**	2	24.37	**<0.001**
Elsanta vs. Sonata	2	15.20	**<0.001**	2	3.20	**<0.001**	2	0.01	0.999
Elsanta vs. Darselect	2	12.45	**<0.001**	2	5.91	**<0.001**	2	-1.42	**<0.001**
Sonata vs. Darselect	2	-2.75	0.669	2	2.70	**<0.001**	2	-1.42	**<0.001**
Community*Cultivar	6	1.08	0.375	6	1.05	0.391	6	1.02	0.409
Error	849			849			849		
Year	1	906.58	**<0.001**	1	225.07	**<0.001**	1	2489.12	**<0.001**
Community*Year	3	5.16	**0.002**	3	3.07	**0.027**	3	2.81	**0.039**
Cultivar*Year	2	3.52	**0.030**	2	7.67	**<0.001**	2	48.38	**<0.001**
Community*Cultivar*Year	6	1.47	0.185	6	1.77	0.102	6	1.17	0.321
Error	681			681			681		

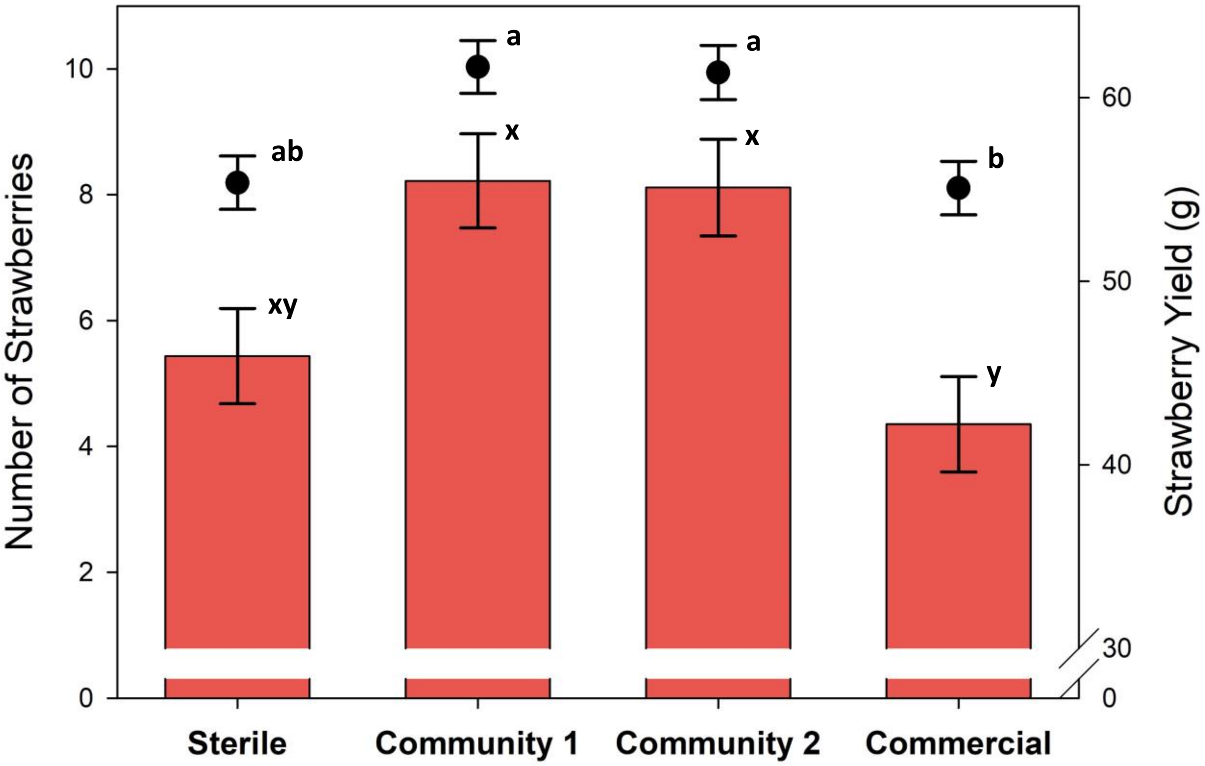


Figure 4.3: Number of strawberries (dots) and total strawberry yield (bars) in the field by AMF community. Values represent least squares means (±SE). Lower case letters adjacent to upper error bars represent post-hoc contrasts between treatments. Treatments that share letters had indicate no significant difference, whereas different letters indicate a significant difference between treatments. As two variables are shown on the same graph, letters ‘a’, ‘b’, and ‘c’ are used for the left x-axis, and ‘x’, ‘y’, and ‘z’ for the right y-axis.

4.4.2 Pollinator visitation frequency and diversity

Over both field seasons we collected a total of 492 individual pollinators – 237 in year 1, and 255 in year 2. Pollinating insects were captured from 5 orders – Hymenoptera, Diptera, Hemiptera, Coleoptera, and Neuroptera, within 16 families (Table 4.3). The majority of pollinators consisted of Diptera (356 individuals, 72% of total captures), followed by Coleoptera (96 individuals, 20% of total captures), Hymenoptera (34 individuals, 7% of total captures), Hemiptera (5 individuals, 1 % of total captures), and Neuroptera (1 individual, 0.2% of total captures). Over half (59%) of the Diptera visitors were made up of hoverflies (*Syrphidae*) (211 out of 356 individuals), and hoverflies comprised 19 species within 8 genera, and 8 families. Three species, within two genera and two families of Coleoptera were captured, and Hymenoptera captures consisted of 6 species, within four genera and three families. Hemiptera captures were all within a single genus, and there was only one capture from Neuroptera. Floral visits were dominated by three species; *Meligethes aeneus* (a pollen beetle) (94 individuals), *Anthomyiidae* spp. (a common nectar feeding fly) (69 individuals), and *Platycheirus manicatus* (a common nectar feeding hoverfly) (64 individuals), constituting nearly half (46%) of all visits (Table 4.3). Pollen beetles were included in the analysis as although they do not actively contribute to pollination, during taxonomic identification they were found to carry pollen grains across their bodies and could contribute to selfing (self-fertilisation within a flower).

Rarefaction curves show overlapping 95% confidence intervals, indicating that similar levels of pollinator abundance and richness were sampled for each treatment (Figure 4.4). Although AMF community and strawberry cultivar did not alter the observed species richness, the expected species richness varied with AMF community, with sterile plants having the highest expected species richness (44 species), followed by Community 1 (34 species) and Community 2 (32 species), and plants with the Commercial Inoculant having the lowest expected species richness (29 species). The number of expected pollinator species did not vary to the same degree across strawberry cultivars, with Elsanta and Sonata predicted to have 38 species, and Darselect 42 species (Figure 4.4, Tables 4.2 & 4.3).

Table 4.2: Total captures of pollinators across both years. Figures are separated by pollinator order, and totals at the bottom of each order indicate the total number of individuals and species for each AMF community / strawberry cultivar, with totals in the far-right column indicating the total number of captures for the pollinator within the row. Colours show a heat map of captures, on a scale from yellow (lowest number of captures) to green (highest number of captures). Heatmaps are calculated separately for each order and main effect.

				Number of Individuals							
				AMF Community				**Strawberry Genotype**			
Order	**Family**	**Genus**	**Species**	**Sterile**	**Community 1**	**Community 2**	**Commercial**	**Elsanta**	**Sonata**	**Darselect**	**Total**
Hymenoptera	*Halictidae*	*Lasioglossum*	*leucopus*	0	1	0	1	0	2	0	*2*
	Apidae	*Apis*	*mellifera*	1	2	0	2	2	2	1	*5*
		Bombus	*lapidarius*	1	3	1	5	4	5	1	*10*
			lucorum	2	1	1	0	0	1	3	*4*
			terrestris	6	3	0	3	3	6	3	*12*
	Vespidae	*Vespula*	*vulgaris*	0	0	0	1	1	0	0	*1*
			Species:	4	5	2	5	4	5	4	*6*
			Individuals:	*10*	*10*	*2*	*12*	*10*	*16*	*8*	*34*
Diptera	*Anthomyiidae*		*sp.*	20	15	16	18	23	29	17	*69*
	Bibionidae		*sp.*	2	1	1	0	2	1	1	*4*
	Ceratopogonidae		*sp.*	4	2	1	5	1	8	3	*12*
	Muscidae		*sp.*	10	9	7	4	9	11	10	*30*
	Phoridae		*sp.*	0	0	0	1	0	0	1	*1*
	Sarcophagidae		*sp.*	0	1	0	0	1	0	0	*1*
	Sciaridae		*sp.*	0	1	2	0	1	2	0	*3*
	Simuliidae		*sp.*	6	11	4	4	10	9	6	*25*
	Syrphidae	*Episyrphus*	*balteatus*	11	12	6	7	11	14	11	*36*
		Eristalis	*tenax*	0	2	3	1	3	2	1	*6*
		Eupeodes	*corollae*	4	6	7	5	9	6	7	*22*
			latifasciatus	0	0	2	0	0	0	2	*2*
			luniger	0	0	1	2	1	1	1	*3*
			manicatus	1	0	0	0	1	0	0	*1*
			nitens	1	1	0	0	0	1	1	*2*
		Lejogaster	*metallina*	1	1	0	0	1	0	1	*2*
			tarsata	1	0	0	0	0	1	0	*1*
		Platycheirus	*albimanus*	5	4	1	3	6	5	2	*13*
			clypeatus	0	0	0	1	0	0	1	*1*
			manicatus	17	14	18	15	25	25	14	*64*
			nielseni	1	1	0	1	2	1	0	*3*
			peltatus	6	2	3	4	3	9	3	*15*
			scambus	5	2	2	2	5	4	2	*11*
			tarsalis	3	2	3	4	5	4	3	*12*
		Sphaerophoria	*interrupta*	1	1	2	4	4	1	3	*8*
		Syritta	*pipiens*	3	0	1	2	2	2	2	*6*
		Syrphus	*ribesii*	1	0	2	0	2	0	1	*3*
			Species:	*20*	*19*	*19*	*18*	*22*	*20*	*22*	*27*
			Individuals:	*103*	*88*	*82*	*83*	*127*	*136*	*93*	*356*
Hemiptera	*Aphididae*	*Aphis*	*sp.*	1	2	2	0	3	1	1	*5*
			Species:	*1*	*1*	*1*	*0*	*1*	*1*	*1*	*1*
			Individuals:	*1*	*2*	*2*	*0*	*3*	*1*	*1*	*5*
Coleoptera	*Cantharidae*	*Cantharis*	*livida*	0	1	0	0	0	1	0	*1*
			pellucida	0	1	0	0	0	1	0	*1*
	Nitidulidae	*Meligethes*	*aeneus*	27	25	20	22	23	21	50	*94*
			Species:	*1*	*3*	*1*	*1*	*1*	*3*	*1*	*3*
			Individuals:	*27*	*27*	*20*	*22*	*23*	*23*	*50*	*96*
Neuroptera	*Chrysopidae*		*sp.*	0	0	1	0	1	0	0	*1*
			Species:	*0*	*0*	*1*	*0*	*1*	*0*	*0*	*1*
			Individuals:	*0*	*0*	*1*	*0*	*1*	*0*	*0*	*1*
			Total Species:	26	28	24	24	29	29	28	38
			Total Individuals:	*141*	*127*	*107*	*117*	*164*	*176*	*152*	*492*

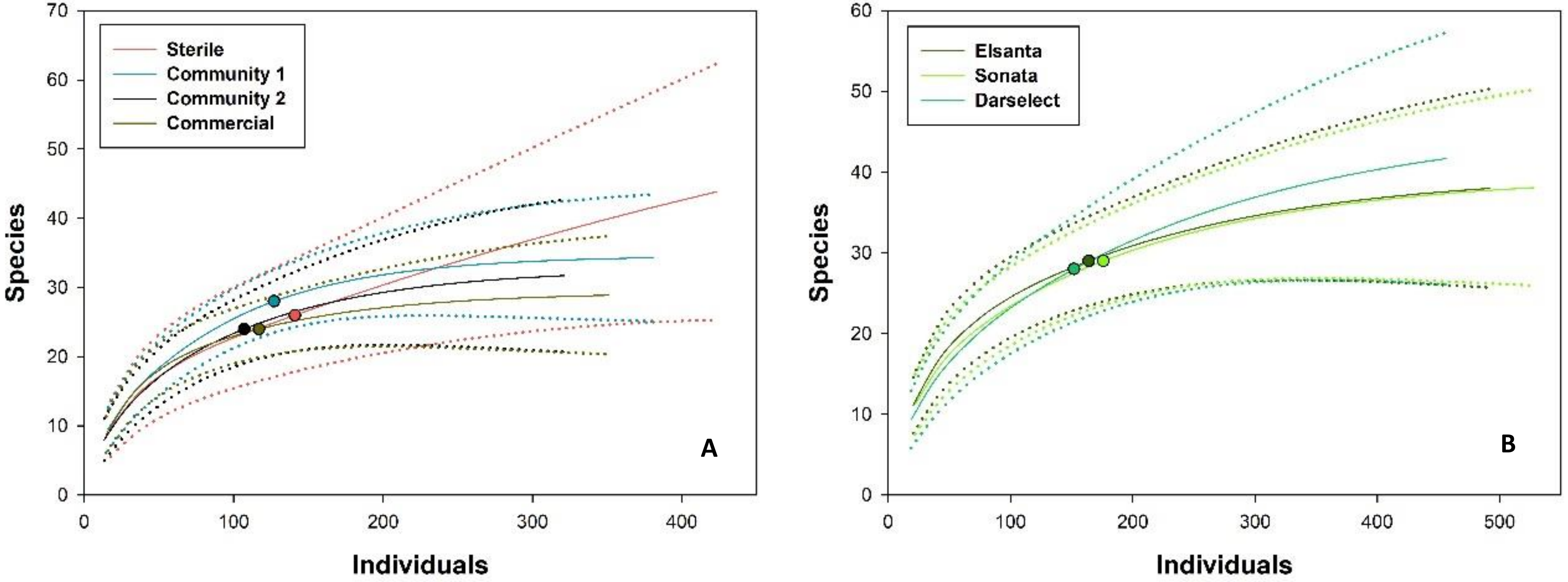


Figure 4.4: Rarefaction curves illustrating the expected species richness for A) AMF Communities, and B) strawberry cultivars. Colours in the legend boxes show each community / cultivar. Solid lines represent expected species richness, and dotted lines show 95% confidence intervals. Large circles on each slope show the observed species richness and abundance following the colour scheme indicated in the legend.

Table 4.3: Summary of observed and expected species richness and sampling completeness for all pollinator captures, and for each AMF community and strawberry cultivar.

		AMF Community				**Strawberry Cultivar**		
Metric	**Full Network**	**Sterile**	**Community 1**	**Community 2**	**Commercial**	**Elsanta**	**Sonata**	**Darselect**
Number of Pollinator Species	38	26	28	24	24	29	29	28
Expected Species Richness	47	44	34	32	29	38	38	42
Sampling Completedness	83%	51.9%	95.6%	78.5%	86.7%	79%	78%	67%

GLMM models found no influence of AMF community or strawberry cultivar on either the frequency of visits or observed species richness for pollinators overall. However, when examining the subsets of pollinator taxa separately, we found that AMF community significantly influenced both the frequency and species richness of visiting Hymenoptera, with fewer visits in plants associated with Community 2, and interacted with strawberry cultivar (Table 4.4, Figures 4.5 & 4.6), although a low number of visits were observed. Similarly, we found no difference between expected species richness for either AMF community ($F_{3,49} = 0.60$, $p = 0.62$) or strawberry cultivar ($F_{2,35} = 0.15$, $p = 0.86$). Year significantly influenced pollinator visitation as environmental conditions were different between the two flowering seasons, and as such influenced the relative abundance of most pollinator taxa (*Syrphidae*) present in the field. Figure 4.6 represents the network of plant-pollinator interactions observed, and illustrates the abundance of each pollinator species and order, as well as which pollinators interacted with each AMF community and strawberry cultivar. Whereas the statistical analysis compares differences in visits from taxonomic groups of pollinators, Figure 4.6 provides a visual illustration of differences in visits to each AMF community / strawberry cultivar from individual pollinator species and orders, of which there were not enough captures of within each group to be compared statistically.

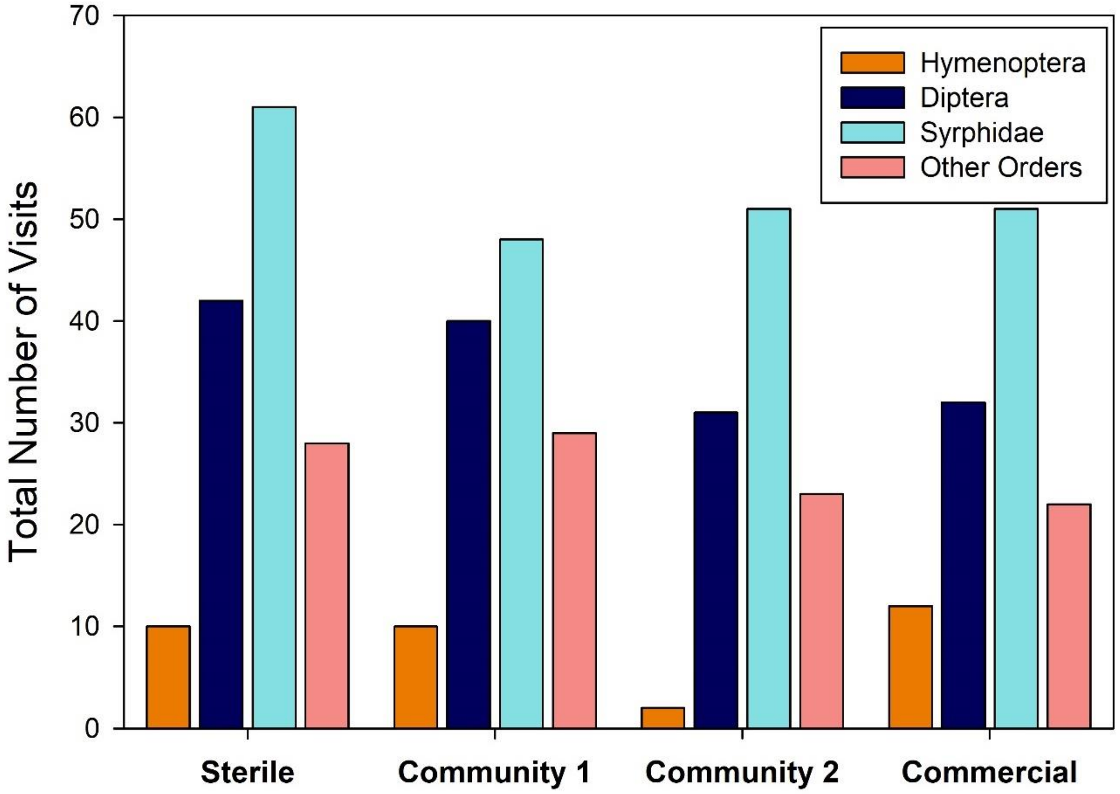


Figure 4.5: Total number of pollinator captures between AMF communities by insect taxa. Insects are grouped as Hymenoptera (orange), Diptera excluding *Syrphidae* spp. (dark blue), *Syrphidae*,spp. (light blue), and 'other orders' (Coleoptera, Hemiptera, and Neuroptera) (pink). Values represent the total number of captures for each treatment.

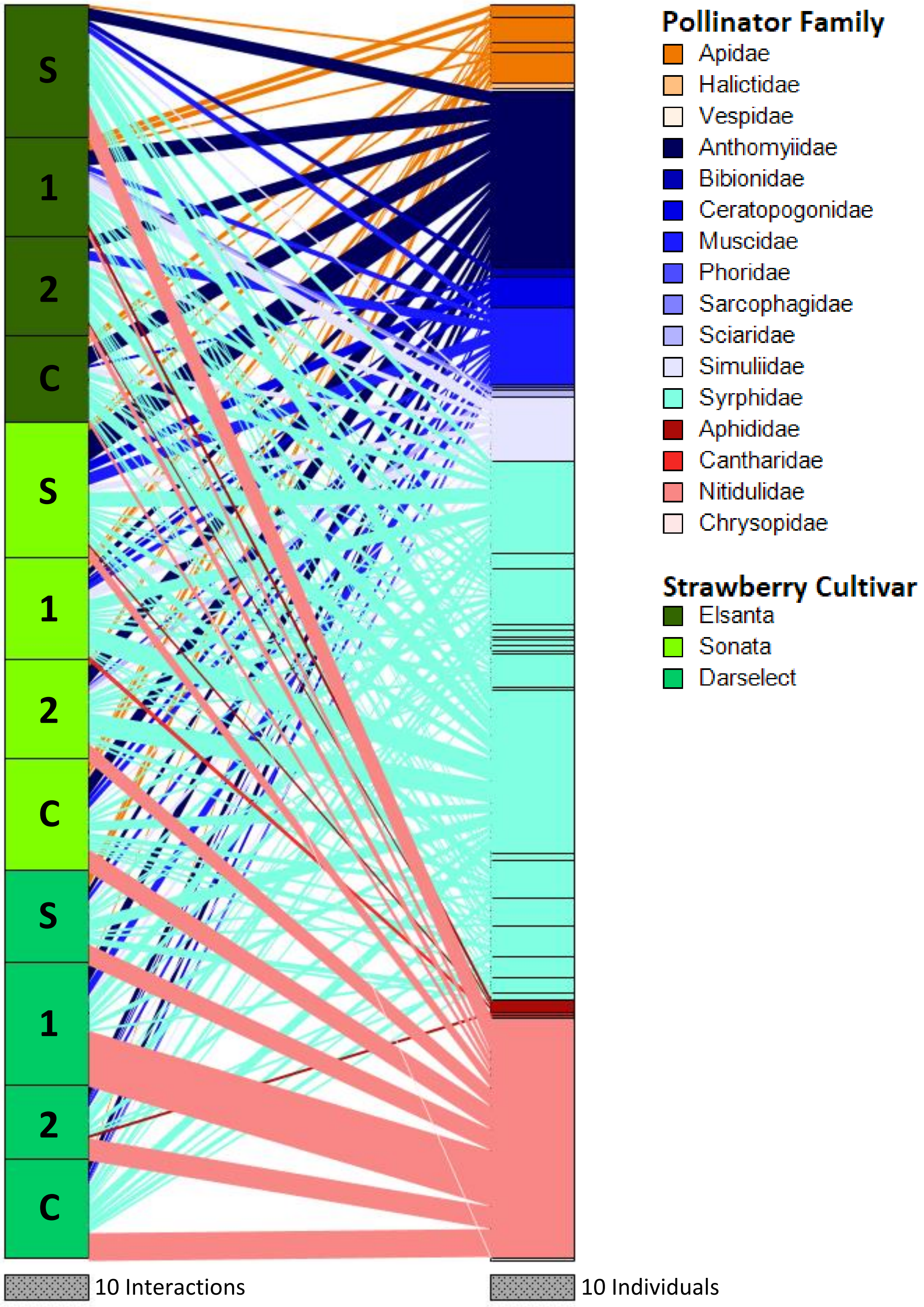


Figure 4.6: Bi-partite network illustrating interactions between pollinators (right column) and strawberry plants (left column). The width of bars on each side show the number of pollinators interacting (right column), and number of interactions received by plants (left column). Chequered gray boxes at the bottom provide a scale for the number of interactions. Each shade of green in the plant column designate a strawberry cultivar (see legend), and letters indicate AMF communities (S = Sterile, 1 = Community 1, 2 = Community 2, and C = Commercial). Colours in the pollinator column indicate pollinator families (see legend), with shades of orange denoting Hymenoptera species, shades of blue denoting Diptera (*Syrphidae* spp. in teal), and other orders in shades of red. The width of bars connecting the two columns shows the number of pollinators from each group interacting with a plant treatment.

Table 4.4: The influence of AMF community and strawberry cultivar on the frequency of visits and observed species richness by all pollinators, and pollinator taxa: Hymenoptera, Diptera, Diptera excluding Syrphidae spp., Syrphidae spp., and other orders (Hemiptera, Coleoptera, and Neuroptera).

Observed Frequency and Richness of Wild Pollinators									
Pollinator subset	**Factor**	**df**	**X^2**	**P**	**Pollinator subset**	**Factor**	**df**	**X^2**	**P**
Total number of visits	AMF Community	3	3.75	0.290	**Total Richness**	AMF Community	3	2.20	0.532
	Strawberry Cultivar	2	0.92	0.630		Strawberry Cultivar	2	0.88	0.645
	Year	1	9.70	**0.002**		Year	1	9.27	**0.002**
	Community*Cultivar	6	2.74	0.840		Community*Cultivar	6	1.05	0.984
Hymenoptera visits	AMF Community	3	608.54	**<0.001**	**Hymenoptera Richess**	AMF Community	3	608.54	**<0.001**
	Strawberry Cultivar	2	3.83	0.147		Strawberry Cultivar	2	3.83	0.147
	Year	1	4.38	**0.036**		Year	1	4.38	**0.036**
	Community*Cultivar	6	2808.46	**<0.001**		Community*Cultivar	6	2808.46	**<0.001**
Diptera visits	AMF Community	3	2.13	0.545	**Diptera Richness**	AMF Community	3	1.94	0.585
	Strawberry Cultivar	2	2.56	0.278		Strawberry Cultivar	2	2.15	0.341
	Year	1	1.95	0.162		Year	1	2.05	0.152
	Community*Cultivar	6	2.47	0.872		Community*Cultivar	6	1.90	0.928
Diptera visits (excluding *Syrphidae* spp.)	AMF Community	3	0.51	0.917	**Diptera Richness (excluding *Syrphidae* spp.)**	AMF Community	3	0.67	0.881
	Strawberry Cultivar	2	4.07	0.131		Strawberry Cultivar	2	4.38	0.112
	Year	1	6.91	**0.009**		Year	1	6.92	**0.009**
	Community*Cultivar	6	5.16	0.524		Community*Cultivar	6	5.63	0.466
***Syrphidae* spp. visits**	AMF Community	3	3.98	0.264	***Syrphidae* spp. Richness**	AMF Community	3	4.22	0.238
	Strawberry Cultivar	2	2.41	0.300		Strawberry Cultivar	2	3.51	0.173
	Year	1	0.13	0.715		Year	1	0.11	0.739
	Community*Cultivar	6	3.76	0.709		Community*Cultivar	6	5.01	0.542
Visits from other orders	AMF Community	3	1.17	0.761	**Other orders Richness**	AMF Community	3	3.05	0.383
	Strawberry Cultivar	2	0.21	0.902		Strawberry Cultivar	2	0.57	0.750
	Year	1	22.39	**<0.001**		Year	1	33.93	**<0.001**
	Community*Cultivar	6	1.98	0.922		Community*Cultivar	6	5.90	0.435

4.5 Discussion

Pollinators play important roles in improving the yields of crops (Gallai *et al.*, 2009), and provide important ecosystem services, and here we have shown that within a single crop species, concurrent interactions with other mutualists such as AMF do not influence wild pollinator visitation overall, but can influence visitation rates of some pollinator taxa. In addition, the AMF community a strawberry plant was associated with significantly influenced the yield of the plant, with the two natural communities providing the greatest benefit. Despite a low number of visits from bees, AMF communities influenced yield in the same way as in the greenhouse, indicating a significant degree of functional redundancy in the wild pollinator community.

4.5.1 Strawberry yield

AMF influenced all measures of strawberry yield, and we found a similar pattern of changes in yield to those found in the greenhouse (Chapter 3), with Community 1 having the greatest benefit. Similarly, AMF community did not interact with strawberry cultivar, indicating that the effects of each AMF community were the same across all cultivars.

Both of the natural AMF communities provided the greatest yield, and to a greater degree than in the greenhouse. We extracted these communities from field soils 250 m (Community 1) and 900 m (Community 2) from the experimental site, and as such both communities were adapted to local conditions. These communities may have outperformed plants with the Commercial Inoculant as by being associated with plants exposed to the environmental and biotic stresses in the local area, co-evolutionary pressures may have selected for AMF species and strains that were adapted to support plants with the specific set of environmental conditions and stresses present in the region. AMF are thought to have co-evolved with plants since the Devonian period with bryophytes before the evolution of true roots (Brundrett, 2002), and these processes are thought to be ongoing, creating adaptive differences within AMF and plants on a local scale (Hoeksema, 2010). The interaction between species can cause evolutionary change resulting in adaptive differentiation among populations (Ehrlich and Raven, 1964; Thompson, 1994; Thompson, 2005), and selective pressures may promote traits within AMF communities that improve plant performance in response to the local environmental conditions (Hoeksema, 2010).

In addition, in Chapters 2 and 3 we found that AMF Community 1 increased both the duration of nectar foraging visits (through an increase in nectar production) and strawberry yield. In the field, insects were only captured when they were observed to be feeding from the flower, and increasing the amount of time nectar feeding visitors spent on each flower could have promoted pollen deposition and in turn yields.

In the greenhouse (Chapter 3), we found that Community 1 produced higher yields than the Commercial Inoculant, whereas in the field we found that both Communities 1 and 2 had higher yields than the Commercial Inoculant. In addition, the magnitude of differences in yield between AMF communities was larger in the field than in the controlled environment of the greenhouse. Previous studies have shown that AMF can improve plant growth to a greater degree when plants are exposed to stresses on plants such as nutrient (Johnson *et al.*, 2015) or water (Al-Karaki *et al.*, 2004) availability in isolation, here we have shown that improvements can also be achieved when plants are exposed to the full range of biotic and abiotic stresses in the field that are not present in controlled environments such as glasshouses.

The number of strawberries a plant produces is largely determined by the number of flowers it produces, which results from how vigorous the plant is. We found a similar pattern between the number of fruits and strawberry yield, indicating that AMF induced changes in yield may be due to AMF promoting plant growth, however this increase in vigour may have also promoted nectar production, and the duration of visits from nectar feeding pollinators. Wild pollinators can sufficiently fulfil the pollination requirements of crops in the majority of production systems (Garibaldi et al., 2013), and our results support the value of wild pollinators by showing that even with a low number of visits from bees, the wild pollinator community had a high degree of functional redundancy and was able to sufficiently fulfil crop pollinator requirements.

4.5.2 Wild pollinator visitation

Studying the influences of wild pollinators is important as the composition of pollinator communities plays an important role in determining crop yields through multiple methods. First, wild pollinators have been shown to successfully fulfil the pollination of crops in the majority of production systems (Garibaldi *et al.*, 2013). Diptera dominated the floral visits we observed (72% of total captures), and have important roles in crop pollination. For example, *Syrphidae* species can carry similar pollen loads to Hymenoptera (Orford *et al.*, 2015), making significant contributions to crop pollination. Although non-syrphid Diptera carry smaller pollen loads, their abundance and frequency of visits can contribute substantially to crop pollination (Orford *et al.*, 2015). In addition, visits by a diverse community of pollinators can promote functional complementarity through differences in foraging techniques (Chagnon *et al.*, 1993), which can improve the likelihood of pollen being deposited on all stigmas, and in turn determining overall pollination efficiency.

Second, the efficiency of individual pollinator visits has been shown to vary between pollinator species and taxa, with Hymenoptera previously being reported to be the most efficient (Schemske and Horvitz, 1984; Fishbein and Venable, 1996; Bingham and Orthner, 1998; Ivey *et al.*, 2003), and bee pollination can improve yield quantity and quality in strawberry, producing larger fruits of a higher commercial grade and in turn market value (Klatt *et al.*, 2014).

To date, only three studies have examined the influence of AMF on wild pollinators, with two finding that AMF influenced the frequency of some pollinator taxa while others were unaffected (Varga and Kytöviita, 2010b; Barber *et al.*, 2013), whereas the third showed that AMF did influence overall visitation rates, but the changes in the visitation of pollinator taxa depended on the plant species (Gange and Smith, 2005). However, these studies utilised either single AMF species, or mixes of several AMF species. In studies that use mixes of AMF species, treatments with species mixtures tend to consist of a few dominant AMF species, and do not capture the influence of whole AMF communities that crops associate with in the field.

Our results show that manipulating AMF communities does not change the number and frequency of wild pollinators to strawberry overall. While we found that AMF community influenced the frequency and richness of Hymenoptera, visits by these highly efficient pollinators were relatively low (and may have been an effect of sampling completeness), and changes in their visitation rates did not influence yield (see Section 4.5.1). Our results support the importance of wild pollinators in crop production systems, and with recent declines in domesticated pollinators (Biesmeijer *et al.*, 2006; Potts *et al.*, 2010; Potts *et al.*, 2015), highlighting the importance of wild pollinators and their contribution to crop yields to growers and stakeholders in the agricultural industry is vital.

Gange and Smith (2005) showed that the influences of AMF on wild pollinators depended on plant species, and in order to further understand the influences of AMF on wild pollinators in agro-systems, studies in a range of crop species are required. In addition to plant species mediating AMF induced changes in pollinator visits, Poulton *et al.* (2001), showed that AMF and crop phenotype can interact to influence pollinator visitation, and the inherent traits of crop cultivars may play important roles. However, although we found that the strawberry cultivars we tested did not influence wild pollinator visits as a main effect, our results illustrate that they can influence the visitation of some pollinator taxa when interacting with AMF, indicating that the influence of AMF on pollinator visitation can potentially be mediated by the traits of crop cultivars. As such, there is scope for AMF communities to be paired with crop cultivars to provide maximised pollination for each cultivar in commercial production.

4.5.3 Increasing the resolution of plant-pollinator interactions

Interactions between plants and pollinators are widely considered to be shaped by the coevolution of plants and pollinators (Crepet, 1983), however, the evolution of these interactions may also be shaped by concurrent evolutionary processes between plants and other mutualists that promote or hinder reproductive traits which affect pollinator efficiency. The frequency and efficiency of pollinator visits can be influenced by pollinator foraging preferences, with changes in the plant species within a community resulting in alterations to conspecific pollen loads and fertilisation (Brown and Mitchell, 2001; Moragues and Traveset, 2005; Larson *et al.*, 2006), leading to changes in the evolutionary success of plants. However, as AMF communities may influence plant-pollinator interactions, we suggest that future studies examine if the indirect influences of AMF on plant-pollinator interactions can shape these coevolutionary processes, and if the evolutionary success of plant species or genotypes may be shaped by tri-partite AMF-plant-pollinator networks.

Although we utilised a single plant species and were not able to quantitatively compare network metrics, we have shown that on a localised level there are important dynamics at play that shape plant-pollinator interaction networks within a single plant species. Plant-pollinator networks are generally thought to be generalised when spatial and temporal variation is taken into account (Waser et al., 1996), here we have shown that the indirect influences of a plant's interactions with other mutualists, such as AMF, as mediated by the inherent traits of plant genotypes, could play a role in determining the frequency and richness of visits from some pollinator taxa, influencing the generalisation / specialisation of plant-pollinator interaction networks in agro-ecosystems.

Utilising community based approaches is vital when studying the evolution of mutualisms (Jordano, 1987), and taking into account the influences of AMF communities and crop cultivars offers the potential to increase the resolution of our understanding of these processes. By studying AMF-plant-pollinator interactions with wild pollinators using a community based approach we can further understand the dynamics that influence ecological networks, as well as informing management decisions in agro-ecosystems, such as controlling which AMF communities associate with crops, in order to maximise yields.

4.6 Conclusion

Pollinators provide a key ecosystem service, and are responsible for improving crop yields, however declines in domesticated pollinators may lead to an increased reliance on wild pollinators in the future. We have shown that AMF community does not influence the total number of pollinator visits in strawberry, but that functional redundancy was provided by the wild pollinator community, and AMF affected yields in the same manner as in the greenhouse. As such, crop yield was likely determined by the influences of AMF on plant vigour, or as in Chapter 2, changes in pollinator behaviour. The two natural AMF communities extracted from soils surrounding the experimental site provided the greatest benefit to yield, and as such, utilising AMF communities that are adapted to the environment in which they are utilised could provide the greatest benefits to crop production. These results provide support for the importance of wild pollinators for crop production, and the potential application of AMF in crop production systems. However, in order to improve crop production through promoting wild pollinators and the implementation of AMF as a novel biotechnology, it is vital to determine the perceptions of growers and stakeholders on the importance they place on wild pollinators, and their views on utilising AMF to improve yields.

4.7 Supplementary Information

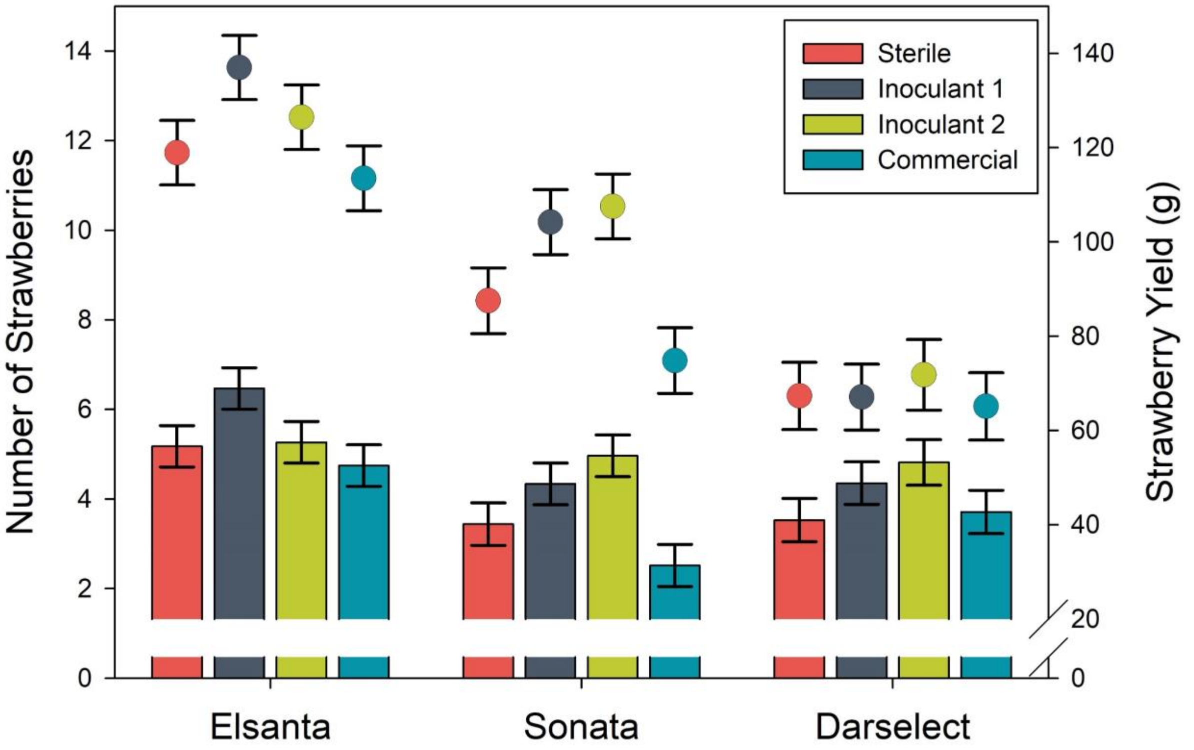


Figure S4.1: Number of strawberries (dots) and total strawberry yield (bars) in the field by AMF community and strawberry cultivar. Values represent least squares means (±SE).

Chapter 5: Perceptions on the Introduction of AMF as a Novel Biotechnology in the Production of Soft Fruit:
An Analysis Using Q-Methodology

Abstract

There are increasing concerns over food security with a rapidly rising population and increasing fertiliser prices. Whilst novel biotechnologies may form part of a toolset to mitigate these problems, and are already present in some crop production systems (such as the use of rhizobia in legumes), AMF have yet to be implemented in agricultural production systems. The successful introduction of new biotechnologies is ultimately decided by end users, however, the value that agricultural stakeholders place on the ecosystem services provided by AMF and wild pollinators, or their motivations for improving these services remain unknown. In order for the successful uptake of new technologies that improve agricultural production, it is vital to understand the perceptions and range of viewpoints present amongst end users. In this chapter, I move from investigating the fundamental biology of above-belowground interactions to determine the impacts of these interactions for humans. I utilise an innovative method from social science (Q-methodology) that has previously been used to determine viewpoints on sustainability efforts, but has yet to be used to investigate the introduction of a novel biotechnology. I measure the perceptions of growers and experts on the value they place on the ecosystem services provided by AMF and wild pollinations, and determine their motivations for maximising these interactions. I find three predominant viewpoints, 'progressive thinkers' who have a strong interest in new techniques, and are motivated by intrinsic rewards, 'profit centred traditionalists' who would try new methods with the aim of increasing profits, and 'knowledgeable growers' who are well informed but place less importance on sustainability. In addition, strawberry growers supplement their production with commercial beehives, and I found only the 'progressive thinkers' place strong importance on maintaining diverse wild pollinator communities. As such, while AMF may improve crop yields, and wild pollinators could be further utilised in strawberry production systems, efforts to maximise the ecosystem services they provide must take into account the perspectives and motives of stakeholders.

5.1 Introduction

Rising global populations are expected to reach 9.7 billion by 2050 (United Nations, 2017), and feeding this rising population poses a significant challenge (Godfray *et al.*, 2010). In order to meet growing food demands, agricultural production must be expanded, however intensive agriculture uses high amounts of resources (Williams *et al.*, 2006). Intensive production also causes pollution from fertilisers (Vitousek *et al.*, 1997; Carpenter *et al.*, 1998; Correll, 1998; Sims *et al.*, 1998; Tilman *et al.*, 2002) and agro-chemicals (Zhang *et al.*, 2011), increases pressures on biodiversity (Tscharntke *et al.*, 2005), is responsible for land use change of half of global usable land, leading to significant habitat loss (Tilman *et al.*, 2001), as well as being a major contributor to greenhouse gas emissions (Robertson *et al.*, 2000), and agricultural expansion using conventional methods will exacerbate these problems (Tilman *et al.*, 2001).

To mitigate these impacts, sustainable intensification techniques can be implemented, which aim to produce more food from the same area of land, whilst simultaneously reducing the environmental impacts of intensive techniques (Baulcombe *et al.*, 2009). For example, expanding production through conventional methods will result in a significant increase in global greenhouse gas emissions (CO_2) of $\sim 3 \text{ Gt y}^{-1}$, however sustainable intensification techniques are estimated to mitigate these increases by 1/3, as improvements in production techniques will require significantly less land clearance (Tilman *et al.*, 2011). Tilman *et al.* (2002) argue sustainable intensification can be achieved through increasing nutrient and water use efficiency, maintaining and restoring soil fertility, and improving disease and pest control. For example, crops take up only 45% of phosphorus fertilisers applied, but precision application of fertilisers, crop rotations or intercropping, or improving microbial nutrient acquisition could improve this efficiency (Tilman *et al.*, 2002; Baulcombe *et al.*, 2009; Roy-Bolduc and Hijri, 2011). To change production methods, however, requires the development of appropriate novel technologies, disseminating knowledge to stakeholders (persons or organisations who have key roles in an industry), and providing appropriate incentives is necessary in order to foster their successful uptake (Tilman *et al.*, 2002).

Implementing changes to improve sustainability within agriculture is promoted through the use of governmental policies, and major producers, such as the EU, USA, Australia, and Canada have all implemented forms of subsidies to growers who utilise sustainable production techniques (Tilman *et al.*, 2002). However, policies will be unlikely to achieve goals and have minimal impact if they are based on incorrect assumptions about stakeholders and their opinions are not reflected in the policy. People do not make changes based on monetary rewards alone, and intrinsic motivations can play a vital role in their decision making (Frey, 1997). For example, a policy for reducing nutrient inputs may provide growers with financial subsidies as an incentive to implement a new technique, whereas the growers may be motivated more by an intrinsic care for sustainability than financial rewards. Frey suggests that in such situations, offering highly rewarding incentives can actually ‘crowd out’ the intrinsic desire to improve sustainability, creating apathy towards the new technology, a feeling of being forced to use it, and in turn low adoption of the technology. As an analogy to explain the difference between intrinsic vs. extrinsic rewards (i.e. monetary), paying someone to complete a hobby on a regular basis (an extrinsic monetary reward) can decrease their enjoyment of it, and in turn how often they participate in it, whereas providing improved tools to complete the hobby can increase their enjoyment (an intrinsic reward), and in turn uptake of the hobby. In such cases, policies based on financial incentives alone are unlikely to achieve their goals or have a significant impact. As such, understanding the perceptions of stakeholders, and their motivations is vital both in generating successful policies and developing novel biotechnologies that will improve sustainable intensification.

Increasing financial pressures exist in the agricultural industry, and in the USA, the price of fertilisers more than tripled between 2002 and 2012, yet the prices received by farmers for crops did not match this increase (Bureau of Labour Statistics, 2017; USDA-NASS, 2017). This difference creates a disparity where the use of traditional chemical fertilisers is reducing the profit margins of growers. Regulations on agricultural practices are increasing, and land managers no longer have the same degree of freedom with increased governmental control on farming practices (Dwyer and Hodge, 2001). In the UK, to prevent environmental impacts from the use of phosphorus fertilisers, regulations state that farmers must prove a need for adding Phosphorus (P) fertilisers by analysing soil samples, along with additional restrictions on when and where these fertilisers can be applied (DAERA, 2008; DAERA, 2016).

5.1.1 Utilising beneficial microbes to support sustainable intensification

One method for improving fertiliser efficiency is the use of novel biotechnologies that exploit beneficial microbes as biological amendments to conventional production systems. Arbuscular mycorrhizal fungi (AMF) are soil-dwelling fungi that associate with the majority of plant species, including many commercially important crops. These fungi act as a secondary root system, providing plants with increased access to nutrients and water resources (Smith and Read, 2008). By providing increased uptake of nutrients (Marschner and Dell, 1994), AMF could improve fertiliser use efficiency, and in turn reduce application rates. These reductions could lead to improved profit margins for growers and environmental benefits. As a result, AMF are a potential tool to improve the sustainable intensification of agricultural systems (Baar, 2008; Baulcombe *et al.*, 2009; Roy-Bolduc and Hijri, 2011; Orrell and Bennett, 2013). For example, AMF increased cassava yield under a 50% reduction in phosphorus application (Ceballos *et al.*, 2013), and maintained strawberry yield despite a 25% reduction in phosphorus application (Sharma and Adholeya, 2004) (also see Chapter 3).

Despite other beneficial organisms, such as rhizobia being widely utilised in leguminous crops since the 1900s to provide nitrogen fertilisation (Catroux *et al.*, 2001), mycorrhizae, which can improve phosphorus fertilisation (Marschner and Dell, 1994; Roy-Bolduc and Hijri, 2011), have yet to be widely utilised in mainstream agriculture. Mycorrhizal inoculants are available commercially, and have been used in developing countries and Canada, however evidence quantifying the scale of their uptake or success is lacking (Roy-Bolduc and Hijri, 2011). By proposing the introduction of this biotechnology into mainstream agriculture, it is vital to understand and quantify the current perceptions of stakeholders in order to foster successful uptake.

5.1.2 Improving Pollination Services

AMF may also provide additional benefits to crop production, and in addition to reducing fertiliser application, AMF may benefit crop production and improve sustainable intensification through other means, such as increasing pest defence (Gehring and Bennett, 2009), improving drought tolerance (Al-Karaki *et al.*, 2004), or enhancing plant-pollinator interactions (Barber and Soper Gorden, 2014, and see Chapter 2). Providing crops with optimal nutrition allows the potential for high production rates, however crops such as soft fruit rely on efficient and effective pollination to maximise yields. Pollinators provide an important ecosystem service and make a significant contribution to the yield of 70% of the main crops used for human consumption (Klein *et al.*, 2007). Pollinators contribute 9.5% of the value of global agricultural production of human food (€153 billion), and pollinator dependent crops are worth five times more than non-dependent crops (Gallai *et al.*, 2009).

Both wild and domesticated pollinators are currently in decline, and colonies of honey bees (*Apis mellifera*) declined 59% in the USA between 1947 and 2005 (National Research Council, 2007; Hayes Jr *et al.*, 2008), and 25% in Europe between 1985 and 2005 (Potts *et al.*, 2010). In the case of soft fruit production, the loss of pollinators is expected to drop fruit production below current levels of consumption (Gallai *et al.*, 2009). In addition to the previously described financial pressures and regulations on fertiliser usage, regulations in other areas of agriculture are increasing. For example, the use of three commonly used neonicotinoid pesticides, have been temporarily banned in the EU (European Commission, 2013), and their application is regulated in the USA (U.S. Environmental Protection Agency, 2017) in order to mitigate declines in pollinators.

Beneficial belowground microbes, such as AMF, have been shown to influence pollinators in numerous ways (Barber and Soper Gorden, 2014, and see Chapter 2), including plant reproductive traits (the quality and abundance of pollen and nectar in flowers (Koide, 2000; Gange and Smith, 2005; Varga and Kytöviita, 2010b), the size and number of inflorescence (Koide, 2000; Gange and Smith, 2005; Varga and Kytöviita, 2010a), the number of flowers (Koide, 2000), and the timing of reproduction (Koide, 2000)). Pollinators respond to these changes in floral rewards (Lau *et al.*, 1995; Koide, 2000; Poulton *et al.*, 2001; Poulton *et al.*, 2002; Gange and Smith, 2005; Varga and Kytöviita, 2010a; Willmer, 2011), and floral display (Gange and Smith, 2005; Cahill *et al.*, 2008), influencing the frequency of visits and the efficiency of the pollination services provided (Lau *et al.*, 1995; Koide, 2000; Poulton *et al.*, 2001; Poulton *et al.*, 2002; Gange and Smith, 2005; Cahill *et al.*, 2008; Varga and Kytöviita, 2010b; Willmer, 2011), which in turn affects crop yields (Jauker *et al.*, 2012).

Changes in the AMF community may also influence both the species of visiting pollinator (Gange and Smith, 2005; Wolfe *et al.*, 2005), and the frequency of their visits (Gange and Smith, 2005; Wolfe *et al.*, 2005; Cahill *et al.*, 2008; Varga and Kytöviita, 2010b) (also see

Chapter 4). The species of pollinator plays an important role in determining both the quantity of yield in strawberries, and in determining the quantity of misshapen fruits. Chagnon *et al.* (1993) found that strawberries visited by wild bees alone tended to result in misshapen fruits, whereas perfectly shaped fruits were more likely to develop from pollination by both wild bees and honeybees, and due to different foraging strategies, wild bees and honey bees can have complementary roles. Pollinator diversity has been shown to influence crop yields (Greenleaf and Kremen, 2006; Hoehn *et al.*, 2008; Garibaldi *et al.*, 2016), improving diversity can lead to increased crop production; for example, increasing wild pollinators has been found to improve fruit set by twice the amount of a similar increase in honey bees (Garibaldi *et al.*, 2013). As such, maintaining a diverse community of pollinators and a robust plant-pollinator interaction network increases the likelihood of effective pollination and maximising yields. Additionally, Klatt *et al.* (2014) found that in comparison to wind pollination and selfing, bee pollination increased not only the yield, but also improved fruit shape, redness, firmness, sugar-acid ratios, and improved shelf life and commercial grades of strawberry. As soft fruit crops are highly pollinator dependent (Gallai *et al.*, 2009), improving plant-pollinator interactions holds the potential to improve yields and increase yield quality, and if AMF can influence the community of visiting pollinators, further improvements in yield may be obtained.

If mycorrhizal fungi can both reduce fertiliser inputs as well as indirectly improve pollination services, high quality yields may be achieved under a reduced fertiliser regime that improves profits for growers, increases fruit quality, and provides environmental benefits. However, introducing a novel biotechnology that has multiple benefits – improved profit margins, environmental benefits (from a reduction in fertiliser use), and improved yield quality (a higher percentage of class A fruits from improved pollination) has significant complications. In order to effectively market a product based on this technology, or design a policy that supports the introduction of technology it must be determined: 1) if the concerns of growers lie predominantly in profitability or sustainability, and if intrinsic or external rewards are most important; 2) if growers are interested in adopting new techniques, to determine if there is demand to move from the status quo; 3) if growers already have any knowledge of the emerging technology, to evaluate how much information must be disseminated by experts; 4) which benefits provided by AMF are most important for growers; and 5) if the sets of viewpoints amongst growers are similar, or if several distinct and opposing viewpoints are present, to determine if the technology should be promoted in multiple ways to target multiple groups of growers.

As well as growers, scientific experts are considered to be key stakeholders, as they play an important role in developing new biotechnologies, as well as beginning the process of disseminating findings to growers and illustrating the potential of these new technologies, and as such, it is vital to also consider their viewpoints. If experts have a different set of viewpoints to the end users, or if they have incorrect assumptions about the views of growers, they may develop new technologies in a way that does not align with the needs and desires of growers. As such the viewpoints of both end users (growers) and those who develop new technologies (scientists) must be taken into account. However, in order to answer these questions and to effectively promote a novel biotechnology, we must quantify the subjective opinions of stakeholders.

5.1.4 Q-methodology

To reveal and examine commonalities and divergences in stakeholder attitudes and perceptions we applied the Q-method which originally was originally developed for use in psychology (Stephenson, 1963). This method incorporates elements of behavioural studies and uses quantitative tools to analyse stakeholder opinions (Brown, 1993). Q methodology offers the ability to quantify the perceptions of stakeholders through combining qualitative and quantitative methods. Studies utilising Q methodology come from a broad range of subject areas, ranging from determining perceptions of how solar power systems affect landscapes (Naspetti *et al.*, 2016) to the influence of cultures in tourism (Wijngaarden, 2017), and viewpoints of fairtrade carbon projects (Howard *et al.*, 2016) to viewpoints on water security (Strickert *et al.*, 2016), and land use changes (Nijnik *et al.*, 2009; Nijnik *et al.*, 2014; Nijnik *et al.*, 2017). These studies often have little in common besides an underlying goal to quantify and understand subjective stakeholder opinions (Herrington and Coogan, 2011). Q methodology allows the viewpoints of stakeholders (respondents) on a range of subjects to be analysed as whole sets of responses, and respondents are placed into groups with shared overall views (factors) (see Table 5.1 for a glossary of terms used in Q-methodology) (Brown, 1980; McKeown and Thomas, 1988; Herrington and Coogan, 2011). This is achieved by presenting respondents with a set of statements on multiple subjects, which they order from strong agreement to strong disagreement. Respondents are then grouped into factors based on how their opinions correlate with each other. A set of viewpoints from each factor is then produced that describes the typical viewpoint of that factor (a composite sort), and illustrates statements on which respondents across all factors agree (consensus statements), and those which factors significantly disagreed on (distinguishing statements).

Table 5.1: Glossary of terms used in Q-methodology studies

Term	Definition
Respondent	A participant who shares their viewpoints on the topic of the study
Statement	An opinion on a study topic which respondents rank their agreement
Q-sort	A grid in which respondents rank their agreement of statements (Figure 1). Also refers to a respondents completed entry
Factor	A group of respondents who quantitatively share viewpoints
Factor Loading	The correlation of a respondent to the overall (composite) views of a factor
Consensus Statements	Statements which all factors have a shared view
Distinguishing Statements	Statements which have significantly different views between factors
Composite Sort	A summary of the overal viewpoint of a factor

Thus, Q-methodology can be used to ask respondents how they feel about sustainability, AMF, and pollination services, and determine groups (factors) who have shared overall opinions on all three subjects. The strength of this method allows us to determine two key pieces of information. Firstly, as opposed to other methods which simply determine how respondents tend to feel based on their traits (i.e. do stakeholders of a greater age have more concern for sustainability), we can measure how opinions are shared across multiple subject areas, and secondly, determine if there are a small number of dominant viewpoints shared by the majority of stakeholders, or if there are multiple distinct and opposing sets of views (Barry and

Proops, 1999; Herrington and Coogan, 2011). The combination of these two pieces of information is key in the introduction of a novel biotechnology. For example, different biotechnology introduction strategies are needed if a single dominant group of respondents has a strong intrinsic interest in sustainability, but little knowledge on AMF or pollinators, versus a case where two main groups of respondents, one with a primary concern for profitability, and a second with greater concerns over sustainability.

Q-methodology has previously been employed to analyse viewpoints in multiple areas of sustainability (Strickert *et al.*, 2016; West *et al.*, 2016; Armatas *et al.*, 2017, etc.), particularly agricultural sustainability (Davies and Hodge, 2007; Pereira *et al.*, 2016, etc.). These analyses typically find that growers fall into two categories: first, environmentalists who strongly promote sustainability, and second, production and profit oriented growers with a focus on profitability. Other identified factors had fewer respondents, and depended upon the type of agriculture study focus. These studies, however, all examined perceptions of existing sustainability efforts, and no studies have yet focused on an emerging technology.

In order to improve agricultural systems through the application of novel biotechnologies, such as AMF, it is vital to determine and understand the viewpoints of end users (growers) and influencers (scientists). Here we quantitatively analyse the subjective opinions of soft fruit growers and scientists involved in soft fruit research from both the UK and USA on the introduction of mycorrhizal fungi to improve soft fruit production through a reduction in fertiliser usage, and improvements in pollination services. To our knowledge there have been no studies analysing the opinion of agricultural stakeholders on the introduction of a promising biotechnology, utilising Q methodology or otherwise, nor have the perceptions of the agricultural sector on the adoption of mycorrhizal fungal amendments been quantified. Here we aim to determine in strawberry production:

1) The current level of knowledge of growers on arbuscular mycorrhizal fungi and plant-pollinator interactions
2) The perceptions and motives of growers on the use of arbuscular mycorrhizal fungi
3) The value growers place on the ecosystem service provided by wild pollinators
4) If growers and experts share the same viewpoints

Based on previous studies that have utilised Q-methodology to determine perceptions on sustainability efforts, I predict that stakeholders will fall into two predominant viewpoints: those with an intrinsic care for sustainability, and those who are production and profit focused, and their motives in utilising AMF will be different between these two groups. Because growers commonly use commercial beehives in their production systems, I predict that they will not place a high degree of value on wild pollinators.

5.2 Methods

5.2.1 Survey

Respondents were identified through online searches for strawberry grower's co-operatives and individual farms producing strawberries in the UK and USA. Respondents were selected based on the criteria of being either growers or scientific experts in mycorrhizal fungi and soft fruit in the UK and USA. The selection of growers was not constrained by any factors such as their farm size, any indications of production methods, advertised level of sustainability, demographics, or indicators of previous knowledge of any subject area in order to avoid biasing the results by selecting a subset of the industry. Similarly, neither the potential benefits of AMF, pollinators, sustainability, or the environmental impacts and increasing costs of fertilisers were discussed in the introduction to the survey (see Figure S5.1) to avoid influencing the results or bias those who would respond to the survey invitation.

Q methodology surveys were conducted by providing respondents with a set of statements on multiple issues, with statements derived from conducting a preliminary 'concourse' stage with stakeholders, in which stakeholders are asked their opinions on the topics in order to derive statements for the main 'discourse' stage (explained below) (McKeown and Thomas, 1988; Herrington and Coogan, 2011). 10 participants were selected for this initial discourse – 5 UK strawberry growers, and 5 US strawberry growers (who all also completed the main 'discourse' stage as described below). During this concourse, a selection of stakeholders were asked to provide their views, by writing a three to five sentence paragraph on each subject of profitability, sustainability, mycorrhizal fungi, pollinators, introducing new techniques, and fertiliser use. Each paragraph was then broken down into a list of statements, and statements from all respondents in the concourse were pooled. The final set of statements used in the main ‘discourse’ survey stage (Table 5.2) was then derived by removing duplicate statements, and the remaining statements generalised (i.e. ‘I recently switched a few tunnels to a new variety of strawberry and noticed they were attracting lots of wild bees’ becomes ‘I am aware that growing different varieties of a crop may attract different pollinators into the field’ (Statement 10)), and split if needed (i.e. ‘I know that mycorrhizal fungi live in the soil and that they are supposed to help plants, but I don’t know what they do or how.’ becomes ‘I am aware of what mycorrhizal fungi are’ (Statement 1) and ‘I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake’ (Statement 2)). Additional statements were added to determine for what purpose growers would use AMF if introduced as a new technology (Statements 8, 9, 13, 16, 17). A total of 25 statements were generated, covering the subjects of mycorrhizal fungi, pollinators, and sustainability (Table 5.2), with statements either containing a single subject (i.e. Statement 1), or an intersection of two subjects (Statement 13).

Table 5.2: Statements derived from the concourse stage and used in the main discourse survey stage.

Statement Number	Statement
1	I am aware of what mycorrhizal fungi are.
2	I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake.
3	I know that mycorrhizal fungi can potentially change plant floral traits, and influence crop pollination.
4	I am aware that many crop plants are colonised with mycorrhizal fungi.
5	Maintaining a plant-pollinator network with a variety of pollinator species is not important to me.
6	I am aware that some pollinators can pollinate a crop very efficiently, whereas others are not so efficient.
7	I am aware that efficient pollination is required for a good quantity and quality of yield.
8	Mycorrhizal inoculants should be used to improve the quality of yields.
9	We should harness interactions between above and belowground organisms to improve crop production.
10	I am aware that growing different varieties of a crop may attract different pollinators into the field.
11	It is important to ensure that crops become colonised with the most suitable type of mycorrhizal fungi.
12	I am aware that altering the belowground organisms present may influence the pollinators visiting the crop.
13	Crop pollination should be managed aboveground through increased numbers of hives or attractants, etc. rather than utilising belowground organisms.
14	It does not matter if growers farm in an environmentally friendly way.
15	Maintaining profits should be thought about first before considering how sustainable a new approach is.
16	Mycorrhizal inoculants should be utilised if they could cut down usage of chemical fertilisers.
17	Mycorrhizal inoculants should only be used if there was a significant improvement to profits.
18	Profitability is the primary goal of an agricultural business.
19	It is more important to increase profits than worry about sustainability
20	It is best to stick to tried and tested techniques of farming.
21	Growers should be interested in trialling new techniques.
22	I am concerned that the cost of chemical fertilisers are increasing.
23	Chemical fertilisers will solely be relied on for the foreseeable future.
24	Biological amendments are not as reliable as chemical fertilisers.
25	I am concerned about current declines in pollinators.

After the final set of statements were generated, a platform (a method of inputting data) was provided to respondents in order for them to indicate their agreement or disagreement with each statement. Statements were initially sorted into broad groups of ‘Disagree’, ‘Neutral’, and ‘Agree’, in order to assist respondents (Watts and Stenner, 2012) in completing the main stage of specifically ranking each statement into a ‘Q-sort’ – a grid where statements are ordered from strong disagreement (-4) to strong agreement (+4) (Figure 5.1).

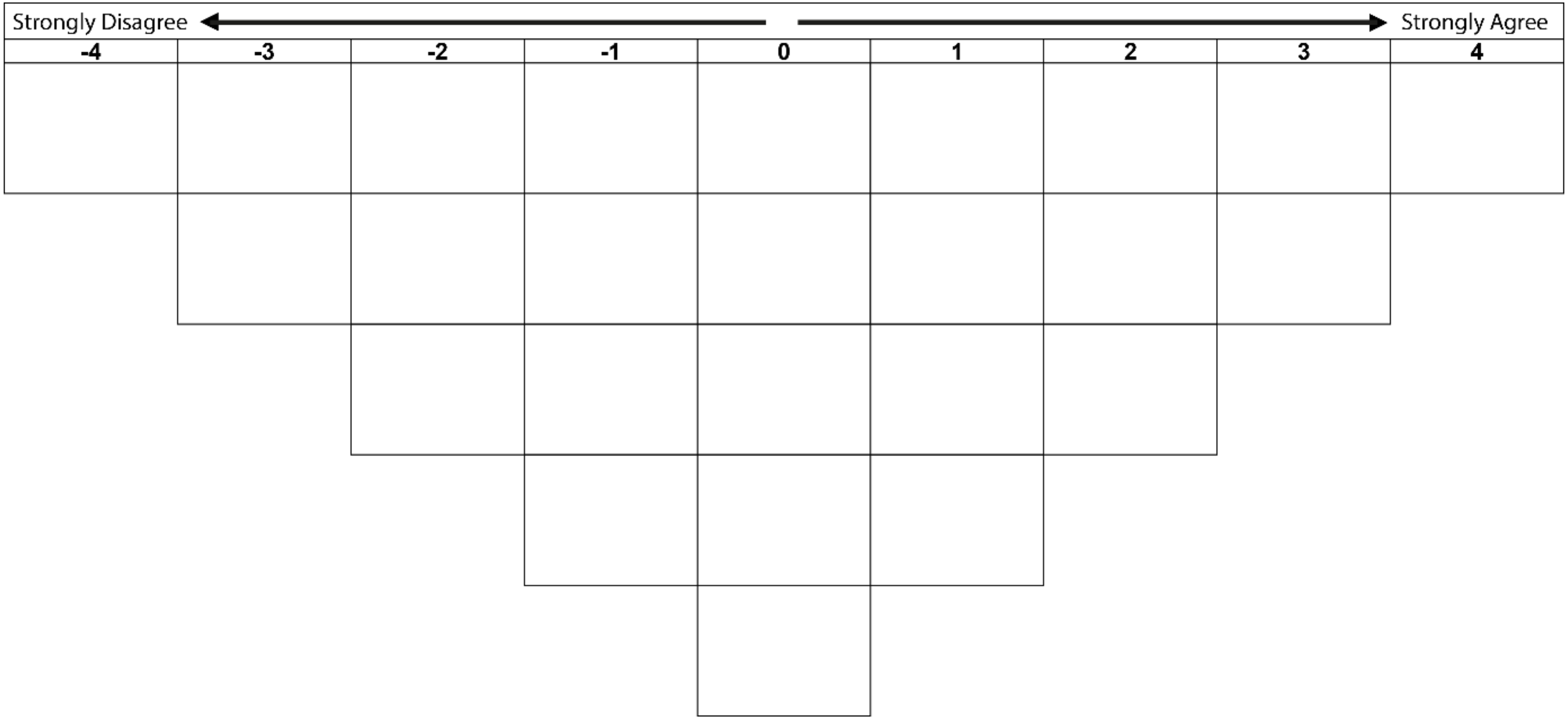


Figure 5.1: Example Q-sort grid. Respondents are presented with a set of statements, which are sorted into broad categories of 'Agree', 'Neutral', and 'Disagree'. Respondents then move these statements from the broad categories into a Q-sort grid, with a single statement placed in each box. Boxes range from strongly disagree (-4) on the left side, neutral (0) in the middle, to strongly agree (4) on the right side. Numbers along the top represent a range of disagreement (-4) to agreement (4), and the shape of the grid determines how many statements can be placed into each value of agreement / disagreement.

This study utilised a recently developed online web application tool (Q-sortware, Pruneddu, 2016), that enables respondents to easily rank statements and complete the Q-sort, and allows respondents from multiple countries to complete the test. The online survey was formatted into four stages: 1) a general introduction to the study, 2) an initial sort, in which participants placed statements into either general 'agree', 'neutral', or 'disagree' categories, 3) a final sort, where statements were moved from the two general categories into the full Q-sort, and 4) questions on demographic information, including standard characteristics, such as age, gender, income, etc., and specific questions on respondents' knowledge and experience of each subject (see Table S5.4). A splash screen (a screen that provides introductory information) was provided at the beginning of the survey with a general overview, and instructions on how to complete each stage were provided at the top of the screen for each stage (see Figure S5.1 for a screenshot of each stage of the survey). Participants were free to change their answers at any time before final submission, could spend any amount of time progressing through each stage, and were given a final decision of if they wanted to submit their responses at the end of the survey. Demographic information was also collected, and was asked as either multiple choice questions (e.g. for income scales), boolean entries (yes or no questions, e.g. 'Have you tried alternatives to chemical fertilisers?'), or free text ('What crops do you grow…?').

5.2.2 Statistical Analysis

A statistical analysis can be performed using Q-methodology with as few as 12 participants (Barry and Proops, 1999), as Q-methodology aims not to determine the distribution of opinions within the population of all potential respondents, but rather to illustrate the diversity of viewpoints present (Valenta and Wigger, 1997; Zabala and Pascual, 2016). Furthermore, the strength of this methodology is the large amount of data contained within each respondent's Q-sort. The level at which a respondent agrees or disagrees with a given statement is compared with all other respondents to determine how similar their agreement or disagreement is, before being repeated for each of the other statements, and then for each of the other respondents generating a large dataset of comparisons (Barry and Proops, 1999).

Q-sorts returned from respondents were analysed using the Ken-Q Analysis software package (Banasick, 2017). Initially, a correlation matrix is created, which describes the relationship of each Q-sorts with the others, however this matrix only describes how closely Q-sorts are related overall, and does not look at the relationships of individual statements with all other statements.

Utilising factor analysis, we can determine a set of ‘factors’ (groups of respondents with shared viewpoints), in which respondents are ‘loaded’ based on how they have ordered the set of statements (Webler *et al.*, 2009). Q methodology offers two methods of extracting factors from respondents Q-sorts. First, centroid extraction, a theoretical approach, which provides indeterminate factor groupings (respondents placed into groupings, but each not assigned to specific factors) for the researcher to make theoretical judgements on, or secondly, a statistical approach, utilising principal component analysis which mathematically forms a determinate set of factors (respondents placed into groups and assigned to factors based on how closely their views are related) (Brown, 1993; Webler *et al.*, 2009). Although factor extraction through either principal components or centroid extraction typically produces similar results, especially in studies of environmental topics (Webler *et al.*, 2009), due to its indeterminacy centroid extraction lends itself to judgemental factor rotation (described below), and is typically used when a researcher has *a priori* expectations of the viewpoints of respondents and leaves them free to explore rotation solutions in which they believe to be theoretically correct (Brown, 1980; Brown, 1993; Watts and Stenner, 2005; Webler *et al.*, 2009).

Initially, eight factors are extracted through principal components, however retaining all factors typically produces a scattered fit, where respondents are allocated to additional factors for having marginally different viewpoints. The number of factors to be kept results from how many sets of distinct viewpoints are present within the respondents – many distinct viewpoints result in the need for an increased number of factors, whereas if there is a high degree of shared views, fewer factors will be extracted. Brown (1980) suggests utilising a combination of statistical and theoretical methods to determine which factors to keep. First, in the statistical approach, eigenvalues are calculated (the sum of a factor’s squared factor loadings), and these values indicate a factor’s explanatory strength, with eigenvalues of >1 and more than two respondents loading onto a factor are considered to indicate significant factors (Brown, 1980; Watts and Stenner, 2012). Secondly, in the theoretical approach, respondents loaded into factors with eigenvalues <1 (which typically contain only a single respondent) are checked manually to determine if the opinion of the respondent is distinct from all other factors (and therefore considered important), or if there is any theoretical reason as to why they may have a different opinion (for example, a grower that has been farming for a substantially longer time than all others and has more experience) (Brown, 1980; Watts and Stenner, 2005).

Once factors have been extracted and the number of groups of shared viewpoints determined, these factors are then rotated (described in Figure 5.2). Initial factor extraction only determines the number of groups of shared opinions, and does not align groups of respondents with similar views to fit into the same factor (see Figure 5.2). In order to place the groupings of respondents into defined factors, we must rotate the placements of these groups of respondents to fit distinctly within, and not between factors (see Figure 5.2). In a similar manner to how families and link functions can be altered in a generalised linear model to create a model that best fits the data set, factor rotation determines a structure in which the most number of Q-sorts fit into defined factors (Brown, 1980). Similar to factor extraction, this can be completed by two methods, either using judgemental rotation, which relies on a researcher's theoretical assumptions, or varimax rotation, which uses mathematical iterations of multiple rotations to explain as much variance as possible, and has a higher degree of exploratory power than judgemental rotation (Brown, 1980; Akhtar-Danesh and Mirza, 2017).

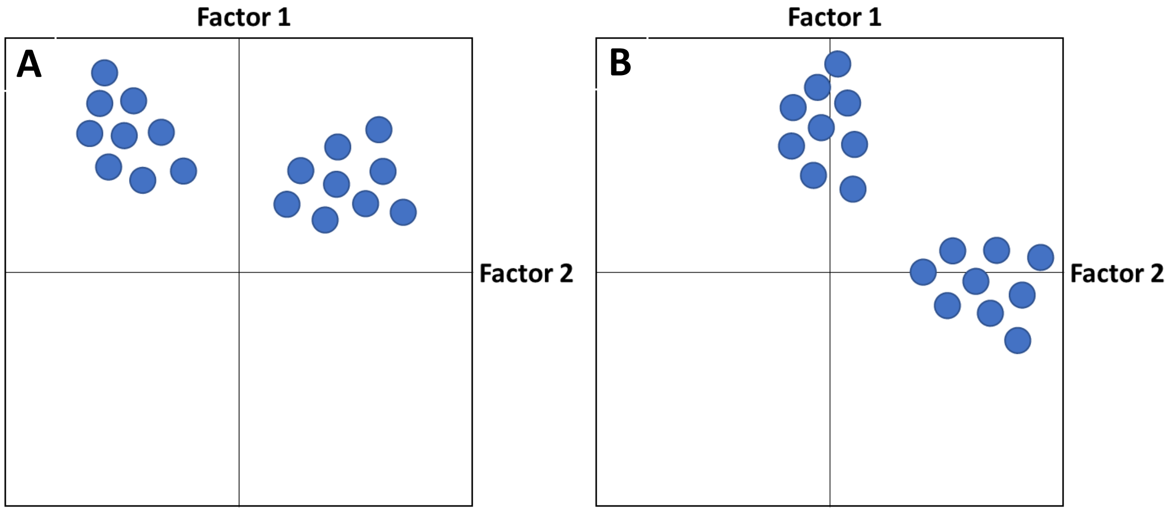


Figure 5.2: Illustrative example of factor rotation. A) After factors are extracted, distinct groups of respondents emerge (blue circles) representing sets of shared opinions, but are not aligned to factors (axes), and would load to the factor that they are closest too, creating a situation where respondents with similar viewpoints load on to separate factors. B) After factor rotation, the groupings of the respondents remain the same, however the groups are now clearly aligned to factors, and will load into each factor based on their groups of shared viewpoints.

Following rotation, factor loadings are used to determine which respondents fit into each factor. Factor loadings describe the degree of correlation between a respondent and the overall views of a factor, Q-sorts with factor loadings >0.5 are considered significant, and respondents are allocated to a factor based on their highest factor loading (Brown, 1980; Zabala and Pascual, 2016). Respondents with all loadings <0.5 are considered non-significant, and to have opinions that do not fall into any distinct factor, and are excluded.

Finally, once respondents have been loaded (allocated) to a factor, composite Q-sorts are created. Composite Q-sorts describe an overall summary of viewpoints of the respondents within each factor (see below). These composite sorts also illustrate ‘consensus statements’ – statements on which all factors similarly agree or disagree, and ‘distinguishing statements’ – statements which separate the viewpoint of a factor from the others. Z-scores are generated (a weighted average of the values that all the respondents within a factor give to a statement), to indicate how much a factor agrees or disagrees with a statement. Statements are then determined to be consensus or distinguishing statements, based on if the z-scores across factors are statistically different, and p-values are determined through standard error of differences (see Brown, 1980 p. 245; Zabala and Pascual, 2016).

5.3 Results

A total of 388 invitations to complete the survey were sent to growers and experts in the UK (176 invitations) and the USA (212 invitations), with 20 respondents completing the test - 11 strawberry growers from the UK, 5 strawberry growers from the US, 3 experts from the UK, and 1 expert from the US (see Table S5.1 for codes assigned to respondents). Q-analyses studies can be completed with as few as 12 participants, as the methodology aims not to describe the distribution of opinions across the population of all potential participants, but rather to show the diversity of viewpoints present (Brown, 1980; Valenta and Wigger, 1997; Zabala and Pascual, 2016).

As we had no *a priori* expectation of the viewpoints of respondents, factors were extracted as principal components. Three factors had eigenvalues >1, and cumulatively explained 80% of the total variance (Tables 5.3 & 5.4, Figure 5.3). Additional factors explained ≤ 5% of additional variance, and including additional factors resulted in a reduction of distinguishing statements.

Using the criteria discussed in Section 5.2.2 (keeping factors with eigenvalues >1 and at least two significant loadings, with no theoretical reason for respondents to load into additional factors (Brown, 1980)), three factors were kept for rotation. As we had no *a priori* expectation of how respondent's opinions would group, varimax rotation was applied. All respondents were clearly separated into a factor with significant factor loadings >0.5, except for a single respondent (UKFRGR3), who had similar factor loadings for factors 1 and 2: 0.477, and 0.457 respectively, and as such was not associated with any factor. Factor correlations illustrate how similar or dissimilar viewpoints were between factors, and factors 1 and 3 were relatively well correlated with each other, whereas factor 2 was more distinct, indicating a substantially different set of opinions and outlook from respondents in the other two factors (Table 5.3).

Table 5.3: Factor correlations, showing how similar overall views are between factors. A value of 1 indicates factors that highly related views, and a value of zero shows factors that do not share any views.

	Factor 1	Factor 2	Factor 3
Factor 1	1	0.1456	0.7591
Factor 2		1	0.01
Factor 3			1

Table 5.4: Eigenvalues and variance explained by each factor. Eigenvalues are used to determine how many factors should be included, generally constrained to including factors with a value >1. Variance explained shows how much of the variance is explained by each factor, and the cumulative variance shows how much additional variance is explained by including additional factors.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
Eigenvalues	11.4	3.482	1.269	0.901	0.692	0.523	0.466	0.305
Variance Explained	57%	17%	6%	5%	3%	3%	2%	2%
Cumulative Variance Explained	57%	74%	80%	85%	88%	91%	93%	95%

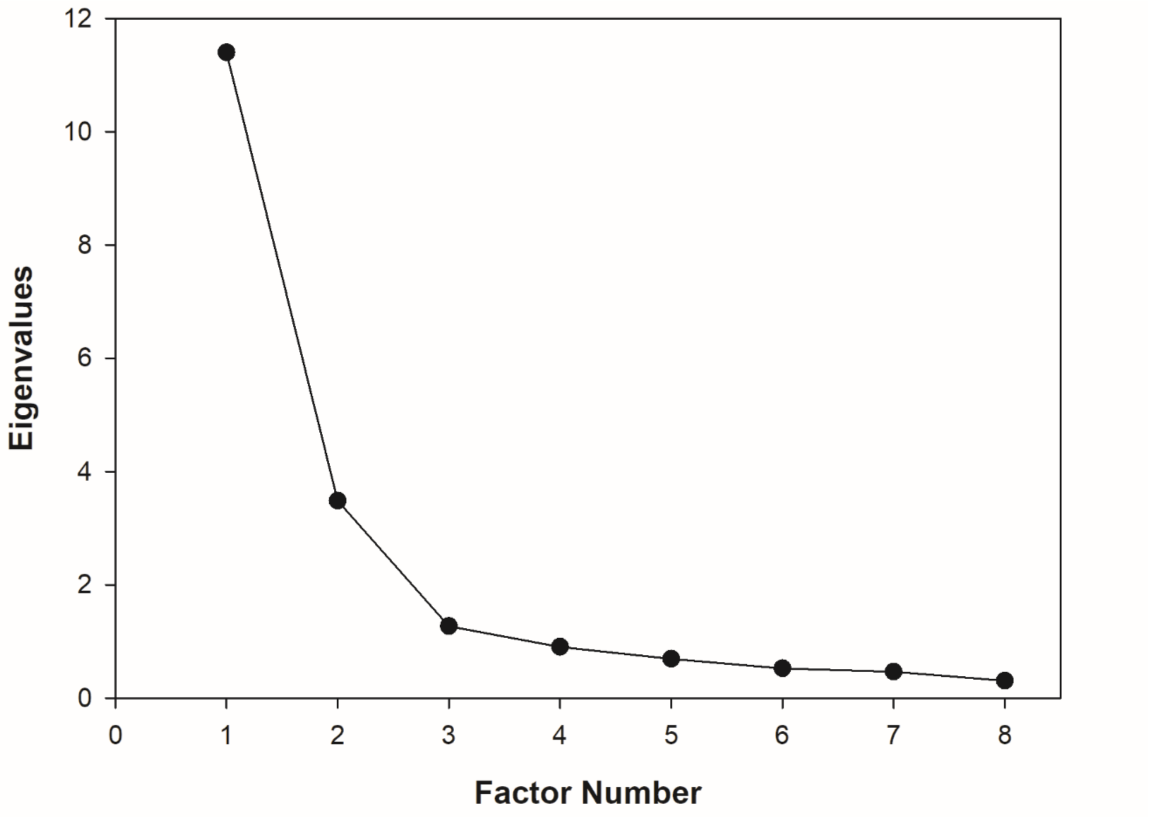


Figure 5.3: Graphical representation of the eigenvalues of each factor, creating an illustrative view of how including each factor helps to explain the variance in responses of respondents.

5.3.1 Consensus Statements

All respondents agreed on three statements regardless of which factor they belonged to, with no significant difference in agreement or disagreement between factors (Table 5.5). These results show that respondents from all factors agree that growers should farm in an environmentally friendly way (statement 14), that chemical fertilisers will not be relied on exclusively in the future (statement 23), and that they indicated neutral to slight agreement on having knowledge that different varieties of crops attract different pollinators (statement 10).

Table 5.5: Consensus statements: statements in which all respondents had similar opinions. Composite sort values show the typical score for a statement from respondents within a factor, and z-scores are a weighted average of the values that all the respondents within a factor give to a statement.

			Factor 1		Factor 2		Factor 3	
Statement Number	**Statement**	**Significance**	**Composite Q-sort Value**	**Z-score**	**Composite Q-sort Value**	**Z-score**	**Composite Q-sort Value**	**Z-score**
10	I am aware that growing different varieties of a crop may attract different pollinators into the field	P > 0.05	1	0.71	0	0.16	1	0.35
14	It does not matter if growers farm in an environmentally friendly way	P > 0.05	-4	-1.94	-4	-1.72	-4	-1.93
23	Chemical fertilisers will solely be relied on for the foreseeable future	P > 0.05	-2	-0.97	-1	-0.50	-2	-1.12

5.3.2 Distinguishing Statements and Demographic Analysis

Factor names are derived by analysing the prevailing viewpoints that distinguish respondents within a factor from other factors, based on their distinguishing statements. P values indicate statements which a factor had a significantly different opinion from other factors. Composite Q-sort values are reported on a scale from -4 to +4, with -4 = strong disagreement, -3 = disagreement, -2 = moderate disagreement, -1 = slight disagreement, ±0 = neutral, +1 = slight agreement, +2 = moderate agreement, +3 = agreement, and +4 = strong agreement.

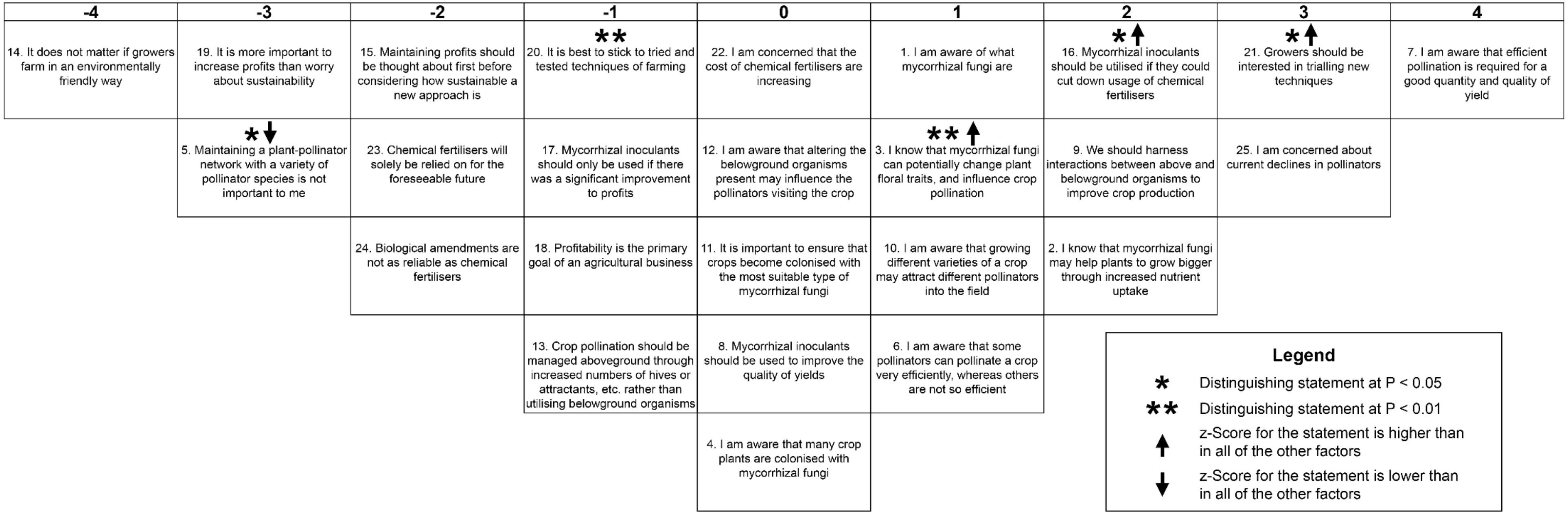
5.3.3 Factor 1 – 'Progressive thinkers'

Factor 1 had five distinguishing statements ($P < 0.05$), with two of these being highly significant ($P < 0.01$) (Table 5.6, Figure 5.4). This factor contained over half of the total number of respondents (n=11), including half of all growers – 4 out of 11 UK growers and 4 out of 5 US growers, three out of four of the experts - 2 UK experts, and 1 expert from the USA (Table S5.1). This factor had the widest range of correlation of opinions between its respondents, ranging from 49-95% ($\bar{x}$ = 73.00% ±0.88) (Table S5.4), indicating a wider variation of opinions compared to other factors, although more variation in opinion is to be expected with a higher number of respondents.

Respondents in this factor were distinguished by agreeing (+3) that growers should be testing new techniques (statement 21), somewhat disagreeing (-1) with sticking to tried and tested techniques (statement 20) (though less strongly than factor 3), and moderately agreeing (+2) that mycorrhizal fungal inoculants should be utilised if they can reduce fertiliser usage (statement 16), indicating a large willingness to try new solutions, but still retaining faith in tried and tested methods to a higher degree than factor 3. As such, due to their willingness to try new techniques and interest in using a novel biotechnology to reduce fertiliser use, these respondents in this factor are termed 'progressive thinkers'. In addition, they somewhat agreed (+1) that they were aware that mycorrhizal fungi may influence plant reproductive traits (statement 3), and disagreed (-3) that maintaining a diverse plant-pollinator network was not important to them (statement 5) (i.e. wild pollinators were important to them).

Table 5.6: Distinguishing statements – Factor 1: statements in which separated opinions between respondents in Factor 1 and other factors. Composite sort values show the typical score for a statement from respondents within a factor, and z-scores are a weighted average of the values that all the respondents within a factor give to a statement. Statements from Factor 1 are highlighted in bold.

			Factor 1		Factor 2		Factor 3	
Statement Number	**Statement**	**Significance**	**Composite Q-sort Value**	**Z-score**	**Composite Q-sort Value**	**Z-score**	**Composite Q-sort Value**	**Z-score**
21	Growers should be interested in trialling new techniques	P < 0.05	**3**	1.25	1	0.63	1	0.66
16	Mycorrhizal inoculants should be utilised if they could cut down usage of chemical fertilisers	P < 0.05	**2**	0.84	0	0.13	0	0.19
3	I know that mycorrhizal fungi can potentially change plant floral traits, and influence crop pollination	P < 0.01	**1**	0.54	-2	-0.84	-1	-0.22
20	It is best to stick to tried and tested techniques of farming	P < 0.01	**-1**	-0.93	1	0.38	-3	-1.73
5	Maintaining a plant-pollinator network with a variety of pollinator species is not important to me	P < 0.05	**-3**	-1.32	1	0.34	-1	-0.70



-4	-3	-2	-1	0	1	2	3	4
14. It does not matter if growers farm in an environmentally friendly way	19. It is more important to increase profits than worry about sustainability	15. Maintaining profits should be thought about first before considering how sustainable a new approach is	** 20. It is best to stick to tried and tested techniques of farming	22. I am concerned that the cost of chemical fertilisers are increasing	1. I am aware of what mycorrhizal fungi are	* ↑ 16. Mycorrhizal inoculants should be utilised if they could cut down usage of chemical fertilisers	* ↑ 21. Growers should be interested in trialling new techniques	7. I am aware that efficient pollination is required for a good quantity and quality of yield
	* ↓ 5. Maintaining a plant-pollinator network with a variety of pollinator species is not important to me	23. Chemical fertilisers will solely be relied on for the foreseeable future	17. Mycorrhizal inoculants should only be used if there was a significant improvement to profits	12. I am aware that altering the belowground organisms present may influence the pollinators visiting the crop	** ↑ 3. I know that mycorrhizal fungi can potentially change plant floral traits, and influence crop pollination	9. We should harness interactions between above and belowground organisms to improve crop production	25. I am concerned about current declines in pollinators	
		24. Biological amendments are not as reliable as chemical fertilisers	18. Profitability is the primary goal of an agricultural business	11. It is important to ensure that crops become colonised with the most suitable type of mycorrhizal fungi	10. I am aware that growing different varieties of a crop may attract different pollinators into the field	2. I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake		
			13. Crop pollination should be managed aboveground through increased numbers of hives or attractants, etc. rather than utilising belowground organisms	8. Mycorrhizal inoculants should be used to improve the quality of yields	6. I am aware that some pollinators can pollinate a crop very efficiently, whereas others are not so efficient			
				4. I am aware that many crop plants are colonised with mycorrhizal fungi				

Legend

*	Distinguishing statement at P < 0.05
**	Distinguishing statement at P < 0.01
↑	z-Score for the statement is higher than in all of the other factors
↓	z-Score for the statement is lower than in all of the other factors

Figure 5.4: Composite q-sort for Factor 1, illustrating a summary of opinions for respondents within this factor. Statements that distinguish this factor from others are indicated as significant or highly significant and arrows indicate if these statements were significantly higher or lower than other factors. Distinguishing statements without arrows fall at a score between the scores in the two other factors

5.3.4 Factor 2 – ‘Profit Centred Traditionalists’

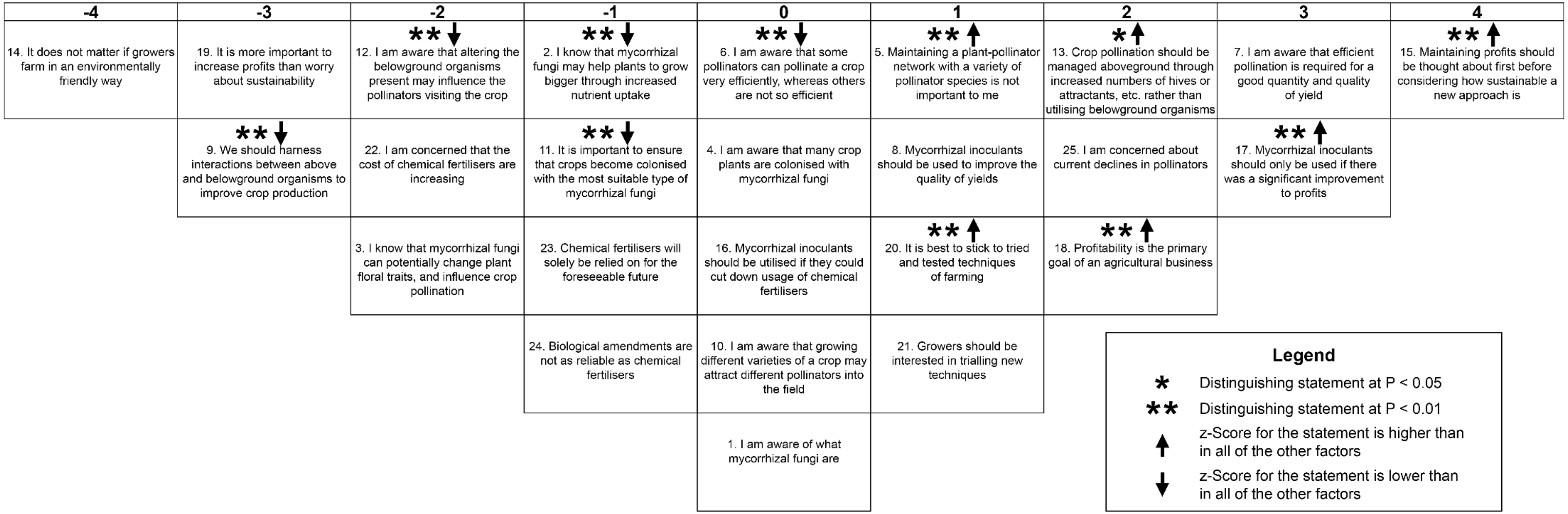
Factor 2 had 11 distinguishing statements ($P < 0.05$), with 10 of these highly significantly different ($P < 0.01$) from respondents in the other factors (Table 5.7, Figure 5.5). This factor had the lowest number of respondents (n=3) - one UK fruit grower, one US grower, and a UK expert. These respondents showed a high degree of correlation in their opinions with each other, ranging from 90-95% correlation ($\bar{x}$ = 93.00% ± 0.97) (Table S5.4).

Respondents in this group were distinguished by a focus on profitability and strongly agreed (+4) that maintaining profits should be the first consideration over sustainability (statement 15), as well as moderately agreeing (+2) that profitability is the main goal of agriculture (statement 18). They showed slight agreement (+1) that it is best to stick to tried and tested methods (statement 20), slightly disagreed (-1) that it is important to ensure crops are colonised with suitable mycorrhizal fungi (statement 11), and agreed (+3) that we should not use above-belowground interactions to improve crop production (statement 9), but agreed (+3) on the use of a mycorrhizal fungal inoculant if it improved profits (statement 17), indicating motivation driven by external financial rewards.

Compared with the other two factors, these respondents indicated a relatively low level of knowledge of the subject areas of the survey, with slight disagreement (-1) that they were aware that mycorrhizal fungi can improve crop growth through increased nutrient uptake (statement 2), and moderate disagreement (-2) of awareness that belowground organisms can influence pollinators (statement 12). They had neutral agreement/disagreement (±0) awareness of pollination efficiency varying between pollinator species (statement 6), slightly agreed (+1) that a diverse plant-pollinator community was not important to them (statement 5), and moderately agreed (+2) pollination should be managed through aboveground means, rather than by utilising belowground organisms (statement 13). Due to their strong focus on profitability and unwillingness to try new techniques without financial rewards, respondents within this factor are termed ‘profit centred traditionalists’.

Table 5.7: Distinguishing statements – Factor 2: statements in which separated opinions between respondents in Factor 2 and other factors. Composite sort values show the typical score for a statement from respondents within a factor, and z-scores are a weighted average of the values that all the respondents within a factor give to a statement. Statements from Factor 2 are highlighted in bold.

			Factor 1		**Factor 2**		**Factor 3**	
Statement Number	**Statement**	**Significance**	**Composite Q-sort Value**	**Z-score**	**Composite Q-sort Value**	**Z-score**	**Composite Q-sort Value**	**Z-score**
15	Maintaining profits should be thought about first before considering how sustainable a new approach is	P < 0.01	-2	-1.14	**4**	1.88	-2	-0.71
17	Mycorrhizal inoculants should only be used if there was a significant improvement to profits	P < 0.01	-1	-0.57	**3**	1.63	-2	-1.06
18	Profitability is the primary goal of an agricultural business	P < 0.01	-1	-0.52	**2**	1.16	0	-0.05
13	Crop pollination should be managed aboveground through increased numbers of hives or attractants, etc. rather than utilising belowground organisms	P < 0.05	-1	-0.49	**2**	0.88	0	0.01
20	It is best to stick to tried and tested techniques of farming	P < 0.01	-1	-0.93	**1**	0.38	-3	-1.73
5	Maintaining a plant-pollinator network with a variety of pollinator species is not important to me	P < 0.01	-3	-1.32	**1**	0.34	-1	-0.70
6	I am aware that some pollinators can pollinate a crop very efficiently, whereas others are not so efficient	P < 0.01	1	0.73	**0**	-0.13	2	1.05
11	It is important to ensure that crops become colonised with the most suitable type of mycorrhizal fungi	P < 0.01	0	0.19	**-1**	-0.63	1	0.32
2	I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake	P < 0.01	2	1.17	**-1**	-0.66	3	1.67
12	I am aware that altering the belowground organisms present may influence the pollinators visiting the crop	P < 0.01	0	0.07	**-2**	-1.00	0	-0.06
9	We should harness interactions between above and belowground organisms to improve crop production	P < 0.01	2	1.12	**-3**	-1.63	2	1.08



-4	-3	-2	-1	0	1	2	3	4
14. It does not matter if growers farm in an environmentally friendly way	19. It is more important to increase profits than worry about sustainability	** ↓ 12. I am aware that altering the belowground organisms present may influence the pollinators visiting the crop	** ↓ 2. I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake	** ↓ 6. I am aware that some pollinators can pollinate a crop very efficiently, whereas others are not so efficient	** ↑ 5. Maintaining a plant-pollinator network with a variety of pollinator species is not important to me	* ↑ 13. Crop pollination should be managed aboveground through increased numbers of hives or attractants, etc. rather than utilising belowground organisms	7. I am aware that efficient pollination is required for a good quantity and quality of yield	** ↑ 15. Maintaining profits should be thought about first before considering how sustainable a new approach is
	** ↓ 9. We should harness interactions between above and belowground organisms to improve crop production	22. I am concerned that the cost of chemical fertilisers are increasing	** ↓ 11. It is important to ensure that crops become colonised with the most suitable type of mycorrhizal fungi	4. I am aware that many crop plants are colonised with mycorrhizal fungi	8. Mycorrhizal inoculants should be used to improve the quality of yields	25. I am concerned about current declines in pollinators	** ↑ 17. Mycorrhizal inoculants should only be used if there was a significant improvement to profits	
		3. I know that mycorrhizal fungi can potentially change plant floral traits, and influence crop pollination	23. Chemical fertilisers will solely be relied on for the foreseeable future	16. Mycorrhizal inoculants should be utilised if they could cut down usage of chemical fertilisers	** ↑ 20. It is best to stick to tried and tested techniques of farming	** ↑ 18. Profitability is the primary goal of an agricultural business		
			24. Biological amendments are not as reliable as chemical fertilisers	10. I am aware that growing different varieties of a crop may attract different pollinators into the field	21. Growers should be interested in trialling new techniques			
				1. I am aware of what mycorrhizal fungi are				

Legend

*	Distinguishing statement at $P < 0.05$
**	Distinguishing statement at $P < 0.01$
↑	z-Score for the statement is higher than in all of the other factors
↓	z-Score for the statement is lower than in all of the other factors

Figure 5.5: Composite q-sort for Factor 2, illustrating a summary of opinions for respondents within this factor. Statements that distinguish this factor from others are indicated as significant or highly significant and arrows indicate if these statements were significantly higher or lower than other factors.

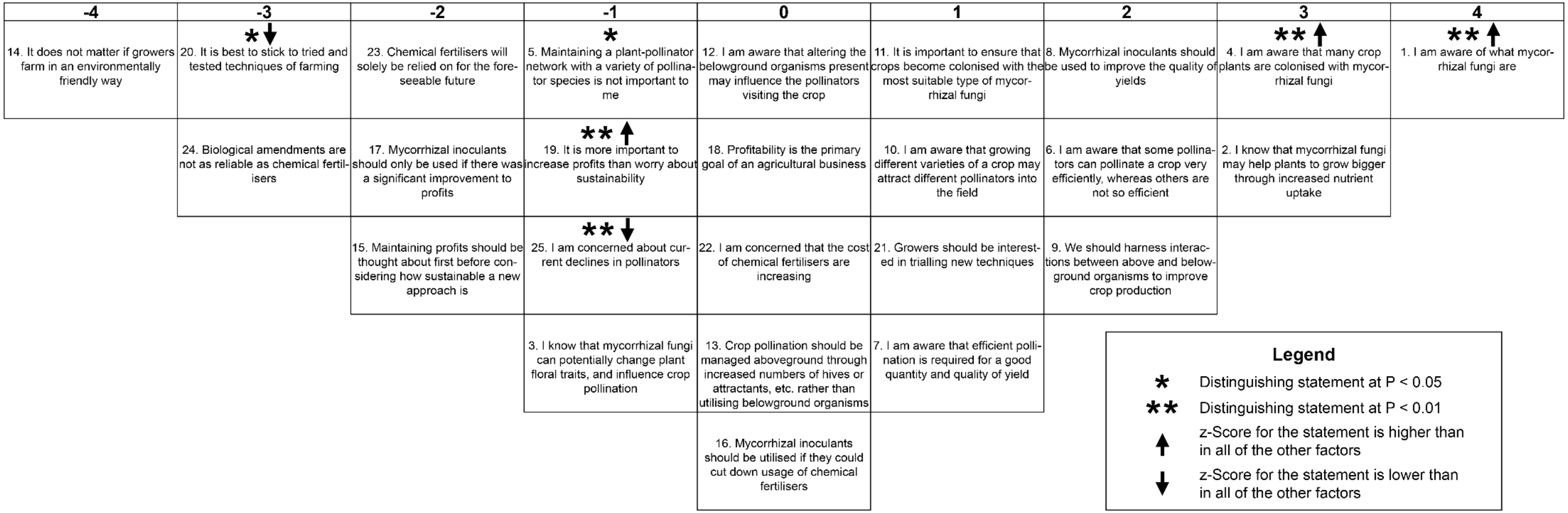
5.3.5 Factor 3 – 'Knowledgeable Growers'

Factor three contained six distinguishing statements ($P < 0.05$), with five of these highly significantly different ($P < 0.01$) (Table 5.8, Figure 5.6). This factor contained five respondents, all of which were UK fruit growers, whose opinions correlated between 56-95% ($\bar{x}$ = 74.90% ± 2.71).

Respondents in this group were separated from other factors by strongly agreeing (+4) that they were aware of mycorrhizal fungi (statement 1), and agreed (+3) that they colonise a wide range of crop plants (statement 4). These respondents had mixed viewpoints on pollinators, and were the only factor that showed any disagreement (slight disagreement (-1)) that they are concerned over current pollinator declines (statement 25), but did slightly disagree (-1) that maintaining a diverse plant-pollinator community was not important (statement 5). Out of all the factors, they also disagreed (-3) most strongly that it is best to stick with tried and tested methods of farming (statement 20), but only slightly disagreed (-1) that profits were more important than sustainability (statement 19), indicating that although they were willing to make changes, their intrinsic desire for change was weaker than factor 1. Due to showing a significantly higher level of knowledge of the subjects of the survey, this factor is termed 'knowledgeable growers'.

Table 5.8: Distinguishing statements – Factor 3: statements in which separated opinions between respondents in Factor 3 and other factors. Composite sort values show the typical score for a statement from respondents within a factor, and z-scores are a weighted average of the values that all the respondents within a factor give to a statement. Statements from Factor 3 are highlighted in bold.

			Factor 1		**Factor 2**		**Factor 3**	
Statement Number	**Statement**	**Significance**	**Composite Q-sort Value**	**Z-score**	**Composite Q-sort Value**	**Z-score**	**Composite Q-sort Value**	**Z-score**
1	I am aware of what mycorrhizal fungi are	P < 0.01	1	0.45	0	0.16	**4**	1.67
4	I am aware that many crop plants are colonised with mycorrhizal fungi	P < 0.01	0	0.34	0	0.00	**3**	1.22
25	I am concerned about current declines in pollinators	P < 0.01	3	1.48	2	1.00	**-1**	-0.29
19	It is more important to increase profits than worry about sustainability	P < 0.01	-3	-1.51	-3	-1.66	**-1**	-0.66
5	Maintaining a plant-pollinator network with a variety of pollinator species is not important to me	P < 0.05	-3	-1.32	1	0.34	**-1**	-0.70
20	It is best to stick to tried and tested techniques of farming	P < 0.01	-1	-0.93	1	0.38	**-3**	-1.73



-4	-3	-2	-1	0	1	2	3	4
14. It does not matter if growers farm in an environmentally friendly way	* ↓ 20. It is best to stick to tried and tested techniques of farming	23. Chemical fertilisers will solely be relied on for the foreseeable future	* 5. Maintaining a plant-pollinator network with a variety of pollinator species is not important to me	12. I am aware that altering the belowground organisms present may influence the pollinators visiting the crop	11. It is important to ensure that crops become colonised with the most suitable type of mycorrhizal fungi	8. Mycorrhizal inoculants should be used to improve the quality of yields	** ↑ 4. I am aware that many crop plants are colonised with mycorrhizal fungi	** ↑ 1. I am aware of what mycorrhizal fungi are
	24. Biological amendments are not as reliable as chemical fertilisers	17. Mycorrhizal inoculants should only be used if there was a significant improvement to profits	** ↑ 19. It is more important to increase profits than worry about sustainability	18. Profitability is the primary goal of an agricultural business	10. I am aware that growing different varieties of a crop may attract different pollinators into the field	6. I am aware that some pollinators can pollinate a crop very efficiently, whereas others are not so efficient	2. I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake	
		15. Maintaining profits should be thought about first before considering how sustainable a new approach is	** ↓ 25. I am concerned about current declines in pollinators	22. I am concerned that the cost of chemical fertilisers are increasing	21. Growers should be interested in trialling new techniques	9. We should harness interactions between above and belowground organisms to improve crop production		
			3. I know that mycorrhizal fungi can potentially change plant floral traits, and influence crop pollination	13. Crop pollination should be managed aboveground through increased numbers of hives or attractants, etc. rather than utilising belowground organisms	7. I am aware that efficient pollination is required for a good quantity and quality of yield			
				16. Mycorrhizal inoculants should be utilised if they could cut down usage of chemical fertilisers				

Legend

*	Distinguishing statement at P < 0.05
**	Distinguishing statement at P < 0.01
↑	z-Score for the statement is higher than in all of the other factors
↓	z-Score for the statement is lower than in all of the other factors

Figure 5.6: Composite q-sort for Factor 3, illustrating a summary of opinions for respondents within this factor. Statements that distinguish this factor from others are indicated as significant or highly significant and arrows indicate if these statements were significantly higher or lower than other factors. Distinguishing statements without arrows fall at a score between the scores in the two other factors.

Although multiple factors may show agreement or disagreement with individual statements, the degree to which they agree or disagree, may differ, and as such overall opinions may be shared across factors, but within a different degree of the strength of the opinion/rank provided (Table S5.3). Four statements (7, 8, 22, and 24) were neither significantly distinguishing or consensus statements but had similar trends in agreement / disagreement. All factors believed that biological amendments can be as reliable as chemical fertilisers (statement 24), opinions ranged from neutral to moderate agreement that mycorrhizal inoculants should be used to improve yield quality (statement 8), had neutral opinions to moderate disagreement that they were concerned about the cost of fertilisers increasing, and were aware that efficient pollination is required for good yields (statement 7) (Table S5.3, Figure S5.3).

5.3.6 Similarity of factor viewpoints

Factor 3 was the only factor that showed strong agreement that they were aware of mycorrhizal fungi (**S1**), and that most crops are colonised by mycorrhizal fungi (**S4**) whereas factors 1 and 2 had neutral to slight knowledge on both statements. Both factors 1 and 3 agreed that they knew that mycorrhizal fungi improve nutrient uptake, while factor 2 disagreed (S2). Factor 1 showed some agreement that they knew that mycorrhizal fungi could change plant reproduction, but factors 2 and 3 were not aware of this (**S3**).

All factors had knowledge that efficient pollination is required for good yields (though to different degrees) (S7), however other knowledge about pollinators varied. Factors 1 and 3 agreed that they knew that some pollinators are more efficient than others, but factor 2 had a neutral view (**S6**). There was neutral to slight agreement that different crop varieties attract different pollinators (*S10*), and there was neutral to some disagreement on knowledge that belowground organisms can influence pollinators (**S12**). Although factors 1 and 2 had concerns over pollinator declines, factor 3 disagreed (**S25**).

Respondents had a range of different motivations for the use of AMF. First, no factors were concerned about rises in fertiliser costs (S22). However, all factors agreed that chemical fertilisers will not be solely relied on in the future (**S23**), and growers should farm in environmentally friendly ways (*S14*). Factors 1 and 3 felt that the wider remit of harnessing interactions between above and belowground organisms should be used to improve crop production, whereas factor 2 disagreed.

Factor 1 agreed that mycorrhizal fungi should be used to reduce the need for chemical fertilisation (**S16**). Coupled with their belief that growers should farm in an environmentally friendly way (*S14*), and their desire to try new techniques (**S21**), and disagreement that profitability is the main goal of agriculture (**S17**), or that mycorrhizal inoculants should only be used if profits were increased (**S17**) shows that intrinsic desires, more than monetary rewards motivate respondents in this factor.

Conversely, Factor 2 felt that mycorrhizal fungi should only be used if there was a significant improvement in profits (**S17**), and that profitability is the main goal of an agricultural business (**S18**). Although they did agree that growers should farm in an environmentally friendly way (*S14*), this is not as important to them as profits, and as such are motivated by financial rewards.

AMF can improve crop quality (Kapoor *et al.*, 2002; Kapoor *et al.*, 2004; Castellanos-Morales *et al.*, 2010; Baslam *et al.*, 2011a; Baslam *et al.*, 2011b; Hart *et al.*, 2014), and although factor 3 did not favour utilising mycorrhizal fungi to reduce fertiliser use (**S16**), they were interested in using AMF to improve yield quality (S8). Although farming in an environmentally friendly way was important to them (*S14*), these respondents had a neutral opinion on the relative importance of sustainability and profitability (**S18**), and showed less concern over considering sustainability over increasing profits (**S19**), indicating that their motivation for utilising AMF to improve yield quality is for financial reasons.

Improving plant-pollinator interactions has the potential to increase grower's profits, and AMF are able to alter these interactions, however, respondents showed varying degrees of motivation regarding pollinators. Factor 3 was not concerned about declines in pollinators, whereas both factors 1 and 2 indicated that they were concerned (**S25**) (Figure S5.3). Factors 1 and 3 felt that maintaining pollinator diversity was important to them, however factor 2 disagreed.

5.4 Discussion

Although we had a small sample size compared to empirical studies in ecology, by utilising Q-methodology we are able to determine sets of viewpoints are present in the strawberry production industry on the introduction of AMF as a novel biotechnology. Here we have shown for the first time significant interest in introducing mycorrhizal fungi in soft fruit production, however we found three distinct viewpoints, and found levels of knowledge, motivations, and perceptions varied across the groups. In addition, the views of experts only aligned with two out of the three factors.

5.4.1 Stakeholder knowledge on AMF and pollinators

Despite growing interest and coverage in the media on arbuscular mycorrhizal fungi (BBC, 2012; Fleming, 2014; Macfarlane, 2016; Erizanu, 2017), both factors 1 and 2 did not indicate that they had substantial knowledge about arbuscular mycorrhizal fungi, or which plants AMF colonise, while respondents in factor 3 showed a significant degree of knowledge on both of these statements. Despite this belief, most respondents (factors 1 and 3) could knowledgeably answer questions regarding specific aspects of mycorrhizal fungi and indicated that they were aware that these fungi could benefit plant growth through improved nutrition.

Although media attention on arbuscular mycorrhizal fungi has increased, the level at which it features is still relatively low, and may not have been received by the respondents. However, there has been substantially more coverage of pollinators in the face of concerns over current declines on all major news networks in the UK and US (Ansari, 2016; Garnier, 2017; Morelle, 2017; Senkul, 2017), and articles have featured in specialist publications in the agricultural industry (Davies, 2016; Davidson, 2017). The knowledge gap hypothesis proposes that with increasing media attention, knowledge on a topic also increases (Tichenor *et al.*, 1970), and those with a higher socio-economic status (measured by education level) will tend to have a higher level of knowledge of a subject when featured in the media (Hwang and Jeong, 2009). In this study 18 of the 20 respondents in this study had a university education, however the only subject on which any respondents indicated substantial knowledge of pollinators was that efficient pollination is required for good yields. Different pollinator taxa have been shown to exhibit distinct levels of efficiencies in crop systems (Canto-Aguilar and Parra-Tabla, 2000;

Jauker *et al.*, 2012), but all factors were not confident that they were aware of this, and had similar neutral to slight agreement that they were aware that crop varieties influence the pollinators that visit, indicating that there is a need to disseminate more information on plant-pollinator interactions to growers.

This information is available in academia, for example Barber and Soper Gorden (2014) reviewed evidence illustrating that belowground organisms are able to modulate plant reproductive traits, and that pollinator behaviour responds to these changes. However, we found that only factor 1 showed slight knowledge that mycorrhizal fungi are able to influence plant floral traits, and no factor had knowledge that belowground organisms can influence pollinators, and as such more work needs to be done to disseminate this field of research from academia to growers. If AMF are able to help growers through improving both plant nutrition, and pollination, research in this field by experts needs to be shared with growers for this biotechnology to be implemented effectively.

In issues related to sustainability in particular, the level of knowledge one has can determine how likely they are to adopt a new technology, with those with higher knowledge on the subject more likely to adopt new technologies (Scott, 1997; Mills and Schleich, 2012). There is, however, a difference between knowledge (informal personal experience) of a topic and education (a formal process) on a topic, and basic knowledge of what opportunities exist to save money and improve sustainability are not as effective as education on the subject (Mills and Schleich, 2012). As such, simply advertising that a product is available and its potential benefits will not be as effective as building a foundation of education on the subject.

If the adoption of novel technologies can be improved through disseminating research, the most appropriate techniques must be employed. There are multiple methods of disseminating scientific research and increasing education, with varying levels of effectiveness. For example, some studies have found information campaigns to be successful (Reiss and White, 2008), whereas the OECD finds these campaigns to not be as effective as expected (Ferrara and Serret, 2008). Mass marketed information is often not as valuable as directly targeted information schemes (Lutzenhiser, 1993), and as this biotechnology relies on relatively specialised subjects to a specific audience, a direct connection of experts to growers through industry events may prove most efficient. This direct connection need not always be in person, and utilising modern technology could help to improve education on AMF, pollinators, and sustainability.

Novel methods of disseminating information to growers have been developed, such as using mobile applications for smartphones to improve grower knowledge. A wide range of applications have been developed, and often suggest the use of specific products, however these typically focus on weeds, pests, pathogens, planting, and crop varieties (De Silva *et al.*, 2013a; Bayer CropScience UK, 2016; PEAT, 2017; Studio Noframe, 2017). Although applications do exist specifically related to fertilisers (Kverneland Group, 2016; Ag PhD, 2017), these contain application advice for existing chemical products, and do not provide information on alternatives. Similar applications could be developed to focus on emerging technologies such as AMF, providing relevant information and sharing the findings of research, as well as promoting the use of AMF as a new agricultural product. In order for education to be increased, we must understand the current perception of growers on utilising AMF.

5.4.2 Perceptions on and motivations for the use of AMF in strawberry production

Understanding the perceptions and motivations of stakeholders is vital to the success of introducing a new technology in agriculture. 37% percent of the globe is devoted to agricultural production (World Bank, 2017), and land managers play a large role in determining the sustainability of these production systems, and ecosystem conservation. Studies have shown that introducing new agricultural techniques often fails if the perceptions and motivations of the end users are not understood (Newmark and Hough, 2000; Oba *et al.*, 2000; Quinn *et al.*, 2003). For example, development projects to improve livelihoods in rural agricultural communities in Africa have often failed as they did not recognise the perceptions or needs of these communities, but rather provided solutions did not solve the end users most important problem, and were not retained after the end of the project (Newmark and Hough, 2000; Oba *et al.*, 2000; Quinn *et al.*, 2003). In situations where there are multiple problems that can solved, it is vital to design a solution that addresses the most relevant problem to the end users.

AMF are able to improve fertiliser use efficiency, which can improve profit margins and provide environmental benefits, as well as improving pollination. Factors in this study had differing motivations for the use of AMF. Factor 1 was driven by an intrinsic desire to improve sustainability, whereas factors 2 and 3 showed motivations driven by financial rewards. Specific motivations ranged from reducing fertiliser use, to improving yields, and increasing profits, indicating that motivations include both intrinsic motivations and external financial rewards (see section 5.3.6).

Pereira *et al.* (2016) illustrated that perceptions amongst Brazilian beef farmers that cattle farming and conservation could exist simultaneously were present across multiple factors of farmers. Similarly, our results show that in the soft fruit industry, although Factor 2 was particularly profit driven, respondents from all factors favoured farming sustainably to some degree, agreed (to different degrees) that sustainability is more important than profits, and that chemical fertiliser will not be the sole source of plant nutrition in the foreseeable future. Although 'fertilisers' were not specifically stated, Davies and Hodge (2007) found that 10 years ago arable farmers in the UK from all factors had neutral to agreeable views that chemical use is vital and will probably always be so, but that resources should be used as sparingly as possible, and disagreed that pollution caused by agricultural chemicals is insignificant compared to their benefits. Statements in our study had a more specific focus on chemical fertilisers, and we found similar views on the over the use of resources and the relationship between profits and sustainability, but the respondents in our study did not believe that agrochemicals are the only option for the foreseeable future. Davies and Hodge looked at a broader range of arable farmers, and this difference in opinion may result from either the specific views in the strawberry production industry, or a change in opinions over time.

Parkins *et al.* (2015) found that respondents felt ingenuity and innovation will add sustainability to the energy sector, and here we show similar viewpoints in soft fruit production. For example, two viewpoints (factors 1 and 3) thought first about sustainability before profits, that growers should trial new techniques, and new farming techniques should be explored. However, if new technologies are to be implemented, it is vital to understand how well the viewpoints of the experts developing the technology align with the views of growers.

5.4.3 Do growers and experts share the same viewpoints?

Building linkages between growers and experts can prove to be vital in the innovation of and adoption of new technologies. For example, in developing new innovations for water use, growers who participated in hands on workshops that encouraged them to try their own experimentation tended to adopt and retain the new technology, and those who experimented on their own farms developed better production systems overall due to decisions based on trial and error observations (De Silva *et al.*, 2013b). In addition, these direct interactions between experts and growers help experts to recognise and understand the views of growers, so that that they are working towards a common goal, as well as providing insight into which efforts from the research community are effective and have impact for growers.

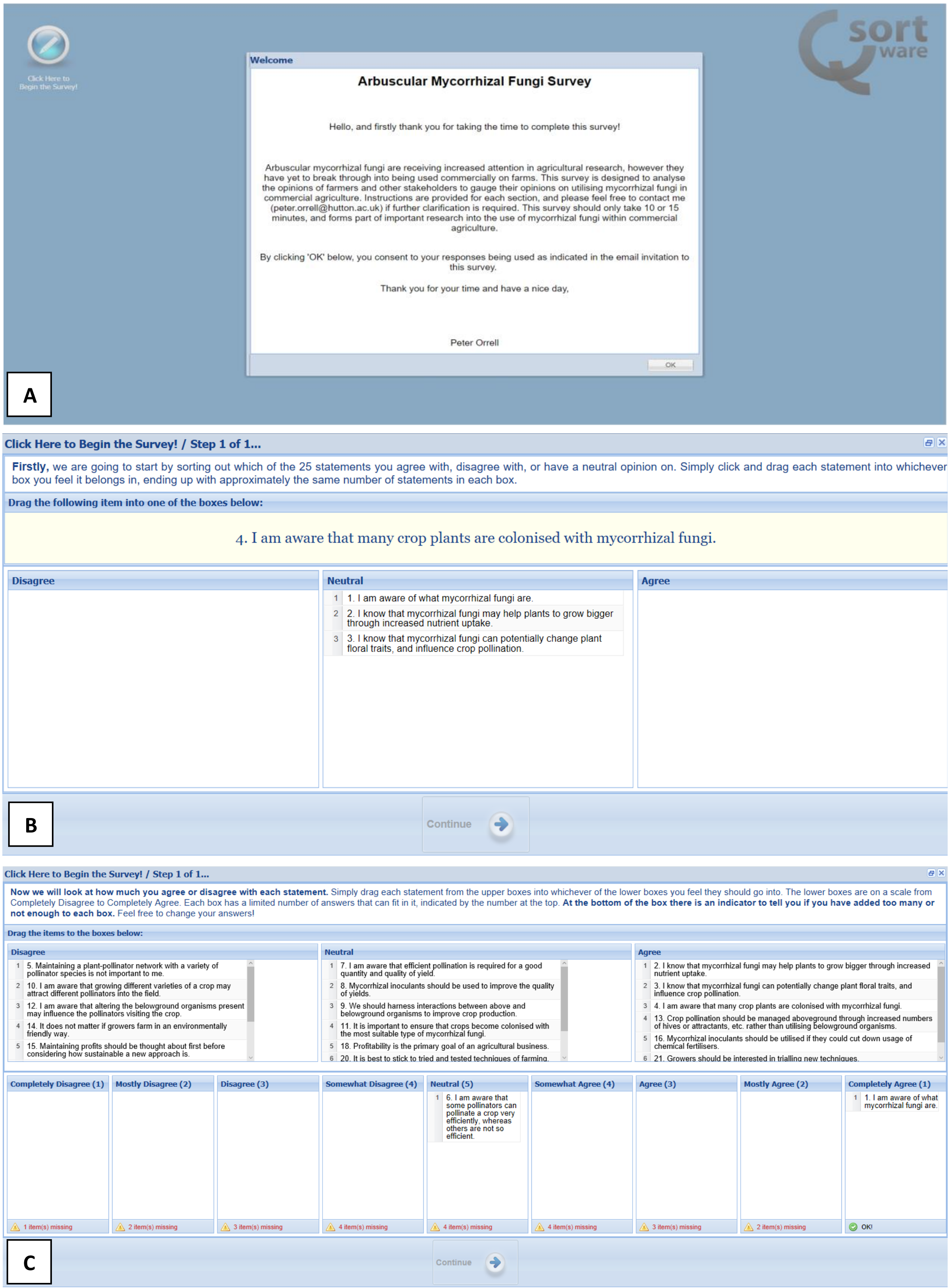
The experts in this study shared both intrinsic and financial viewpoints with growers, but their views aligned only with factor 1 and 2, with none loading into factor 3. Although we had a low sample size of experts, these experts did not have a completely different viewpoint (which would have formed their own factor), but only shared two out of the three viewpoints expressed by respondents. Holding a different viewpoint to end users often leads to situations where solutions are developed that do not match the needs of the end users, and as such fail (Newmark and Hough, 2000; Oba *et al.*, 2000; Quinn *et al.*, 2003).

Experts are key in developing new technologies, and multiple forms of discourse analysis on perceptions of wind farms have found that the relationships between experts and laypersons play a key role in determining if new wind farm proposals are accepted (Ellis *et al.*, 2007). The roles of experts can be particularly important, for example, a previous study utilising Q-methodology in natural resource management found that one factor specifically valued the opinions of experts and considered them to be key facilitators and catalysts (Gruber, 2011). Social analyses such as these can provide vital information to experts about the views of growers, enabling them to form trusting relationships. Trust of institutions and grower's attitudes towards them have been shown to be key in disseminating knowledge and in turn adopting new techniques (Meinzen-Dick *et al.*, 2003). Sharing visions and viewpoints have been shown to play an important role in knowledge transfer, both within and between organisations (Li, 2005), and as such experts who share the same viewpoints as growers will be more successful in sharing key information that leads to the successful adoption of a new technology.

5.5 Conclusion

The successful uptake of improvements to agricultural production systems are ultimately decided by end users and their motivations, however to date studies have not explored the perceptions of stakeholders on the introduction of new techniques in agricultural production systems. Here we have explored for the first time the value that stakeholders place on the ecosystem services provided by AMF and wild pollinators. We measured the viewpoints of key stakeholders on the introduction of a novel biotechnology in strawberry production, as well as the value they place on wild pollinators, and found three predominant viewpoints. The 'progressive thinkers' in factor 1 were interested in testing new techniques, would use AMF inoculants to reduce fertiliser use, and were intrinsically motivated, and felt that maintaining a diverse pollinator community was important. Similarly, the 'knowledgeable growers' in factor 3 disagreed with sticking to tried and tested methods of farming, and had a relatively high degree of knowledge on AMF, but showed little concern over sustainability and pollinators, indicating less intrinsic interest. Factor 2, the 'profit centred traditionalists' felt that profits were the primary goal of an agricultural business, which should be considered before sustainability, and AMF inoculants should be used if profits could be improved, indicating a strong desire for financial rewards, and were motivated by extrinsic rewards. Factors 1 and 3 were well correlated, and as such our results illustrate a predominantly progressive view amongst growers who are interested in trialling new techniques, care about the sustainability of their production systems, and have a degree of knowledge of about the potential of this biotechnology. In addition, we found that only factor 1 showed that they strongly valued wild pollinators. All experts we surveyed shared these same viewpoints and loaded into the same factors. However, we found that more dissemination of research on AMF and pollinators from academia is required to increase grower knowledge before AMF can be introduced as a biotechnology. For this dissemination to be effective, experts must understand or share the viewpoints of growers to develop trust, and we found that the views of experts aligned with two of the three perspectives amongst growers. The key findings from our research (i.e. the heterogeneity of attitudes) could be helpful in understanding the uptake of new technologies, as well as in the design of policy and practice related measures, targeting projects more effectively, and formulating plans and decisions on the introduction of AMF as a novel biotechnology in the production of soft fruit. Utilising new technologies such as beneficial microbes presents a new horizon in the improvement of the sustainable intensification of agricultural systems, however, as we have demonstrated, motivations differ between end users, and understanding the complex viewpoints of key stakeholders in the industry presents an opportunity to introduce these new applications in the most effective manner. As interest in AMF in the agricultural community grows and new products are developed, further studies utilising Q-methodology can be used to assess the effectiveness of education campaigns and changes in opinions, therefore identifying opportunities and challenges of the uptake of novel biotechnology in the production of soft fruits.

5.6 Supplementary Information



Arbuscular Mycorrhizal Fungi Survey

Hello, and firstly thank you for taking the time to complete this survey!

Arbuscular mycorrhizal fungi are receiving increased attention in agricultural research, however they have yet to break through into being used commercially on farms. This survey is designed to analyse the opinions of farmers and other stakeholders to gauge their opinions on utilising mycorrhizal fungi in commercial agriculture. Instructions are provided for each section, and please feel free to contact me (peter.orrell@hutton.ac.uk) if further clarification is required. This survey should only take 10 or 15 minutes, and forms part of important research into the use of mycorrhizal fungi within commercial agriculture.

By clicking 'OK' below, you consent to your responses being used as indicated in the email invitation to this survey.

Thank you for your time and have a nice day,

Peter Orrell

Firstly, we are going to start by sorting out which of the 25 statements you agree with, disagree with, or have a neutral opinion on. Simply click and drag each statement into whichever box you feel it belongs in, ending up with approximately the same number of statements in each box.

4. I am aware that many crop plants are colonised with mycorrhizal fungi.

Neutral:

1 1. I am aware of what mycorrhizal fungi are.
2 2. I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake.
3 3. I know that mycorrhizal fungi can potentially change plant floral traits, and influence crop pollination.

Now we will look at how much you agree or disagree with each statement. Simply drag each statement from the upper boxes into whichever of the lower boxes you feel they should go into. The lower boxes are on a scale from Completely Disagree to Completely Agree. Each box has a limited number of answers that can fit in it, indicated by the number at the top. **At the bottom of the box there is an indicator to tell you if you have added too many or not enough to each box.** Feel free to change your answers!

Disagree:

1 5. Maintaining a plant-pollinator network with a variety of pollinator species is not important to me.
2 10. I am aware that growing different varieties of a crop may attract different pollinators into the field.
3 12. I am aware that altering the belowground organisms present may influence the pollinators visiting the crop.
4 14. It does not matter if growers farm in an environmentally friendly way.
5 15. Maintaining profits should be thought about first before considering how sustainable a new approach is.

Neutral:

1 7. I am aware that efficient pollination is required for a good quantity and quality of yield.
2 8. Mycorrhizal inoculants should be used to improve the quality of yields.
3 9. We should harness interactions between above and belowground organisms to improve crop production.
4 11. It is important to ensure that crops become colonised with the most suitable type of mycorrhizal fungi.
5 18. Profitability is the primary goal of an agricultural business.
6 20. It is best to stick to tried and tested techniques of farming.

Agree:

1 2. I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake.
2 3. I know that mycorrhizal fungi can potentially change plant floral traits, and influence crop pollination.
3 4. I am aware that many crop plants are colonised with mycorrhizal fungi.
4 13. Crop pollination should be managed aboveground through increased numbers of hives or attractants, etc. rather than utilising belowground organisms.
5 16. Mycorrhizal inoculants should be utilised if they could cut down usage of chemical fertilisers.
6 21. Growers should be interested in trialling new techniques.

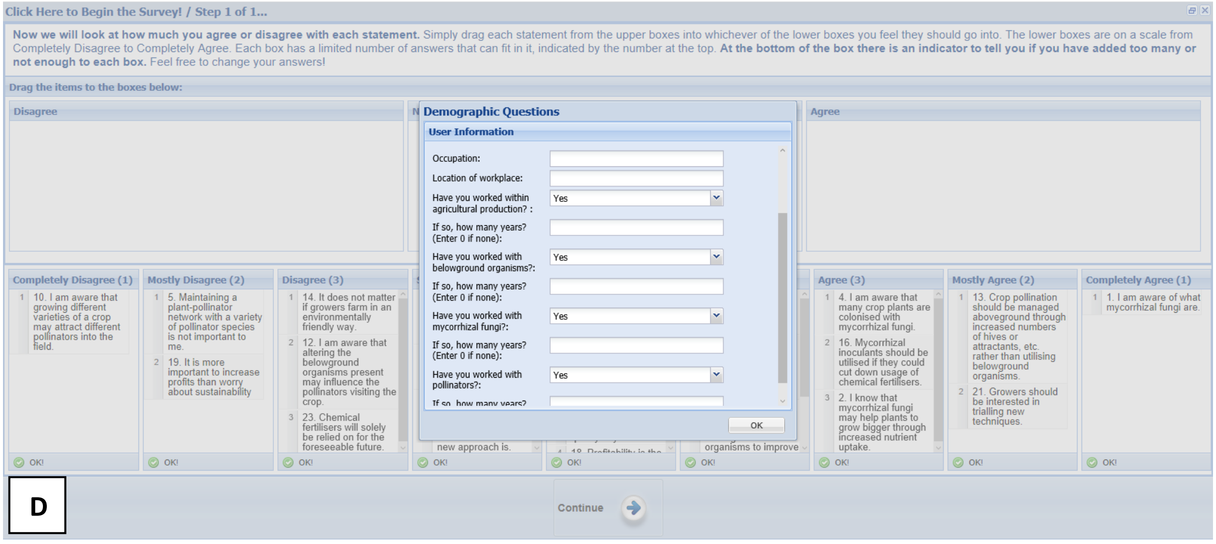
Neutral (5):

1 6. I am aware that some pollinators can pollinate a crop very efficiently, whereas others are not so efficient.

Completely Agree (1):

1 1. I am aware of what mycorrhizal fungi are.

Figure S5.1: Screenshots of the online survey illustrating A) the main intro splash screen, B) the initial sort, in which respondents place statements into the broad categories of agree/neutral/disagree, and C) the main sort, where statements are moved from the broad categories into specific groups ranging from 'completely disagree' to 'completely agree'.



Click Here to Begin the Survey! / Step 1 of 1...

Now we will look at how much you agree or disagree with each statement. Simply drag each statement from the upper boxes into whichever of the lower boxes you feel they should go into. The lower boxes are on a scale from Completely Disagree to Completely Agree. Each box has a limited number of answers that can fit in it, indicated by the number at the top. **At the bottom of the box there is an indicator to tell you if you have added too many or not enough to each box.** Feel free to change your answers!

Drag the items to the boxes below:

Demographic Questions

User Information

Occupation:
Location of workplace:
Have you worked within agricultural production? : Yes
If so, how many years? (Enter 0 if none):
Have you worked with belowground organisms?: Yes
If so, how many years? (Enter 0 if none):
Have you worked with mycorrhizal fungi?: Yes
If so, how many years? (Enter 0 if none):
Have you worked with pollinators?: Yes

Completely Disagree (1)
1. 10. I am aware that growing different varieties of a crop may attract different pollinators into the field.

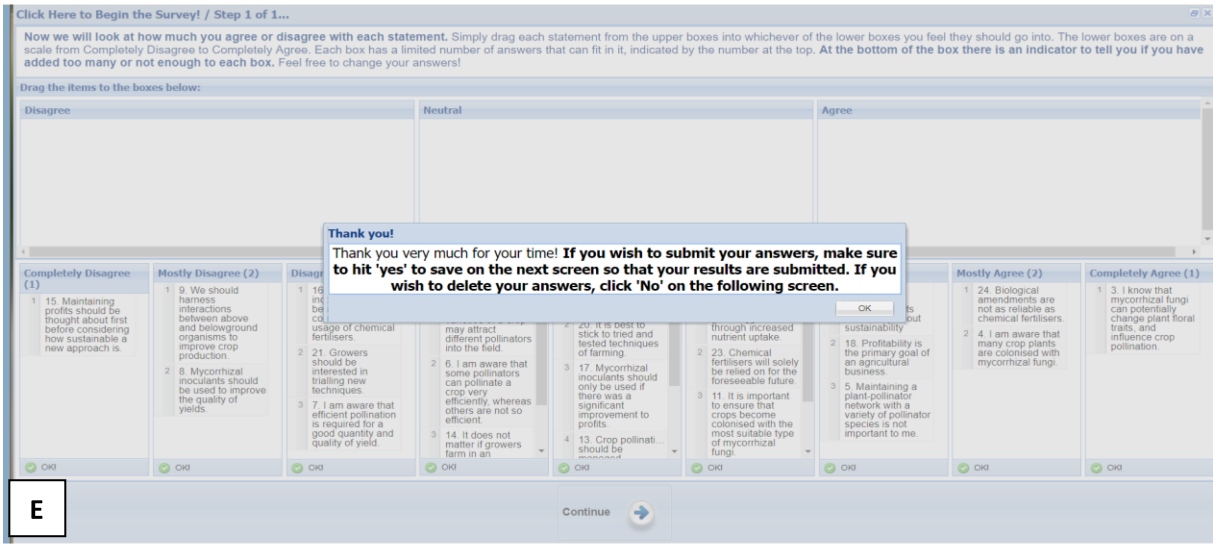
Mostly Disagree (2)
1. 5. Maintaining a plant-pollinator network with a variety of pollinator species is not important to me.
2. 19. It is more important to increase profits than worry about sustainability

Disagree (3)
1. 14. It does not matter if growers farm in an environmentally friendly way.
2. 12. I am aware that altering the belowground organisms present may influence the pollinators visiting the crop.
3. 23. Chemical fertilisers will solely be relied on for the foreseeable future.

Agree (3)
1. 4. I am aware that many crop plants are colonised with mycorrhizal fungi.
2. 16. Mycorrhizal inoculants should be utilised if they could cut down usage of chemical fertilisers.
3. 2. I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake.

Mostly Agree (2)
1. 13. Crop pollination should be managed aboveground through increased numbers of hives or attractants, etc. rather than utilising belowground organisms.
2. 21. Growers should be interested in trialling new techniques.

Completely Agree (1)
1. 1. I am aware of what mycorrhizal fungi are.



Click Here to Begin the Survey! / Step 1 of 1...

Now we will look at how much you agree or disagree with each statement. Simply drag each statement from the upper boxes into whichever of the lower boxes you feel they should go into. The lower boxes are on a scale from Completely Disagree to Completely Agree. Each box has a limited number of answers that can fit in it, indicated by the number at the top. **At the bottom of the box there is an indicator to tell you if you have added too many or not enough to each box.** Feel free to change your answers!

Drag the items to the boxes below:

Thank you!

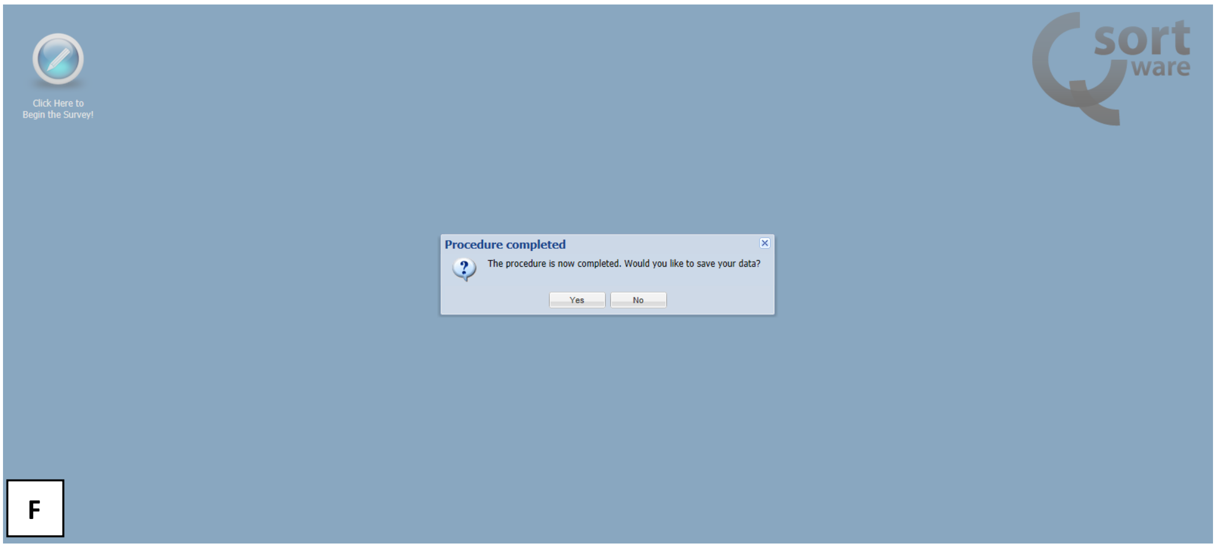
Thank you very much for your time! **If you wish to submit your answers, make sure to hit 'yes' to save on the next screen so that your results are submitted. If you wish to delete your answers, click 'No' on the following screen.**

Completely Disagree (1)
1. 15. Maintaining profits should be thought about first before considering how sustainable a new approach is.

Mostly Disagree (2)
1. 9. We should harness interactions between above and belowground organisms to improve crop production.
2. 8. Mycorrhizal inoculants should be used to improve the quality of yields.

Mostly Agree (2)
1. 24. Biological amendments are not as reliable as chemical fertilisers.
2. 4. I am aware that many crop plants are colonised with mycorrhizal fungi.

Completely Agree (1)
1. 3. I know that mycorrhizal fungi can potentially change plant floral traits, and influence crop pollination.



Procedure completed

The procedure is now completed. Would you like to save your data?

Figure S5.1 (continued): Screen shots of the online survey illustrating D) collection of demographic information, E) thank you and reminder to save the results, and F) final save screen.

Table S5.1: Number of respondents in each group and

Code	Respondent Group	n
UKFRGR	UK Fruit Grower	11
USFRGR	US Fruit Grower	5
UKEX	UK Expert	3
USEX	US Expert	1

Table S5.2: Heterogeneity of stakeholder attitudes, factor loadings, and factor groupings. The variance explained by each factor when restricted to three factors is indicated below each factor.

Respondent	Factor Group	Factor 1	Factor 2	Factor 3
UKEX1	F2-1	0.0485	0.9724 *	0.0232
UKEX2	F1-9	0.695 *	0.0242	0.5661
UKEX3	F1-7	0.7635 *	0.1306	0.3813
USEX1	F1-8	0.7318 *	-0.1914	0.4062
UKFRGR1	F1-2	0.891 *	0.0995	0.2826
UKFRGR2	F3-3	0.5333	-0.1349	0.6722 *
UKFRGR3	None	0.4772	0.4565	-0.4535
UKFRGR4	F1-11	0.6217 *	-0.0431	0.597
UKFRGR5	F3-4	0.5887	-0.1311	0.6696 *
UKFRGR6	F3-5	0.4806	0.078	0.6425 *
UKFRGR7	F3-1	0.3692	0.0534	0.8803 *
UKFRGR8	F3-2	0.412	0.0151	0.8263 *
UKFRGR9	F2-2	0.0196	0.9623 *	0.0104
UKFRGR10	F1-10	0.6751 *	0.4083	0.3592
UKFRGR11	F1-5	0.7913 *	0.2078	0.2985
USFRGR1	F1-3	0.8573 *	-0.032	0.2802
USFRGR2	F1-6	0.7646 *	0.3432	0.4229
USFRGR3	F1-4	0.8008 *	0.0228	0.2948
USFRGR4	F2-3	0.0561	0.9531 *	-0.0602
USFRGR5	F1-1	0.8914 *	0.0875	0.2911
Percent of Explained Variance		40%	17%	23%

Table S5.3: Ranks and composite scores for statements within each factor. Ranks indicate where the average response fell for each factor, on a scale from 1 ('strongly agree') to 25 ('strongly disagree'), and are used to construct a composite q-sort for each factor. Composite scores indicate the position of the statement on the composite Q-sort for each factor.

Statement Number	Statement	Factor 1		Factor 2		Factor 3	
		Rank	Score	Rank	Score	Rank	Score
1	I am aware of what mycorrhizal fungi are.	10	1	11	0	1	4
2	I know that mycorrhizal fungi may help plants to grow bigger through increased nutrient uptake.	4	2	19	-1	2	3
3	I know that mycorrhizal fungi can potentially change plant floral traits, and influence crop pollination.	9	1	20	-2	16	-1
4	I am aware that many crop plants are colonised with mycorrhizal fungi.	11	0	14	0	3	3
5	Maintaining a plant-pollinator network with a variety of pollinator species is not important to me.	23	-3	10	1	19	-1
6	I am aware that some pollinators can pollinate a crop very efficiently, whereas others are not so efficient.	7	1	15	0	5	2
7	I am aware that efficient pollination is required for a good quantity and quality of yield.	1	4	3	3	8	1
8	Mycorrhizal inoculants should be used to improve the quality of yields.	12	0	9	1	6	2
9	We should harness interactions between above and belowground organisms to improve crop production.	5	2	23	-3	4	2
10	I am aware that growing different varieties of a crop may attract different pollinators into the field.	8	1	12	0	9	1
11	It is important to ensure that crops become colonised with the most suitable type of mycorrhizal fungi.	13	0	18	-1	10	1
12	I am aware that altering the belowground organisms present may influence the pollinators visiting the crop.	14	0	22	-2	15	0
13	Crop pollination should be managed aboveground through increased numbers of hives or attractants, etc. rather than utilising belowground organisms.	16	-1	6	2	12	0
14	It does not matter if growers farm in an environmentally friendly way.	25	-4	25	-4	25	-4
15	Maintaining profits should be thought about first before considering how sustainable a new approach is.	22	-2	1	4	20	-2
16	Mycorrhizal inoculants should be utilised if they could cut down usage of chemical fertilisers.	6	2	13	0	11	0
17	Mycorrhizal inoculants should only be used if there was a significant improvement to profits.	18	-1	2	3	21	-2
18	Profitability is the primary goal of an agricultural business.	17	-1	4	2	14	0
19	It is more important to increase profits than worry about sustainability	24	-3	24	-3	18	-1
20	It is best to stick to tried and tested techniques of farming.	19	-1	8	1	24	-3
21	Growers should be interested in trialling new techniques.	3	3	7	1	7	1
22	I am concerned that the cost of chemical fertilisers are increasing.	15	0	21	-2	13	0
23	Chemical fertilisers will solely be relied on for the foreseeable future.	21	-2	17	-1	22	-2
24	Biological amendments are not as reliable as chemical fertilisers.	20	-2	16	-1	23	-3
25	I am concerned about current declines in pollinators.	2	3	5	2	17	-1

5.6.1 Demographic information

Table S5.4: Demographic information of each factor. Years of experience in each subject and family history in agriculture are provided for growers only. Age and income means are provided as mid-range averages. Variation is ± standard error.

	Factor 1	Factor 2	Factor 3
Number of Respondents	11	3	5
UK Growers	4	1	5
US Growers	4	1	-
UK Experts	2	1	-
US Experts	1	-	-
Respondent correlation	49 - 95% x̄ = 73.00% (±0.88)	90 - 95% x̄ = 93.00% (±0.97)	56-95% x̄ = 74.90% (±2.71)
Age range (years)	40 - 70, x̄ = 50-60	20 - 59, x̄ = 40-49	30 - 70, x̄ = 40-49
Gender	55% Male, 45% Female	100% Male	100% Male
Educational experience	Secondary school - Postgraduate Degrees	Undergraduate - Master's	Undergraduate - Master's
Income range	<£14,999 - 74,999, x̄ = £25,000-34,999	<£14,999 - 34,999, x̄ = £15,000-24,999	£15,000 - >85,000, x̄ = £35,000-44,999
Married	90%	100%	100%
Have children	64%	100%	80%
Years Farming	4 - 50, x̄ = 26 (±5.85)	15 - 30, x̄ = 22.5	2.5 - 38, x̄ = 23.9 (± 5.70)
Experience with mycorrhizal fungi (years)	(n=2), 0 - 50, x̄ = 7.50 (±6.20)	(n=0), 0 x̄ = 0	(n=2), 0 - 2 x̄ = 0.80 (± 0.49)
Experience with pollinators (years)	(n=8), 3 - 50, x̄ = 22.86 (±6.23)	(n=2), 10 - 20 x̄ = 15	(n=5), 1 - 38 x̄ = 20.80 (± 6.15)
Family history in agriculture (generations)	1 - 10, x̄ = 5.13 (±1.41)	4 - 7, x̄ = 5.50	2 - 10, x̄ = 4.20 (± 1.46)
Growers that trade internationally	75%	50%	20%
Growers that have tried alternatives to chemical fertilisers	100%	50%	80%
Growers that had success with alternatives to chemical fertilisers (if attempted)	100%	100%	100%

Within factor 1 group, respondents all fell in the age range of 40-70 years, with an average age of 50-60, did not include any of the three respondents who were under 40, and all five female respondents were loaded into this factor. Growers in this factor had 4-50 years' experience in farming ($\overline{x}$ = 26 ±5.85 years), and all had experience working with pollinators, from 3-50 years ($\overline{x}$ = 22.86 ± 6.23 years). In addition, this factor contained two out of the four growers who had experience working with mycorrhizal fungi (10 and 50 years' experience respectively). Furthermore, all of the growers in this group had previously tried some form of alternative to chemical fertilisers, and all felt that these were successful. Educational experience did not seem to play a role in the demographic distribution of this factor, with educations ranging from secondary school to postgraduate degrees. Similarly, income levels also varied highly, ranging from <£14,999 - 74,999, ($\overline{x}$ = £25,000 – 34,999). Growers in this group all had 1 - 10 previous generations of family farmers, with an average of 5.13 ±1.41 generations. In addition, six of the 8 growers in this factor trade internationally. Three of the four experts surveyed were in this group, indicating that experts typically share the opinions of growers, and did not have a separate set of viewpoints (Table S5.4).

Respondents from factor 2 were all male, between the ages of 20-59 ($\overline{x}$ = 40-49), education levels ranged from undergraduate to master's degrees, income levels were from £14,999 - 34,999 range ($\overline{x}$ = £15,000 – 24,999), and their location varied from Scotland to the US. The two growers had been farming from 15 and 30 years, with the same number of years of experience in working with pollinators, however neither of them had any experience working with mycorrhizal fungi. Both growers had 4 and 7 previous generations of farmers in their families, and were both married with children. One farmer trades internationally, and had successfully tried alternatives to chemical fertilisers, whereas the other grower neither trades internationally, nor had tried alternative fertilisers (Table S5.4).

Respondents in factor 3 had a wide age range of 30-70 ($\overline{x}$ = 40-49), and were all from the UK. They had been farming from from 2.5 to 38 years ($\overline{x}$ = 23.9 ±5.7). Only two of these growers had experience with mycorrhizae, both for two years. All growers had experience working with pollinators, ranging from 1 to 38 years, with an average of 20.80 years ($\overline{x}$ = 20.80 ±6.15). All growers had undergraduate or Master's degree, and income levels ranged from £15,000 - >85,000 ($\overline{x}$ = £35,000 – 44,999). All growers were married, and all but for one had children, and their families had been farming for 2 to 10+ generations ($\overline{x}$ = 4.20 ±1.46). Only one grower trades internationally, and four out of five have previously tried alternatives to chemical fertilisers, with all four of them being successful (Table S5.4).

Chapter 6: General Discussion

In this thesis I utilised an interdisciplinary approach starting in the greenhouse and ending with the consumer, in order to measure the impacts of above- and belowground interactions in agro-ecosystems. I utilised multiple techniques to explore the fundamental biology of these interactions, as well as determining the consequences not only from the perspectives of the organisms studied, but also the perspectives of humans who either consume the crops or those who utilise the ecosystem services I studied. I accomplished this by conducting an experiment in the controlled environment of a greenhouse, before examining interactions in the field, and finally engaged with stakeholders to determine the views of the agricultural industry on above-belowground interactions. Below, I detail a brief overview of our findings.

6.1 Overview of results

6.1.1 Chapter 2: Belowground mutualists and crop cultivars influence pollinator foraging behaviour

In Chapter 2, I measured the influences of multiple entire AMF communities and strawberry cultivars on plant reproductive traits and pollinator visitation by *Bombus terrestris* Audax in the controlled environment of a greenhouse. To determine the effects on plant reproductive traits, I measured floral display (number of flowers produced), and male (pollen production), and female (nectar production) reproductive traits. I found that AMF community influenced flower production, with Community 1 producing more flowers than plants with the commercial inocula. AMF influenced nectar production in the afternoon, but not pollen production. To measure influences on pollinator visitation, I examined the frequency and duration of bumblebee visits overall, and measuring differences in pollinator foraging behaviour by observing if bees were foraging for pollen or nectar. AMF influenced the frequency and duration of pollinator visits overall, as well as for each foraging type. Community 1 had fewer visits than plants with the Commercial Inoculant, and were of a shorter duration for pollen foraging visits, but longer for nectar foraging visits than sterile plants. I predicted that the natural AMF communities would be better adapted to promote plant reproduction than the Commercial Inoculant, however whilst Community 1 had an increased number of flowers, the number and duration of visits per flower were less than the Commercial Inoculant. I predicted that as AMF plant-pollinator interactions vary across plant species, and AMF influences would depend on crop cultivar, however I found that AMF and strawberry cultivar only interacted to influence the number of anthers per flower, and did not interact to influence pollinator visitation or foraging behaviour.

6.1.2 Chapter 3: Mycorrhizal fungi influences strawberry yield with no loss in fruit quality across multiple cultivars

In Chapter 3, I continued the greenhouse experiment to determine the influences of multiple AMF communities on the yield of several crop cultivars. As well as examining the quantity of yield produced by measuring the number and weight of fruits, I also determined the consequences of these interactions from a human perspective by conducting a taste test. I found that AMF community influenced the number of fruits and total production per plant, with Community 1 producing more fruits and a greater total yield than the Commercial Inoculant. However, fruit quality was maintained and there was no reduction in the average size of fruits, their sugar content (Brix), or in any measure of human perceived fruit quality. I predicted that the Commercial Inoculant would have the greatest benefit on yield as it is adapted to growth in an intensive environment, however I found that Community 1 improved strawberry production over the Commercial Inoculant. I also predicted that the influence of an AMF community would depend on crop cultivar, due to the difference in traits between strawberry cultivars, but found that AMF and strawberry cultivar only interacted to influence human perceived strawberry appearance.

6.1.3 Chapter 4: The effects of AMF community and strawberry cultivar on interactions with wild pollinators and strawberry yield

In Chapter 4, I utilised the same AMF communities and strawberry cultivars to determine if the differences in pollinator visitation observed in the greenhouse translate to the wider wild pollinator community in the field, and if this resulted in functional consequences for yield. I measured the frequency and species richness of pollinators overall, and for individual taxa of pollinators. I predicted that the natural AMF communities would be better adapted to support plant reproduction and pollinator visitation, but found that AMF did not influence the frequency or richness of overall pollinator visitation. Although Hymenoptera are commonly used in commercial strawberry production, and AMF influenced their visitation, there were a low number of visits from Hymenoptera from the wild pollinator community, and floral visits were dominated by Diptera. To determine if visits by the wild pollinator community could fulfil crop pollination requirements in comparison to hives of pollinators typically used in commercial production (as examined in Chapter 2), I examined yield by measuring the number and weight of strawberries produced. I found the same pattern of yield as in the greenhouse, indicating that the wild pollinator community was able to provide a high degree of functional redundancy, and fulfil strawberry pollinator requirements as well as commercially produced pollinators. In addition, I predicted that the natural AMF communities would be better adapted to the local environmental conditions, and would provide the greatest yield. I found that both Community 1 and Community 2 produced higher yields than the Commercial Inocula, and the strength of this increase was greater than in the greenhouse.

6.1.4 Chapter 5: Perceptions on the introduction of AMF as a novel biotechnology in the production of soft fruit: An analysis using Q-methodology

In Chapter 5, I utilised Q-methodology, an innovative method from social science in order to examine the value placed on the ecosystem services provided by AMF and wild pollinators by stakeholders in the agricultural industry. I measured opinions on the introduction of AMF as a novel biotechnology to improve crop yields, and if these views were motivated by intrinsic or extrinsic factors. In addition, I explored the importance that growers place on wild pollinator communities. Q-methodology determines which distinct sets of viewpoints are present, and I predicted that respondents would fall into two primary groups – those with an intrinsic care for sustainable production systems, and those who are production and profit oriented and motivated by extrinsic rewards. I found three prevailing sets of views: ‘progressive thinkers’, who had a strong interest in new techniques and felt that a diverse pollinator community was important to them; ‘profit centred traditionalists’, who were primary motivated by the extrinsic rewards of improving production to increase profits; and ‘knowledgeable growers’, who were well informed, but had less interest in sustainability than ‘progressive thinkers’.

6.2 The importance of AMF-plant-pollinator interactions in crop yields

Pollinators provide a key ecosystem service by making a significant contribution to crop yields (Gallai *et al.*, 2009). However, whilst there is significant evidence that AMF can influence plant reproductive traits (Bryla and Koide, 1990; Stanley *et al.*, 1993; Lu and Koide, 1994; Lau *et al.*, 1995; Koide, 2000; Pendleton, 2000; Poulton *et al.*, 2001b; Poulton *et al.*, 2001a; Poulton *et al.*, 2002; Scagel, 2004; Gange and Smith, 2005; Wolfe *et al.*, 2005; Perner *et al.*, 2007; Kiers *et al.*, 2010; Varga and Kytöviita, 2010b; Aguilar-Chama and Guevara, 2012), and that pollinators respond to these changes (Buchmann and Cane, 1989; Harder, 1990; Real and Rathcke, 1991; Ashman *et al.*, 2000; Poulton *et al.*, 2001a; Buide, 2006; Cahill *et al.*, 2008; Soto *et al.*, 2013), there are few studies that connect these above-belowground interactions (Gange and Smith, 2005; Wolfe *et al.*, 2005; Cahill *et al.*, 2008; Varga and Kytöviita, 2010b; Barber *et al.*, 2013).

In addition, there are several gaps in our knowledge, for example, there have been no previous studies that have examined how AMF can influence pollinator foraging behaviour (the duration of visits or floral resources foraged for), how multiple belowground AMF communities affect pollinator visitation across several crop cultivars, or the functional consequences on crop yields. Here I have shown in the glasshouse experiment that AMF can influence pollinator foraging behaviour, but this depends on the specific AMF community a plant associates with, as the species that form that community each have unique traits, and as such elicit different influences on plant-pollinator interactions. In addition, the strawberry cultivars I tested were influenced similarly by the AMF community. Crop yield was influenced by AMF community, but this was not dependent on the total number of pollinator visits, as the Commercial Inoculant had the highest number of visits but the lowest yield. Community 1 increased the duration of nectar foraging visits through an increase in nectar production, as well as having the highest yield. This increase in yield may have resulted from both an improvement in plant vigour, and influencing pollinator behaviour to increase the duration of these less efficient visits. As such, although AMF can influence crop-pollinator interactions and pollinator behaviour, these are likely to only significantly influence crop yields when pollination is limited.

Examining these effects in the field allowed us to measure the impacts of above-belowground interactions in an environment where the plants were exposed to similar biotic and abiotic stresses of commercial production systems. Measuring the impacts of AMF on wild pollinators is important, as although commercial strawberry production is supplemented by hives of bumblebees or honeybees, and Hymenoptera are considered to be the most efficient pollinators (Schemske and Horvitz, 1984; Fishbein and Venable, 1996; Bingham and Orthner, 1998; Ivey *et al.*, 2003), recent evidence suggests that wild pollinators can provide a high degree of functional redundancy in the majority of crop production systems, and are able to fulfil the pollinator requirements of crops (Garibaldi *et al.*, 2013). In addition, visits from multiple pollinator species can improve crop yields due to functional complementarity of the different foraging techniques of pollinators (Klatt *et al.*, 2014).

I found that floral visitation was dominated by Diptera, which though less efficient as individuals, are able to fulfil pollination requirements through their abundance and frequency of visits (Orford *et al.*, 2015). Our results support the value of wild pollinators, as I found while AMF did not influence the visitation of wild pollinators, AMF influenced yield in the same pattern as in the greenhouse. Although yield was greater in the glasshouse due to more favourable growing conditions, and fewer biotic and abiotic stresses on the plants, both experiments had similar average fruit weights, indicating that wild pollinators were able to fulfil pollination requirements as well as the commercial hive bees in the greenhouse, where plants were pollinated by the highly efficient *Bombus terrestris* Audax (Figure 6.1). Furthermore, I found that the magnitude of differences between AMF communities was greater in the field, with Community 1 providing the greatest yields in both environments, and Community 2 having a similar benefit in the field. As the two natural AMF communities were extracted from field soils surrounding the experimental site, this may be a result of these communities being adapted to support plant growth under the local environmental condition. In both the field and the greenhouse, I found that AMF and strawberry cultivar largely did not interact, indicating that the influence of an AMF community was similar across all of the cultivars I tested, and that growers can expect the same improvements in yield from an AMF community across multiple cultivars.

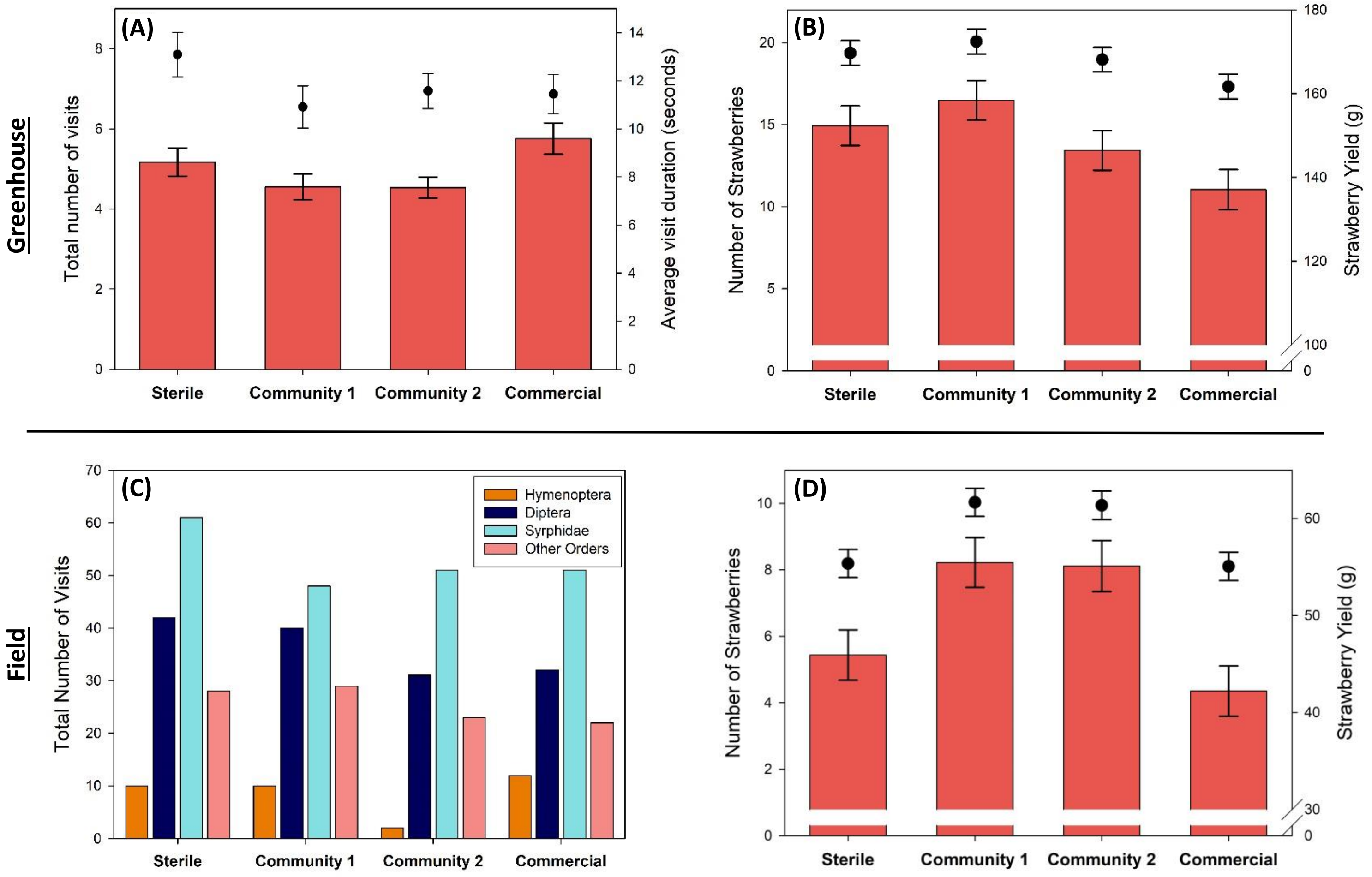


Figure 6.1: Comparison of pollinator visitation and strawberry yield between the greenhouse (top row (Figures a&b)) and the field (bottom row (Figures c&d)): A) total number (dots) and average duration of visits per flower by *Bombus terrestris* Audax in the glasshouse; B) number of strawberries (dots) and yield (bars) per plant in the glasshouse; C) total number of visits per AMF community (see legend) from wild pollinators in the field; and D) number of strawberries (dots) and yield (bars) per plant in the field. Values for Figures A, B, and D represent least squares means (±SE). Values for Figure C represent the total number of captures for each treatment.

6.3 The importance of measuring human perceptions on the consequences of above-belowground interactions

As well as examining the fundamental biology that influences above and belowground interactions, I explored the consequences of these interactions from the perspective of humans through two methods. First, I conducted a human assessment of the quality of strawberries produced from plants associated with each AMF community. While I found that AMF were able to influence fruit production, it is important to measure the quality of fruit produced by these changes, as factors such as fruit colour are used as an indication of quality by consumers and influences purchasing decisions (Caner *et al.*, 2008). I asked human taste testers to rate strawberries based on seven factors, ranging from colour to sweetness (see Chapter 3), and found that while AMF have the potential to increase yields, this does not result in any loss in human perceived quality.

Secondly, I conducted a social analysis of the viewpoints of stakeholders in the agricultural industry, to determine their perceptions on AMF, wild pollinators, and above-belowground interactions, and the value that they place on them as ecosystem services. Utilising biotechnologies such as AMF may offer the opportunity to improve sustainable intensification and improve food security (Tilman *et al.*, 2002; Baulcombe *et al.*, 2009; Roy-Bolduc and Hijri, 2011; Orrell and Bennett, 2013). However, the implementation of new techniques relies on the uptake of end users, and people make choices based on both intrinsic (i.e. a care for sustainability) and extrinsic rewards (i.e. an increase in profits) (Frey, 1997), and the introduction of new technologies relies on appropriate incentives (Tilman *et al.*, 2002). Although I found growers valued the ecosystem service provided by AMF, and were interested in implementing AMF within agriculture, I found distinct sets of viewpoints, and their motivations for AMF as a novel biotechnology differed from intrinsic rewards (improving the sustainability of their production systems) to extrinsic rewards (increasing their profits). Furthermore, the value growers place on wild pollinators varied between groups, and only the ‘progressive thinkers’ indicated that maintaining a diverse plant-pollinator network was particularly important to them. As such, although wild pollinators make important contributions to crop yields, and implementing AMF as a novel biotechnology in agriculture could increase yields, ultimately the decision to manage and improve these ecosystem services are decided by humans. Therefore, the successful implementation of products and policies requires the integration of academics, policy makers, and the agricultural industry in order to agricultural production systems.

6.4 Future Directions

Although this study used a holistic approach, examining the fundamental biology of above-belowground interactions, as well as the functional consequences for end users and their perceptions, inherently, there are limitations. Whilst I utilised multiple AMF communities, strawberry cultivars, and pollinators, these still represent a small subset of all AMF/strawberry/pollinator species and cultivars, and additional information could be added by utilising additional species and cultivars in future studies. Similarly, this work took place in a single environment, and mirroring studies such as this between multiple locations would help us to understand how environmental conditions influence these interactions. In such experiments, including data from a longer range of growing seasons would allow us to separate the influences of the climate of a region from the impacts of the growing conditions within individual seasons. Furthermore, experiments that manipulate temperature, rainfall, and CO_2 would help to determine how these interactions could be influenced by climate change.

In addition, although I found that the wild pollinator community was able to successfully fulfil crop pollination requirements, the assemblage of pollinator communities is highly variable, and there is opposing evidence of their ability to provide the same services as commercial pollinators in agricultural systems (Cardinale *et al.*, 2012; Garibaldi *et al.*, 2013). As such future studies would benefit from testing AMF-plant-pollinator interactions with multiple wild pollinator communities. Furthermore, in this study we were not able to determine the relative contribution of each individual pollinator species or taxa, or how AMF influences the behaviour of each pollinator species during an individual floral visit beyond *Bombus terrestris*. Future studies could explore this further by measuring how AMF influence the duration and behaviour exhibited during floral visits by constituent members of the wild pollinator community by exploring their interactions in a controlled environment.

Similarly, whilst I found that response variables were often influenced by which AMF community a plant was associated with, it was not possible in this study to determine if individual constituent species in the AMF community were responsible for the effects, or if these were the result of synergistic effects of the multiple species within a community colonising the same plant. Future work would benefit from testing the influence of entire AMF communities, and then breaking down this community to explore the effects of each species within it, and combinations of them, to determine which species cause the responses observes.

Furthermore, while for the first time, social science has been used to explore the perceptions of end users and experts on the use of AMF in agriculture, further evidence is required to explore the opinions of a wider range of stakeholders (i.e. inocula producers, policy makers, industry bodies, etc.) to evaluate the overall environment of the market. In addition, this analysis focused on strawberry growers, who often use advanced production methods and multiple biotechnologies for pest and pathogen control. As such, their viewpoints will not represent the overall agricultural industry, and future studies would benefit from exploring the perceptions of growers of other crops, who may have a different set of view, priorities, and motivations. In addition, production methods vary greatly across locations, and although I explored the perceptions of growers in the UK and US, an increased number of views would likely be elucidated by exploring additional countries. The respondents in this study were all from broadly similar backgrounds, and an understanding of perceptions of AMF and pollinators could be improved by comparing intensive commercial farms, small holdings, organic / sustainable based farms, and subsistence farms in multiple locations globally.

Finally, if we are to utilise the potential practical applications of controlling above-belowground interactions, then further work is required to develop suitable AMF inoculants. Currently, many of the products available on the market are blanket solutions, containing either a single species or blend of AMF species that are easy to culture and mass produce. As such, and because the influence of an AMF species or community can range from positive to negative depending on which plant they associate with, there is a degree of unpredictability with commercial inocula, and little evidence is available to indicate that a grower will see improvements in their production system. As discussed in Chapter 5, for the effective uptake of these products, it is vital that novel biotechnologies are able to fulfil the desires of growers, and are promoted in a method that aligns with the motivations of the end user. Furthermore, advances in production methods are required to mass produce cost-effective inocula for use on an intensive agricultural scale. Traditional *in vivo* production methods (culturing AMF in a carrier material over several months in a greenhouse) is prohibitively expensive for large scale use, and although advances have been made in *in vitro* production (Decklerck *et al.*, 2005), there is some evidence that *in vitro* produced spores can be less effective (Calvet, *et al.*, 2013). As such, for AMF to achieve the success of other microbial organisms, such as rhizobia (Catroux *et al.*, 2001), after we have explored the fundamental biology and identified a potential application, it is vital to understand the viewpoints of end users, and develop a product which is cost-effective, meets the needs of end users, and provides reliable effects in the field.

As such, few studies have previously explored AMF-plant-pollinator interactions, and while this thesis adds an important contribution to the body of evidence and explores new aspects, further research is required before we can begin to fully understand and predict the outcomes of above-belowground interactions, and the functional consequences for: 1) consumers of crops, including both pollinators and humans, and 2) the end users of the ecosystem services provided by AMF and pollinators.

6.5 Conclusion

Although AMF-plant and plant-pollinator interactions are well studied in isolation, far fewer studies link above and belowground mutualistic interactions, creating several knowledge gaps. For the first time, utilising an interdisciplinary approach that started in the greenhouse and ended with consumers, I have examined the biology of how plant-pollinator interactions are influenced on a local scale by multiple AMF communities across several crop cultivars, and the functional consequences for crop yields, as well as the consequences of these interactions from a human perspective. I began our analysis in the controlled environment of a greenhouse, where I found that AMF can influence the behaviour of *Bombus terrestris* Audax, but AMF influences on yield were not a result of changes in pollinator visitation. In addition, AMF induced changes in yield did not have any reduction in human perceived strawberry quality. I then examined above-belowground interactions in the field, where I found that the wild pollinator community, with very few visits from Hymenoptera, had a high degree of functional redundancy, and was able to fulfil the pollinator requirement of the crop. Furthermore, both of the natural AMF communities provided a greater benefit to yields than in the glasshouse, indicating that they performed better in the environmental conditions they were adapted to. Finally, our social analysis of stakeholder opinions revealed that not all growers placed importance on wild pollinators. I found interest in utilising AMF in commercial production, however across the three distinct sets of viewpoints I found, motivations ranged from improving sustainability to increasing profits.

As such, the resolution of plant-pollinator interaction networks could be improved by including above-belowground interactions, and measures of pollinator behaviour in order to further our understanding of the dynamics of plant-pollinator interactions. In addition, using measures of crop yield or seed set in wild plant species will help to uncover the functional consequences of altered networks. Furthermore, while AMF can have an important role in determining strawberry yields, and could form part of a toolset to improve sustainable intensification, the use of social science examining the perspectives of end users is needed to aid in the introduction of novel agricultural biotechnologies.

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