University of Reading

MANAGING BIODIVERSITY FOR ECOSYSTEM SERVICES IN APPLE ORCHARDS

Doctor of Philosophy

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Thesis abstract

Conventional intensive agriculture is largely reliant on high agrochemical inputs and has resulted in damaging environmental impacts including large scale biodiversity loss. More environmentally sustainable agricultural production methods are required. Ecological intensification is an approach which aims to sustainably increase production by using biodiversity-derived ecosystem services and replacing agrochemical inputs where possible. This can be achieved through modifying agricultural management practices to support ecosystem service-providing beneficial species. This thesis investigates the ecological intensification of commercial apple orchards in the UK. Apples are one of the most economically and nutritionally important fruit crops globally and their production relies upon a number of ecosystem services including pollination, pest regulation, and soil fertility services.

Alleyway cover crops were trialled as a novel management practice which has the potential to improve a number of orchard ecosystem services. Three different cover crop species mixtures, all based on perennial legumes but each with a different rationale, were compared to a standard mown-grass control. Growing cover crops in the alleyways was provided improved habitat quality for beneficial species, attracting greater numbers of natural enemy taxa including predatory beetles, parasitoids, and active-hunting spiders, without increasing the abundance of crop pests. Greater numbers of pollinators were also observed in alleyways sown with cover crops. Despite the greater numbers of beneficial species recorded in the cover crop treatments, no increases in pest regulation or pollination services were detected and no change in production was observed during the timescale of the study.

A second potential benefit of alleyway cover crops is the production of mulch material. Traditionally, organic mulches were used in orchards to help supress weeds underneath the trees, increase soil nutrients, and retain soil moisture, however many of these functions have now been replaced by agrochemical inputs. The cuttings from alleyway cover crops can provide an in-situ source of mulching material. Alleyway cuttings were compared to two traditional mulch materials, compost and straw, and a standard no-mulch control. Alleyway cover crop cuttings boosted numbers of earthworms and enhanced leaf litter decomposition, whilst the traditional straw and compost mulches improved some soil fertility measures including soil organic matter and moisture when compared to the control.

In the final study of the thesis, the importance of pollination is quantified and the methods used to assess pollinator dependence and pollination deficits are tested, with recommendations made about the scale at which these experiments should be carried out. Following pollinator exclusion apple yields were found to fall to 55% whilst supplementary hand pollination led yields to increase to 167% of current ‘open’ pollination rates, showing that pollination deficits existed in the study orchards. This study also highlights the importance of pollination for fruit quality, a key deciding factor of a crop’s economic value.

Alleyway cover cropping was found to have positive effects on ecosystem service providers both above and below ground. Even relatively inexpensive cover crop mixtures, combined with a reduction in mowing frequency and increase in mowing height, were found to increase numbers of beneficial species. The findings demonstrate the potential benefits of this multi-purpose habitat management method.

Contents

Page

Declaration i

Acknowledgements ii

Thesis abstract iii

Contents v

Chapter 1: **Introduction** 1

1.1 Intensive agriculture and food security 3

1.2 Ecosystem services 5

1.3 Ecological intensification 8

1.4 Opportunities for ecological intensification in apple orchards 11

1.5 Thesis aim and objectives 16

1.6 References 18

Chapter 2: **The ecosystem service benefits of traditional and cover crop-derived mulches** 35

2.1 Abstract 37

2.2 Introduction 37

2.3 Methods 40

2.4 Results 46

2.5 Discussion 53

2.6 Conclusions and management recommendations 56

2.7 References 57

Chapter 3: **Supporting natural enemies with orchard alleyway cover crops** 63

3.1 Abstract 65

3.2 Introduction 65

3.3 Methods 68

3.4 Results 74

3.5 Discussion 79

3.6 Conclusions and management recommendations 82

3.7 References 83

Chapter 4: **Orchard ground cover management for pollinators and pollination services** 91
4.1 Abstract 93
4.2 Introduction 93
4.3 Methods 97
4.4 Results 100
4.5 Discussion 110
4.6 Conclusions and management recommendations 112
4.7 References 114

Chapter 5: **The benefits of insect pollination to apple yield and fruit quality** 125
5.1 Abstract 127
5.2 Introduction 127
5.3 Methods 130
5.4 Results 134
5.5 Discussion 140
5.6 Acknowledgments 145
5.7 References 145

Chapter 6: **Discussion** 153
6.1 Review of thesis aim and objectives 155
6.2 Methodological constraints 158
6.3 Management recommendations and potential issues 159
6.4 Future research 163
6.5 Final conclusions 165
6.6 References 166

Appendix 1: 7.1 Mulch trial experimental design and orchard management details

Appendix 2: 7.2 Cover crop trial experimental design and orchard management details

Chapter 1:

Introduction

(A standard mown-grass alleyway and bare-soil herbicide strip)

Chapter 1:

Introduction

1.1 Intensive agriculture and food security

The increasing growth of the human population and the rising demand for food drives the conversion of natural habitat to agricultural systems; over a third of Earth's land surface is now dedicated to food production (Vitousek *et al.* 1997; Smith *et al.* 2008) and 71% of land in the UK (DEFRA 2016a). This conversion of land cover comes at a high price to biodiversity; habitat destruction for agriculture is considered one of the primary drivers for species extinctions (Pimm & Raven 2000; Foley *et al.* 2005; Barnosky *et al.* 2011). Many of the natural areas currently at risk of conversion are of great biological conservation value (Vitousek *et al.* 1997; Hoekstra *et al.* 2005; Koh & Wilcove 2008; Gibbs *et al.* 2010). These large-scale changes to land cover are having global and regional effects on our environment and are increasing the release of greenhouse gases and adding to climate change (Vitousek *et al.* 1997; Smith *et al.* 2008; Gibbs *et al.* 2010). As well as the global expansion of agricultural land into natural areas, the increasing demand for food and changes in global diets are leading to greater intensification of existing systems (Lambin *et al.* 2001). Intensive agricultural systems aim to produce more food from less land; this is generally achieved by employing highly simplified monoculture cropping and relying on considerable external inputs to maintain yields. These methods can, at least over the short term, allow higher volumes of food to be produced (Tilman *et al.* 2002). However, focusing solely on food production can result in the decline of other ecosystem functions, reducing sustainability (Tilman *et al.* 2002, 2011; Foley *et al.* 2005). The reliance on globally traded finite inputs also reduces food security as prices of these resources fluctuate over time and are expected to increase (Tilman *et al.* 2002; Galloway *et al.* 2008; Robertson & Vitousek 2009; Godfray *et al.* 2010).

The inputs used in intensive agriculture consist largely of agrochemicals, including synthetic fertilisers, pesticides, and a variety of products used to regulate plant growth. The production of these chemicals generally relies on fossil fuels, both for the base ingredients and to supply the high energy demand of production. Mechanical inputs such as ploughing and mowing are also largely reliant on fossil fuel derivatives, particularly diesel (Tilman *et al.* 2002). This dependence on oil and other fossil fuels reduces food security and these non-renewable resources have undergone extreme fluctuations in both price and availability in recent decades (Galloway *et al.*

2008; Godfray *et al.* 2010). The agricultural industry is a significant consumer of oil and its derived products, and it is one of the industries most at risk from the consequences of climate change (Gregory, Ingram & Brklacich 2005; Schmidhuber & Tubiello 2007). Attempts to replace oil with intensively produced biofuels may further drive the loss of natural areas as the demand for cropping land increases, and can result in the net increase in greenhouse gas emission (Jørgensen & Andersen 2012).

As well as the global impacts of climate change, intensive agricultural methods can have severe negative impacts on the environment at regional and local scales. Many intensive agricultural systems are irrigated with water abstracted from aquifers or withdrawn from rivers (Rosegrant 2003; Hanjra & Qureshi 2010). Overuse of these sources has led to increasing soil salinity and the reduction of rivers and lakes in some areas (Micklin 1988; Pitman & Läuchli 2002; Matthaei, Piggott & Townsend 2010). The increasing demand for water from the growing human population, and the risks posed to water supplies by climate change, may make irrigation less feasible in the future (Vorosmarty 2000; Allen *et al.* 2010; Hanjra & Qureshi 2010). There is also a risk that increasingly valuable water resources will be polluted with agrochemicals. Contamination of drinking water sources can harm human health and remediation can have a high financial cost (Carpenter *et al.* 1998; Vörösmarty *et al.* 2010).

Synthetic nitrogen fertilisers are one of the most commonly used fertilisers in agriculture and the addition of these and other inorganic nutrients to soils have allowed huge increases in yields. Synthetic nitrogen fertilisers are produced using natural gas through the Haber-Bosch process (Erisman *et al.* 2008; Galloway *et al.* 2008), a process which has been described as the 'detonator of the population explosion' because of its significance to food production and human population growth (Smil 1999; Galloway *et al.* 2004; Erisman *et al.* 2008). The inappropriate use of fertilisers comes at a financial and environmental cost (Erisman *et al.* 2008; Galloway *et al.* 2008; Robertson & Vitousek 2009). Much of the inorganic nitrogen fertiliser applied to croplands is lost back into the atmosphere, leached into ground water, or passes into waterbodies (Schlesinger 2009). Eutrophication occurs when excess nutrients enter waterbodies and results in severe damage to the ecosystems of rivers, lakes, and seas (Smith, Tilman & Nekola 1998; Conley *et al.* 2009; Stoate *et al.* 2009). The other key group of agrochemicals are pesticides. To control the spread of pests and diseases in crop monocultures, many intensive agricultural systems have become reliant on chemical controls (Tilman *et al.* 2011; Zhang, Jiang & Ou 2011). The over-use and misuse of pesticides, particularly older broad-spectrum products, has caused significant impacts on human health and biodiversity (Carvalho 2006; Sanchez-Bayo 2011; Zhang, Jiang & Ou 2011). Many of the effects of pesticides on non-target species, and the implications of using mixtures of pesticides and the potential synergies which may occur, are still unknown (Sanchez-Bayo 2011; Gill, Ramos-Rodriguez & Raine 2012).

The economic returns of many agrochemical inputs and intensive agricultural practices vary depending on the time scale over which their use is considered, with trade-offs between short-term productivity and longer term sustainability. High-input farming can produce greater short-term economic returns, however, when considered over a longer term the compounding negative effects on the environment may erode the sustainability of such systems and reduce future production (Rasmussen *et al.* 1998; Tilman *et al.* 2002; Pretty 2008). Greater intensification of agricultural landscapes also causes the loss and degradation of those areas of natural habitat which remain, as more land is converted for production; this can result in further loss of biodiversity and ecosystem functioning (Altieri 1999; Benton, Vickery & Wilson 2003; Green *et al.* 2005; Butler, Vickery & Norris 2007; Henle *et al.* 2008). Preventing the loss of habitats and species is important for biological conservation as a whole and for the cultural and human health benefits of natural habitats and biodiversity, but it is also important for food production and the fundamental functioning of the system as many species provide vital ecosystem services.

1.2 Ecosystem services

Ecosystem services are commonly defined as: “the benefits which people obtain from ecosystems” (MEA 2005), though there is some debate over terminology with other definitions including the processes behind these benefits (Daily 1997; Costanza *et al.* 1997). Some definitions also make distinctions between ‘intermediate services’, for example pollination, and ‘final services’, such as improved crop yields (Boyd & Banzhaf 2007; Fisher, Turner & Morling 2009). The most common definition of ecosystem services divides them into four broad categories; supporting services such as soil formation and nutrient cycling, regulating services such as natural pest regulation and pollination, cultural services such as education and recreation, and provisioning services which include food production (MEA 2005). Agricultural systems are essentially ecosystems which have been modified to primarily achieve the ecosystem service of food production (Foley *et al.* 2005). Biodiversity-derived ecosystem services are performed by beneficial species, which are sometimes collectively referred to as the ‘functional diversity’ of a system. Ecosystem services occur throughout agricultural systems and are as diverse as nitrogen fixation in the soil, to the pollination of flowers in a fruit tree (Swinton *et al.* 2007; Zhang *et al.* 2007).

Biological nitrogen fixation in agricultural soils is mainly carried out by bacteria growing in symbiosis with the roots of certain plants, such as legumes, and to a lesser extent by bacteria living freely within the soil (Herridge, Peoples & Boddey 2008). Biological nitrogen fixation was historically the primary route of nitrogen into food production and is still exploited in many extensive and pastoral agricultural systems (Galloway *et al.* 2004; Graham & Vance 2014). However, since the invention of the Haber-Bosch process the availability of cheap synthetic fertiliser has diminished the role of biological nitrogen fixation in many intensive systems (Galloway *et al.* 2004, 2008). Other soil-based ecosystem services include nutrient cycling and decomposition of detritus by soil organisms. Such services are essential to the functioning of all terrestrial ecosystems and are vital for soil fertility and agricultural productivity (Lavelle *et al.* 1997; Jouquet *et al.* 2006; Barrios 2007). Earthworms are an example of valuable soil species and their importance for soil formation and nutrient cycling has long been known (Darwin 1881). Earthworms are considered ‘ecosystem engineers’ because of their importance for soil formation (Blouin *et al.* 2013). The overuse of ploughing and excessive use of herbicides and other pesticides can damage soil functional diversity, including earthworms (Pfiffner & Luka 2007; Tsiafouli *et al.* 2015). These practices can also result in negative effects on important soil physiochemical properties such as organic matter content and structure (Balesdent, Chenu & Balabane 2000; Holland 2004), and have led to erosion and leaching of nutrients (Hansen & Djurhuus 1997; Shi & Shao 2000; Steenwerth & Belina 2010). The functioning of soil ecosystems is complex and involves a diverse array of chemical cycles and microscopic and macroscopic species. Considerable knowledge gaps remain in our understanding of soil processes, despite their fundamental importance to food production (Barrios 2007; Gardenas *et al.* 2011).

Natural enemies form a diverse collection of species, usually predators or parasitoids, which help to limit pest damage by suppressing pest populations (Wilby & Thomas 2002). Natural enemies may be generalists or specialists and the multiple interactions between natural enemies, crop pests, and other species can be both complicated and dynamic, including potential competition, predation, or complementarity between natural enemy species (Rosenheim, Wilhoit & Armer 1993; Cardinale *et al.* 2003; Gontijo, Beers & Snyder 2015). Different natural enemies require a differing range of habitats and both the abundance and diversity of these beneficial species are higher in heterogeneous landscapes which contain natural and semi-natural areas (Landis, Wratten & Gurr 2000; Benton, Vickery & Wilson 2003; Bianchi, Booij & Tscharntke 2006). In intensive systems where monocultures of genetically similar or identical plants, often of varieties which are less robust than their wild-type progenitors, crop pests and diseases are presented with the opportunity to thrive and rapidly spread (Andow 1983; Zhu, Chen & Fan 2000). These systems often become reliant on pesticides for much of their pest control and although these chemical controls can be effective, many have unintended consequences on non-target species, including natural enemies (Wilby & Thomas 2002; Geiger *et al.* 2010).

The loss of natural enemies through habitat destruction and the negative effects of pesticides reduces the biological pest regulation services which natural enemies provide and further increases reliance on chemical controls (Bommarco *et al.* 2011; Meehan *et al.* 2011). The declining biological control services in intensive systems, increasing restrictions on pesticide use, and the evolution of pesticide resistance in some crop pests, have all led to renewed interest in agricultural methods which can support natural enemies (Landis, Wratten & Gurr 2000; Fiedler, Landis & Wratten 2008; Jonsson *et al.* 2008). The management of biological controls falls into four methods; 'classical', 'inoculation', and 'inundation' biological control all involve the introduction of biological control agents which have been reared or collected elsewhere (Eilenberg, Hajek & Lomer 2001). These methods are common in protected cropping systems which are largely isolated from the wider environment (van Lenteren 2012). 'Conservation' biological control differs as it involves supporting naturally occurring populations of natural enemies, through sympathetic management practices; it is therefore considered an ecosystem service (Fiedler, Landis & Wratten 2008; Jonsson *et al.* 2008).

Insect pollination is a key ecosystem service contributing to food production, with 35% of global outputs and 75% of global crop species depending, at least in part, on this service (Klein *et al.* 2007). Pollinator dependent crops are also some of the most valuable in terms of nutritional content and fibre (Eilers *et al.* 2011; Chaplin-Kramer *et al.* 2014), and therefore play an even greater role in nutritional security than the tonnage of their yields suggest. Bees are the most important pollinators globally, though a diverse range of other taxa also contribute to crop pollination (Klein *et al.* 2007; Rader *et al.* 2016). Domesticated honey bees (e.g. *Apis mellifera*) are the most numerous and widespread pollinator species and many intensively produced pollinator dependent crops rely on them for production (Potts *et al.* 2016). Honey bees are facing a range of threats however, and the growing demand for pollination services makes wild pollinators increasingly important (Aizen & Harder 2009; Potts *et al.* 2010a, 2016; Garibaldi *et al.* 2013; Brittain, Kremen & Klein 2014). Unfortunately, as with natural enemies, pollinators have declined in many European agricultural landscapes due to a combination of habitat loss and exposure to pesticides (Kremen, Williams & Thorp 2002; Godfray *et al.* 2014; Stanley *et al.* 2015). The loss of both wild and domesticated pollinators from agricultural systems and the increasing demand for pollination services, due to the expansion of pollinator dependent crops, threatens to reduce yields and crop quality and to weaken food security (Winfree 2008; Aizen *et al.* 2009; Garibaldi *et al.* 2011a; Potts *et al.* 2016).

Declines in biodiversity-derived ecosystem services following beneficial species loss can destabilise crop systems and have significant implications for agricultural production (Altieri 1999; Ricketts *et al.* 2008; Garibaldi *et al.* 2011b; Tscharntke *et al.* 2012). The reliance on finite, potentially polluting inputs, and the neglect of other ecosystem services have caused the economic, environmental, and ecologically sustainability of conventional intensive agriculture to be questioned, and the security of food supply chains to be put in doubt (Matson, Parton & Power 1997; Tilman *et al.* 2002, 2011; Godfray *et al.* 2010). In recognition of this situation there have been calls for more ecologically robust methods of production (Allen-Wardell & Others 1998; Bommarco, Kleijn & Potts 2013; Deguines *et al.* 2014).

1.3 Ecological intensification

Ecological intensification is one approach to the concept of ‘sustainable intensification’, whereby food production is increased by further intensifying production using more sustainable methods (Tilman *et al.* 2011; Garnett *et al.* 2013; Godfray & Garnett 2014). This approach involves enhancing ecosystem services by boosting the populations of beneficial species (Bommarco, Kleijn & Potts 2013). The aim of ecological intensification is not to conserve all biodiversity, though many non-target species are likely to benefit, but to specifically support those species whose activities can enhance production and sustainability. This involves a more holistic approach than that used in conventional intensive production, moving away from highly simplified cropping systems to more diverse agroecosystems. By identifying suboptimal ecosystem services, i.e. those which are creating yield gaps, targeted management practices can be employed to support the relevant beneficial species, and improve production. Although ecological intensification may require, and allow, the reduction in use of specific agrochemicals, it does not demand that these tools are completely replaced. More ecologically intensive methods could help us to reduce our reliance agrochemical inputs and limit the environmental damage which they cause, whilst maintaining or increasing production (Bommarco, Kleijn & Potts 2013; Deguines *et al.* 2014; Pywell *et al.* 2015). For example, if certain pest species are controlled through biological means it may allow the use of more selective chemical control products which are targeted at fewer pest species. The changes in management needed to ecologically intensify production come in two main forms: the cessation of practices which are excessively damaging to ecosystem service providers, and the active enhancement of agricultural systems to benefit service providers.

As the loss of natural habitat from agricultural landscapes is known to be a significant driver of beneficial species decline, the prevention of further losses should be a priority. Agricultural land which is managed as a monoculture with little natural and semi-natural habitat can be a barren landscape for beneficial species, especially when crops are not flowering (Potts *et al.* 2010a; Garibaldi *et al.* 2011b). There are strong economic reasons for maintaining natural habitat within agricultural areas, including increases in yields due to better pollination services; examples include canola (Morandin & Winston 2006), coffee (Klein, Steffan-Dewenter & Tscharntke 2003), and almond (Norfolk, Eichhorn & Gilbert 2016). Inappropriate use of agrochemicals has also been shown to have significant negative effects on beneficial species. Examples of such effects include the decline in important natural enemies such as spiders (Bogya, Marko & Szinetar 2000) and predatory mites (Hill & Foster 1998) from fruit orchards due to the use of broad spectrum pesticides, and the declines in pollinators seen through a range of crops (Kremen, Williams & Thorp 2002; Godfray *et al.* 2014). The use of those products which cause excessive harm to ecosystem-service providers should cease. Methods such as Integrated Pest Management and conservation tillage are examples of practical management methods which are already helping to reduce negative effects on ecosystem service providers (Thomas 1999; Birch, Begg & Squire 2011; van Capelle, Schrader & Brunotte 2012).

Habitat management will be a key tool for actively enhancing agricultural landscapes to increase the abundance of beneficial species, as without appropriate habitat beneficial species survival is reduced (Landis, Wratten & Gurr 2000; Fiedler, Landis & Wratten 2008). Functional diversity is affected by habitat availability and quality at both the local and the landscape scale (Bianchi, Booij & Tscharntke 2006; Kennedy *et al.* 2013; Aviron *et al.* 2016). There is strong evidence to show that areas of natural habitat such as woodland and forests have positive effects on beneficial species and can improve ecosystem service provision in neighbouring agricultural systems (Blanche, Ludwig & Cunningham 2006; Bianchi, Booij & Tscharntke 2006; Eilers & Klein 2009; Carvalheiro *et al.* 2010; Thomson *et al.* 2010; Klein *et al.* 2012; Holzschuh, Dudenhöffer & Tscharntke 2012; Martins, Gonzalez & Lechowicz 2015). More localised habitat management practices include the creation of in-field semi-natural areas such as field margins (Pfiffner & Luka 2000; Smith *et al.* 2007) 'beetle banks' (MacLeod *et al.* 2004), and flower strips (Sutherland, Sullivan & Poppy 2001; Haaland, Naisbit & Bersier 2011). It is important that areas of semi-natural habitat are protected and restored, by reducing fragmentation for example, because they provide sources of food, nesting sites, overwintering sites, and shelter from environmental extremes and pesticide exposure (Benton, Vickery & Wilson 2003; Bianchi, Booij & Tscharntke 2006; Park *et al.* 2015). Flowering plants provide pollen and nectar which is vital for many beneficial species, particularly pollinators but also many natural enemies (Landis, Wratten & Gurr 2000; Garibaldi *et al.* 2011b). Plants which host alternative prey can also help to support natural enemy populations and improve predator fitness, though the presence of other prey species has the potential to disrupt biological control of crop pests (Settle *et al.* 1996; Koss & Snyder 2005; Symondson *et al.* 2006). Other methods for supporting beneficial species include the provision of artificial resources such as nesting tubes for cavity nesting bees (Sheffield 2014), refugia for generalist predators such as spiders and earwigs (Halaj, Cady & Uetz 2000; Logan *et al.* 2007), and supplementary feeding for natural enemies (Wade *et al.* 2008). Many of the current methods for ecological intensification through habitat management are based on agri-environment schemes which have been developed to improve the value of cropland habitat for biodiversity in general (Holland *et al.* 2014; Batáry *et al.* 2015). These methods provide a good starting point for ecological intensification, though they may need to be modified so that they are optimal for ecosystem service providers (Altieri 1999; Tscharntke *et al.* 2005; Ekroos *et al.* 2014).

The greater complexity of ecologically intensive agroecosystems means that an improved understanding of the interactions between species will be needed. It is therefore important that a holistic approach to management is taken, and that the potential synergies and trade-offs between services are appreciated. With increasing complexity comes the potential for unknown consequences, which is why these methods must be well researched before recommendations are made to farmers and growers. If managed correctly, ecologically intensive systems may provide a more sustainable and less environmentally damaging form of crop production without reducing yields. Even in systems where agrochemical inputs remain high there is scope to improve sustainability and production. Considerable knowledge gaps remain regarding how ecological intensification can be achieved, and the development of new management practices through applied research is crucial for progress (Tilman *et al.* 2002; Bommarco, Kleijn & Potts 2013). It is possible that natural systems and traditional agricultural methods may provide ideas for such practices (Altieri 2004; Altieri, Funes-Monzote & Petersen 2012). The methods for ecological intensification will vary between cropping systems depending on which services are required, which beneficial species are present, and how the crop is managed. The studies in this thesis use perennial apple orchards as a model system and investigate how ecological intensification methods can be integrated into this intensively managed crop.

1.4 Opportunities for ecological intensification in apple orchards

Apple trees (*Malus domestica*) have been grown by humans for at least 4,000 years (Cornille *et al.* 2014). Their main progenitors (*M. sieversii*) are found in the hills and mountains of Central Asia, though genetic evidence shows that a number of other wild species found throughout Eurasia, including European crab-apples, have contributed to the genetic make-up of the modern apple (Cornille *et al.* 2012). Apples are now the most widely and commonly grown fruit crops in temperate regions with 5,293,340 hectares used worldwide for apple production in 2016, with 2,383,905 hectares in China alone (FAO 2017) in the UK, Apple production was estimated to be worth £118m to the UK economy in 2015, with 16,512 hectares planted with apple orchards (DEFRA 2016b). Approximately half of the apple orchard area in the UK is dedicated to growing dessert or culinary apples with the rest growing cider apples, the studies in this thesis focus on dessert apple production.

Traditional apple trees were grown on their ‘seedling’ rootstocks and could reach heights of up to 10 m; such trees were planted at densities of 70-300 trees per hectare. Modern orchards contain trees which have been grafted onto dwarfing rootstocks and are planted at far high densities, with 1,000-6,000 trees per hectare (Robinson, Ferree & Warrington 2003). These smaller trees establish and yield more quickly than trees on seedling rootstocks, an important quality as the higher expense of creating an intensive orchard requires more rapid return on investment. Smaller trees also allow easier access for operations such as spraying, pruning, picking, etc. Most modern orchards are expected to remain commercially viable for between 15-20 years (Robinson, Ferree & Warrington 2003). A common dessert apple orchard design contains around 3,000 trees per hectare, grown to a height of approximately 3.5 m and spaced 1 m apart within a row and 3.5 m between rows. The alleyways between tree rows allow access to the trees, air flow, and light penetration. These high density systems can produce higher yields but they demand greater inputs, such as fertiliser and water, and require more ecosystem services. Beneficial species can have a range of positive effects on apple production, and key ecosystem services include pollination, pest and disease regulation, and those services related to soil fertility (Pizl 1992; Dib *et al.* 2010; Cross *et al.* 2015; Blitzer *et al.* 2016).

Many modern orchards have ‘fertigation’ pipes installed along the tree rows, supplying the trees with water and inorganic fertiliser. The majority of dessert apple orchards in the UK are found in the south east of England, an area which has seen rapid population increase and growing demand for water for both domestic and industrial uses. This increasing demand along with the hydrological changes due to climate change may reduce the availability of water for irrigation and so the development of more water-efficient methods of production is important for the future sustainability of production. Competition for water and nutrients can reduce effective soil fertility and weed control is important part of orchard management. Most growers use regular applications of herbicide to maintain a vegetation-free 1.5-2 m wide area known as the ‘weed strip, ‘weed-free strip’, or ‘herbicide strip’ in the tree rows. Improving the sustainability of weed control and soil fertility methods have been identified as an important research area in tree fruit production (Granatstein & Sánchez 2009).

Apple trees suffer from a range of pests and pathogens and the amount of pesticides used per hectare in apple production is one of the highest of all large-scale UK crops (DEFRA 2015). The most commonly used pesticides in apple orchards are fungicides (60-75% by weight), with apple scab (*Venturia inaequalis*) cited as the target of most sprays (Garthwaite *et al.* 2012). Major UK insect pests include tortrix moth species (especially the codling moth, *Cydia pomonella*), rosy apple aphid (*Dysaphis plantaginea*), woolly apple aphid (*Eriosoma lanigerum*), and the apple blossom weevil (*Anthonomus pomorum*). Woolly apple aphids feed on sap through the bark of the trees and the damage which they cause can provide an entry route for apple canker (*Neonectria ditissima*), a fungal pathogen which can ultimately kill the trees. It is likely that fewer pesticides will be available for apple growers in the future as UK and EU legislation moves towards restricting the use of chemicals which cause harm to non-target species (Hillocks 2012). Insecticides commonly used in apple orchards globally include some of the most contentious, namely organophosphates and neonicotinoids (Blacquière *et al.* 2012; Giesy *et al.* 2014). Recent examples of pesticide restrictions in the UK include the withdrawal of the broad-spectrum organophosphate insecticide chlorpyrifos in 2016 and the moratorium on some neonicotinoids (although this currently focuses on those neonicotinoids used in seed dressings of arable crops, rather than the foliar sprays used in orchards). Many growers now also avoid the use of pyrethroid sprays because of their detrimental effects on *Typhlyodrmus* mites, which are important natural enemies of phytophagous mite pests (Cross & Berrie 1994). In organic orchards, pests and pathogens are controlled using a mixture of biological pest control, cultural practices, and organic pesticides including copper and sulphur based fungicides. Sulphur sprays are considered to be only partially effective in controlling apple scab, and the long-term use of copper-base fungicides can have negative effects on soil biota (Wang, Zhou & Cang 2009).

Most apple varieties are self-incompatible (Ramírez & Davenport 2013) and it is common practice to plant ‘polliniser’ trees of different, compatible, varieties at regular intervals within the orchard to facilitate cross pollination (Robinson, Ferree & Warrington 2003). Yields are highly dependent on pollination by insect vectors (Dennis, Ferree & Warrington 2003; Ramírez & Davenport 2013), with poor pollination resulting in lower fruit set and increases in misshapen fruit (Matsumoto, Soejima & Maejima 2012; Garratt *et al.* 2014b; Sheffield 2014). The most important apple pollinators are solitary bees, honey bees, bumble bees, and hoverflies (Gardner & Ascher 2006; Tepedino *et al.* 2007; Garratt *et al.* 2014b) and increasing the abundance of these pollinators during apple flowering has been shown to improve yields (Stern, Eisikowitch & Dag 2001; Ladurner *et al.* 2004). Current pollination services in the UK are estimated to be worth £36.7m to the production of the two commonest dessert apple varieties, Cox and Gala, alone (Garratt *et al.* 2014b; a). A pollination deficit has been detected in some UK apple orchards, however, suggesting potential yield gaps (Garratt *et al.* 2014c). The declines seen in both wild and managed pollinators (Potts *et al.* 2010b; a) may present a threat to future production.

The studies in this thesis focus on methods for ecological intensification which involve changes to groundcover management. The large areas of orchard ground which are not directly cropped present an uncommon opportunity to introduce habitat for beneficial species throughout an intensive agricultural system. Currently, the ground cover in commercial orchards commonly consists of a herbicide strip of bare soil directly underneath the trees and a mown grass sward in the alleyways. Mown grass is a relatively poor habitat for many beneficial species (Bugg, Dutcher & McNeill 1991; García & Miñarro 2014). Alternative ground cover management methods may better support ecosystem service providers and enable growers to reduce agrochemical inputs. The use of alleyway cover crops, sometimes referred to as ‘alley-crops’, which have been chosen specifically for the enhancement of ecosystem services, is a relatively novel management practice which could potentially increase both production and sustainability (Figure 1).

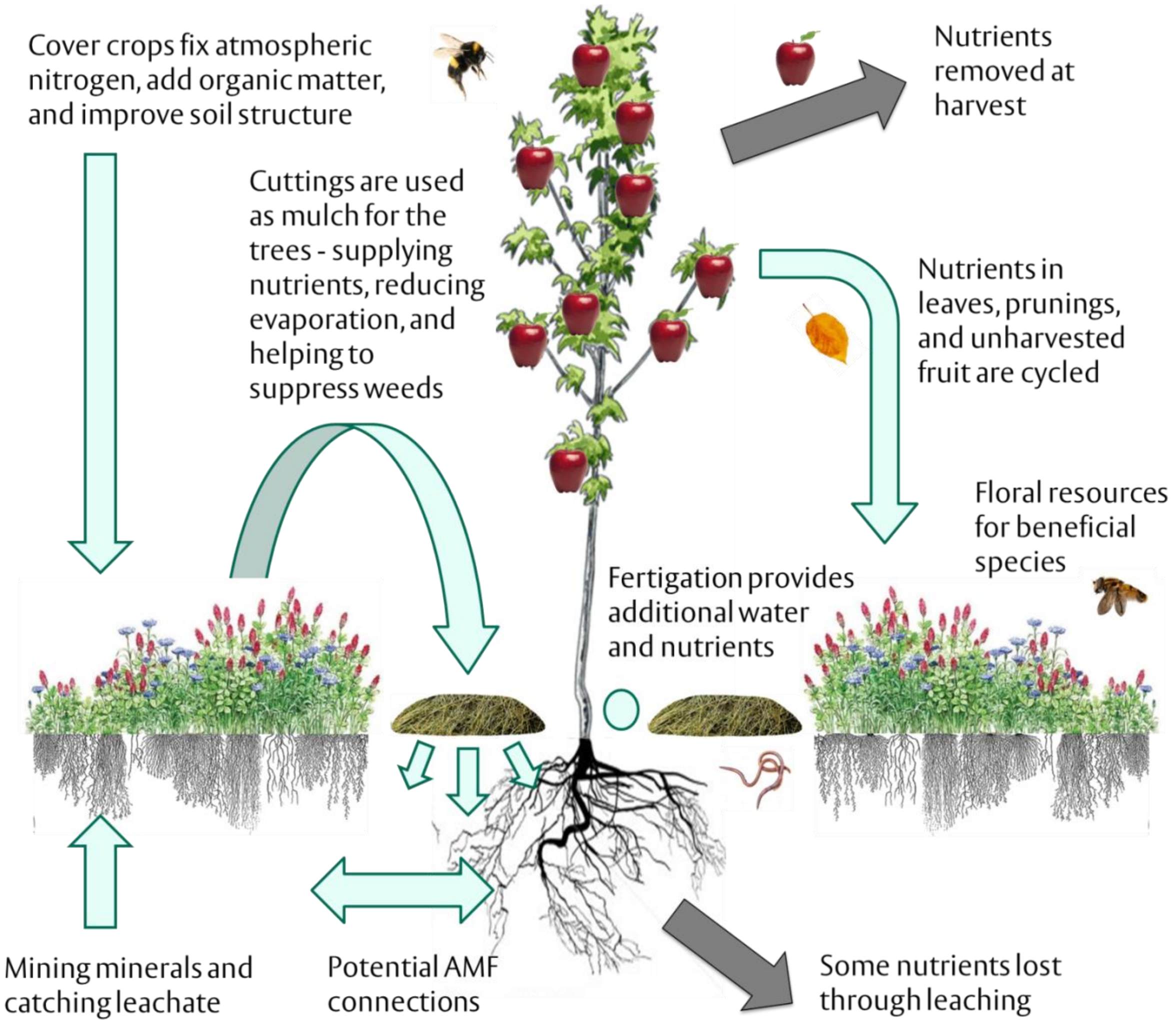

Figure 1. A cross section of an apple orchard showing the potential benefits of alleyway cover cropping. Green arrows indicate movement of nutrients within the system and grey arrow indicate loss of nutrients from the system. “AMF” = arbuscular mycorrhizal fungi.

Research has shown that sowing cover crops which consist of plants that are valuable to beneficial species can enhance a range of ecosystem services in orchards (Bugg and Waddington, 1994; Granatstein and Sánchez, 2009; Simon et al., 2010). Altieri et al. found that that cover crops could support beneficial species in vineyards, orchards (Altieri & Schmidt 1985, 1986; Altieri, Ponti & Nicholls 2005), and vegetable crops (Altieri & Letourneau 1982). Work by McKerchar (2016) also shows that when wildflower plantings establish well in orchards they can improve pollinator visitation to apple flowers and increase numbers of some aphidophagous natural enemies. There is also evidence to show that alleyway vegetation can be used to improve soil fertility: Sanchez et al. (2007) tested legume cover crops in an organic orchard and found they led to improved tree growth and yields. A similar study by Mullinix and Granatstein (2011) found that lucerne (alfalfa, *Medicago sativa*) grown in the alley contributed to orchard nitrogen needs without having a negative impact on the trees. During these studies the cover crop material was mown and left in the orchard alley, a more rewarding use of the cuttings may be to distribute them underneath the trees, onto the herbicide strip, where they can act as mulch and a slow-release fertiliser (Sirrine *et al.* 2008; Kuhn & Pedersen 2009; Fredrikson, Skinkis & Peachey 2011). This technique is sometimes referred to as 'mow and blow' or 'mow and throw' and is a development of previous cover cropping strategies which has so far received relatively little research (though see Sirrine *et al.* 2008; Kuhn & Pedersen 2009; Fredrikson, Skinkis & Peachey 2011).Trialling mixtures of legumes as alley cover crops and investigating the effects of cover crop cuttings as mulch have been suggested as useful areas of future research (Granatstein & Sánchez 2009). Cover crops have been shown to boost population of some natural enemies in apple orchards including some species of predatory mites (Markó *et al.* 2012) and hoverflies (Gontijo, Beers & Snyder 2013).

Although orchard alleyway cover crops have the potential to provide and support multiple ecosystem services the majority of previous studies have concentrated on single service and only limited research has been done on the effects of cover crops on pollination; the potential benefits of cover crops may therefore have been underestimated (Sutter & Albrecht 2016). Alleyway or strip cropping has also been used to enhance pest regulation in annual crops (Haaland, Naisbit & Bersier 2011; Brennan 2013; Tschumi *et al.* 2015, 2016) and studies have shown that sowing flowering plants in areas adjoining crop fields can increase both pollination and pest regulation (Walton & Isaacs 2011; Haaland, Naisbit & Bersier 2011; Blaauw & Isaacs 2014, 2015; Pywell *et al.* 2015). Knowledge gaps remain in how to integrate cover cropping into orchard systems; which plant species to sown, how to manage them, how much they cost, and whether this practice provides an effective method of supporting beneficial species and enhancing ecosystem service provision.

In order to improve understanding of current orchard management practices, a questionnaire was created during the first year of the project (2013) and sent out to members of the Sainsbury's Supermarket Top Fruit Development Group, a collection of UK apple growers. Over 90% of the growers who responded used conventional intensive management methods. Mown grass alleyways with bare soil herbicide strips were the most common method of ground cover management at over 80%, with approximately 10% of growers using mulch. The extent of pest and disease damage was considered very important to production by over 70% of respondents. Codling moth was stated as the most damaging pest, and apple scab and apple canker (*Neonectria ditissima*) the most damaging diseases. Predatory *Typhlodromus pyri* mites were the most commonly cited natural enemy taxa of importance, along with ladybirds (Coccinellidae), lacewings (Neuroptera), pirate bugs (Anthocoridae), and earwigs (Dermaptera). Over 80% of respondents considered pollination to be very important to production, and over 40% considered a lack of pollination an issue in their orchards, with a further 30% considering it an occasional issue. Over 90% of growers said that they maintained areas of natural vegetation to support beneficial species, with approximately 40% sowing areas with flowering plants. Several growers stated that they would like more advice on how to manage pollinators. The findings from this survey were used to help refine the aims and objectives of the project.

1.5 Thesis aim and objectives

Aim: The studies in this thesis investigate and evaluate the use of alleyway cover crops as a method for ecologically intensifying apple production.

Objective 1: Evaluate the effects of different mulches on soil fertility and soil-derived ecosystem services. It is hypothesised that the addition of mulch will improve soil fertility and soil-derived ecosystem services.

Objective 2: Determine the effects of alleyway cover crops on natural enemy abundance and the resulting implications for pest regulation services. Alleyway cover crops are predicted to provide a higher quality habitat than current management, leading to an increase in natural enemy abundance and an enhancement of pest regulation services.

Objective 3: Assess the value of alleyway cover crop habitat for pollinators and evaluate its impact on pollinator abundances and pollinator services. Pollinators are also hypothesised to benefit from the higher quality habitat provided by the cover crops, and increasing their abundance is predicted to improve pollination services.

Objective 4: Quantify the role of pollination in apple production and identify potential pollination deficits. Pollination dependence and pollination deficits are expected to be found, with pollination affecting both yield and fruit quality.

The studies assess the effects of alleyway cover cropping throughout the ecosystem service process by determining the value of the cover crop habitats, the effects these habitats have on beneficial species, the impacts on ecosystem service provision, and finally the implications for production.

Chapter 2. The ecosystem service benefits of traditional and cover crop-derived mulches

This study focuses on Objective 1. It reviews the knowledge of mulching in orchards and determines how both traditional and cover-crop derived mulches affect soil fertility, earthworm abundance, leaf litter decomposition rates, and production.

Chapter 3. Supporting natural enemies with orchard alleyway cover crops.

This study focuses on Objective 2. The habitat quality of different ground cover treatments are assessed and the effects on a range of natural enemies are determined. Pest regulation services are quantified through monitoring of pest species abundance and the use of sentinel prey surveys.

Chapter 4. Orchard ground cover management for pollinators and pollination services

This study focuses on Objective 3. The floral resources of the ground cover treatments are quantified and the abundance of pollinators, both during apple flowering and in the following summer months, are assessed. Pollination services are monitored and the effects on production are determined.

Chapter 5. Benefits of insect pollination on apple yield and fruit quality

This study focuses on Objective 4. Apple pollination dependence and potential pollination deficits are quantified and the methods used to measure these parameters are assessed. The effect of pollination on fruit quality, an important property which largely determines the value of a crop, is investigated.

Chapter 6. Concluding discussion

Here, the objectives are reviewed and the benefits to ecosystem services provided by mulching and alleyway cover cropping are discussed. Methodological limitations and potential integration issues are discussed and recommendations are made for orchard ground cover management and areas of future research.

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Chapter 2:

The ecosystem service benefits of traditional and cover crop-derived mulches

(Mulch trial plots)

Chapter 2:

The ecosystem service benefits of traditional and cover crop-derived mulches

2.1 Abstract

Mulching of fruit trees is a traditional method of groundcover management which can help to suppress weeds, retain moisture, and add nutrients to the soil. This method has, however, become less common in commercial orchards since the increased adoption of herbicides and synthetic fertilisers, and because of the costs of transporting mulch materials. Using cuttings from cover crop plants grown in the alleyway spaces between tree rows may provide an environmentally friendly source of mulch without the need for transport. In this study, cuttings from two legume-based cover crop mixtures and two traditional mulch materials (straw and compost) were trialled alongside a non-mulched control using a randomised complete block design in a conventionally managed commercial 'Gala' apple orchard. After two years, the compost mulch increased soil carbon and nitrogen, and the straw mulch resulted in higher soil moisture and suppressed weeds well, however neither appeared to benefit earthworms. In general, cover crop cuttings showed positive effects on earthworms and increased soil moisture. Though not tested here, increasing the abundance of earthworms and other decomposers and detritivores is expected to help control apple scab (*Venturia inaequalis*) by increasing the removal of leaf litter, which hosts overwintering fungal spores. Higher quantities of cover crop cuttings produced more positive effects, suggesting that the cumulative addition of cuttings over the lifetime of the orchard could be beneficial for several soil-derived ecosystem services. The results from this study highlight some of the potential benefits of mulching and demonstrate that alleyway cover crop cuttings can be used as a beneficial mulch. This study took place in an apple orchard but the findings may be applicable to other orchard and row-grown perennial crops.

2.2 Introduction

Orchard floor management is crucial for maintaining soil health and controlling weeds (Hogue & Neilsen 1987; Merwin, Ferree & Warrington 2003). It can also affect the abundance of fungal diseases of fruit trees through its impact on soil biota. Current orchard management practices involve a variety of agrochemical-intensive methods for maintaining soil fertility; including nutrient sprays, fertiliser spreading, and fertigation lines. Vegetation growing directly underneath the trees is generally controlled with herbicides, or tilling in organic orchards, creating an area of bare soil known as the ‘weed-strip’ or ‘herbicide-strip’. In commercial apple (*Malus domestica*) orchards, a 1-2 m wide herbicide strip centred on the tree row is common, with a mown grass alleyway maintained between the rows (Merwin, Ferree & Warrington 2003). A 2 m^2 vegetation-free area around the trunk is generally considered sufficient to prevent competition with tree roots (Merwin & Ray 1997), though there has been relatively little research in this area. Unfortunately, maintaining areas of bare soil negatively impacts soil health and can result in continual weed pressure (as it provides a potential seed bed for weeds), erosion, loss of soil organic matter, and damaging effects on both the soil’s physiochemical properties and soil biota (Merwin & Stiles 1994; Yao *et al.* 2005; St. Laurent, Merwin & Thies 2008; Gómez *et al.* 2009; van Capelle, Schrader & Brunotte 2012; Keesstra *et al.* 2016). Synthetic geotextile mulches such as polypropylene sheets can be effective at suppressing weeds and increasing soil moisture, but they too can have negative effects on soil biota (Walsh *et al.* 1996; Lipecki & Berbeć 1997; Forge *et al.* 2003; Andersen *et al.* 2013).

Soil biota provide a range of ecosystem services including soil structure improvements (Blouin *et al.* 2013), nutrient cycling (Beare *et al.* 1997; Tagliavini *et al.* 2007; Germer, Dongen & Kern 2017), and pathogen control through the decomposition of plant material (Raw 1962; Jacometti, Wratten & Walter 2007a). Removal of leaf litter from the orchard floor is an important ecosystem service in apple production because it can reduce the prevalence of apple scab (*Venturia inaequlais*), which is one of the most economically important apple diseases globally (MacHardy 1996). Removing leaf litter has been shown to reduce primary inoculum and the amount of damage caused by apple scab (Holb 2006; Gomez *et al.* 2007). The pathogen affects leaves and shoots, but can also damage fruit which may become disfigured and so considerably less valuable to the grower. The majority of apple scab pseudothecia overwinter in fallen leaves and so the removal of this material by decomposers and detritivores is an important, if overlooked, ecosystem service. Techniques for encouraging leaf litter decomposition include urea spraying before leaf-fall, and leaf shredding afterwards (Sutton, MacHardy & Lord 2000; Vincent, Rancourt & Carisse 2004; Holb, Heijne & Jeger 2006; Gomez *et al.* 2007). The urea adds nitrogen to the leaves, making them more palatable for decomposers and detritivores, whilst mowing increases the surface area of the leaves and may aid burying by earthworms. Earthworms are thought to be responsible for much of the leaf litter removal and decomposition in orchards (Raw 1962; Glover, Reganold & Andrews 2000; Holb, Heijne & Jeger 2006), and are considered ecosystem engineers due to their importance in nutrient cycling and formation of soil structure (Fragoso *et al.* 1997; Lavelle *et al.* 1997; Jouquet *et al.* 2006).

Traditionally, organic mulches such as straw, compost, and farm yard manure have been used to suppress weeds and boost soil fertility (Hogue & Neilsen 1987). However, these materials are bulky and can be costly to transport and apply, for this reason their use is limited in commercial orchards. If mulch material could be produced within the orchard system it would reduce many of the costs associated with traditional mulches. One method of producing mulch in situ is the use of 'living mulches', specific plant species which are deliberately grown in the herbicide strip. Although this method has been shown to have some beneficial effects on soil fertility and soil biota, the close proximity of sown plants and trees has generally resulted in excessive competition (Sánchez *et al.* 2007; Hoagland *et al.* 2008; Mullinix & Granatstein 2011; Qian *et al.* 2015; Żelazny & Licznar-Małańczuk 2018) and damage from rodent pests (Wiman *et al.* 2009; Sullivan, Sullivan & Granatstein 2018). An alternative method is the use of alleyway cover crops which are grown only in the alleyway space between tree rows, and not directly underneath them in the herbicide strip. Side-discharging mowers can then be used to spread the cuttings from these cover crops onto the adjacent herbicide strips where they act as mulch for the trees. This method is sometimes referred to as 'mow and blow' or 'mow and throw' (Sarrantonio 1992; Sirrine *et al.* 2008; Granatstein & Sánchez 2009; Pavek & Granatstein 2014) and has been shown to help reduce weed abundance in vineyards (Steinmaus *et al.* 2008). If legumes are used in these mixtures their ability to fix nitrogen from the atmosphere can be exploited and nitrogen can be added to the orchard system, potentially reducing the need for expensive synthetic fertiliser (Sirrine *et al.* 2008; TerAvest *et al.* 2010; Mullinix & Granatstein 2011). This method can help also to support soil biota in the herbicide strip (Nakamoto & Tsukamoto 2006; Thomson & Hoffmann 2007), potentially sustaining a larger community of detritivores and decomposers which can then increase leaf litter decomposition rates following leaf-fall (Jacometti, Wratten & Walter 2007b). Currently alleyway vegetation consists predominately of grasses, cut to a short height, with cuttings left in the alleyways. Only small modifications to existing equipment or the replacement of standard mowers to side-discharging models would allow growers to adopt the cover crop mulch method.

The aim of this study is to test the effects of both traditional and cover crop derived mulches on soil fertility in the tree row, tree growth, fruit production, soil biota, and the orchard sanitation services which soil biota provide. It is hypothesised that the addition of mulch will improve soil fertility, which, in turn, may improve tree growth and yields. It is also predicted that mulching will support a larger and more active community of soil organisms, which will lead to more rapid leaf litter decomposition. The following mulch materials were trialled alongside an un-mulched control: compost, straw, and the cuttings taken from cover crops grown in orchard alleyways. Two rates of two different cover crop mixtures were trialled, both consisting of a mixture of legumes and grasses. The first application rate is representative of the amount of material which would be produced from the adjacent alleyway. The second rate is twice this volume and aims to mimic the effect greater mulch production, in orchard with wider alleyways and/or narrower herbicide strips, as well as the build-up of cuttings which can occur during the lifetime of orchards (c 15 years) (Weibel *et al.* 2003; Yao *et al.* 2005). More details on these cover crops mixtures and their management will be presented in Chapter 3.

2.3 Methods

Study site

Fieldwork took place between June 2015 and May 2017 in a commercial apple orchard (planted in 2012) near Maidstone, Kent, England. The orchard was established on a clay loam soil (33% clay, 46% sand, and 21% silt) with a pH of 7.1-7.5. Top soil available nutrient abundances were; phosphorus at 63.0 mg/l, potassium at 455.0 mg/l, and magnesium at 117.0 mg/l. The crop tree variety was ‘Gala’ grafted onto ‘M9’ rootstocks. The orchard was managed conventionally with drip fertigation lines under each row of trees. Tree spacing was 1 m within the row and 3.5 m between rows. A 2 m wide herbicide strip was centred on the tree row with a 1.5 m wide strip of mown vegetation, predominately perennial ryegrass (*Lolium perenne*), in the centre of the alleyway. The herbicide strips in all plots were sprayed with herbicide in May/June and December/January at commercial rates and alleyways were mown to a height of 5 cm every 7-10 days between March and August, with cuttings left in the alleyways (more management details can be found in Appendix 1). Excluding the application of mulch, the management of trees and groundcover continued as a conventional commercial orchard.

Experimental design

Seven understory treatments (Table 1) were tested using a randomized complete block design. Experimental plots consisted of three trees within a 4 m long and by 2 m wide area of herbicide strip. Treatment plots were replicated in nine blocks across three tree rows, with each row containing three blocks (63 plots in total). Plots were separated by 2 m within a row or by one alleyway width between rows, and blocks were separated by 7 m within the row or one alleyway between rows (see Appendix 1 for more details).

Table 1. Details of mulching treatments. DM = dry mass. Spreading costs are estimated from Nix (2014), compost price is based on figures from WRAP (2008), and straw price based on figures from AHDB. More details on cover crop costs can be found in Chapter 3. Costs are based on orchards with 2 m wide herbicide strips and 1.5 m wide alleyways.

Treatment	**Description**	**Quantity added per application (per m^2 of herbicide strip)**	**C and N content of mulch**	**Mulch applications**	**Estimated cost of application (per hectare)**	**Estimated cost over lifetime of orchard (c 20 years)**
Control	No mulch	NA	NA	NA	NA	NA
Compost	PAS100 municipal compost. Particle diameter 0 - 10 mm	9,750 g DM, 18.75 litres	23.2% C 1.6% N	One – June 2015	Mulch: 56 tonnes at £6-20 per tonne = £336-1120 Spreading: £72-98 Total: £408-1,218	Re-apply every 3 years Total: £2,448-7,308
Straw	Conventionally grown wheat straw	2,062.5 g DM, 18.75 litres	40.7% C 0.9% N	One – June 2015	Mulch: 11.8 tonnes at £65 per tonne = £767 Spreading: £108-147 Total: £875-914	Re-apply every 3 years Total: £5,250-5,484
Oversown-clover	Cuttings from standard grass alleyways oversown with white clover and black medic	Estimated 215 g DM (equivalent to 1 m of alleyway cuttings)	39.7% C 2.1% N	Six (three annually) – June 2015 (double rate applied), August 2015, May 2016, June 2016, August 2016	Ground preparation (disking) and seeding: £61	If allowed to seed re-sowing may not be needed. Total: £61
Oversown-clover2	Double rate application of the oversown-clover treatment	Estimated 430 g DM (equivalent to 2 m of alleyway cuttings)	39.7% C 2.1% N	Six (three annually) – June 2015 (double rate applied), August 2015, May 2016, June 2016, August 2016	NA	NA
Legume-grass	Cuttings from alleyways sown with lucerne, red clover, timothy, and cocksfoot	Estimated 247.5 g DM (equivalent to 1 m of alleyway cuttings)	32.3% C 1.1% N	Six (three annually) – June 2015 (double rate applied), August 2015, May 2016, June 2016, August 2016	Ground preparation (herbicide, disking, harrowing) and seeding: £153	Re-sowing may be needed every 4-5 years. Total: £765
Legume-grass2	Double rate application of the legume-grass treatment	Estimated 495 g DM (equivalent to 2 m of alleyway cuttings)	32.3% C 1.1% N	Six (three annually) – June 2015 (double rate applied), August 2015, May 2016, June 2016, August 2016	NA	NA

The compost used in this study was a commercially available municipal compost adhering to BSI PAS 100 standards (WRAP 2011), with a particle size of 0-10 mm. The straw was conventionally grown wheat straw. For this study cover crops were not grown in the alleyways adjacent to the plots, instead the cuttings were collected from nearby alleyways where the cover crop mixtures had already been established. Cuttings were collected using a modified rotary mower (DR PRO42) with a discharge chute used to collect cuttings in sacks. Cuttings were then moved to the experimental plots and applied by hand on the dates shown in Table 1. In a commercial setting, cover crop cuttings would be directly spread from the alleyway onto the adjacent herbicide strip using side-discharging mowers. The first application of cuttings was planned for May 2014 but due to a delay in site preparation these cuttings were instead applied at the same time as the cuttings in June 2014. Chapter 3 provides further details on these mixtures and their management.

The alleyways were 1.5 m wide with 1 m of herbicide strip on either side so 1 m^2 of herbicide strip received 0.75 m^2 worth of alleyway cuttings. The herbicide strips of the single-rate treatments (‘oversown-clover’ and ‘legume-grass’) had cuttings applied from an equivalent length of alleyway, whilst the double rate treatments (‘oversown-clover2’ and ‘legume-grass2’) had twice this volume of material applied. The mass of mulch applied was estimated by weighing the sacks used to collect fresh cuttings; subsamples were then dried and weighed to establish dry matter content and a dry matter conversion factor. The weights of compost and straw shown in Table 1 are based on supplier estimates. To assess the carbon and nitrogen content of the different mulches, 4 subsamples of each material were dried, milled, and weighed to 10 mg (±0.3 mg) before percentage C and N were measured using a Flash 2000 CN analyser (Thermo Scientific) (Table 1).

Soil carbon and nitrogen

Soil cores were taken at the end of the experiment in May 2017. A soil corer with a diameter of 2.5 cm was used to take 15-20 cores per plot to a depth of 10 cm. Cores from individual plots were pooled and mixed before being air dried for at least 14 days. Samples were then milled and a 10 mg (±0.3 mg) subsample was used to determine percentage carbon and nitrogen for each plot using a Flash 2000 CN analyser (Thermo Scientific).

Soil temperature

Soil temperatures under different treatments were recorded in three of the nine blocks from July 2015 to March 2016, and August 2016 to April 2017. Records were taken at a depth of 5 cm below the soil surface every 1.5 hours using iButton data loggers (Thermocron iButton, Maxim Integrated). The daily mean, minimum, maximum, and range in temperatures were calculated for analysis.

Weed cover

The percentage cover of plants growing in the herbicide strip was recorded using a 1 m x 0.5 m quadrat, positioned to cover the width of the herbicide strip from the alleyway to the tree row. All vascular plants growing in this area were considered weeds; moss cover was recorded but not included in the analysis. The percentage weed cover in one quadrat per plot was recorded in June 2015 (before mulch application) and then in May 2016, June 2016, July 2016, and April 2017. Although weeds were recorded to species this was not included in the analysis, with total vegetation cover used instead.

Soil moisture

Soil moisture measurements were taken alongside soil respiration measurements in August 2015, and April, May, June, July, and August 2016. Measurements were taken using a ML2 ThetaProbe (Delta-T), this produced conductivity readings in mV which were converted to soil moisture percentages using the formula: % soil moisture = mV x 0.05 - 5

Soil respiration

Soil respiration rates were used as a proxy of soil biological activity and were recorded using an infra-red gas analyser (LCi-SD IRGA, LCi Photosynthetic System, ADC Bio Scientific Ltd. UK) with a soil chamber attachment which was placed directly onto the soil surface. Repeated measurements were taken once per month in August 2015, and April, May, June, July, and August 2016. Respiration rates were calculated as the net molar flow of CO_2 into or out of the soil (C_e (p mols s^{-1}) :

$$C_e = u\,(-\Delta c)$$

u = molar air flow in mol s^{-1}

Δc = difference in CO_2 concentration through soil chamber, dilution corrected, *u*mol mol^{-1}.

Leaf decomposition

In December 2015, apple leaves were collected from the alleyways surrounding the experimental plots. The leaves were air dried until a constant weight was reached and then separated into 10g (±0.35 g) samples. The weight of each individual sample was recorded before being put into 20 cm^2 plastic mesh bags with hole diameters of 2mm. Two bags were placed flat on the ground in the herbicide strip of each plot to simulate natural leaf fall (126 bags in total). In October 2016, the bags were collected and their contents were air dried for at least 14 days before being reweighed. In December 2016, this process was repeated, with bags being collected in late April 2017.

In addition to the leaf litter bags put out in December 2016, individually weighed 10 g (±0.5 g) leaf samples (2 samples per plot) were collected and placed underneath 20 cm^2 plastic mesh covers with hole diameters of 20 mm. These covers were pegged down to prevent leaves from being blown away, but allowed unrestricted access from the soil below the leaves. The leaves underneath these covers were therefore exposed to macroinvertebrates such as earthworms, whereas those in the bags were not.

Apple scab prevalence was not monitored on the experimental plots as airborne apple scab ascospores can be highly mobile (Aylor 1998) and the orchard was being managed with a conventional fungicide regime which is likely to have confounded results.

Earthworm abundance

Earthworm sampling was conducted in April 2016. For each plot, a soil pit measuring 25 x 25 x 25 cm was dug in the herbicide strip, half way between the tree line and the edge of the alleyway vegetation. The soil was spread on a 1.8 m x 1.2 m tarpaulin and sorted by hand for 10 minutes; pilot studies in previous years had shown that 10 minutes of sorting resulted in optimal balance between an accurate estimate of earthworm biomass and time allocation. All earthworms from the pits were collected and kept in pots containing moist paper towels for 48 hours to pass their gut contents. The earthworms were then washed, blot-dried with paper towels, counted, and weighed. Pieces of earthworm were included in the weight measurements but were not counted.

Tree growth

In July 2015 digital callipers were used to take two trunk diameter measurements for every tree (the second measurement perpendicular to the first). Measurements were taken 50 cm above ground level at a point which was permanently marked with an oil-based marker. In April 2017 measurements were repeated at these marks to establish trunk expansion as a proxy for tree biomass growth.

Leaf chlorophyll

In July 2016 a chlorophyll meter (Konica Minolta SPAD-502Plus) was used to record the chlorophyll content of 15 leaves per plot (5 leaves per tree). Chlorophyll content is closely related to soil nitrogen content and provides an indication of tree nutrition.

Fruit production

One to two weeks before commercial harvest the total number of fruit on each tree was recorded. Ten apples per tree (30 fruit per plot) were randomly selected and their diameters recorded. Three of these fruit (9 fruit per plot) were collected for further quality assessments. Quality measures included: number of seeds, fresh weight, firmness (using a Silverline penetrometer), soluble solids or Brix (using a Hanna refractometer), and dry weight (entire fruit were cut into 4 pieces and oven dried at 70°C for at least 72 hours before reweighing). In total 1,134 fruit were assess for this study; 9 fruit from each of the 63 plots in both 2015 and 2016.

Statistical analysis

Data were analysed using linear and generalised linear mixed effects models in R (R Core Team 2017) using the “lme4” package (Bates, Mächler & Bolker 2012). Treatment was the main fixed effect in all models, with original measurements included as covariates in the leaf litter weights and the trunk diameter models. Block was used as a random effect in all models, with plot and bag as nested random effects for the leaf litter bag models, and tree as a nested random effect for the trunk diameter and fruit count models. Sampling month was included as a crossed random effect in the soil respiration, soil moisture, and weed cover models and date was used as a crossed random effect for soil temperature. Data from different years for fruit production and leaf litter decomposition were analysed separately. Generalised linear mixed effect models with a Poisson error distribution were used for both fruit number and earthworm number data. To compare between treatments pairwise post hoc least-squares means tests were conducted using the R package “lsmeans” (Lenth 2016). Graphics were produced in R using the package “ggplot2” (Wickham 2009).

2.4 Results

Soil carbon and nitrogen

Both soil carbon and soil nitrogen were significantly higher in the compost treatment compared to all other treatments (Figure 1) (p<0.001). There were no other significant differences between treatments.

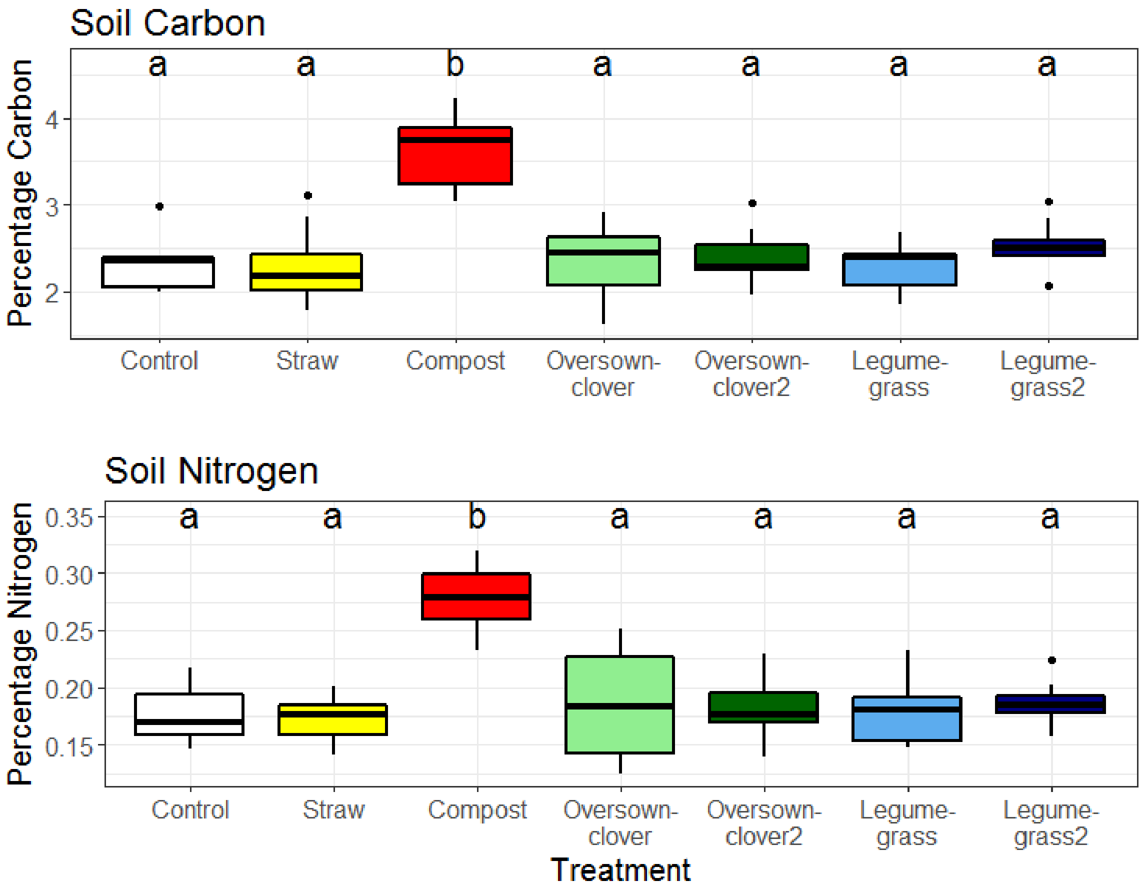

Figure 1. Soil carbon and nitrogen percentages in orchard herbicide strips following mulching (taken at a depth of 0-10 cm). No mulch was applied in the Control treatment. Letters indicate significant differences as calculated by pairwise least-squares means tests (p<0.05).

Soil temperature

There were no statistically significant differences between treatments in the daily mean or maximum soil temperatures. The straw treatment showed significantly lower daily minimum temperatures than the control (p=0.0056), and a significantly reduced range in daily temperatures when compared to the control, ‘oversown-clover’, and ‘oversown-clover2’ treatments (p=0.019, p=0.047, p=0.026 respectively).

Weed and mulch cover

Weed cover was significantly higher in the compost treatment (77.8%), and significantly lower in the straw treatment (4.3%) in comparison to all other treatments (Figure 2). This compares to a mean weed cover of 31.1% in the ‘legume-grass2’ plots (which had the second lowest weed cover) and 37.8% in the control treatment. The most abundant plants growing in the herbicide strip were grasses (predominately perennial ryegrass, *Lolium perenne*), groundsel (*Senecio vulgaris*), cleavers (*Galium aparine*), and common nettle (*Urtica dioica*). Groundsel made up 39% of weed cover across all treatments, and 64% of weed cover in the compost treatment. The analysis of mulch cover shown in Figure 2 does not include the compost treatment as the mixing of compost and soil made percentage cover estimates unreliable. The mulch cover values seen in the control and compost treatments were due to grass cuttings from the adjoining alleyways being blown onto the herbicide strip.

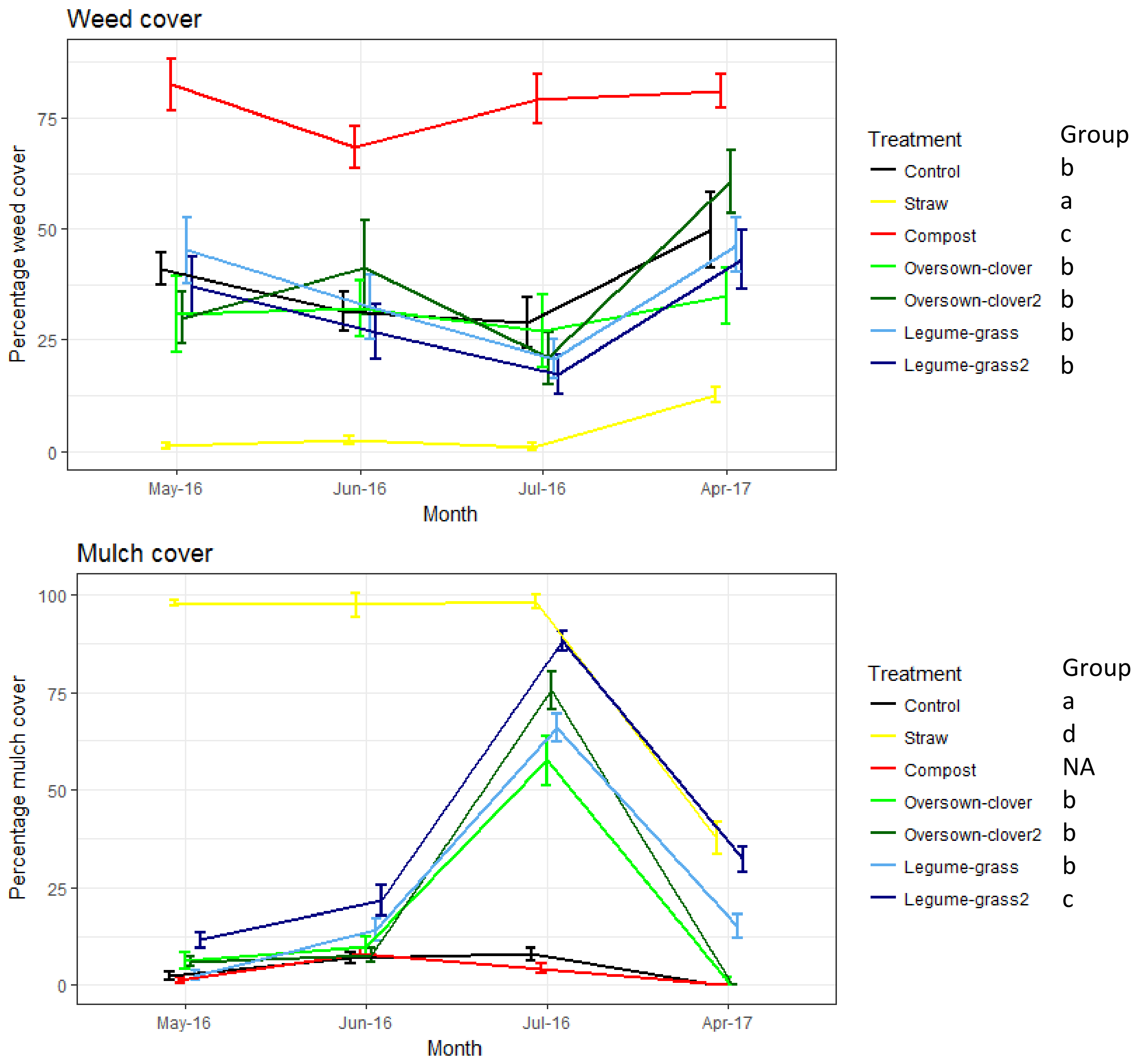

Figure 2. Percentage weed and mulch cover in herbicide strips (mean ± SEM). The compost treatment was not included in the mulch cover analysis as the incorporation of compost into the soil made estimates unreliable. Group letters indicate significance differences across the whole sampling period, as calculated by pairwise least-squares means tests (p<0.05). Straw and compost was applied once in June 2015, and cuttings from the ‘oversown-clover’, ‘oversown-clover2’, ‘legume-grass’, and ‘legume-grass2’ treatments were applied in June and August 2015, and April, June, and August 2016.

Soil moisture

Over the length of the study soil moisture was significantly higher in the straw treatment when compared to all other treatments, whilst soil in the ‘legume-grass2’ treatment had significantly more moisture than both the control (p<0.001) and compost (p=0.0025) plots, which showed the lowest and second lowest moisture levels respectively (Figure 3).

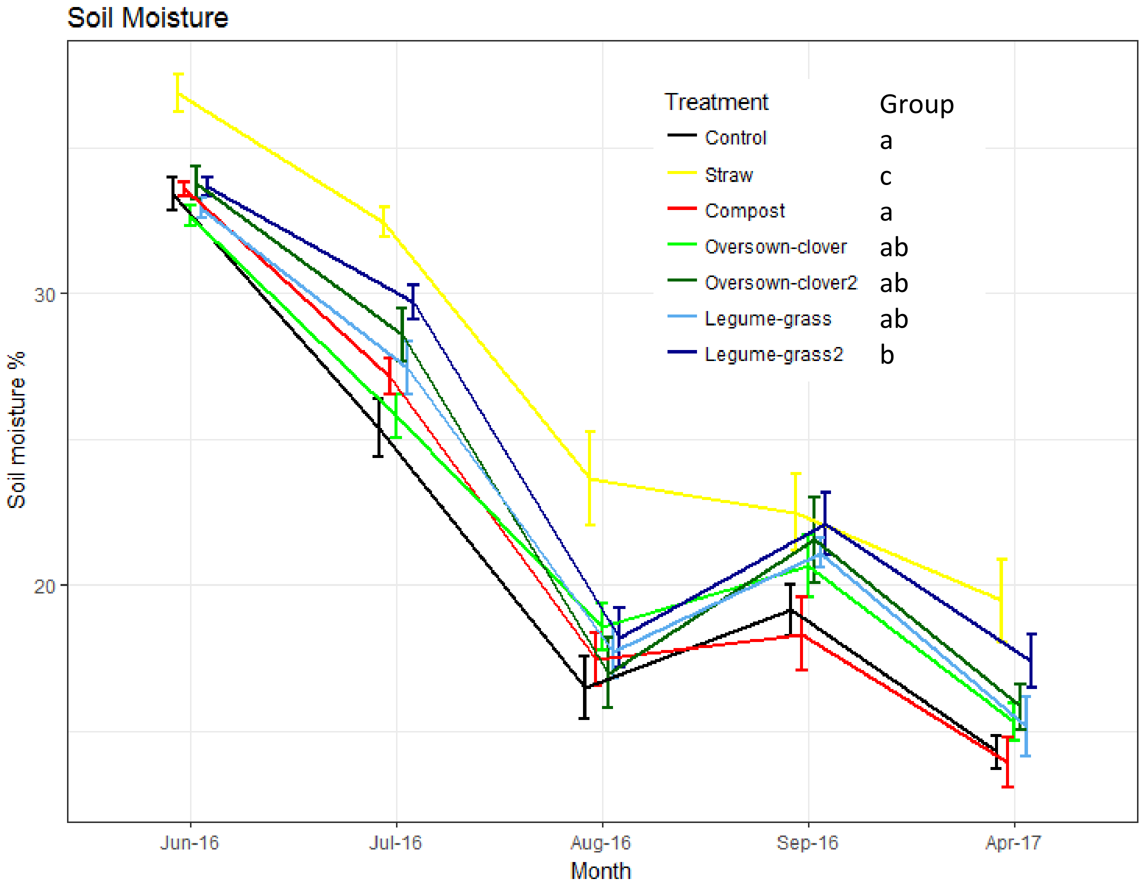

Figure 3. Soil moisture percentages (mean ± SEM) in the herbicide strip (taken at a depth of approximately 6 cm). Group letters indicate significant differences over the whole sampling period, as calculated by pairwise least-squares means tests ($p<0.05$).

Trunk diameter

Tree growth, as measured by trunk diameter, showed no significant differences between the treatments over the two year period of the study. Mean trunk diameter increases were between 4.5 mm and 5.2 mm in individual treatments.

Leaf chlorophyll

No significant differences were seen in leaf chlorophyll content between the treatments.

Yield estimate

No significant differences were detected between treatments in estimated fruit yields or in any of the fruit quality measures (see Table 2).

Table 2. Fruit yield and quality. Each degree Brix is equal to 1 g of sucrose in 100 g of solution. Values are means ± SEM for fruit collected in both 2015 and 2016.

Treatment	Number of fruit	Diameter (mm)	Fresh weight (g)	Dry weight (g)	Seeds	Firmness (kg/cm)	°Brix
Control	111.4±4.2	60.9±0.3	100.9±1.6	14.7±0.3	3.0±0.2	9.2±0.1	12.2±0.07
Straw	115.1±3.4	61.0±0.2	100.0±1.9	14.5±0.3	2.8±0.2	9.1±0.1	12.1±0.07
Compost	108.6±3.2	61.0±0.2	101.5±1.6	14.7±0.2	3.1±0.2	9.0±0.1	12.3±0.07
Oversown-clover	111.2±3.0	61.0±0.2	101.3±1.4	14.8±0.2	2.7±0.2	9.1±0.1	12.2±0.06
Oversown-clover2	111.7±3.6	61.3±0.2	104.6±1.5	15.3±0.3	3.2±0.2	9.0±0.1	12.2±0.06
Legume-grass	109.6±3.6	61.4±0.3	98.1±2.3	14.5±0.3	2.9±0.2	9.1±0.1	12.2±0.06
Legume-grass2	113.3±4.6	61.5±0.3	98.5±2.1	14.5±0.3	3.1±0.2	8.9±0.1	12.2±0.06

Soil respiration

The straw, 'legume-grass2', and compost treatments showed the highest rates of soil respiration during the monitoring period, with respiration in the straw treatments being significantly higher than the remaining 4 treatments (Figure 4).

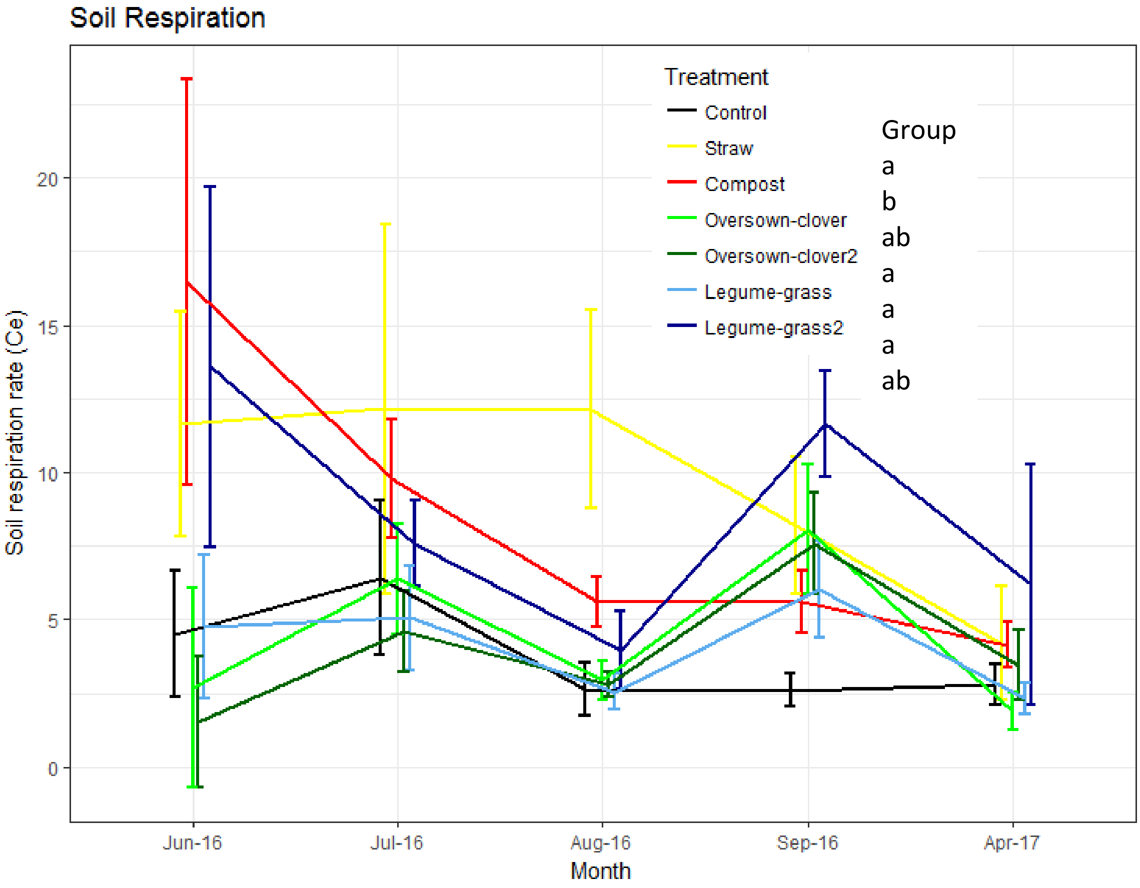

Figure 4. Soil respiration rates (mean ± SEM) following mulching treatments (recorded at the soil surface). Group letters indicate significant differences over the whole sampling period, as calculated by pairwise least-squares means tests (p<0.05).

Leaf litter decomposition

For litter bags incubated between December 2015 and October 2016 (Figure 5) the greatest reduction in leaf mass was seen in the ‘legume-grass2’ and compost treatments (with a mean of 2.16 g and 2.17 g remaining respectively). These two treatments showed significantly less leaf mass remaining when compared to the control and straw treatments (with 3.11 g and 3.35 g respectively). The ‘oversown-clover2’ treatment, which had an average of 2.44 g leaf mass remaining, also showed significantly more decomposition than the straw treatment. The bags that were incubated between December 2016 and April 2017 did not show any statistically significant differences between treatments though those in the ‘legume-grass2’ plots again had the least leaf litter remaining. In both years the ‘oversown-clover’, ‘oversown-clover2’, and ‘legume-grass’ treatments showed similar amounts of leaf decomposition.

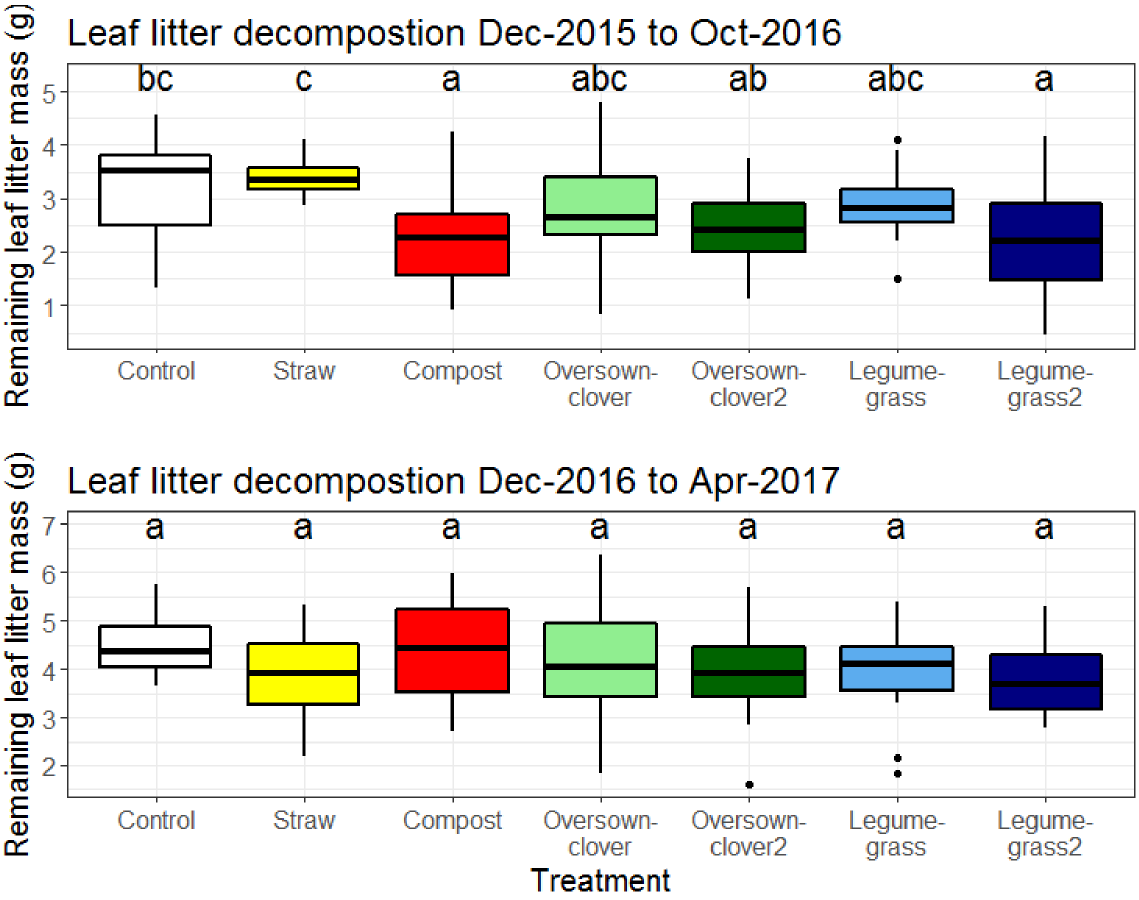

Figure 5. Mass of leaf litter remaining in litter bags after 10 months exposure (Dec-15 to Oct-16) and 5 months exposure (Dec-16 to Apr-17) on the surface of orchard herbicide strips under different mulching treatments. Original weights of 10g (±0.5g). Group letters indicate significant differences as calculated through pairwise least-squares means tests (p<0.05).

The leaf litter covers, which allowed macro fauna access to the leaves, showed 100% removal of recoverable leaf litter fragments in all treatments between December 2016 and April 2017. This compares to an average reduction in mass of 40.4% (4.07 g remaining) for leaves in litter bags over the same period.

Earthworm abundance

Earthworm abundance varied considerably between the treatments, with the 'legume-grass2' treatment having 2.4 times the number and 3.4 times the weight of earthworms compared to the straw treatment, and 1.7 times the number and 1.8 times the weight when compared to the control treatment (Figure 6). Earthworms were significantly more numerous in both of the double rate cuttings treatments ('legume-grass2' and 'oversown-clover2') compared to the control, straw, and compost treatments. Significantly more worms were also found in the 'legume-grass' treatment compared to the straw and compost treatments, with the straw treatment showing the fewest worms overall.

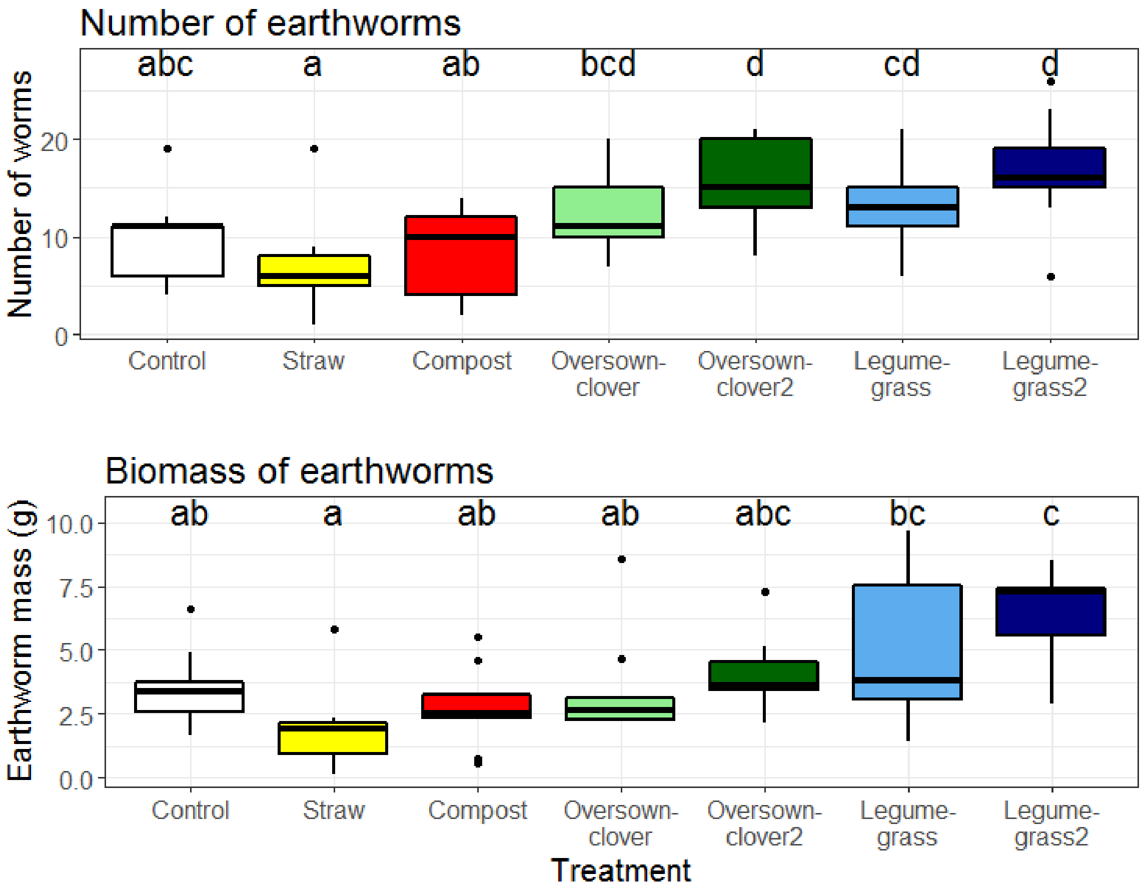

Figure 6. Numbers and of biomass of earthworms per 25 x 25 x 25 cm soil pit taken from the herbicide strips of orchard plots under different mulching treatments. Group letters indicate significance differences as calculated by pairwise least-squares means tests (p<0.05).

Summary of results

Table 3. Summary of results showing effect of mulching treatment compared to a bare-ground control. Leaf litter decomposition refers to the bags incubated in 2015-2016 only. Symbols indicate significance as calculated using pairwise least square means tests (*p*=0.05): '-' = lower than the control, '+' = greater than the control, and '0' = not significantly different from control.

Treatment	Straw	Compost	Oversown -clover	Oversown -clover2	Legume -grass	Legume -grass2
Soil Carbon	0	+	0	0	0	0
Soil Nitrogen	0	+	0	0	0	0
Weed suppression	+	-	0	0	0	0
Soil moisture	+	0	0	0	0	+
Tree growth	0	0	0	0	0	0
Fruit yield and quality	0	0	0	0	0	0
Soil respiration	+	0	0	0	0	0
Leaf litter decomposition	0	+	0	0	0	+
Number of earthworms	0	0	0	+	0	+
Mass of earthworms	0	0	0	0	0	+

2.5 Discussion

The results from this study show that whilst mulching can indeed improve measures of soil fertility and soil biological activity, the effects vary depending on the materials used (Table 3). Mulching generally had a positive effect on soils, though these benefits were not seen in all treatments; nor were the effects detectable in measures of tree growth or yields within the timescale of the study. The compost treatment showed significantly higher soil carbon and soil nitrogen content when compared to all other treatments, whilst the straw treatment appeared to have little effect on these parameters, despite having the second greatest mass of mulch material added after the compost treatment. This may be due to the degree of mixing with the soil which took place; the small particle size of the compost would have allowed easier incorporation into the soil compared to the long stalks of the straw. Soil moisture levels were higher in the straw and 'legume-grass2' treatments when compared to the control, but there were no statistically significant differences in the other treatments. Other studies have also shown that mulching can increase soil moisture (Merwin, Stiles & van Es 1994; Byers, Ferree & Warrington 2003; St. Laurent, Merwin & Thies 2008; Stefanelli 2009). As well as the highest soil moisture levels, the straw treatment showed the lowest minimum soil temperature and the least range in daily soil temperatures, this is likely to be due to the insulating effect of the straw.

It is possible that the pale coloured straw may have increase light reflection, which may have led to cooler soils but could also have increased colouring of fruit (Blanke 2008; Meinhold, Damerow & Blanke 2011), although no evidence of this effect was found in this study. The straw treatment provided significantly improved weed suppression, which is another likely cause for the high soil moisture; as transpiration from weeds would have been reduced. The enhanced weed suppression shown in the straw treatment was despite the fact that herbicide strips were already being controlled with herbicide. The application of compost, on the other hand, led to higher weed cover and had the second lowest soil moisture content. Although the weed cover was higher in the compost treatment the most common species was groundsel (*Senecio vulgaris*), a shallow-rooted summer-annual, which may be less competitive with the trees than other weed species.

The alleyway cuttings did not show improvements in weed suppression compared to the control, however it is possible that if cuttings were repeatedly applied over several years a layer may build up which could smother weeds in a similar way to the straw (Yao *et al.* 2005; Granatstein & Sánchez 2009). Layers of mulch covering bare soil may also help to reduce rain-splash, which is considered a potential route of soil and leaf litter pathogens (including apple canker, *Neonectria ditissima*) movement onto trees (Weber 2014). During the time frame of this study mulching did not have a detectable effect on tree growth, leaf nitrogen, or yields, though other studies have shown that the addition of compost and wood-chips the herbicide strip can positively affect tree growth and yields (Autio, Greene & Schupp 1991; Smith, Carroll & Cheary 2000; TerAvest *et al.* 2010).

The hypothesis that mulching would increase soil biological activity and leaf litter decomposition also received some support from the results. Only the straw treatment showed significantly higher soil respiration rates when compared to the control, although this did not translate into greater leaf litter decomposition rates as predicted. This may be because the leaves were lying on the top layer of straw whilst the rapid biological activity indicated by the higher respiration rates was occurring in the damper, lower layers of straw and soil. The fastest leaf litter decomposition rates were seen in the 'legume-grass2', 'oversown-clover2', and compost treatments which may be due to the microbe communities in these plots being more abundant or active, although respiration rates were not significantly higher than in the control plots. In the case of the 'legume-grass2' and 'oversown-clover2' treatments it may be that the microbe communities were better adapted to decomposing fresh plant material. Other studies have shown that mulching can increase soil biological activity and positively alter the composition of soil microorganism communities (Yao *et al.* 2005; Forge *et al.* 2008; St. Laurent, Merwin & Thies 2008). By providing resources and favourable habitat for decomposer and detritivore communities, mulch may enable improved leaf litter decomposition rates following leaf-drop in autumn. Mulching has been shown to reduce the sporulation of fungal pathogens, increase resistance to disease, and improve yields in vineyards (Jacometti, Wratten & Walter 2007a; b). The control treatment, where no mulch had been applied, showed the lowest soil moisture content, the lowest rates of soil respiration, and the least leaf litter decomposition, though not all of these difference were statistically significant. The lack of significant differences in leaf litter decomposition seen in 2016-2017 may be due to the reduced length of time that they were in the orchard; ten months in 2015-2016 versus just five in 2016-2017. The leaves under the leaf covers showed a far more rapid loss of mass over the same period, with no recoverable leaf litter fragments remaining. This supports the notion that macroinvertebrates, such as earthworms, are key for leaf litter removal and therefore for the biological control of apple scab.

The higher earthworm abundances seen in some of the mulched plots suggest that adding organic material to the herbicide strips can indeed help to support beneficial soil biota. The higher earthworm abundances seen in the ‘legume-grass2’ and ‘oversown-clover2’ treatments suggest that fresh vegetation cuttings are more valuable to earthworms than compost or straw mulches, although other studies have found that straw mulch can have a positive effect on earthworm numbers (Thomson & Hoffmann 2007; Andersen *et al.* 2013). The edibility of different mulch materials is likely to vary for different earthworm species depending on their feeding niches; with epigeic (surface dwelling) and anecic (burrowing) species (Bouch 1977) more likely to feed on fresh material such as cover crop cuttings, and apple leaves, than those species which generally feed on soil or compost (Curry & Schmidt 2007; Blouin *et al.* 2013). As well as supporting soil biota, mulching can affect above-ground species; and has been seen to boost the number of some natural enemies and reduce the number of some pest species (Mathews, Bottrell & Brown 2002). The effects on ground beetles have been more varied however, with mixed results on how mulching affects their diversity and numbers (Miñarro & Dapena 2003; Tuovinen *et al.* 2006). There is also the possibility that mulching will reduce the availability of nest sites for ground nesting bee species, as these species often prefer bare soil (Potts *et al.* 2005; Sardinas & Kremen 2014), although they are less likely to nest underneath trees than more open areas (Grundel *et al.* 2010). Mulching is also likely to affect earwigs, which are important predators of apple pests (Suckling *et al.* 2006), as they also nest in the soil. As well as the mulch that they produce, the living cover crops themselves may benefit soil functional diversity through their nutrient-rich root exudates (Jiao *et al.* 2013) and interactions with mycorrhizal fungi (Baumgartner, Smith & Bettiga 2005). Cover crops also have the have the potential to increase the numbers of natural enemies and pollinators, along with the ecosystem services which they provide; this will be discussed further in Chapters 3 and 4.

2.6 Conclusions and management recommendations

The traditional mulch materials became increasingly degraded and dispersed and by the end of the experiment they were visibly reduced, and the straw treatment's percentage cover and effectiveness at suppressing weeds appeared to be waning by the end of the experiment. If the effects of the two traditional mulches were to continue throughout the lifetime of the orchard they would need to be reapplied, probably on a three year basis. Transportation and application of these mulches is costly and it is likely that their use would only be feasible if materials were locally available. One of the major benefits of the alleyway cuttings is that they do not require transportation, though they too may need replenishing. Whilst white clover can survive indefinitely if allowed to seed it is likely that stands of lucerne will need to be re-sown every 4-5 years (AHDB 2014). The fact that the cuttings from cover crops do not need to be transported and can applied repeatedly to the herbicide strip over many years makes them a low-cost alternative to traditional mulches (Table 1). Cuttings from current alleyway vegetation, which is usually predominately grasses, could also be used to produce mulch, though the nitrogen content is likely to be lower than for a legume based cover crop.

If cover crops were sown during orchard establishment, just as grass alleyways currently are, they would require minimal change on management. Mulching with cover crop cuttings would require side-discharging mowers however. The yield of cuttings from cover crops will vary between sites according to soil fertility, climate, and management, but cover crops have the potential to provide a small but regular source mulch and nitrogen for the tree roots in the herbicide strip. The alleyway cover crops used in this study were producing 4.3 and 4.9 tons of dry mass per hectare annually for the 'oversown-clover' and 'legume-grass' treatment respectively. These yields are towards the lower end of expected clover-grass and lucerne yields and if the cover crops were managed more efficiently the yield of cuttings may be increased. Using a combination of mulching options may be the most effective method for newly established orchards: one potential method could be to apply straw to the herbicide strips in newly planted orchards then as the trees are growing; the straw could then be ‘topped-up’ with cuttings from the alleyway. Growing alleyway cover crops could prove to be a cost-effective and sustainable way of producing mulching material and adding nitrogen; negating transport costs, reducing mowing frequency, and possibly allowing a reduction in herbicides and fertiliser applications (Patrick *et al.* 2004; Sirrine *et al.* 2008; Mullinix & Granatstein 2011).

Whilst the effects of traditional mulches have been well studied, and some of the longer-term benefits have been shown (Hogue & Neilsen 1987; Merwin, Ferree & Warrington 2003; Neilsen *et al.* 2003), the use of alleyway cuttings is more novel and still requires further research. This experiment was conducted in a commercial orchard under conventional management; fertiliser was being applied and the herbicide strip was being managed with bi-annual herbicide applications. The differences between treatments found in this study were seen despite the effects of intensive management, which included irrigation and fertiliser applications. Surveys for apple scab damage on trees were not conducted because of the proximity between plots and because the orchard was being conventionally managed with fungicides, which is likely to have masked any treatment effect. Although some studies have seen competition between cover crops and trees for water and nutrients (Teravest *et al.* 2011; Du, Bai & Yu 2015) this will depend on the climate, cover crop type and management, and the width of the herbicide strip, and other studies have shown improved soil fertility and production with cover cropping even in arid and semi-arid conditions (Sánchez *et al.* 2007; Ramos *et al.* 2011). The water conserving ability of mulches may be of increasing importance as droughts are made more likely due to climate change and the demand for water increases due to continued human population growth (Vorosmarty 2000; Allen *et al.* 2010). Quantifying the effects of cover crops and other management practices in orchards can be difficult because orchards may be commercially viable for decades (Jackson 2003; Nix 2014), whilst most studies will only run for a few years. It may be that only large scale, long term studies are capable of revealing the true effects on production, but the results from this study demonstrate that mulching, and mulching with cover crop cuttings can be a beneficial management practice which may enhance ecosystem services and improve soil health and sustainability.

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Chapter 3:

Supporting natural enemies with orchard alleyway cover crops

(‘Floral-rich’ plot, June 2016)

Chapter 3:

Supporting natural enemies with orchard alleyway cover crops

3.1 Abstract

Increasing the biological control of crop pests by natural enemies will help to improve the ecological sustainability of agricultural systems by reducing the need for pesticides. In this study, different mixtures of orchard alleyway cover crops were tested to establish their capacity to boost natural enemy populations and enhance pest regulation services in apple orchards. Three cover crop mixtures were tested: the first consisting of a range of legumes and wildflowers designed to provide abundant floral resources, the second of legumes and tussock-forming grasses designed to increase the structural complexity of alleyway vegetation, and the third comprising two legumes species over-sown into existing alleyway vegetation which was intended as a low-cost option. These mixtures were trialled in conventional commercial orchards alongside a mown-grass standard control using a randomised complete block design with plots averaging 0.25 ha in size. All three mixtures had positive effects on several important natural enemy groups, including predatory beetles, parasitoids, and active-hunting spiders, without increasing the abundance of apple crop pests. However, no significant changes in pest abundance or in measures of predation rates, using pea aphid sentinel cards, were detected. The results suggest that whilst florally diverse mixtures are the most effective for attracting a range of natural enemies into orchards even the addition of simple, low-cost seed mixtures can lead to an increase in populations of natural enemies.

3.2 Introduction

Improving pest regulation services by providing habitat for natural enemies is an important method for ecologically intensified production and to reduce our reliance on pesticides (Landis, Wratten & Gurr 2000; Fiedler, Landis & Wratten 2008; Bommarco, Kleijn & Potts 2013). Reducing pesticide use is, in turn, key to reducing biodiversity and improving sustainability in many agricultural systems, including fruit orchards (Reganold *et al.* 2001; Godfray *et al.* 2014; Connelly, Poveda & Loeb 2015). If natural enemies are to provide pest regulation services they must have access to the resources that they need; sources of nutrition and energy such as prey species (either crop pests or alternative prey), pollen, and nectar. Physical resources including shelter, web-building sites, nesting sites, and overwintering sites are also essential. These resources must be available at the correct times during the species lifecycle. Natural enemies are generally more abundant in diverse agricultural landscapes where natural and semi-natural areas provide a range of habitats and resources (Bianchi, Booij & Tscharntke 2006). However, many intensively managed agricultural systems are homogenous monocultures, lacking the resources needed to sustain the populations of many natural enemy species to the extent that they meet current pest control demands (Meehan *et al.* 2011).

Commercial orchards commonly have groundcovers consisting of weed-strips of bare soil and alleyways of regularly mown grasses (Merwin, Ferree & Warrington 2003). This leaves little room for the plants which provide the additional resources required by natural enemies and other beneficial species (Horton *et al.* 2003). Alleyways can found in many perennial systems and make up a significant amount of the land area. They provide the space needed to access the trees and allow the passage of air and light , whilst the alleyway vegetation helps to provide traction for vehicles and reduces erosion and run-off when compared to bare soils (Keesstra *et al.* 2016; García-Díaz *et al.* 2017). The majority of alleyway vegetation consists of closely mown grasses (Merwin, Ferree & Warrington 2003), which can be poor habitat for many beneficial species and adds little to the orchard agroecosystem (Horton *et al.* 2003). Growing flowering plants in the alleyways could provide a significant resource for beneficial species in close proximity to the crop (Bugg & Waddington 1994; Wyss, Niggli & Nentwig 1995). Having floral resources immediately adjacent to the crop should reduce the amount of energy that natural enemies need to expend travelling between food sources, potentially increasing their effectiveness (Lavandero *et al.* 2005). Improved alleyway habitat may also help natural enemies move into the orchards from any semi-natural areas surrounding the crop; reductions in the abundance of some natural enemies have been seen at greater distances from orchard edges (Thomson & Hoffmann 2009, 2013).

Habitat management forms a key part of conservation biological control (Landis, Wratten & Gurr 2000) and targeted habitat creation can be used to increase the numbers of natural enemies and other beneficial species which provide ecosystem services to crops (Ramsden *et al.* 2015; Tschumi *et al.* 2016). Adding areas of flowering plants to agricultural systems is one method which can help to support a range of beneficial species (Pywell *et al.* 2005; Haaland, Naisbit & Bersier 2011; Ditner *et al.* 2013). Creating strips of wildflowers and flowering cover crops alongside crops has been shown to boost natural enemy numbers and in some cases improve pest regulation services in a range of crops including grapes (Berndt, Wratten & Hassan 2002;

Altieri, Ponti & Nicholls 2005), lettuce (Gillespie *et al.* 2011), lucerne (Jacometti, Jørgensen & Wratten 2010), blueberry (Blaauw & Isaacs 2015), olives (Tschumi *et al.* 2016), wheat (Hatt *et al.* 2017), and apples (Markó *et al.* 2012; Gontijo, Beers & Snyder 2013). Flowers provide an important source of carbohydrates to many natural enemies and access to flowers can increase the fecundity and longevity of important natural enemies such as parasitoid wasps, lacewings, and hoverflies (Berndt, Wratten & Scarratt 2006; Vattala *et al.* 2006; Robinson *et al.* 2008; van Rijn, Kooijman & Wäckers 2013). The addition of wildflowers and tussock-forming grasses to farmland can also increase the structural complexity of available habitats at both the landscape and plant scale; providing overwintering sites, microclimates, nesting sites, and places where webs can be made (Langellotto & Denno 2004; MacLeod *et al.* 2004; Woodcock *et al.* 2007; Ramsden *et al.* 2015). Such habitats also help to support a diverse range of other species, including alternative prey, and can limit the loss of farmland biodiversity in general (Benton, Vickery & Wilson 2003; Haaland, Naisbit & Bersier 2011).

Not all flowers are of equal value to natural enemies and most natural enemies lack the specialised flower-feeding mouthparts evolved by many pollinators. Natural enemy species therefore typically prefer flowers with simple, open structures (Wäckers 2004; Vattala *et al.* 2006). Providing mixtures of plants with a range of flower structures and corolla lengths may be needed to support a range of beneficial species (Campbell *et al.* 2012). Considerable knowledge gaps exist regarding which habitat enhancements, and which plant species, can provide the most benefits in different crop systems. The complex interactions found in agroecosystems can be hard to predict and there is a risk that introducing new plant species such as cover crops may inadvertently support more pests (Lavandero *et al.* 2006; Bone *et al.* 2009). It is therefore essential to study the effects of habitat management practices on the whole agroecosystem before recommendations are made.

This study details a trial of three orchard alleyway cover crop mixtures planted with the aim of supporting beneficial natural enemy species: the 'floral-rich' treatment was based on a commercially available 'pollen and nectar' mixture and was focused on providing a large volume of floral resources from a variety of flower structures. The 'legume-grass' treatment included legumes and grasses and focused on increasing structural complexity and producing a large volume of vegetation. The 'oversown-clover' treatment was focused on producing a large volume of flowers, but at minimal cost, and used a low-diversity legume mixture over-sown into the existing vegetation. These treatments were trialled alongside a commercial standard control of closely-mown predominately grass alleyways. Perennial legumes were chosen as the basis for these mixtures because they are commercial available, relatively inexpensive, and can produce a large volume of flowers. They also have the ability to add nitrogen to the orchard system, as discussed in Chapter 2. It is hypothesized that the increased floral resources and structural complexity in the cover crop treatments would support greater numbers of natural enemies, which would, in turn, provide greater pest regulation services. The dense and diverse range of flowering plants in the 'floral-rich' treatment was predicted to support the greatest abundance of natural enemies. The 'legume-grass' treatment was predicted to increase both structural complexity and floral abundance, though floral abundance was expected to be lower than that in the 'floral-rich' and 'oversown-clover' treatments and so a different community of species may be seen. The 'oversown-clover' treatment is expected to attract beneficial species but to a lesser extent due to its lower-growing plants and lower diversity in plant species and flower structures.

3.3 Methods

Study site

The study took place between June 2014 and September 2016 on two farms (located roughly 16 km apart) near Maidstone, Kent, England. Apple orchards used in the study were aged between 4 and 8 year old and contained the variety 'Gala', grafted onto 'M9' rootstocks. Tree spacing was 1 m within the row and 3.5 m between rows. A 2 m wide weed-strip was centred on the tree row with a 1.5 m wide swathe of vegetation in the centre of the alleyway. The orchard was managed conventionally with drip fertigation lines under each row of trees. The weed-strips in all plots were sprayed with glyphosate at commercial rates and, excluding the introduction and mowing of the alleyway cover crops, management of trees continued as standard for a conventional commercial orchard (details on management can be found in Appendix 1). The orchard blocks were managed as units and so that each treatment plot received the same management as the other plots in the same block.

Experimental design

Four orchard alleyway treatments were tested using a randomised complete block design, with six blocks split evenly between the two farms (24 experimental plots in total). Experimental plots consisted of six orchard alleyways containing five rows of trees, plots varied between 75 and 205 metres in length (0.14 to 0.39 ha in size). Plots were separated from each other by at least 40 m (fourteen tree rows) and from the outside of the orchard by a minimum of 10 m. Blocks were at least 200 m apart. In early May 2014 plots were randomly assigned to the treatments described in Table 1. Weeds were controlled in some of the plots in July and August 2014 with a strimmer and areas where cover crop germination was low were reseeded in August 2014.

Table 1. Treatment species composition, management, and estimated costs. All alleyways were cut at 15 cm with a side-discharging mower. Ground preparation and sowing took place early May 2014 and ground preparation costs are estimated using figures from Nix, 2014. NB - Costs are based on orchards with 2 m wide weed-strips and 1.5 m wide alleyways. Sowing weights and costs are relative to alleyway widths.

Treatment (percentage seed weight)	**Ground preparation and management**	**Estimated costs per hectare, longevity, and life time cost (with an orchard lifespan of 20 years)**
Mown-grass - (standard practice control) - Current vegetation, predominately perennial ryegrass (*Lolium perenne*). (in new orchards sown at 10.7 kg/ha)	No ground preparation. Cut at 5 cm every 7-10 days between March and September with a tractor mounted mower. Cuttings left in alleyways.	Established orchards: £0 New orchards: seed cost £46 Ground preparation £77 Total: £123, Lifetime cost: £123
Floral-rich - (sown at 5.6 kg/ha): Red clover (*Trifolium pratense*) 41.4% Birdsfoot trefoil (*Lotus corniculatus*) 19.7% Sainfoin (*Onobrychis viciifolia*) 18.6% Alsike clover (*Trifolium hybridum*) 10.1% Lesser knapweed (*Centaurea nigra*) 3% Musk mallow (*Malva moschata*) 3.1% Red campion (*Silene dioica*) 2.1% Oxeye daisy (*Leucanthemum vulgare*) 1.1% Yarrow (*Achillea millefolium*) 1.1%	Alleyways sprayed with glyphosate one week before disc harrowing twice and power harrowing once. Seed broadcast by hand and alleyways ring rolled to ensure good seed to soil contact. Mown in July and August in each year. In July only alternate rows were mown to encourage late flowering. Alleyways cut at 15 cm with a side-discharging mower.	Seed cost: £94 Ground preparation: £77 Total per hectare: £171 Estimated longevity: 3-4 years Lifetime cost: £855
Oversown-clover - sown at 3.4 kg/ha: Current vegetation, predominately perennial ryegrass (*Lolium perenne*), over-sown with: White clover (*Trifolium repens*) 75% (3 varieties) Black medic (*Medicago lupulina*) 25%	Alleyways disc harrowed once and seed broadcast by hand. Mown in June, July, and August in 2015, and July and August in 2016. Alleyways cut at 15 cm with a side-discharging mower.	Seed cost: £33 Ground preparation: £28 Total per hectare: £61 (for over sowing into existing swards) If allowed to seed re-sowing may not be needed.
Legume-grass - sown at 10.7 kg/ha: Lucerne (*Medicago sativa*) 72% Red clover (*Trifolium pratense*) 8% Cocksfoot grass (*Dactylis glomerata*) 12% Timothy grass (*Phleum pratense*) 8%	Alleyways sprayed with glyphosate one week before disc harrowing twice and power harrowing once. Seed broadcast by hand and alleyways ring rolled to ensure good seed to soil contact. Mown in June, July, and August 2015, and July and August in 2016. Alleyways cut at 15 cm with a side-discharging mower.	Seed cost: £77 Ground preparation: £77 Total per hectare: £154 Estimated longevity: 4-5 years Lifetime cost: £765

'Mown-grass' standard control

'Floral-rich'

'Oversown-clover'

'Legume-grass'

Figure 1. Alleyway cover crop mixtures. Experimental swards sown in May 2014, photographed here in June 2016. The control treatment was mown at a height of 5 cm once every 7-10 days between March and August. The three other treatments were mown at a height of 15 cm 2-3 times per year between April and August.

Vegetation cover and floral cover

Alleyway vegetation was surveyed using a 0.75 x 0.5 m quadrat which covered half of the alleyway width. Six randomly placed quadrats were taken in each plot every month between April and August in 2015 and 2016. The percentage cover of each plant species and the percentage floral cover for each plant species (i.e. the percentage cover of a species flowers) were visually estimated. and four measurements of vegetation height were taken for each quadrat using the direct measure method

(Stewart, Bourn & Thomas 2001). Sward height was recorded because it has been shown to affect hoverfly abundance and diversity (Sjödin, Bengtsson & Ekbom 2008).

Alleyway temperatures

One concern with growing taller, denser vegetation in alleyways is that it may reduce airflow and increase humidity, thereby increasing the risk of frost damage (Snyder & Connell 1993). To test whether the cover crops affected the temperatures in the orchards, thermometer data-loggers (Thermocron iButton, Maxim Integrated) were suspended in the middle of the central tree row at 50 cm above ground and were covered by upturned polystyrene cups to ensure they were shaded from the sun. Temperatures were recorded from March 2015 to June 2016, and December 2016 to May 2017. Daily mean, minimum, and maximum temperatures were calculated for analysis.

Invertebrate sampling

The abundance of natural enemies and pest species in the trees was ascertained by ‘tap’ sampling (also known as ‘beat’ sampling) where a funnel shaped net (70 x 43 x 50 cm) was held underneath a branch, and the branch struck twice with a padded stick. Falling invertebrates were collected in sample bags and frozen for later identification. Eighty branches per plot were sampled in this manner, one branch per tree at a height of roughly 1 metre. Sampling was conducted once per month from April 2015 to July 2015, and from April 2016 to August 2016. Invertebrates in the alleyways were sampled using the sweep net method, where a funnel net (36 x 25 x 50 cm) was swept back and forth through the alleyway vegetation over a 20 m transect, making two sweeps for every metre of alleyway. Invertebrates were collected in sample bags and frozen for later identification. Samples were taken monthly between May and August 2015, and April and August 2016. ‘Vortis’ suction sampling was trialled in April 2015 but was discontinued as capture rates were low. All sampling was conducted in dry conditions, with all plots within a block sampled within a few hours of each other. Invertebrate surveys were conducted before mowing in the cover crop plots.

Specimens were identified to family where possible, with parasitoid Hymenoptera grouped as Parasitica. Individuals belonging to taxa identified as important apple pests in the Horticultural Development Company Apple crop walker’s guide (Fountain & Saville 2013) were identified to species where possible. Herbivores belonging to species which were not considered apple pests were grouped together for analysis (families included Aphididae, Lygaeidae, Miridae, Acanthosomatidae, Triozidae, Delphacidae, Psyllidae, Cicadellidae, Apionidae, Curculionidae, and Chrysomelidae). A minority of the species belonging to the Miridae and Lygaeidae are predatory but the families were included as herbivores for simplicity. Predatory invertebrates were allocated to the following groups: predatory beetles (consisting of Carabidae, Cantharidae, Cocinellidae, and Staphylinidae), lacewings (Chrysopidae, Hemerobiidae), and earwigs (Forficulidae). Spiders were separated into two groups according to their hunting mode; web-forming spiders (Linyphiidae, Araneidae, Tetragnathidae, Theridiidae), and cursorial or active-hunting spiders (Gnaphosidae, Salticidae, Philodromidae, Lycosidae, Thomisidae, and Clubionidae) (Wise 1993). Detritivores were also identified to family where possible and mites were grouped as Acari, though these taxa were not included in the analysis.

Predation monitoring

Pea aphids (*Acyrthosiphon pisum*) were used as sentinel prey to estimate biological control services in the different treatments. Live aphids were attached to white plastic cards (85 x 55 mm) with odourless PVA glue, with an average of 13.3 aphids per card. Cards were attached to trees at a height of 1.5 m above ground level and were positioned so that the aphids were on the underside to reduce exposure. Six cards were attached to trees spread evenly throughout each plot and at least 15 m from the orchard edge. The number of aphids present on each card was recorded at the point of deployment and then again after being exposed to predators for 24 hours. Sentinel card surveys were carried out in August 2015, April 2016, May 2016, June 2016, and July 2016.

Statistical analysis

Data were analysed using linear mixed effects models and generalised linear mixed effects models (GLMMs) in R (R Core Team 2017) using the “lme4” (Bates, Mächler & Bolker 2012) and “glmmADMB” packages. Treatment was the main fixed effect with block nested within farm as random effects in all models. Vegetation height was log transformed and analysed using a linear model. Floral cover was analysed using a zero-inflated GLMM with negative binomial errors. Random effects were as above with quadrat nested within plot and block, and survey month as a crossed random effect. Temperatures were analysed with linear models with date as a crossed random effect. Invertebrate abundance data from tap sampling and sweep sampling were modelled using GLMMs with either Poisson or negative binomial error families depending on dispersion statistics. If dispersion statistics were greater than 2 with Poisson errors, negative binomial errors were used instead. Sampling month was nested within year as crossed random effects. Zero-inflated models were used where this resulted in a lower AIC. Aphid predation data were modelled using a binomial GLMM comparing the number of aphids present at the start of 24 hour period to the number remaining for each card in a two-column integer matrix. Random effects included card, nested within plot, block, and farm, with survey month as a crossed random effect. Treatments were compared with each other in all of these analyses using post hoc pairwise least-squares means tests with a Tukey adjustment in the R package “lsmeans” (Lenth 2016). Graphics were produced in R using the package “ggplot2” (Wickham 2009).

3.4 Results

Vegetation and floral cover

When considered over the whole year, floral cover was significantly higher in the ‘floral-rich’ and ‘oversown-clover’ treatments compared to the ‘legume-grass’ and ‘mown-grass’ control. Vegetation height was also significantly greater in all of the cover crop treatments when compared to the control (Figure 1). Further details about plant species percentage cover and flower cover can be found in Chapter 4.

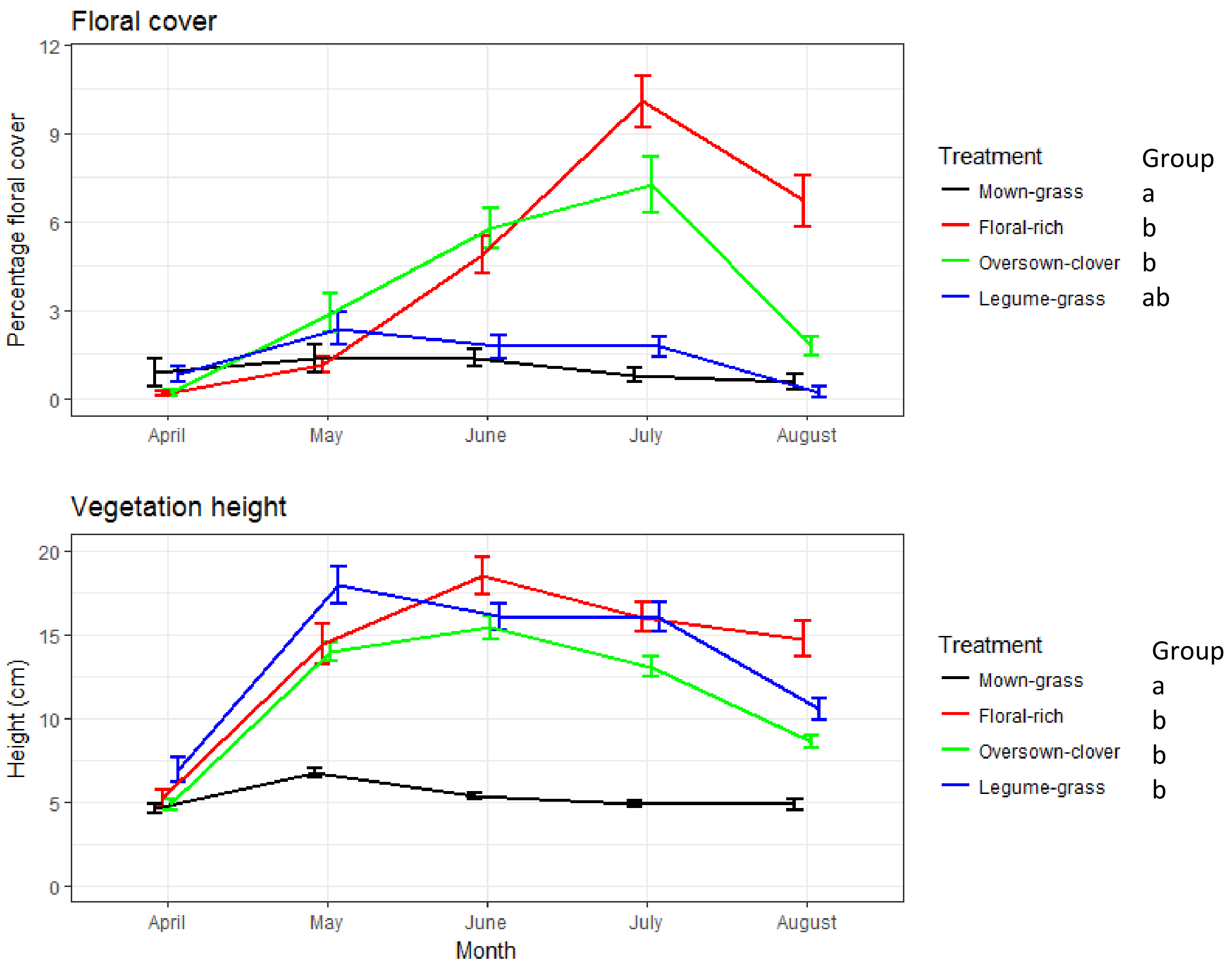

Figure 1. Mean percentage floral cover and vegetation height (mean ± SEM) in orchard alleyways with different sward mixtures (using combined data from 2015 and 2016). Group letters indicate significant differences over the whole survey period as calculated by post hoc least-squares means tests ($p<0.05$).

Temperature

Analyses of alleyway temperatures were conducted for two periods: spring (March, April, and May), when the risk of frost damage is greatest (Snyder & Connell 1993; Rodrigo 2000), and the year as a whole. No significant differences were seen between treatments, in either period, in the mean, minimum, or maximum daily temperatures at 50 cm above ground level. The diurnal temperature amplitude did not differ significantly either.

Invertebrate sampling

Several invertebrate taxa showed significant differences in abundance between the treatments, with variation seen in the results between the orchard alleyways (sweep netting) and the trees (tap sampling); the results of the analyses are shown in Table 2. The abundance of web-forming spiders did not differ significantly between treatments in either the trees or the alleyways. Active-hunting spiders were significantly more abundant in the trees of the ‘floral-rich’ and ‘legume-grass’ plots when compared to the ‘mown-grass’ and were significantly more abundant in the alleyways of all cover crop treatments when compared with the control. Lacewing abundance did not differ between treatments in the trees but was significantly higher in the ‘floral-rich’ and oversown-clover’ alleyways. Earwig abundance also showed no significant differences in the trees and numbers were too low for analysis in the alleyways. In comparison to the ‘mown-grass’ control predatory beetles were significantly more common in the trees of the ‘legume-grass’ treatments, and alleyways of the ‘floral-rich’ and ‘oversown-clover’ treatments. The number of Parasitica in the trees did not significantly differ between treatments whilst in the alleyways significantly higher numbers were found in the ‘floral-rich’ and ‘oversown-clover’ treatment than the ‘mown-grass’ control. Apple pests did not differ significantly between treatments in the trees, and did not occur in great enough numbers in the alleyways to allow meaningful analysis. Those herbivores which were not considered apple pests were significantly more abundant in all three cover crop treatments when compared to the ‘mown-grass’ control, in both the alleyways and the trees. No significant differences in any of the taxa discussed were found between the three cover crop treatments.

Table 2. Mean abundance of arthropod groups from monthly tree tap sampling (April to August, 2015 and 2016) and alleyway sweep sampling (May to August 2015 and April to August 2016). Statistically significant differences between treatments are indicated by bold values, as calculated by post hoc pairwise least-squares means tests (p<0.05).

Tree samples	**Treatment (mean ± SEM)**				***P* - values**					
Species group	**Mown-grass**	**Floral-rich**	**Oversown-clover**	**Legume-grass**	**Mown-grass/ Floral-rich**	**Mown-grass/ Oversown-clover**	**Mown-grass/ Legume-grass**	**Floral-rich/ Oversown-clover**	**Floral-rich/ Legume-grass**	**Oversown-clover/ Legume-grass**
Web-forming spiders	23.6 ± 2.5	27.6 ± 3.6	22.5 ± 2.6	29.4 ± 3.6	0.70	0.70	0.067	0.43	0.77	0.058
Active-hunting spiders	2.1 ± 0.4	3.9 ± 0.5	3.28 ± 0.5	4.0 ±0.4	**0.001**	0.069	**0.0002**	0.83	0.99	0.64
Lacewings	1.0 ± 0.2	1.5 ± 0.3	1.4 ± 0.4	1.4 ± 0.4	0.60	0.78	0.73	1.0	1.0	1.0
Earwigs	3.1 ± 0.8	2.1 ± 0.6	5.8 ± 1.5	2.7 ± 1	0.98	0.68	1.0	0.726	0.99	0.87
Predator beetles	0.9 ± 0.2	1.7 ± 0.4	1.4 ± 0.3	1.8 ± 0.4	0.085	0.67	**0.023**	0.86	0.99	0.63
Parasitica	0.9 ± 0.6	1.7 ± 0.8	1.45 ± 0.6	1.8 ± 0.6	0.68	0.51	0.99	0.99	0.93	0.86
Herbivores	8.7 ± 0.9	11.8 ± 1.3	13.0 ± 1.7	12.5 ± 1.9	**0.038**	**0.0012**	**0.019**	0.87	1.0	0.94
Pest aphids	10.7 ± 5.2	36.7 ± 18.7	25.0 ± 11.9	46.1 ± 13.3	0.27	0.94	0.26	0.83	1.0	0.81

Alleyway samples	**Treatment (mean ± SEM)**				***P* - values**					
Species group	**Mown-grass**	**Floral-rich**	**Oversown-clover**	**Legume-grass**	**Mown-grass/ Floral-rich**	**Mown-grass/ Oversown-clover**	**Mown-grass/ Legume-grass**	**Floral-rich/ Oversown-clover**	**Floral-rich/ Legume-grass**	**Oversown-clover/ Legume-grass**
Web-forming spiders	2.6 ± 0.4	3.9 ± 0.7	4.0 ± 0.6	3.3 ± 0.5	0.34	0.20	0.95	1.0	0.85	0.74
Active-hunting spiders	0.1 ± 0.04	1.1 ± 0.3	1.5 ± 0.6	0.6 ± 0.1	**0.0049**	**0.0035**	**0.0096**	0.99	1.0	0.91
Lacewings	0.07 ± 0.04	1.7 ± 0.5	0.5 ± 0.2	1.1 ± 0.3	**0.0001**	0.22	**0.0012**	0.42	0.96	0.61
Predator beetles	0.5 ± 0.1	1.3 ± 0.2	1.4 ± 0.3	0.9 ± 0.1	**0.0004**	**0.0006**	0.13	0.99	0.72	0.65
Parasitica	33.5 ± 5.6	75.9 ± 12.0	92.1 ± 13.7	55.5 ± 8.0	**0.0003**	**<0.0001**	0.083	0.54	0.61	0.05
Herbivores	18.6 ± 9.8	19.4 ± 2.8	27.46 ± 6.0	18.7 ± 5.4	**0.0002**	**0.0001**	**0.0076**	1.0	0.91	0.85

Predation monitoring

Pea aphid sentinel cards did not show significant differences in predation rates between the treatments (Figure 3). Aphid removal rates appeared to be higher later in the growing season. When collecting the sentinel cards the most commonly observed predators still feeding on the aphids were lacewings larvae (Neuroptera), spiders (particularly Philodromidae), and earwigs (Forficulidae).

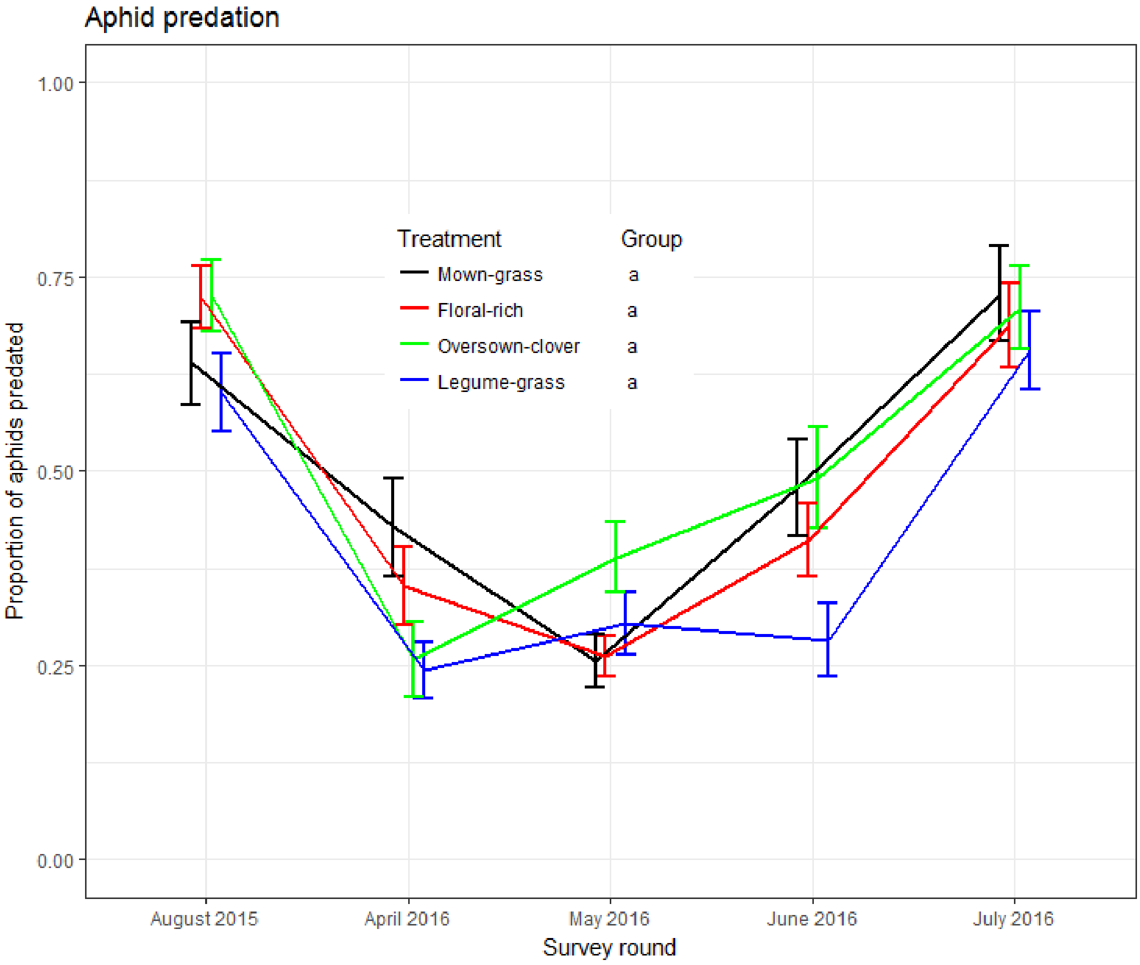

Figure 3. Proportion of pea aphids predated from sentinel cards (mean ± SEM) attached to trees in orchard plots with different alleyway sward mixtures. Group letters indicate significant differences over the whole survey period as calculated by post hoc least-squares means tests ($p<0.05$).

3.5 Discussion

This study shows that alleyway cover crops can provide habitat which attracts a range of natural enemies to orchards, leading to an increase in the numbers of natural enemies in both the alleyways and the trees. The ‘floral-rich’ and ‘oversown-clover’ treatments produced significantly more floral cover than the ‘mown-grass’ control, despite variability in establishment success across blocks. The floral cover was lower than expected in the ‘legume-grass’ treatment, whilst in the ‘oversown-clover’ treatment it was higher than expected and showed no significant difference with the more expensive ‘floral-rich’ treatment. The ‘oversown-clover’ treatment also grew higher than expected relative to the other cover crops; this may be because the movement of tractors through the alleyways reduced the height of the taller plants in the ‘floral-rich’ and ‘legume-grass’ treatments, where much of the growth was restricted to the centre of the alleyways between the paths of tractor wheels. The mean vegetation heights in all three cover crop treatments were still significantly greater than in the regularly mowed control plots which displayed very little seasonal variation in height. Regular mowing of grasslands can directly increase mortality rates in invertebrates (Humbert *et al.* 2010), and reducing mowing frequency and leaving areas uncut has been shown to increase the abundance of beneficial species and other biodiversity (Morris 2000; Cizek *et al.* 2012; Humbert *et al.* 2012; Buri, Arlettaz & Humbert 2013; Bruppacher *et al.* 2016). A reduction in mowing frequency in orchard alleyways has also been shown to increase the numbers of parasitoids and predators and may improve pest regulation services (Horton *et al.* 2003, but see Marliac *et al.* 2015).

As hypothesised, some natural enemy groups were present in significantly higher numbers in the cover crop treatments, however not all taxa showed this response, and the effect was more pronounced in the alleyway vegetation than in the trees. Increasing floral density and diversity is known to have a positive effect on hoverflies, parasitoids, and other flower-feeding natural enemies such as some of the predatory beetles, with sown wildflower strips proving an effective way to increase both their abundance and diversity (Haenke *et al.* 2009; Haaland, Naisbit & Bersier 2011; Markó *et al.* 2012; Jönsson *et al.* 2015). For this reason the higher abundances of these taxa seen in the alleyways of the relatively floristically diverse and abundant ‘floral-rich’ treatment was expected. The higher abundances seen in the ‘legume-grass’ and ‘oversown-clover’ treatments are less likely to be due to their floral cover. The floral cover of the ‘legume-grass’ treatment did not significantly differ from that of the ‘mown-grass’ control and although more flowers were present in the ‘oversown-clover’ treatment the majority were white clover flowers, which have long-corollas which are not favoured by many natural enemies such as parasitoids (Branquart & Hemptinne 2000; Wäckers 2004; Vattala *et al.* 2006; Wäckers & van Rijn 2012; Campbell *et al.* 2012). Greater structural, or architectural, complexity can also increase the habitat value of a sward for a range of species, including important natural enemies and their prey species (Langellotto & Denno 2004; Woodcock *et al.* 2007; Monzó *et al.* 2011). The greater abundances of natural enemies seen in the cover crop treatments is likely to have been at least in part due to the increase in herbivore prey species. Many of the natural enemies which showed increases in abundance are generalist, opportunistic predators which feed on a range of prey species. These natural enemies are capable of providing significant pest regulation services, though inter- and intraspecific predation and competition is likely to be common, which may make predicting biological control effectiveness difficult (Symondson, Sunderland & Greenstone 2002; Östman 2004; Markó *et al.* 2012). Spiders are known to be important for pest regulation in orchards and they were the most common natural enemies found in the trees, which is consistent with a number of other studies (Wyss, Niggli & Nentwig 1995; Marc & Canard 1997; Markó & Keresztes 2014). The different hunting behaviour employed by a spider species can affect its impact on the wider ecosystems; active-hunting spiders, which were significantly more abundant in the cover crop plots, have been shown to reduce the numbers of aphid pests in wheat crops (Birkhofer *et al.* 2008) and to increase primary productivity in grasslands (Schmitz 2008). Supporting a diversity of spider species and hunting types can increase predation of pest species in orchards (Marc & Canard 1997). Ants were not included in the analysis because, although they can be effective predators of some apple pests (Mathews, Bottrell & Brown 2004), they can also be detrimental to apple production as they adopt mutualistic relationships with a number of aphid pests (Cross *et al.* 2015).

The number of aphid pests was not affected by ground cover treatments, nor did the sentinel aphids show significant differences in predation rates. Woolly apple aphids were by far the most numerous species of pest aphid found during the study; more than 92% of all aphids. This species forms colonies which can persist for many years and the vast majority of the specimens collected were from two neighbouring orchard blocks. Pilot tap sample surveys conducted in 2014 showed that similar numbers were present before the cover crops were established. The higher numbers of herbivores seen in the cover crop treatments may explain why, despite the higher abundances of some natural enemies, no differences were detected in the predation of pea aphids: the natural enemies may have been feeding on other species. It seems likely that most of these herbivores were feeding on the cover crops rather than the apple trees as they are not considered to be apple pests. This study took place in a commercial orchard which was being managed with a conventional pesticide programme (more details can be found in Appendix 1); this is likely to have affected the abundance of many species including pests and natural enemies (Dib, Sauphanor & Capowiez 2016). The differences observed in arthropod abundances between the treatments were therefore seen despite the potentially confounding effects of intensive management.

Pesticides can have unintended negative impacts on agroecosystems, including harmful effects on important natural enemies such as earwigs and spiders (Pekár 2012; Fountain & Harris 2015). A number of studies have shown that reducing pesticide sprays can help to boost numbers of natural enemies in orchards (Pekár 1999; Miliczky, Calkins & Horton 2000; Markó *et al.* 2009; Cárdenas *et al.* 2015). A modified pesticide regime may therefore be necessary if the natural enemy populations supported by habitat management practices are to provide an effective and resilient pest regulation service. It is possible that the populations of natural enemies and pests in this study may have responded differently to the cover crops under less intensive management. It is also possible that if the cover cropped orchards had been observed over a longer timescale the populations of natural enemies and other orchard species may have changed further and an increase in the more slowly reproducing species may have been observed. If natural enemy populations are not given the resources needed to establish suitably large populations, or the time needed to recover from pesticide-intensive management, reductions in sprays could lead to rapid increases in pest species.

Other studies have also found that adding areas of flowering plants and increasing the diversity of ground covers can have a positive effect on natural enemies in a range of fruit crops including blueberries (O’Neal *et al.* 2005; Blaauw & Isaacs 2015), vineyards (English-Loeb *et al.* 2003; Danne *et al.* 2010), and olive groves (Paredes, Cayuela & Campos 2013). Cover crops have not always been observed to increase the abundances of natural enemies however, and in a minority of studies an increase in pest species has been observed with the addition of flowering cover crops (Bone *et al.* 2009). Even in those studies where natural enemy numbers have increased, this has not always resulted in a detectable improvement in pest regulation services. Methods of detecting pest regulation services, beyond measuring the abundance of pest species, often involve the use of sentinel prey similar to those used in this study. Whilst this method has the advantage of producing clear outcomes in terms of number of prey eaten, it does have some drawbacks and may not be a fully reliable measure of pest regulation services as it creates aggregates of immobile prey which may be consumed by a single large predator (Furlong & Zalucki 2010). Sweep sampling and tap sampling methods also have some flaws; sweep sampling can be affected by vegetation height and density and both methods may over- and underestimate the abundances of some species depending on their behaviour. Nocturnal species, which include several important natural enemy taxa such as earwigs, and many carabid and active-hunting spider species, may well be underestimated with these sampling methods unless surveys are conducted during the night.

3.6 Conclusions and management recommendations

The results demonstrate that sowing cover crops, coupled with a reduction in the frequency of mowing, can have a positive effect on orchard biodiversity and boost the numbers of beneficial species. If habitat management interventions such as cover crop strips are to be optimal it is likely that they will need to provide both floral resources and structural complexity. Optimal species mixtures will depend on which pest species are an issue and so which natural enemies need to be supported. Although the height of the cover crops did not appear to reduce orchard temperatures, suggesting that they would not increase the risk of frost damage, other studies have found that vegetation in the alleyways can reduce temperatures and this potential effect should be considered in areas where frost damage is a risk (Snyder & Connell 1993; O'Connell & Snyder 1999). Tall vegetation in the alleyways may impede some orchard management practices, especially those which are carried out on foot (e.g. harvesting). It has also been suggested that taller vegetation could help to suppress the movement of apple scab ascospores (Aylor 1998). In this study, the cover crops were mown at the end of August, in part to make movement in the alleyways easier during harvesting. Mowing could also be used to reduce cover crop height during times of increased frost risk. The timing of mowing is also important to orchard fauna and generally mowing later in the season is better for arthropod communities (Potts *et al.* 2009; García & Miñarro 2014). Mowing trials in flower rich grasslands have found that performing the first cut in mid-July or later, and removing the cuttings if possible, can help to encouraging flowering species (Wheeler & Wilson 2016). Spreading cuttings onto the weed-strip using side-discharging mowers may be an effective way to mulch the trees, see Chapter 2.

Although this work was conducted in an apple orchard, the findings may be relevant to other perennial row-grown crops. It should be noted however, that the varied management requirements, pest regulation needs, and pollination demands of different crops means that if cover crops and other habitat management practices are to produce optimal results they may need to be tailored to suit these conditions (Bugg & Waddington 1994; Fiedler, Landis & Wratten 2008; Bone *et al.* 2009; Hogg, Bugg & Daane 2011). Although further work is needed to determine the most suitable plant mixtures and management practices, this study shows that alleyway cover cropping, and generally improving the habitat value of alleyways, may be an effective method for the ecological intensification of orchards.

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Chapter 4:

Orchard ground cover management for pollinators and pollination services

('Floral-rich' plot during apple blossom, May 2016)

Chapter 4:

Orchard ground cover management for pollinators and pollination services

4.1 Abstract

Pollinator-dependent crops are becoming increasingly important in global agricultural production, though the pollinator species responsible for their pollination are facing a number of threats. Many of the causes of wild pollinator declines are due to intensive management methods and the loss of habitat and floral resources from agricultural landscapes. In this study, flowering alleyway cover crops were trialled as a method of habitat management to enhance floral resources for pollinators in commercial apple orchards. A randomised complete block design with an average plot size of 0.25 ha was used to compare three alleyway cover crop mixtures with a standard control consisting of closely mown grass. Cover crops were based on perennial legume species: 1) a commercially available 'pollen and nectar' mixture, 2) a legume and tussock-forming grass mix, and 3) a low-cost, low-diversity mix over-sown into existing alleyway vegetation. Alleyway cover crops provided a significant increase in floral resources throughout the summer months and boosted the numbers of pollinator taxa in the alleyways of orchards during this period. However, no significant changes in pollinator abundance or pollination service were observed during apple blossom. The lack of effect of alleyway cover crops on pollination services may have been due to the early-season, mass-flowering nature of apple trees: cover crops did not come into full flower until after apple blossom had ended, and the highly mobile pollinator species did not appear to show fidelity to cover cropped areas early in the season. The size of the treatment plots and duration of the study may also have not been great enough to produce a detectable response in pollinator populations. The results from the study suggest that the addition of even relatively inexpensive flowering plants to orchard alleyways, coupled with a reduction in mowing frequency, can create an attractive source of forage plants and increase the numbers of foraging pollinators in areas containing highly pollinator-dependent crops.

4.2 Introduction

The production of pollination dependent mass-flowering crops is increasingly globally, and the demand for pollination services is increasing with it (Klein *et al.* 2007; Aizen *et al.* 2008; Breeze *et al.* 2011; FAO 2017). Pollinating species are therefore becoming increasingly important to global agriculture (Klein *et al.* 2007; Aizen & Harder 2009; Garibaldi *et al.* 2011a). Some pollination dependent crops, including apples, may be suffering from pollination deficits (Aizen *et al.* 2009; Isaacs & Kirk 2010; Garibaldi *et al.* 2014; Garratt *et al.* 2014b). Ecologically intensifying production by supporting wild pollinator species and enhancing pollination services may allow these yield and crop quality gaps to be closed (Bommarco, Kleijn & Potts 2013).

The floral resources provided by mass-flowering crops can be beneficial for pollinators (Westphal, Steffan-Dewenter & Tscharntke 2003; Holzschuh *et al.* 2013). However, the effect that these crops have on pollinator populations and other beneficial species will depend on the intensity of their management: intensive use of pesticides can negatively effects pollinators and other beneficial species (Gill, Ramos-Rodriguez & Raine 2012; Whitehorn *et al.* 2012; Mallinger, Werts & Gratton 2015), and if mass-flowering crops are produced in monocultures their benefits to pollinators may be short lived (Hanley *et al.* 2011). The end of the crop flowering period may bring a seasonal gap in food availability in homogenous landscapes, as once the abundance of pollen and nectar from crop flowers has finished there may be few other flowering plants available (Westphal, Steffan-Dewenter & Tscharntke 2009; Hanley *et al.* 2011). Natural and semi-natural areas are important habitat for pollinators and other beneficial species, providing alternative sources of food, shelter, and nesting sites (Kremen *et al.* 2004). They can also act as refuges from environmental extremes, and from pesticide exposure (Park *et al.* 2015a). The intensification of agricultural land and the loss of habitat has been shown to lower the abundance and diversity of wild bees and reduce the pollination service which they provide (Kremen, Williams & Thorp 2002; Hendrickx *et al.* 2007; Ricketts *et al.* 2008; Garibaldi *et al.* 2011b; Marini *et al.* 2012; Kennedy *et al.* 2013).

Modern apple orchards commonly contain 3000 trees per hectare, with hundreds or thousands of flowers per tree, and a flowering period (blossom) of only a few weeks. This creates a huge short-term demand for pollination and may result in competition with other crops (Garratt *et al.* 2014c; Grab *et al.* 2017). Since most commercial apple varieties are self-incompatible they require pollen from different varieties to develop seeds (Ramírez & Davenport 2013). For this reason, trees of a different variety are often planted throughout an orchard to provide sources of compatible pollen, these trees are known as 'pollinisers' (Barden & Neilsen 2003). Unfertilised flowers often fail to produce fruit, or may produce fruit of a lower quality with a greatly reduced market value (Garratt *et al.* 2014a). Apples are primarily insect-pollinated (Free 1964; Dennis, Ferree & Warrington 2003) and rely on a range of taxa including bumble bees (*Bombus* spp.), solitary bees, honey bees (*Apis mellifera*), and hoverflies (Syrphidae) (Ramírez & Davenport 2013). Many of these species are currently under threat from habitat loss, harmful pesticides, climate change, and a variety of pests and diseases (Potts *et al.* 2016). Honey bees are some of the most numerous pollinators of apple but the number of both honey bees hives and honey bee keepers has shown declines in Europe and North America (Aizen & Harder 2009; Neumann & Carreck 2010; Potts *et al.* 2010b). Bee keepers also often charge growers for the use of their hives and hive availability may vary between regions and years (Carreck, Williams & Little 1997; Sumner & Boriss 2006). It is crucial for food production, therefore, that wild pollinator communities are supported (Klein *et al.* 2007). Wild pollinators can enhance the pollination of crops despite the presence of honey bees (Garibaldi *et al.* 2013) and more diverse wild bee communities have been shown to improve pollination services in apple orchards and other crops (Klein *et al.* 2003; Hoehn *et al.* 2008; Holzschuh, Dudenhöffer & Tscharntke 2012; Martins, Gonzalez & Lechowicz 2015; Blitzer *et al.* 2016). A diversity of wild pollinator species can also help to insure against climate change (Rader *et al.* 2013; Bartomeus *et al.* 2013) and further declines in honey bee abundance (Winfree *et al.* 2007). To maintain healthy, stable pollinator communities it is important that the resources which they need are available to them throughout their active seasons (Roulston & Goodell 2011). Pollinators need food sources which will provide them with both nutrients and energy, in most cases this means pollen and nectar, but for some taxa such as hoverflies it also means prey species for their larval stages.

As with natural enemies and pest regulation services (Chapter 3), one way in which pollinator populations and pollination services can be enhanced is through habitat management, particularly the creation and restoration of areas of flowering plants (Pywell *et al.* 2005; Albrecht *et al.* 2007; Carvell *et al.* 2011; Wratten *et al.* 2012). The loss of flowering plants is thought to be a significant factor in the decline of pollinators in agricultural landscapes (Nicholls & Altieri 2013), with the decline in species such as red clover (*Trifolium pratense*) being one of the main drivers behind bumble bee declines in the UK (Carvell *et al.* 2006). The creation of flower strips and flower-enhanced grass margins is a common method for supporting pollinator communities and is supported by several agri-environment schemes (Pywell *et al.* 2007; Albrecht *et al.* 2007). However there are still knowledge gaps regarding the efficacy and management of flower strips and flowering cover crops for supporting pollinator communities and enhancing ecosystem services, though they have shown potential in both arable and fruit systems (Pywell *et al.* 2005; Carvalheiro *et al.* 2012; Blaauw & Isaacs 2014; Feltham *et al.* 2015). One of the key aims of managing habitat for pollinators is to maintain floral resources throughout their active seasons. The relatively early and short flowering period of apple trees means that the crop does not provide floral resources during the summer and autumn. Orchard ground cover plants have the potential to provide an important food source to pollinators and other arthropods through these seasons (Saunders, Luck & Mayfield 2013; García & Miñarro 2014). However, ground cover management in many commercial orchards currently favours bare soil weed-strips and closely mown alleyways (Merwin, Ferree & Warrington 2003). The alleyways between rows make up a significant amount of the orchards land area, but because their management is focused almost exclusively on controlling weeds they usually provide little in the way of habitat for beneficial species. If flowering plants could be grown in these alleyways they could provide considerable and widespread resources for beneficial species without requiring a significant change in management. More knowledge is needed, however, both on which plant species are the most valuable to beneficial arthropods needed in orchards, and which plants are suitable for growing in alleyways; orchard alleyways are subjected to regular vehicle traffic and may be shaded by the neighbouring trees.

In this study, three alleyway cover crop mixtures were tested and their effects on pollinators and pollination services are assessed. Each of the cover crop mixtures were designed with a different focus: the 'floral-rich' mixture was based on a widely available 'pollen and nectar' mixture to provide maximum floral resources from a variety of plant species and flower structures. The 'legume-grass' mixture was designed to increase structural complexity and produce a large volume of vegetation. The 'oversown-clover' mixture is a low-diversity budget option which requires less change to current management. These mixtures were compared to the standard practice of regularly mown, grass-dominated alleyways. Perennial legumes were chosen as the basis for the cover crop mixtures because they can produce a large volume of flowers which are known to be attractive to pollinators (Carvell *et al.* 2007, 2011, Pywell *et al.* 2007, 2011). These species are also often less expensive than other wildflowers, and being perennial they should require less management than annual species. Legumes also have the ability to add nitrogen to the orchard system, as discussed in Chapter 2. Other perennial wildflower species are included in the 'floral-rich' treatment, at lower seed densities, as they have open flower structures which are favoured by short-tongue bees, hoverflies, and natural enemies (Campbell *et al.* 2012; Balzan, Bocci & Moonen 2014).

It is hypothesized that the treatment with the greatest floral diversity and abundance, i.e. the 'floral-rich' treatment, will attract the greatest abundance and diversity of pollinators. The improved habitat expected in the 'legume-grass' and 'oversown-clover' treatments are also predicted to increase pollinator abundance compared to the 'mown-grass' control treatment. The greater pollinator abundances predicted in the cover crop treatments are expected to result in improved pollination services and an increase in apple production and fruit quality.

4.3 Methods

Study site

This study took place between June 2014 and September 2016 on two farms (located roughly 16 km apart) near Maidstone, Kent, England. Apple orchards used in the study were aged between 4 and 8 years old and contained the variety 'Gala', grafted onto 'M9' rootstocks. Polliniser trees were a mixture of crab apples (*Malus spp.)* and the apple variety 'Golden delicious'. Tree spacing was 1 m within the row and 3.5 m between rows, with polliniser trees planted between each tenth and eleventh tree (at a ratio of 1:10 with the crop variety), staggered between rows so that pollinisers were evenly spaced. A 2 m wide weed-strip was centred on the tree row with a 1.5 m wide strip of vegetation in the centre of the alleyway where the cover crops were sown. The weed-strips in all plots were sprayed with glyphosate at commercial rates and, excluding the introduction and mowing of the alleyway cover crops, management of trees continued as standard for a conventional commercial orchard. The orchard blocks were managed as units and so that each treatment plot received the same management as the other plots in the same block.

Experimental design

The four orchard alleyway treatments were tested using a randomised complete block design, with six blocks split evenly between the two farms (24 experimental plots in total). Experimental plots consisted of six consecutive orchard alleyways containing five rows of trees, plots varied between 75 and 205 m in length (0.14 to 0.39 ha in size). Plots were separated from each other by at least 40 m and from the outside of the orchard by a minimum of 10 m. Blocks were at least 200 m apart. In early May 2014 plots were randomly assigned to the treatments described in

Table 1. Weeds were controlled in some of the plots in July and August 2014 with a strimmer and areas where cover crop germination was low were reseeded in August 2014. For more details on cover crop species composition, management, and costs please see Table 1 in Chapter 3.

Vegetation cover and floral cover

Alleyway vegetation was surveyed using a 0.75 x 0.5 m quadrat which covered half of the alleyway width. Six quadrats were taken in each plot every month between April and August in 2015 and 2016. Vegetation cover and floral cover were recorded for each plant species by visually estimating their percentage area cover in each quadrat. Four measurements of vegetation height were also taken for each quadrat using the direct measure method (Stewart, Bourn & Thomas 2001). Sward height was recorded because it has been shown to affect hoverfly abundance and diversity (Sjödin, Bengtsson & Ekbom 2008).

Pollinator transects

Pollinator transects were conducted in 2015 and 2016 during apple blossom (April and May) and in the summer months of June (2016 only), July, and August. At the study sites apple blossom peaked in late April in 2015 and early May in 2016. Four transects per plot were walked during each blossom period and two transects per plot in each non-blossom month, with half of the transects conducted before noon and half afterwards. Each transect consisted of a 10 minute, 100 m walk where all pollinators observed on the trees and within the alleyway were recorded to morphospecies, or species where possible. Transects were conducted between 0900 h and 1730 h, in dry, warm conditions (>13°C), where wind speeds were below 2 on the Beaufort scale. If a pollinator was seen on a flower, the species of plant was recorded. During blossom a relative estimate of apple flower numbers was calculated for each transect by counting the number of open flowers on one side of two randomly selected trees. For analysis pollinators were divided into four taxa; bumble bees (*Bombus* spp.), solitary bees, honey bee (*Apis mellifera*), and hoverflies (Syrphidae). The number of other flies seen in the alleyways was also recorded but due to their wide variety of feeding behaviours, and with little evidence to suggest that they are important apple pollinators (Garratt *et al.* 2014b; a), they were not included in the analysis. The potential contributions of different taxa to apple pollination are discussed further in Chapter 5.

Honey bee hives were not present within the orchard blocks used in this study, though they were present in neighbouring blocks on one of the farms.

Pollination service monitoring and apple quality

Pollination service was monitored by recording the fruit set on thirty branches in each plot; one branch per tree in three sets of ten trees spread evenly throughout the plot (a total of 720 branches from all plots in each year). This method was adapted from previous studies where pollination services to apples and other tree fruit were investigated and where fruit set has been shown to be an strong indicator of pollination (Volz, Tustin & Ferguson 1996; Isaacs & Kirk 2010; Garratt *et al.* 2014a). During blossom the number of flowers present on each branch was recorded and the branch was tagged. In June, the number of fruitlets that had formed on each branch was recorded. Approximately one week before commercial harvest the number of fruit on each branch was recorded again. To determine fruit size the diameters of eight fruit (or all fruit if less than eight were present) were recorded on each branch. To determine seed set six branches per plot were randomly selected and three fruit (or all fruit if less than three were present) were taken from each branch. Apple quality measures were taken on the fruit including fruit diameter, firmness (using a Silverline penetrometer), soluble solids or Brix (using a Hanna refractometer), fresh weight, and dry weight (entire fruit were cut into 4 pieces and oven dried at 70°C for at least 72 hours before reweighing). In total 1,170 fruit were collected for seed set and further fruit quality assessments.

Statistical analysis

Data were analysed with linear mixed models and generalised linear mixed models (GLMMs) in R (R Core Team 2017) using the "lme4" (Bates, Mächler & Bolker 2012) and "glmmADMB" packages. Treatment was the main fixed effect with block nested within farm as random effects in all models. Percentage floral cover was analysed using GLMMs with a negative binomial error family. The random effects in these models were: quadrat, nested within plot, nested within block, with month as a crossed random effect. Pollinator abundance data from transects conducted during blossom (April and May) were pooled and analysed separately to those from the summer transects (June, July and August). Pollinator counts were also pooled for each summer month, and sampling month was used as a random effect in the summer pollinator models, with survey year was included as a fixed effect. Models were given either Poisson or negative binomial error families, depending on over-dispersion statistics: if dispersion statistics were greater than 2 with Poisson errors, negative binomial errors were used instead. Fruit set data were modelled using a GLMM with a binomial error family, comparing the number of flowers which produced fruitlets to the number which did not in a two-column integer matrix.

Seed set data were modelled using GLMMs with a negative binomial error family. All other fruit quality measures were analysed using linear mixed models with apple number nested within branch, plot, block, and farm. Year (either 2015 or 2016) was included as a random effect in all fruit yield and quality models. Comparisons between cover crop treatments in all of these analyses were conducted using post hoc pairwise least-squares means tests with a Tukey adjustment in the R package “lsmeans” (Lenth 2016). Graphics were produced in R using the package “ggplot2” (Wickham 2009).

4.4 Results

Vegetation and floral cover

The floral cover and vegetation height of the alleyways are shown in Figure 1. The floral cover of the alleyways showed no significant differences between treatments during the blossom period, whilst in the summer months the ‘floral-rich’ and ‘oversown-clover’ treatments showed significantly more flowers than the ‘legume-grass’ and ‘mown-grass’ control. The ‘legume-grass’ treatment had significantly higher vegetation than the control during blossom and all three cover crops showed significantly higher vegetation than the control during the summer months. A breakdown of alleyway vegetation cover by species, floral cover by species, and the number of pollinators (bumble bees, solitary bees, honey bees, and hoverflies) observed feeding on different plant species during transects is shown in Table 1.

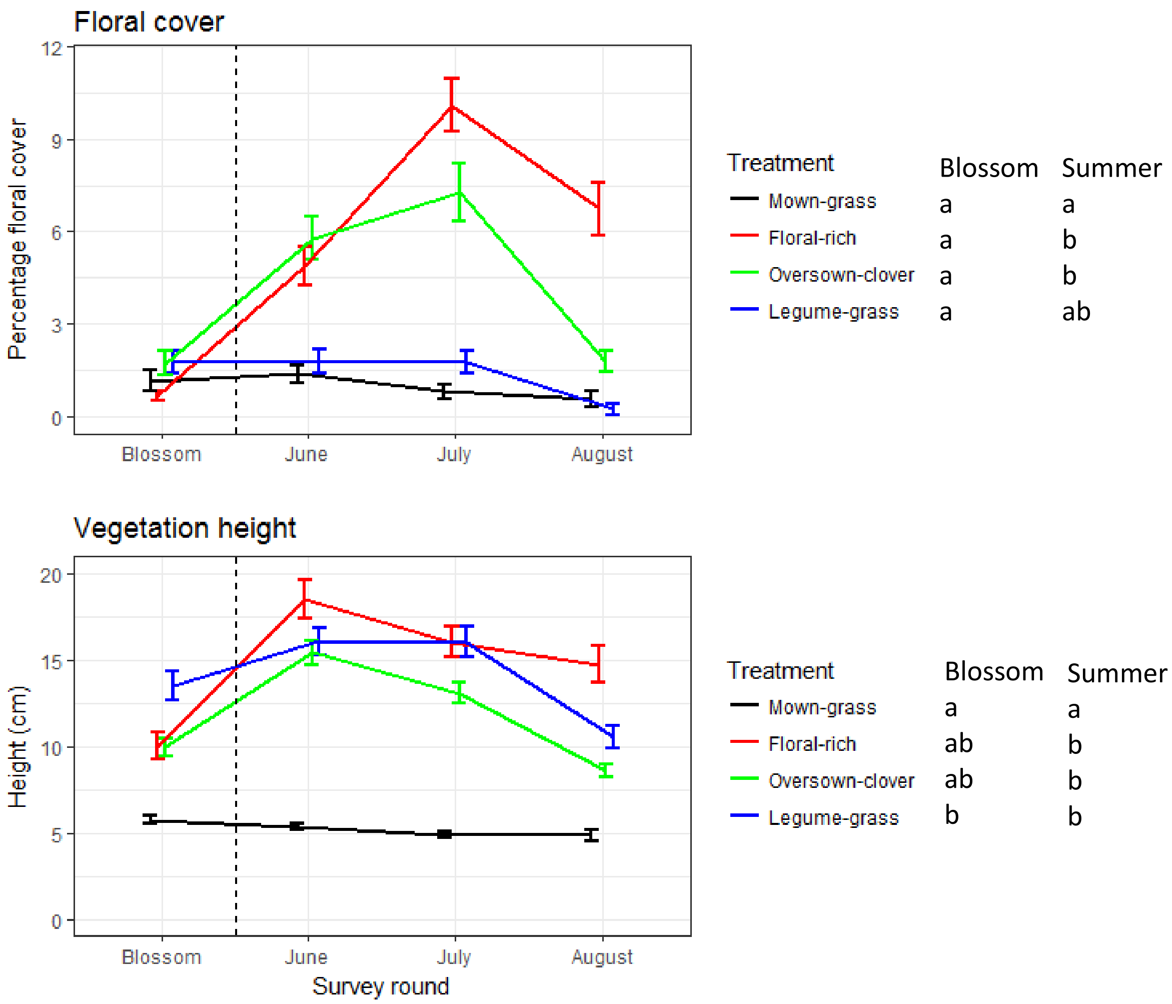

Figure 1. Floral cover and vegetation height of orchard alleyways with different sward mixtures during apple blossom (April and May), and summer months (June, July, and August) using combined data from 2015 and 2016. Letters indicate significant differences as calculated by least square means tests (p=0.05).

Table 1. Mean alleyway vegetation species cover and floral cover between April and August. Only sown species and species showing more than 0.1% vegetation cover or 0.01% floral cover when averaged over the two year are shown here. Total visit count indicates the total number of pollinators observed feeding on alleyway flowers during transects, ‘Visits by pollinators, % of total’ shows percentage breakdown of which flowers were being visited. These include visits made by bumble bees, solitary bees, honey bees, and hoverflies. Data were collected between April and August, 2015 and 2016.

Treatment and species	**Seed weight %**	**Species cover %**				**Floral cover %**				**Visits by pollinators % of total**		
		2015	**2016**	**Average**		**2015**	**2016**	**Average**		**2015**	**2016**	**Average**
Mown-grass (control)					**Total floral cover**	**1.3**	**0.8**	**1**	**Total visit count**	**38**	**13**	**51**
Grasses (Poaceae)		85.5	83.6	**84.6**								
White clover (*Trifolium repens*)		14.1	4.6	**9.4**		0.4	0.03	**0.2**		64.3	14.3	**39.3**
Buttercup (*Ranunculus* spp.)		3.7	3.8	**3.8**		0.1	0.05	**0.08**		0	14.3	**7.1**
Common daisy (*Bellis perennis*)		2.8	4.1	**3.5**		0.7	0.7	**0.7**		7.1	33.3	**20.2**
Mosses (Bryophyta)		1.1	2.5	**1.8**								
Dock (*Rumex* spp.)		1.9	1	**1.5**								
Greater plantain (*Plantago major*)		0.9	0.6	**0.8**								
Dandelion (*Taraxacum officinalis*)		0.9	0.5	**0.7**		0.02	0.01	**0.01**		6.1	19	**12.6**
Black medic (*Medicago lupulina*)		0.02	0.3	**0.2**		0.01	0.03	**0.02**		0	0	**0**
Hawkbits (*Leontodon* spp.)		0.1	0.05	**0.08**		0.04	0	**0.02**		18.4	4.8	**11.6**
Bare soil		4.2	7.3	**5.8**								
Floral-rich - sown at 13 kg/ha:					**Total floral cover**	**5.1**	**4**	**4.6**	**Total visit count**	**369**	**357**	**726**
Red clover (*Trifolium pratense*)	**41**	16.3	4.3	**10.3**		1.2	0.2	**0.7**		11.8	7.9	**9.4**
Birdsfoot trefoil (*Lotus corniculatus*)	**20**	8.1	3.5	**5.8**		1.9	0.5	**1.2**		24.6	10.9	**16.4**
Sainfoin (*Onobrychis viciifolia*)	**19**	6	0.01	**3**		0.04	0	**0.02**		0.7	0	**0.3**
Alsike clover (*Trifolium hybridum*)	**10**	0.2	0.4	**0.3**		0.04	0.1	**0.07**		16.2	5.6	**9.8**
Lesser knapweed (*Centaurea nigra*)	**3**	1	2.5	**1.8**		0.01	0.08	**0.05**		0	4.1	**2.4**
Musk mallow (*Malva moschata*)	**3.1**	5.4	5	**5.2**		0.7	0.6	**0.7**		11	22.2	**17.7**
Red campion (*Silene dioica*)	**2.1**	1.2	0.5	**0.9**		0.09	0.06	**0.08**		3.8	0	**1.5**

Oxeye daisy (*Leucanthemum vulgare*)	**1.1**	3.7	6.3	**5**		0.4	0.8	**0.6**		17.1	20	**18.8**
Yarrow (*Achillea millefolium*)	**1.1**	4.3	6.8	**5.6**		0.07	0.3	**0.2**		6	7	**6.6**
Grasses (Poaceae)		62.2	62.2	**62.2**								
Dock (*Rumex* spp.)		4.5	7.3	**5.9**								
Buttercup (*Ranunculus* spp.)		3.2	3.8	**3.5**		0.3	0.5	**0.4**		1.1	3.5	**2.5**
Greater plantain (*Plantago major*)		2.5	3	**2.8**								
White clover (*Trifolium repens*)		0.03	4.5	**2.3**		0.01	0.4	**0.2**		0.4	13.5	**8.2**
Common daisy (*Bellis perennis*)		0.3	0.9	**0.6**		0.1	0.3	**0.2**		0	1.2	**0.7**
Ribwort plantain (*Plantago lanceolata*)		0.8	0.1	**0.5**								
Black medic (*Medicago lupulina*)		0.04	0.7	**0.4**		0.01	0.1	**0.06**		0	0	**0**
Dandelion (*Taraxacum officinalis*)		0.3	0.2	**0.3**								
Thistles (*Cirisum* spp.)		0.2	0.2	**0.2**		0.01	0.01	**0.01**		0	0	**0**
Hawkbits (*Leontodon* spp.)		0.3	0.1	**0.2**		0.2	0.03	**0.1**		6.9	2.1	**11.6**
Sowthistle (*Sonchus* spp.)		0	0.2	**0.1**								
Mayweed (*Matricaria* spp.)		0.1	0	**0.05**		0.03	0	**0.02**		0	0	**0**
Bare soil		5.5	9	**7.3**								
Oversown-clover - over-sown at 8 kg/ha:					**Total floral cover**	**2.9**	**5**	4	**Total visit count**	**282**	**246**	528
White clover (*Trifolium repens*)	**75**	36.5	37.6	**37.1**		2.1	3.1	**2.6**		70.1	68	**68.8**
Black medic (*Medicago lupulina*)	**25**	0.6	1.7	**1.2**		0.01	0.5	**0.3**		1.6	1.4	**1.5**
Grasses (Poaceae)		79.7	70.1	**74.9**								
Buttercup (*Ranunculus* spp.)		5.8	7.6	**6.7**		0.5	0.9	**0.7**		2.5	13.3	**9**
Dock (*Rumex* spp.)		1.1	1.5	**1.3**								
Common daisy (*Bellis perennis*)		0.2	1	**0.6**		0.07	0.5	**0.3**		6.3	0.3	**2.7**
Dandelion (*Taraxacum officinalis*)		0.7	0.2	**0.5**								
Ribwort plantain (*Plantago lanceolata*)		0.6	0.4	**0.5**								
Greater plantain (*Plantago major*)		0.3	0.3	**0.3**								
Hawkbits (*Leontodon* spp.)		0.2	0.1	**0.1**		0.2	0.03	**0.1**		0	0	**0**
Thistles (*Cirisum* spp.)		0.2	0.04	**0.1**								
Bare soil		3.4	6.5	**5**								

Legume-grass - sown at 25 kg/ha:					**Total floral cover**	**1.1**	**2.2**	**1.7**	**Total visit count**	**33**	**65**	**98**
Cocksfoot grass (*Dactylis glomerata*)	**12**	38.3	42.1	**40.2**								
Lucerne (*Medicago sativa*)	**72**	10.2	7.7	**9**		0.2	0.2	**0.2**		0	21.6	**14**
Timothy grass (*Phleum pratense*)	**8**	0.3	4.8	**2.6**								
Red clover (*Trifolium pratense*)	**8**	3.5	2.3	**2.9**		0.02	0.1	**0.06**		0	8.9	**5.7**
Unsown grasses (Poaceae)		42.9	29.9	**36.4**								
Greater plantain (*Plantago major*)		3.9	3.2	**3.6**								
Dock (*Rumex* spp.)		2.6	2.5	**2.6**								
Common daisy (*Bellis perennis*)		1.4	2.6	**2**		0.8	1.2	**1**		16.9	4.5	**8.9**
Buttercup (*Ranunculus* spp.)		1.3	1.3	**1.3**		0.03	0.2	**0.1**		25	11.6	**16.3**
Dandelion (*Taraxacum officinalis*)		0.3	0.2	**0.3**								
Thistles (*Cirisum* spp.)		0.2	0.2	**0.2**								
Black medic (*Medicago lupulina*)		0.02	0.3	**0.1**		0.02	0.3	**0.2**		4.1	0	**1.5**
Yarrow (*Achillea millefolium*)		0.3	0	**0.1**								
Hawkbits (*Leontodon* spp.)		0.1	0.05	**0.08**		0.04	0.01	**0.03**		36.1	5.2	**16.1**
White clover (*Trifolium repens*)		0.01	0.1	**0.06**		0.01	0.1	**0.06**		0	8	**5.2**
Mallow (*Malva* spp.)		0	0.1	**0.05**		0	0.1	**0.05**		0	16.1	**10.4**
Bare soil		9.3	11.2	**10.3**								

The alleyway quadrats and records of pollinator visits to alleyway flowers show that some plant species in the experimental mixtures established more successfully and appeared to attract more pollinators than others. Birdsfoot trefoil, alsike clover, musk mallow, yarrow, and oxeye daisy were some of the most successful species in the ‘floral-rich’ mixture, all producing relatively high floral cover and attracting a high proportion of pollinators. Red clover produced less floral cover and attracted fewer pollinators than may have been expected by its relatively high proportion of seed weight in the mixture. White clover established well in many of the ‘oversown-clover’ plots and produced a high floral cover which was attractive to pollinators, whilst black medic was less successful and provided relatively little in return for the amount of seed sown. White clover was also found as an attractive volunteer plant in the other treatments where it was not sown. The alleyway floral cover and flower visitation in the ‘legume-grass’ treatment was relatively low, despite the high seed sowing rate of legumes, suggesting that lucerne may not be as valuable a species as some of those in the ‘floral-rich’ mixture, or that the inclusion of grasses lead to competition which impeded flowering. Red clover also showed relatively low establishment and flowering in the ‘legume-grass’ treatment. No pollinators were observed on alleyway flowers whilst apple trees were in blossom.

Pollinator transects

When considered across the whole sampling period (April to August, 2015 and 2016 combined) the total pollinator abundance was highest in the ‘floral-rich’ treatment (35.4 ± 3.2) and the ‘oversown-clover’ treatment (27.0 ± 3.7), which both showed significantly greater numbers of pollinators than the ‘mown-grass’ control (10.9 ± 2.0, $p<0.0001$ and $p<0.001$), but did not differ significantly from each other. The ‘legume-grass’ treatment (16.7 ± 1.8) also showed significantly greater abundances over the whole sampling period when compared to the ‘mown-grass’ control ($p=0.0043$) (Figure 2). By separating the results by taxa and by season a more nuanced effect of cover crops and pollinators can be seen.

Bumble bee abundance did not differ significantly between the ground cover treatments during apple blossom, however, in the summer months the ‘floral-rich’ and ‘oversown-clover’ treatments attracted significantly more bumble bees than the ‘legume-grass’ and ‘mown-grass’ treatments (Figure 3). Bumble bees were significantly less abundant during the summer of 2016 than the same months in 2015 (4.1 ± 0.7 and 8.4 ± 1.7 respectively, $p=0.018$) though no significant change was observed during the blossom periods.

Solitary bees showed a similar pattern, with no significant differences between treatments during blossom, but then becoming significantly more common in the ‘floral-rich’ and ‘oversown-clover’ treatments when compared to the ‘mown-grass’ in the summer (Figure 4). In contrast to bumble bees, solitary bees were significantly more abundant in the summer of 2016 than that of 2015 (2.5 ± 0.4 and 1.7 ± 0.4 respectively, p=0.046). Though again, no significant difference in abundance was found during blossom between the years.

Honey bees also showed no differences in abundance between treatments during blossom. They then became significantly more numerous in the ‘floral-rich’ treatment when compared to both the ‘legume-grass’ treatment and the ‘mown-grass’ control during the summer months. The ‘oversown-clover’ plots also hosted significantly more honey bees during the summer months that the ‘mown-grass’ plots (Figure 5). In 2016 honey bees were present in significantly fewer numbers than in 2015 during both blossom (9.0 ± 1.8 and 21.0 ± 4.2 respectively, p=0.006) and the summer months (2.5 ± 0.6 and 8.2 ± 2.6 respectively, p=0.012).

Hoverflies showed a marginally significantly greater abundance in the ‘floral-rich’ treatment when compared to the ‘mown-grass’ during the blossom period (p=0.073), with the mean number of hoverflies at 3.9 ± 1.0 in the ‘floral-rich’ treatment, and 1.6 ± 0.5 in the ‘mown-grass’ control. All three cover crop treatments showed higher abundances during the summer months (Figure 6). As with the honey bees, hoverflies showed a significant decrease in abundance between blossom in 2016 and blossom in 2015 (2.1 ± 0.5 and 3.9 ± 0.6 respectively, p=0.031), by the summer however, this had turned into a significant increase on the previous year (9.3 ± 1.1 and 4.8 ± 0.8 respectively, p<0.001).

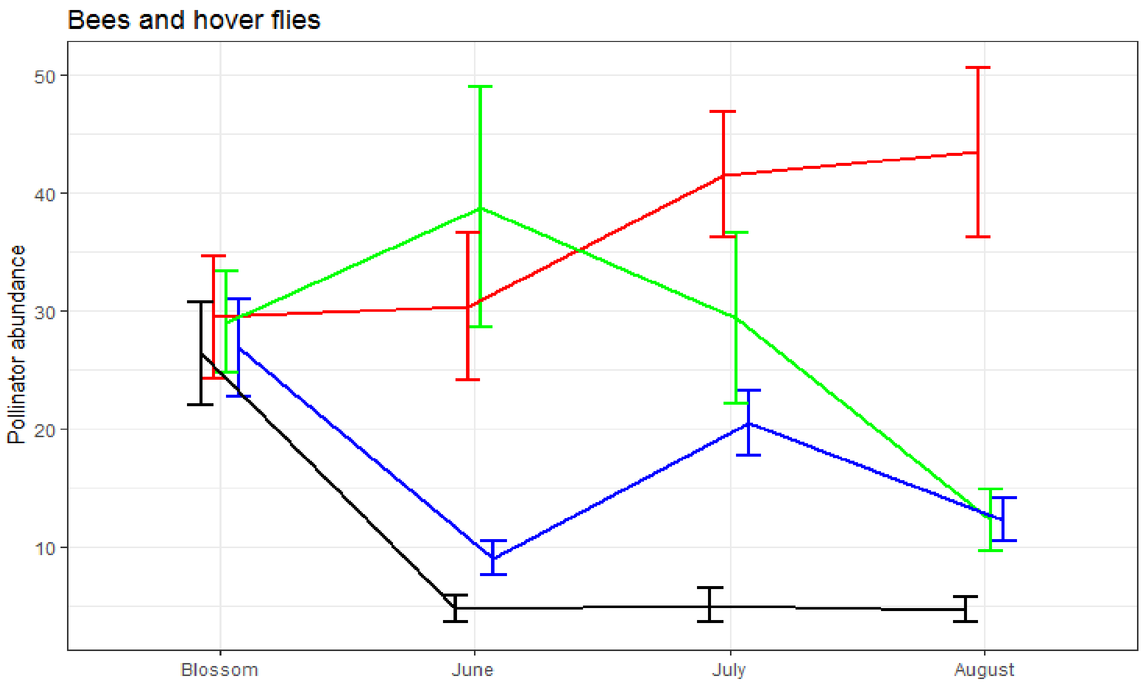

Figure 2. Abundance of bees and hoverflies (Syrphidae) in orchards with different alleyway sward mixtures during apple blossom (April and May) and summer months (June, July, and August). Data are from 2015 and 2016 combined. Letters indicate significant differences (p<0.05). Summed abundance over two 10 minute x 100 m transect walks.

Treatment	Blossom and summer
Mown-grass	a
Floral-rich	c
Oversown-clover	c
Legume-grass	b

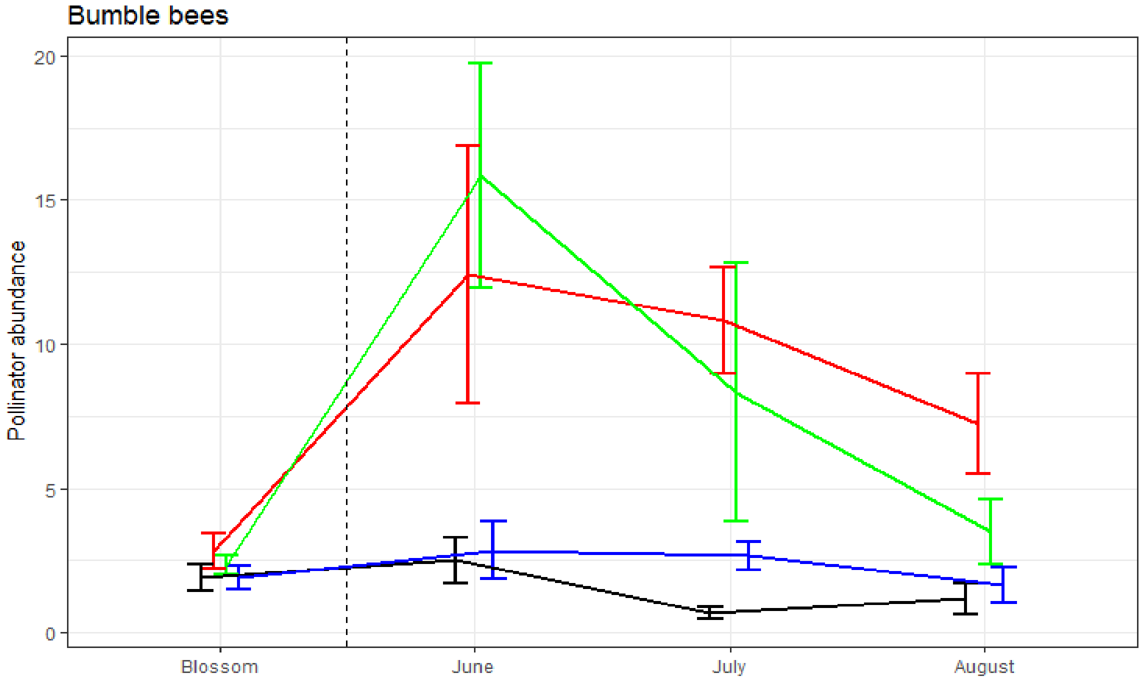

Figure 3. Abundance of bumble bees (*Bombus* spp.) observed in orchards with different alleyway sward mixtures during apple blossom (April and May) and summer months (June, July, and August). Data are from 2015 and 2016 combined. Letters indicate significant differences (p<0.05). Dotted line indicates separation between Blossom and Summer analyses.

Treatment	Blossom	Summer
Mown-grass	a	a
Floral-rich	a	b
Oversown-clover	a	b
Legume-grass	a	a

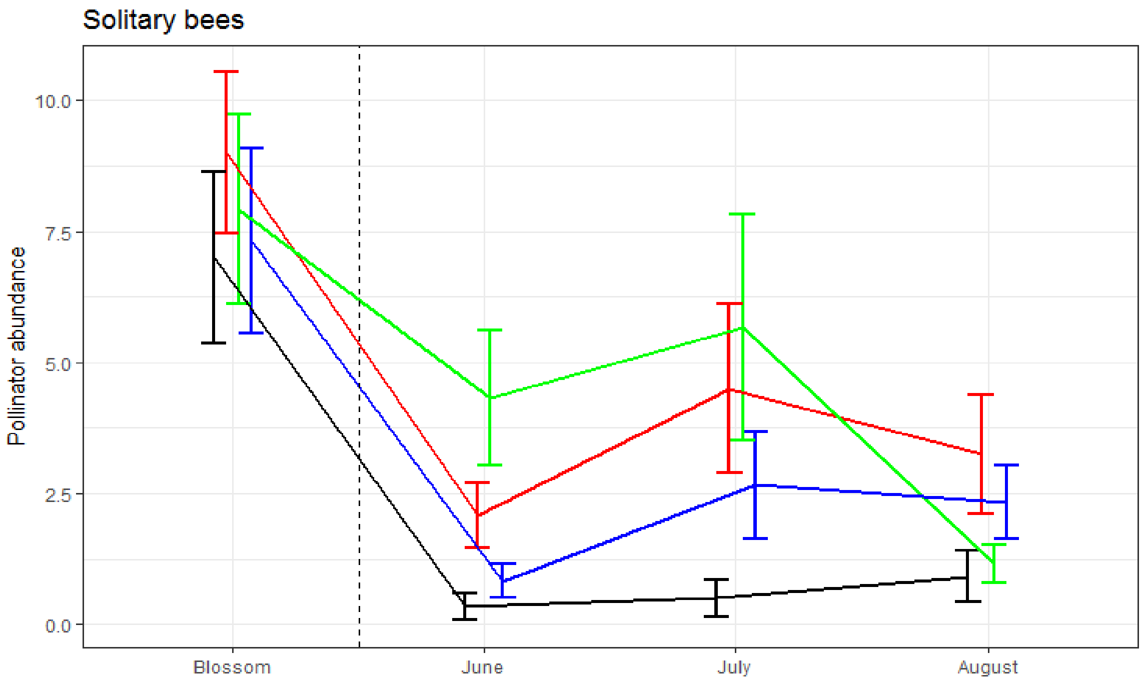

Figure 4. Abundance of solitary bees observed in orchards with different alleyway sward mixtures during apple blossom (April and May) and summer months (June, July, and August). Data are from 2015 and 2016 combined. Letters indicate significant differences (p<0.05). Dotted line indicates separation between Blossom and Summer analyses.

Treatment	Blossom	Summer
— Mown-grass	a	a
— Floral-rich	a	b
— Oversown-clover	a	b
— Legume-grass	a	ab

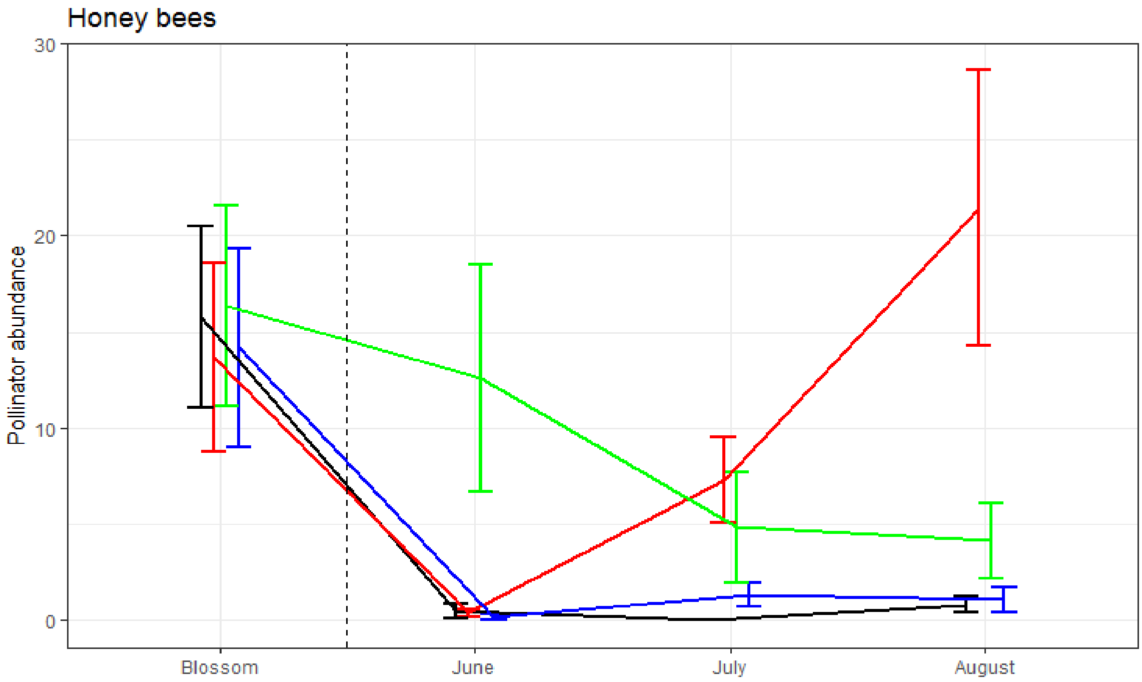

Figure 5. Abundance of honey bees (*Apis mellifera*) observed in orchards with different alleyway sward mixtures during apple blossom (April and May) and summer months (June, July, and August). Data are from 2015 and 2016 combined. Letters indicate significant differences (p<0.05). Dotted line indicates separation between Blossom and Summer analyses.

Treatment	Blossom	Summer
— Mown-grass	a	a
— Floral-rich	a	c
— Oversown-clover	a	bc
— Legume-grass	a	ab

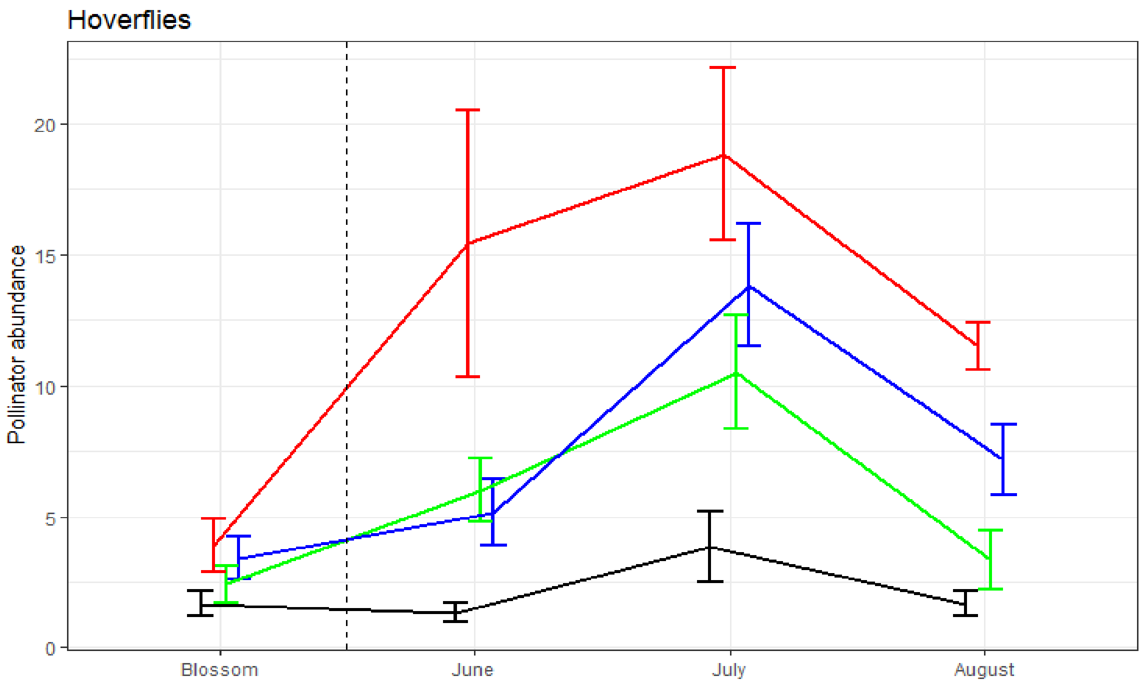

Figure 6. Abundance of hoverflies (Syrphidae) observed in orchards with different alleyway sward mixtures during apple blossom (April and May) and summer months (June, July, and August). Data are from 2015 and 2016 combined. Letters indicate significant differences (p<0.05). Dotted line indicates separation between Blossom and Summer analyses.

Pollination service

No significant differences were detected between treatments in the proportion of flowers that developed into fruit, the proportion of fruit that reached harvest, or the number of seeds set. The fruit quality measures; diameter at harvest, fresh weight, dry weight, firmness, and sugar content (brix), also showed no statistically significantly differences between treatments (Table 2). The effects of pollination on fruit quality in general will be discussed further in Chapter 5.

Table 2. Pollination service measures and fruit quality under different ground cover treatments. Fruit set is the proportion of flowers which developed into a fruitlet, fruit set at harvest is the proportion of flowers at which resulted in a harvestable fruit. Each degree Brix is equal to 1 g of sucrose in 100 g of solution. Mean ± SEM. Data are from 2015 and 2016 combined. No significant differences were found between treatments.

Treatment	Fruit set	Fruit set at harvest	Seed set	Diameter (mm)	Fresh weight (g)	Dry weight (g)	Firmness (kg/cm)	°Brix
Mown-grass	0.36±0.01	0.21±0.01	2.8±0.1	60.1±0.2	99.5±1.4	14.1±0.2	1.7±0.5	11.7±0.1
Floral-rich	0.36±0.01	0.20±0.01	2.6±0.1	60.2±0.2	100.0±1.6	14.4±0.2	3.9±1.0	11.9±0.1
Oversown-clover	0.37±0.01	0.20±0.01	3.2±0.1	60.8±0.2	101.5±1.3	14.1±0.2	2.4±0.7	11.7±0.1
Legume-grass	0.35±0.01	0.21±0.01	2.9±0.1	59.8±0.2	97.4±1.5	14.0±0.2	3.4±0.8	12.0±0.1

4.5 Discussion

The results from this study show that alleyway cover crops, relative to current practices, can substantially increase both the floral resources available in orchards and the abundance of key pollinator taxa through the year. As hypothesised, the more florally abundant cover crop alleyways showed significantly higher numbers of pollinators. The flower-rich 'floral-rich' treatment showed the highest mean pollinator abundance overall, with the less diverse but still florally abundant 'oversown-clover' treatment also showing significant increases. The higher numbers of pollinators in cover crops plots were only observed during the summer months however; no significant differences were observed during apple blossom, it follows that there were no significant differences in the amount of pollination service.

Honey bees were the most numerous taxa observed during apple blossom: accounting for 54% of the total count of monitored pollinators, with solitary bees accounting for 28%, hoverflies 10%, and bumble bees 8%. During the summer months hoverflies became the most abundant species observed on transects at 37%, with bumble bees at 29%, honey bees at 24% and solitary bees at 10%. The sharp decline in honey bee and solitary bee abundance seen in 'mown-grass' control plots after apple blossom contributed to this seasonal demographic shift and reflects the lack of floral resources in these plots during the summer months. The 'floral-rich' and 'oversown-clover' treatments on the other hand appeared to maintain or increase their populations of all four taxa during the summer. The 'floral-rich' treatment displayed the highest abundance of pollinators overall but was only significantly different from the 'oversown-clover' treatment in the number of hoverflies. This was despite the fact that the 'floral-rich' treatment was sown with far more diverse species mixture and cost 2.9 times as much as the 'oversown-clover' treatment. Like the 'mown-grass' control the 'legume-grass' treatment also showed a decline in bee abundance after blossom, although the numbers of hoverflies increased. A study by Sjödin, Bengtsson and Ekbom (2008) found that the abundance and diversity of hoverflies was greater in less intensively managed grassland with a higher sward height. This may explain why numbers were significantly higher in the 'oversown-clover' and 'legume-grass' treatments when compared to the 'mown-grass' control, despite a similar percentage cover of the open structured flowers which these insects are thought to prefer, due to their recued ability to feed on long-corolla flowers (Vattala *et al.* 2006; Campbell *et al.* 2012). Some species of hoverflies are known to feed on grass pollen (Branquart & Hemptinne 2000) which may have been more abundant in the less frequently mown cover crop plots; it is also possible that the taller vegetation played host to more prey species for the hoverfly species with predatory larval stages (Ramsden *et al.* 2015). As well as pollinating apple flowers the predatory larvae of some hover fly species can help to control pest aphids in orchards (Brown 2004; Bergh & Short 2008). Wild bee species also require specific habitats to produce the next generation; it is possible that the orchard alleyways and weed strip could provide nesting habitat for a subset of bee species, and small numbers of mining bees (Andrenidae spp) were observed nesting in sunnier areas of bare soil in the study orchards. For other species nesting and overwintering often occurs in areas of natural or semi-natural habitat including hedgerows, banks, and areas with tussocks of grass (Svensson, Lagerlof & Svensson 2000; Kells & Goulson 2003; Williams & Kremen 2007). The effects of cover crops and other habitat enhancements will depend on the density and diversity of resources such as suitable nesting sites in the surrounding landscape (Scheper *et al.* 2015), and on the existing pollinator communities. Increasing the floral resources and nesting sites available to pollinator populations may allow them to become larger and more stable (Sheffield *et al.* 2008; Oliver *et al.* 2010; Mallinger, Gibbs & Gratton 2016), which may improve the pollination service in following years. The numbers of both hoverflies and honey bees varied between the years, with significantly fewer of both taxa seen in 2016 compared to 2015. Similar fluctuations in pollinator abundance between years have been seen in apple orchards and may be due to differences in weather during blossom (Vicens & Bosch 2000a). This highlights the importance of maintaining a diverse range of pollinator species; species diversity can help to buffer ecosystem service provision against weather and climatic changes (Brittain, Kremen & Klein 2014). Some wild bee species can forage in temperatures and wind speeds that would deter honey bees (Corbet *et al.* 1993; Vicens & Bosch 2000b) and for a crop with a short pollination window such as apple, having a diversity of pollinators available which can forage under different weather conditions could make a significant difference to production if adverse weather were to occur during flowering (Polce *et al.* 2014). Different pollinator taxa have also been found to have synergistic effects on the pollination of crops, including apples (Brittain *et al.* 2013; Sapir *et al.* 2017).

Honey bees and bumble bees regularly cover several kilometres during foraging trips and even small solitary bees may cover many hundreds of metres in search of food (Gathmann & Tscharntke 2002; Greenleaf *et al.* 2007; Wolf & Moritz 2008; Zurbuchen *et al.* 2010). The highly mobile nature of these species may have masked any positive effects that cover crops had on pollinator populations as during the blossom period individuals are unlikely to show fidelity to these plots when huge floral resources are available throughout the landscape. Apple blossom also occurs relatively early in the year when many pollinator species are less abundant, with bumble bee colonies often just establishing for example. A study by Blaauw and Isaacs (2014) showed that it can take several years for a measureable increase in pollinator abundance to be detected following the addition of floral resources, and other studies have found that creating areas of floral resources near to fruit crops can increase both pollinator numbers and the pollination services which they provide (Carvalheiro *et al.* 2012; Blaauw & Isaacs 2014). Although competition for pollinators between alleyway vegetation and apple trees has been considered a potential issue in apple orchards (Free 1968) the result of this study do not support this, in fact no pollinators from the four main taxa were observed on alleyway flowers during apple blossom, despite many hours of surveying. There were also no significant differences in floral cover or pollinator abundance between the treatments during blossom, nor any significant differences in fruit set or seed set. This is likely to be due largely to the huge volumes of apple flowers available, but also to the behaviour of pollinators which can become focused on a single flower type during foraging (Hill, Wells & Wells 1997; Goulson 2000). Apple trees blossom relatively early in the season, commonly April and May in the UK, whilst the alleyway cover crops only began to show an increase flower cover in mid-May and June. There are few commercially available flowering plant species which will flower before apple trees, if floral resources were to be made available to pollinators before apple blossom the addition of flowering trees such as native willows (*Salix* spp.) to windbreaks and hedgerows may be appropriate (Ostaff *et al.* 2015; Park *et al.* 2015b).

4.6 Conclusions and management recommendations

The results suggest that whilst commonly available legume-based ‘pollen and nectar’ mixtures can boost pollinator numbers in orchards, more targeted mixtures may have greater success. An optimal species mixture would be one which provided floral resources throughout the active-seasons of beneficial species (Haaland, Naisbit & Bersier 2011; Peters *et al.* 2013), and did not require re-sowing or further ground management; perennial species are therefore likely to be more suitable than annuals. The seeds for a cover crop must be commercially available and not prohibitively expensive; legume seeds are often less expensive than other wildflower seeds and may form a good base for a mixture. Non-legume wildflowers have the potential to persist for longer than many legumes however, though if properly managed some legumes (e.g. white clover) can naturally reseed, therefore there may be trade-offs between seed price and mixture longevity. Low-growing legumes such as white clover and birdsfoot trefoil combined with long-living wildflower species such as oxeye daisy, musk mallow, and yarrow may form a suitable mixture. Although red clover did not establish particularly well in this study it is known to be an important forage plant for bees, particularly bumble bees (Carvell *et al.* 2006) and it is possible that wild-type varieties, rather than the agricultural varieties as used in this study, may prove more successful as orchard alleyway plants. Resident, or volunteer, plant species, particularly white clover and hawkbits but also buttercups, dandelions, and common daisies, were seen to attract a number of pollinators and such species are known to be important for conserving pollinators in orchards (García & Miñarro 2014). Using a diversity of plants with a range of flower structures can be important when creating habitat because different beneficial species have a variety of feeding abilities and preferences (Haenke *et al.* 2009; Campbell *et al.* 2012), and the establishment and growth of different plant species will vary depending on soils and management.

In newly established orchards the ground is generally cleared of vegetation and flattened, providing a suitable seed bed for sowing cover crop. In this situation the addition of fine-leaved grasses may help to reduce encroachment of more competitive grass species (Pywell *et al.* 2011). The mowing management of alleyways is likely to be instrumental to the success of a cover crop or any wildflower area and will help to determine plant species composition, which in turn decides arthropod species composition. The results of this study and others suggest that reducing the frequency of mowing, possibly to once or twice per year, and raising the height of cuts, to 10-15 cm, and increasing floral diversity and abundance will benefit beneficial species (Buri, Humbert & Arlettaz 2014; García & Miñarro 2014; Wastian, Unterweger & Betz 2016). A reduction in mowing frequency is also less likely to favour competitive grass species which can reduce the growth of wildflowers. Mowing should be avoided when bees are foraging on alleyways as this can result in significant mortality (Humbert *et al.* 2010), and by not mowing entire orchards at the same time, perhaps by cutting only every second row, the unmown areas can act as refugia (Bruppacher *et al.* 2016). This practice may also help to prevent sharp changes in floral resources from disrupting pollinators and other beneficial species (Cizek *et al.* 2012). If growers find that alleyway plants are attracting pollinators during the blossom period, mowing the alleyways may help to push pollinators to the crop flowers. The removal of cuttings is recommend for maintaining areas of flowering plants as they can smother growth, and increase the fertility of the soil which is often detrimental to the success of wildflowers (Pywell *et al.* 2011). Using side-discharging mowers would allow the cuttings to be spread onto the bare soil of the weed-strip where they would act as beneficial mulch for the trees, boosting soil fertility and soil-derived ecosystem services, as discussed in Chapter 2.

It should be noted that whilst providing pollinators with floral resources in orchard alleyways is likely to boost populations, it also means attracting them into an intensively managed environment where their exposure to pesticides may be increased. Although the use of the many of the more harmful broad-spectrum pesticides is being restricted, pesticides are still a major source of pollinator decline and can negatively affect pollination services (Potts *et al.* 2010a; Vanbergen & Garratt 2013; Godfray *et al.* 2014). Many of the pesticides used in orchards are known to having damaging effects on pollinators (Whitehorn *et al.* 2012; Godfray *et al.* 2014; Stanley *et al.* 2015). Even products considered to be less immediately damaging, such as fungicides, can cause harm and their synergistic and species-specific impacts are not fully understood (Brittain & Potts 2011; Biddinger *et al.* 2013). Further research is needed into how orchard pesticides affect pollinators and whether providing floral resources within the orchard system could lead to a damaging increase in pesticide exposure.

In conclusion, orchard alleyways cover large areas of land in agricultural landscapes which have high pollination demands, with the correct management they could potentially increase the area of floral resources, boost pollinator populations, and potentially enhance pollination services. The effects of mowing management and the sowing and over-sowing of flowering plants species into orchard alleyways, and other areas of low-diversity grassland, is an promising method for pollinator conservation which would benefit from further research. This study has shown that the addition of even relatively inexpensive plant species and slight changes in ground cover management have the potential to significantly enhance the value of orchard alleyways to pollinators.

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Chapter 5:

The benefits of insect pollination to apple yield and fruit quality

(*Andrena* sp. foraging on ‘Gala’ apple flowers)

Chapter 5:

The benefits of insect pollination to apple yield and fruit quality

5.1 Abstract

The majority of crop species depend on biotic pollination for improved yields. Whilst there is growing evidence for the extent of pollination dependency in different crops, there are still knowledge gaps in varietal differences and the importance of pollination on crop quality, despite its importance to commercial value. There are also knowledge gaps relating to potential pollination deficits, which will vary depending on the pollination demand of the crop and the ability of the surrounding pollinator community, both managed and wild, to provide a pollination services. Here the pollination dependence and pollination deficits of a commercially important apple variety, 'Gala', are investigated. The methods commonly used to test for pollination dependence and pollination deficits, pollinator exclusion and supplementary pollination, are tested at three experimental scales: the inflorescence, the branch, and the entire tree. 'Gala' apple yields were shown to be highly pollinator dependent; grand means of the scale experiments showed that following pollinator exclusion yields fell to 55%. Supplementary hand pollination led yields to increase to 167% of current rates, showing that pollination deficits existed in the study orchards. Seed set was also significantly affected by pollination rates, and seed numbers were shown to significantly improve a number of fruit quality measurements, and to result in greater production of Class 1 fruit. This shows that apple quality is also dependent on pollination and that seed count may be used as an indicator for fruit quality. Growers are recommended to record seed count as part of their fruit quality monitoring programmes to give an indication of potential pollination deficits. Recommendations are made on the scale at which pollination dependence and deficit studies are conducted and the potential pollinating effectiveness of a suite of apple flower visitors is discussed.

5.2 Introduction

Pollinator dependent crops represent 75% of global crop species and are some of our most valuable foodstuffs both in terms of financial worth and nutritional content (Klein *et al.* 2007; Aizen *et al.* 2009; Eilers *et al.* 2011; Chaplin-Kramer *et al.* 2014). The degree to which pollinator dependent crops rely on insect pollinators varies; oilseed rape can receive an 18% boost to yields when pollinated (Bommarco, Marini & Vaissière 2012), canola yields can be improved by 46% (Sabbahi, De Oliveira & Marceau 2005), and macadamia yields can be 185% greater following pollination (Grass *et al.* 2018). Pollination is also important for crop quality; as well as coffee fruit numbers being enhanced by 49%,fruit weight was found to increase by an additional 7% following pollination (Roubik 2002). Strawberry yields have been shown to increase by 39% with bee pollination, with significant improvements in fruit quality and crop value (Klatt *et al.* 2014). For some other crops, including many fruit crops, pollination may be even more essential to production (Klein *et al.* 2007). There is now also growing evidence for varietal differences in pollination dependence, this has been observed in oilseed rape (Hudewenz *et al.* 2014), strawberries (Klatt *et al.* 2014), blueberry (Benjamin & Winfree 2014), and apples (Garratt *et al.* 2014a, 2016). The increased production of pollination dependent crops raises the demand for pollination services, however the declines seen in pollinator communities mean that there may be a growing risk of pollination deficits (Aizen *et al.* 2008; Winfree 2008; Garibaldi *et al.* 2011a; Potts *et al.* 2016, 2017). Despite the importance of pollinator dependent crops and the potential impacts of pollination deficits, there are relatively few studies which have investigated this phenomenon, though deficits have been detected in some fruit crops including apple (Garratt *et al.* 2014a, 2016), strawberries (Benjamin & Winfree 2014), custard apple (Pritchard & Edwards 2006), and coffee (Klein *et al.* 2003). Pollination deficits will vary regionally and locally and improving our knowledge of where they occur, and to what severity, could help to target efforts to support pollinator services. A better understanding of the effects of pollination on fruit yields and on fruit quality, a critical factor in determining the value of many fruit crops including apples, may also help to inform policy and improve production practices (Breeze *et al.* 2016).

One method to quantify pollinator dependence is through the use of pollinator exclusion experiments. Mesh bags have been used in studies testing pollination dependence in a number of crops including coffee (Roubik 2002; Steffan-Dewenter & Leschke 2003), apples (Garratt *et al.* 2014a), strawberries (Klatt *et al.* 2014), and macadamia nuts (Grass *et al.* 2018). For some flowering crops wind can be responsible for a portion of pollen transfer, but it is not considered an important vector of apple pollen (Free 1964), therefore the exclusion of insect pollinators from apple flowers effectively inhibits pollination. To establish whether a pollination deficit exists for a crop its flowers can be given supplementary pollination; for experimental studies this is usually done by hand, using paintbrushes and pollen collected from a suitable donor plant (Hopping & Simpson 1982; Button & Elle 2014; Garratt *et al.* 2014c; a; Hudewenz *et al.* 2014). The production from these supplementary pollinated flowers is then compared with others which were exposed to current or 'background' levels of pollination. The difference in production between the two can then be inferred as the pollination deficit. For studies of tree crops, these manipulations have generally been carried out at the scale of the inflorescence or the branch (Hopping & Simpson 1982; Klein *et al.* 2003; Garratt *et al.* 2014c; a; Sheffield 2014; Grass *et al.* 2018). For treatments which involve covering flowers with mesh and hand pollinating this may be the most logistically feasible method, however there is little evidence to show that the results produced are representative of the effects of different pollination levels for the whole tree. Trees are able to selectively abscise fruit under low crop loads (Dennis, Ferree & Warrington 2003) and this adaptive reallocation of resources may alter the effects of poor pollination. Commercial apple trees also generally set more fruit than is desirable and growers often 'thin' apple crops, removing excess, small or misshapen fruit to create an optimal crop load.

Apple flowers are grouped in clusters, or inflorescences, of approximately 5 flowers, though the average number may vary between varieties. Flowers are hermaphroditic and a typical flower has 5 sepals and 5 petals, with the centre of the flower having approximately 20 stamen surrounding 5 stigma and styles. Within the flower ovary there are 5 locules, each of which contains 2 ovules; the majority of apples flowers can therefore set up to a maximum of 10 seeds (Jackson 2003). If an ovule is fertilised by compatible pollen it will produce a seed which releases a number of hormones, triggering the development of a fruitlet (Jackson 2003). The majority of apple varieties are self-incompatible (Ramírez & Davenport 2013) and require pollen from a different apple variety to produce seeds. In many modern orchards 'polliniser' varieties are planted amongst the crop variety with the sole purpose of providing compatible pollen. The transfer of pollen between trees is carried out by a variety of insect vectors, the composition of which will vary both spatially and temporally, though wild bees and honey bees are thought to be the principal pollinators (Klein *et al.* 2007; Garratt *et al.* 2014a). Other species such as hoverflies and other flies may also play a more minor role in apple pollination and can be important pollinators of other crops (Rader *et al.* 2016). Poor apple pollination and low seed set can reduce both yields (Stern, Eisikowitch & Dag 2001; Garratt *et al.* 2014c) and fruit quality, leading to smaller fruit (Garratt *et al.* 2014a), increased asymmetry (Sheffield 2014), and reduced mineral content (Volz, Tustin & Ferguson 1996). Fruit quality is a critical deciding factor for the value of fruit crops and can have a significant impact on the value of apple production (Garratt *et al.* 2014a).

In this study, the pollination dependence and local pollination deficits of 'Gala' apples were tested using three pollination treatments: pollinator exclusion or 'closed' pollination, 'open' pollination (where insects were free to visit flowers), and 'supplementary' pollination (where as well as insect pollination flowers were pollinated by hand using a paintbrush and pollen collected from nearby polliniser varieties). To test if the observed effects of pollination intensity are affected by the scale at which it is monitored the three pollination treatments were tested at three different scales: the 'inflorescence', the 'branch', and the whole 'tree'. The effects of the pollination treatments on initial fruitlet set, fruit set at harvest, and seed set were monitored at each experimental scale. The influence of seed set on fruit quality was investigated, using fruit from this experiment and those discussed in chapters 2 and 3, and the behaviour of potential pollinators during apple blossom is discussed. It was hypothesised that greater pollination will result in improved fruitlet set, which in turn is expected to result in more fruit at harvest. It is predicted that the influence of pollination treatment on fruitlet set, and fruit set at harvest will be lower at the larger scales due to the moderating effects of fruitlet abscission and thinning. A higher number of seeds is predicted to lead to improvements in fruit quality (e.g. size and shape).

5.3 Methods

Study sites

This study took place in 2014 and 2015 on a conventionally managed commercial fruit farm near Maidstone, Kent, England. Apple orchards used in the study were aged between 4 and 8 years old and contained the variety 'Gala', grafted onto 'M9' rootstocks. Polliniser trees were a mixture of crab apples (*Malus spp.)* and the apple variety 'Golden delicious'. Tree spacing was 1 m within the row and 3.5 m between rows with polliniser trees planted between each tenth and eleventh tree, at a ratio of 1:10 with the crop variety. The blocks and plots used in this study were the same as those from one of the two farms described in Chapters 3 and 4 (see Appendix 2 for more details on management).

Pollinator abundance and behaviour

Observations of insect pollinator apple flower visits were conducted in April 2014, before the ground preparation or sowing of alleyway cover crops discussed in chapters 2, 3, and 4. Apple blossom watches were observations of one side of a single apple tree for 5 minutes. The number of apple flowers being visited by individual insects (touching flower petals) and the number of times individuals came into contact with the centre of the flower (touching any part of the stamen or pistil) was recorded. Taxa were grouped as bumble bee, solitary bee, honey bee, hoverfly, other fly, or other insect. Six crop watches were conducted in each of the 24 plots at randomly selected trees which were at least 15 m away from the orchard edge. Crop watches were conducted between 0900 h and 1730 h, in dry, warm (>13°C) conditions, where wind speeds were below 2 on the Beaufort scale.

Pollination dependence treatments

The three experimental scales varied considerably in the number of flowers which they contained: the single 'inflorescence' scale treatments had a mean of 5.7 ± 0.2 flowers; the 'branches' had 6.9 ± 0.4 inflorescences and 37.4 ± 2.3 flowers, and the whole 'trees' had 133.3 ± 5.3 inflorescences with an estimated 741.4 ± 29.3 flowers. Flower numbers for whole trees were estimated by counting the number of inflorescences and multiplying by the average number of flowers seen per inflorescence in the 'inflorescence' scale and 'branch' scale treatments (5.55 ± 0.02). Pollination treatments at the 'inflorescence' scale were applied using methods adapted from (Garratt *et al.* 2014a).

In 2014, six trees were selected from 12 plots spread across 3 orchard blocks on one farm. Trees were evenly spaced through the plots and a minimum of 15 m from the orchard edge. Before blossom, five inflorescences of a similar developmental stage, each on different branches, were selected and randomly assigned to a pollination treatment. For the 'closed' treatment PVC mesh bags with 1.2 mm^2 diameter holes were used to cover two inflorescences per tree, these bags were removed once flowering had finished approximately 3 weeks later. Three inflorescences were left 'open' to insect pollination, and one of these inflorescences received 'supplementary' pollination. Supplementary hand pollination was conducted at peak blossom, with dehisced anthers being collected from the flowers of nearby polliniser trees; anthers were shaken in a petri dish to release their pollen which was then applied fresh to the all of the stigmas of the target flower using a fine paintbrush. Two inflorescences per tree were assigned to the 'closed' and 'open' treatments because yields were expected to be lower in these treatments and sufficient numbers of apples were needed for fruit quality analysis. Each inflorescence was tagged with a coloured marker to denote its treatment and the number of flowers present was recorded: 360 inflorescences were monitored in 2014. In 2015, this experiment was repeated using a further 36 inflorescences (1 tree per plot), and was expanded to include 'branch' and 'tree' scale experiments. Along with the experimental inflorescences, 3 branches and 3 trees were monitored in each plot, all evenly spaced between through the plot and a minimum of 15 m from the orchard edge. Pollinator excluded 'branches' were covered with mosquito netting with 2.2 mm^2 diameter holes, and pollinator excluded 'trees' were covered with commercially available mosquito nets of the same material measuring 2.6 m high and with a base diameter of 2.6 m. Netting and nets were removed at petal fall in mid-May. Supplementary hand pollination of the whole trees was carried out up to a height of 3 m. Data from 2014 and 2015 were combined for analysis.

For all treatments the initial fruitlet set was recorded approximately four weeks after blossom had ended. The fruit set at harvest was recorded approximately one week before commercial harvest took place. At this time all fruit from the 'inflorescence' and 'branch' scale pollination treatments were collected along with a randomly selected subset of 5 fruit from each of the 'tree' scale pollination treatments. In this part of the study, 396 'inflorescences' (360 from 2014 and 36 from 2015), 36 'branches' (with 247 inflorescences), and 36 'trees' (with 4,697 inflorescences) were monitored. A total of 537, 194, and 175 apples were collected for seed set counts respectively.

Seed set and fruit quality

The seed set and fruit quality data from the pollination dependence experiment were combined with data from previous experiments to Increase the power of the statistical analyses. In total, 3,196 fruit were included in the analysis; 652 from the pollinator dependence experiment, 1,119 from the mulch experiment (Chapter 2), 1,171 from the cover crop experiment (Chapter 4), and 254 from a pilot experiment. All of these fruit were from 'Gala' apple trees grown on two commercially managed fruit farms in Kent, England (see Appendix 2 for further details on management). Fruit quality measures included: seed number, fresh weight, diameter, firmness (using a Silverline penetrometer), defects (scored as either minimal, moderate, or excessive for defects in shape or development), sugar content or Brix (using a Hanna refractometer), and dry weight (entire fruit were cut into 4 pieces and oven dried at 70°C for at least 72 hours before reweighing). Due to the combination of data sets from different experiments not all fruit quality measures were recorded for all fruit: dry weight was not measured for the fruit in the 2014 inflorescence pollination treatment, Brix and firmness were not measured for the branch or tree scale pollination treatments, and height was not measured for the fruit in the mulch or cover crop experiments.

Fruit were sorted into commercial grades based on standards produced by the Food and Agriculture Organisation (UN) standards (FAO 2010), where fruit must be greater than 60 mm in diameter or 90 g in weight, or must exceed 10.5°Brix and not be smaller than 50 mm or 70 g. Fruit which fulfilled these criteria and which showed minimal defects were scored as 'Class 1', those which fulfilled the criteria but showed more moderate defects were scored as 'Class 2', and those which did not fulfil the criteria or which displayed excessive defects were scored as 'Class 3' and were unmarketable as dessert fruit. Colour was not included as a quality measure as it is thought to be largely determined by light exposure (Corelli-Grappadelli 2003).

Statistical analysis

Data were analysed with linear mixed models and generalised linear mixed models (GLMMs) in R (R Core Team 2017) using the "lme4" (Bates, Mächler & Bolker 2012) and "glmmADMB" (Fournier *et al.* 2012) packages. For the pollinator dependence experiment separate GLMMs were created for pollination treatments at each experimental scale. Initial fruitlet set was analysed as a two-column integer matrix containing the number of flowers (at the relevant experimental scale) which developed into fruitlets compared to the number which failed to set. Fruit set at harvest was analysed as a two-column integer matrix containing the number of flowers (at the relevant experimental scale) which produced fruit still present at harvest compared to the number which failed to do so. Seed set was measured as a count. Pollination treatment was the main fixed effect in all of these models and the random effects were: tree, nested within plot, nested within block. Alleyway groundcover treatment (described in Chapters 3 and 4) was included as the nested random effect 'plot': groundcover treatments were not explicitly included in the models as previous analyses had shown that they had no significant effect on fruit production. Year of harvest was included as a fixed effect for the inflorescence scale models to account for variations between 2014 and 2015. Observation-level random effects were added to reduce overdispersion in the initial fruitlet set, fruit at harvest, and seed set models for the tree scale and for the fruit at harvest model for the branch scales (Harrison 2014). Error families were binomial for the initial fruitlet set and fruit set at harvest models, Poisson for the inflorescence and branch scale seed set models, and negative binomial for the tree scale seed set model.

The initial fruitlet set, fruit set at harvest, and seed set data collected at the three experimental scales were also compared between pollination treatments to ascertain whether the pollination treatments produced different result at different scales. The data were modelled with separate GLMMs with treatment scale now as the main fixed effect. The same random effects were used as above, error families were either binomial or Poisson, and observation-level random effects were included for the ‘excluded’ pollination treatment to reduce overdispersion.

The effect of seed number on fruit quality and class was assessed using linear mixed model regressions. Each fruit quality measure was modelled separately with seed number as the main fixed effect and tree nested within plot (groundcover treatment), block, and farm as random effects. The experiment of origin and the year of harvest were included as crossed random effects.

5.4 Results

Pollinator abundance and behaviour

Solitary bees were the most abundant pollinator taxa seen on apple flowers, with honey bees observed making the highest number of contacts with flower centres. Not all flower visitors were seen to make contact with the centre of a flower. On average, each honey bee visitor came into contact with the centre of more than 2 flowers, non-Syrphid flies on the other hand showed a far lower visitor to contact ratio of 0.33 (Figure 1).

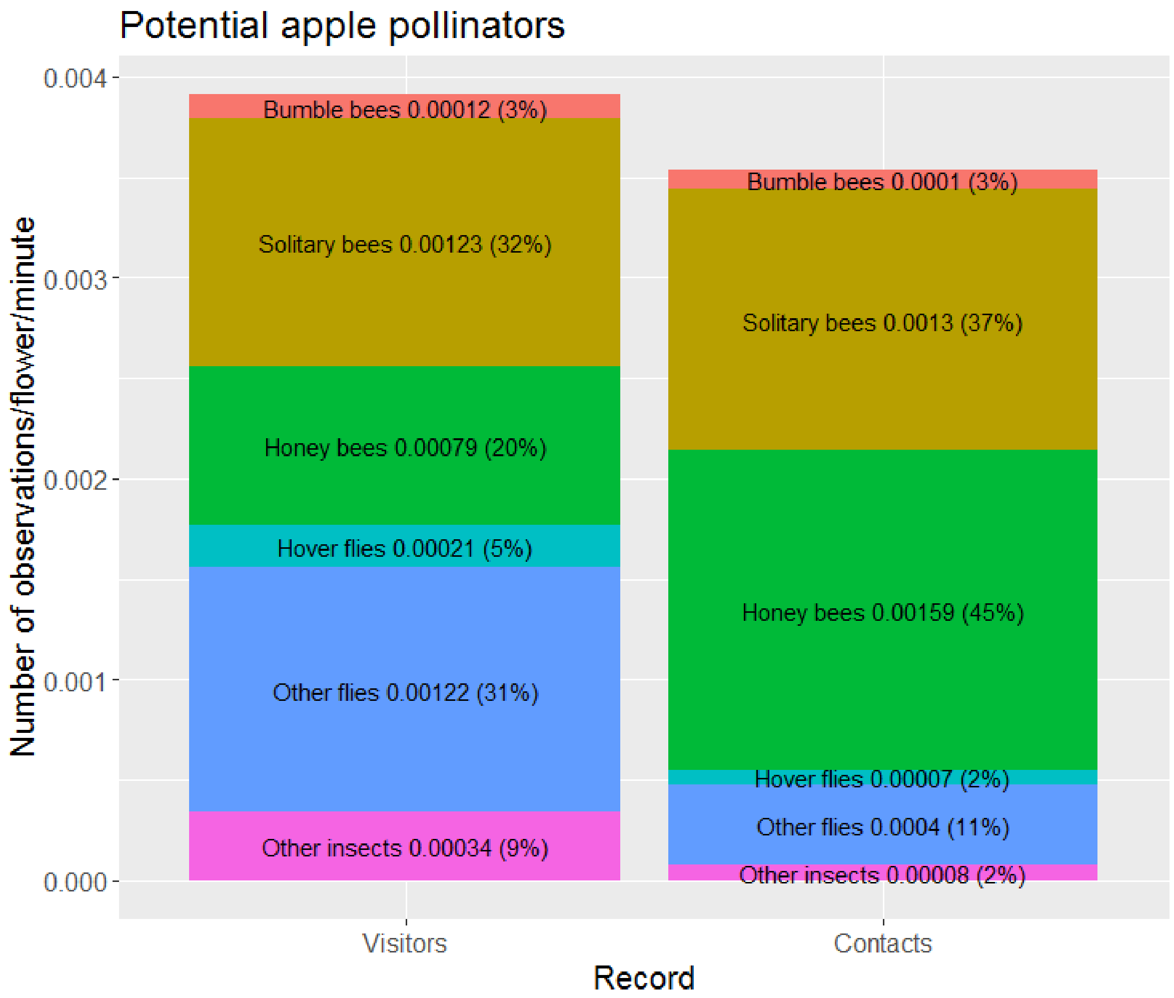

Figure 1. Potential pollinators observed on apple flowers during blossom. “Visitors” indicates the number of individuals observed on apple flowers during crop watches. “Contacts” indicates the number of flowers which individuals appearing to feed from, making contact with the central structures of the flower (pistil or stamens).

Pollination dependence treatments

Manipulating pollination levels showed a trend of greater fruitlet set, fruit set at harvest, and seed set at every experimental scale; inflorescence, branch, and tree (Figure 2). Year showed no significant effect in the ‘inflorescence’ scale models on fruitlet set or fruit set at harvest, but a significant effect was seen in the seed set model. Grand means of the pollination treatments at all three experimental scales showed that, when compared to the ‘open’ treatments, fruitlet set increased to 207.4% with supplementary pollination, and decreased to 54.9% in the pollinator exclusion treatments. Fruit set at harvest increased to 167.3% following additional hand pollination and declined to 55.4% if pollinators were excluded. Seed set showed similar results; with supplementary pollination leading to 149.9% of ‘open’ treatment seed numbers and pollinator exclusion to just 22.8%.

Pollinator exclusion showed a significant reduction in fruitlet set with 21.8%, 72.8%, and 64.6% for the inflorescence, branch, and trees scale experiments respectively. By harvest the numbers of fruit had changed to 12.7%, 75.4%, and 78.6%. Supplementary pollination also showed a significant difference in fruitlet set, and the same trend of decreasing treatment effect at greater scale, with 340.7% at the inflorescence scale, 174.1% at the branch scale, and 124.8% at the tree scale. For fruit set at harvest these percentages had changed to 217.7%, 171.5%, and 116.8% respectively. Seed set was also significantly lower in the excluded treatments at 10.6%, 27.0%, and 31.1% and significant higher in the supplementary treatments at 193.2%, 122.6%, and 135.3% for the inflorescence, branch, and tree scales respectively. Although pollination treatments all showed the same trend for the benefits of increase pollination the effects were not significant at all scales (Table 1).

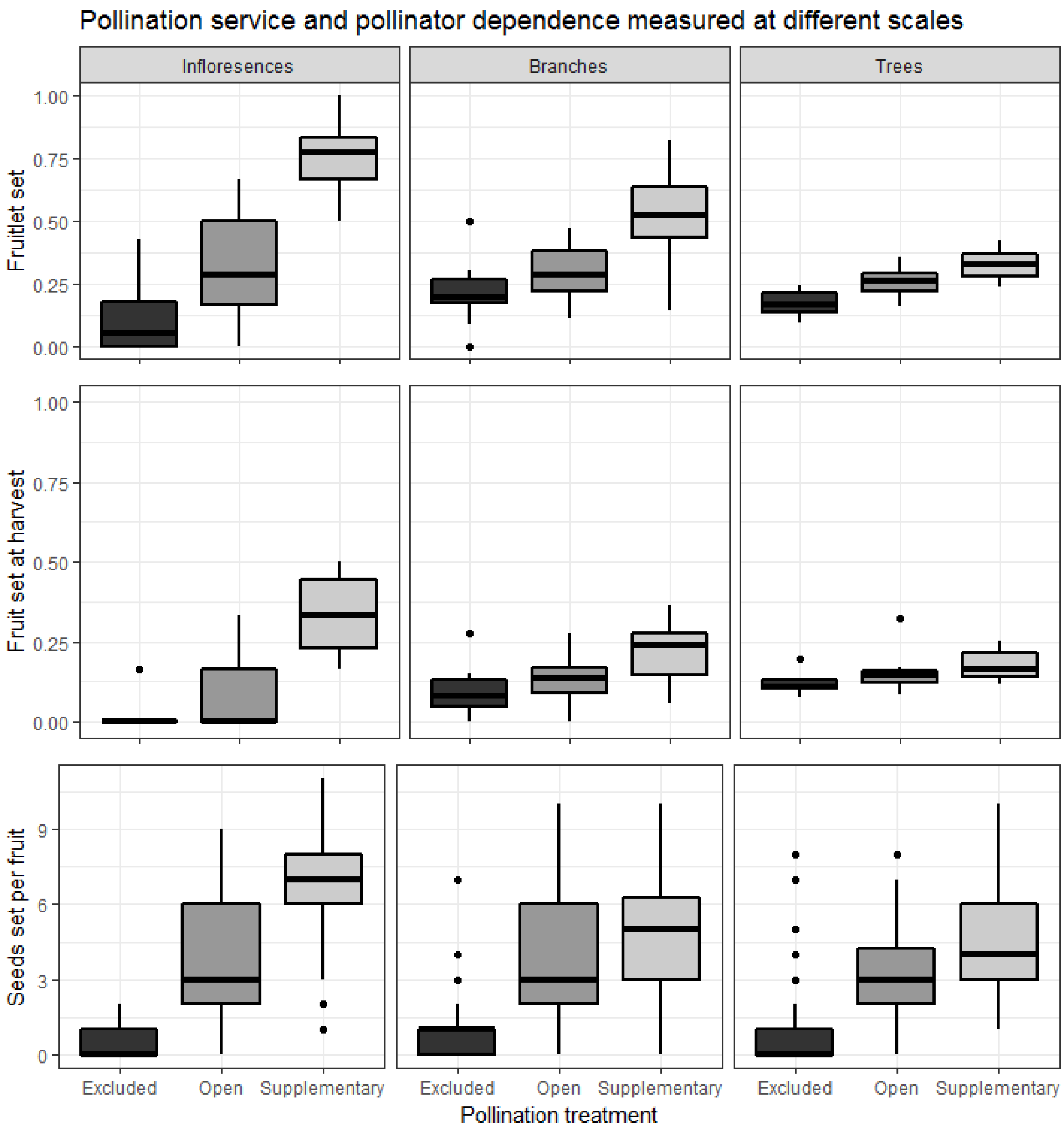

Figure 2. Pollination treatment effect on apple fruit set, fruit set at harvest, and seed set at three scales: the inflorescence (with a mean of 5.7 flowers), the branch (with a mean of 37.4 flowers), and the whole tree (with an estimated mean of 741.4 flowers). Mesh was used to prevent insect pollinators from coming into contact with flowers in the Excluded treatment. The Open treatment allowed insects free access to flowers and the Supplementary combined insect pollination with hand pollination.

p-values	**Initial fruitlet set**			**Fruit set at harvest**			**Seed set**		
Scale	**Open - Excluded**	**Open - Suppl**	**Excluded - Suppl**	**Open - Excluded**	**Open - Suppl**	**Excluded - Suppl**	**Open - Excluded**	**Open - Suppl**	**Excluded – Suppl**
Inflor	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Branch	**0.0074**	**<0.0001**	**<0.0001**	0.416	**0.0271**	**0.0006**	**<0.0001**	**0.0079**	**<0.0001**
Tree	**<0.0001**	**0.017**	**<0.0001**	0.0991	0.2608	**0.001**	**<0.0001**	0.39	**<0.0001**

Table 1. The results of least square means test comparing the effects of pollination treatments on initial fruitlet set, fruit set at harvest, and seed set at three experimental scales. “Inflor” = inflorescence, “Suppl” = supplementary pollination. The treatment with the greater level of pollination (Supplementary > Open > Excluded) produced the highest result in all cases.

Comparing the outcomes of the pollination treatments between the different experimental scales shows that there were some statistically significant differences (Table 2). At the inflorescence scale initial fruitlet set was significantly lower in the ‘excluded’ treatment (21.8%) than for the same treatment at the branch (72.82) and tree (64.6%) scales, suggesting lower flower fertilisation, or possibly more selective fruitlet setting. Significant differences were found comparing fruit set at harvest from the supplementary pollination treatment between the inflorescence (340.7%) and the branch (171.5%) and tree (116.8%), with a declining effect size of supplementary pollination with increasing scale. Seed set was significantly higher in the supplementary pollination treatment at the inflorescence scale (193.2%) than at the branch (122.5%) or tree (135.25%) scales. It is possible that this was because a greater proportion of flowers were receptive at the time of hand pollination: flowering is not completely synchronous within the inflorescence or within the tree.

***P*-values**	**Initial fruitlet set**			**Fruit set at harvest**			**Seed set**		
Treatment	**Inflor - Branch**	**Inflor - Tree**	**Branch - Tree**	**Inflor - Branch**	**Inflor - Tree**	**Branch - Tree**	**Inflor - Branch**	**Inflor - Tree**	**Branch - Tree**
Excluded	**<0.0001**	**<0.0001**	0.1813	**<0.0001**	**<0.0001**	0.3402	0.0840	0.1597	0.8577
Open	0.2160	0.5163	0.7872	0.8483	0.7716	0.5245	0.9226	0.9410	0.9980
Suppl	**<0.0001**	**<0.0001**	**0.0001**	**0.0094**	**<0.0001**	0.2409	**<0.0001**	**<0.0001**	0.7388

Table 2. The results of least square means tests comparing the effects pollination treatments between experiments conducted at different scales. “Inflor” = inflorescence, “Suppl” = supplementary pollination. P-values are calculated by least square means tests.

Seed set and fruit quality

Fruit with higher seed numbers had a significantly greater diameter ($p<0.0001$), height ($p<0.0001$), fresh weight ($p<0.0001$), and dry weight ($p<0.0001$), though the effects were slight (Figure 3). Fruit firmness was not affected by seed number ($p=0.682$), and sugar content showed a significant though slight trend for lower sweetness with more seeds ($p<0.0001$). Fruit set also had a significant positive effect on fruit class, the key deciding factor of a fruits value ($p<0.0001$) (Figure 4).

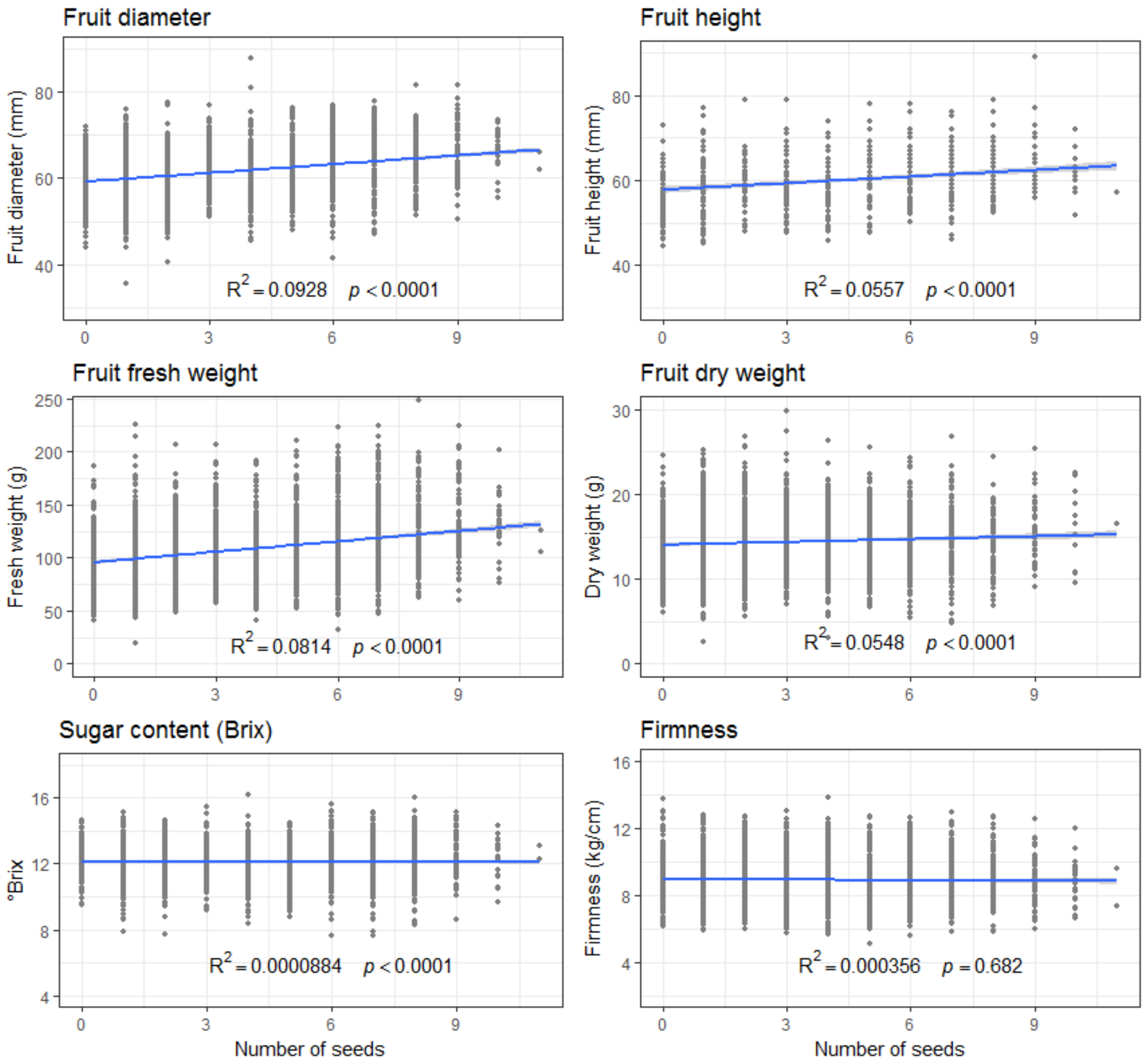

Figure 3. The relationship between seed number and measures of apple fruit quality. Regression lines and R^2 values were calculated using simple linear models.

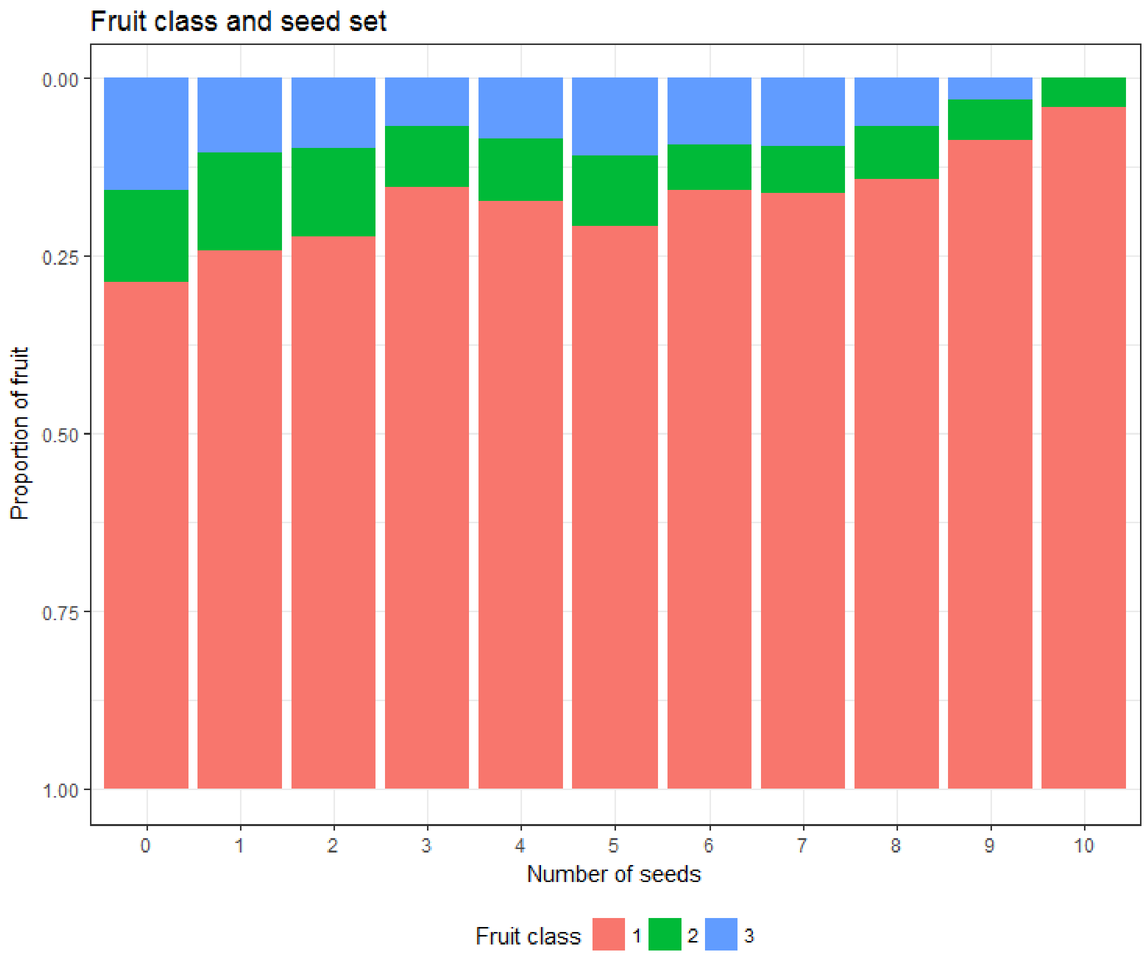

Figure 4. Apple fruit commercial class in relation to seed numbers (based on FAO standards). Class 1 is the highest class with Class 3 being unsuitable for sale as desert fruit. The number of seeds had a significant positive effect on fruit class (p<0.0001). These data are from ‘Gala’ apples which had been commercially thinned prior to harvest.

5.5 Discussion

The results show that ‘Gala’ apples are dependent on pollination for both yield and fruit quality, and that pollination deficits exist in the study orchards. The results also suggest that the scale at which pollination dependence and deficits are measured will affect the extent of the effect observed. A grand mean of the experimental scales showed that yields fell to 55.4% in the absence of pollinators, though these values ranged between 21.8%, 75.4%, and 78.6% when measured at the inflorescence, branch, and tree scales respectively. Pollination deficits were also shown to exist in the study orchards; with a grand mean yield of 167.3% following supplementary pollination. Variation existed depending on the scale at which it was measured: at the inflorescence scale yields were 217.7% of current pollination, whilst at the branch and tree scales the increase was less pronounced at 171.5% and 116.8% respectively. Supplementary pollination also resulted in increased seed set, with seed numbers at 149.9% of current pollination levels when averaged across the experimental scales. Seed set ranged from 193.2% at the inflorescence scale, to 122.6% and 135.3% at the branch and tree scale, respectively. Greater seed set was shown to have a positive effect on several measures of fruit quality and to increase the proportion of Class 1 fruit being produced. These results concur with those of a number of other studies and further highlight the importance of pollination services to apple production (Ladurner *et al.* 2004; Garratt *et al.* 2014c; a; Sheffield 2014).

The positive effects of pollination on fruitlet set, fruit set at harvest, and seed set were seen at all three experimental scales, though there were some significant differences in the degree of their impact. As hypothesised, the effects of decreased and increased pollination appeared to be less pronounced when measured at greater scales. There are many reasons why this may be the case, including biological, cultural, and experimental factors. Fruitlets are more likely to be abscised if they have received poor pollination (Dennis, Ferree & Warrington 2003), however if a plant has a low overall fruit set the chances of abscission are reduced (Stephenson 1981; Jackson 2003). This may affect the representativeness of pollination treatments at different scales: if a single inflorescence is poorly pollinated it will have less of an effect on the overall fruit set than if a branch had received poor pollination, and less effect still when compared to the entire tree. The 'June drop' is a period roughly 4-6 weeks after blossom when trees abscise a proportion of their fruitlets, often those which have received insufficient pollination or which have suffered pest damage. The proportion of the fruit which undergo this process is thought to depend on the level of pollination received by the tree as a whole, the resources within a tree, and weather conditions (Wertheim 1973; Stephenson 1981).

Thinning of fruitlets will also affect the proportion and size of fruit at harvest; thinning is carried out to prevent a trees resources being wasted on overly small or misshapen fruit and to optimise crop loads (Byers, Ferree & Warrington 2003). If a tree has set a large number of fruit, a greater proportion of these fruit may be thinned, as unsustainably high crop loads in one year can induce biennial cropping, where trees enter boom-bust cycles of production which can reduce overall yields and make output unreliable (Jonkers 1979). Excessive crop loads may also increase the risk of branches breaking under the weight of the fruit. It is possible therefore, that a high fruit set could result in increased thinning costs, particularly in varieties which are considered to be heavy cropping, such as 'Gala', and any financial assessment should take this into account. Because hand thinning focuses on smaller, less well formed fruit, which previous studies suggest are more likely to have low seed numbers (Garratt *et al.* 2014c; a), it may lead to an underestimate of the influence of pollination on fruit quality as these fruit do not reach harvest as so are not assessed for quality. Both the thinning process and the natural abscission of fruit are likely to have a moderating effect on extremes of pollination, and they may explain some of the differences observed between the treatments at different scales.

Although the trends of increased pollination were common amongst all of the scales tested, larger scale measurements of pollination service may be better at taking into account the effects of thinning and resource allocation within the tree. However, excluding pollinators from standard trees or those with wire supports may be unfeasible, and the unequal development times of flowers, along with their potential inaccessibility, means that hand pollination may not be as complete at the tree scale. Although apple tree blossoming takes place over a relatively short period flowering phenology is not completely synchronous. Within the inflorescence the apical or ‘king flower’ develops before the lateral flowers and is more likely to set fruitlets and produce larger fruit in many cultivars (Dennis, Ferree & Warrington 2003). Flowering times also vary between inflorescences, depending on various internal and external factors including hormone expression, temperature, light, and pruning (Landsberg 1974; Ferree & Warrington 2003). The results of the branch scale experiment were generally similar to those of the other scales, suggesting that branch scale measurements may be an appropriate compromise between feasibility and representativeness. For tree crops it is therefore recommend that pollination manipulation experiments are carried out at the branch scale if entire trees cannot be manipulated. A protocol has been developed by the FAO which provides further recommendations for conducting pollination deficit assessments (see Vaissière, Freitas & Gemmill-Herren 2011).

Fruit quality is a key deciding factor of a crop’s worth, with Class 1 fruit achieving a significant premium (Garratt *et al.* 2014a). The improvements in quality and higher proportions of Class 1 fruit seen with increasing seed numbers shows that pollination is important for quality as well as yields. Fruit firmness was the only quality trait to show a significant, albeit very slight, negative trend with seed set. Similar results have been found in another study (Garratt *et al.* 2014a) and it is likely that the greater firmness is due to fruit with lower seeds numbers often being smaller and denser. It is also possible that fruit with higher seed numbers developed and ripened more quickly. Seed set will affect fruit morphology not only in terms of how many seeds there are but also how they are distributed amongst the carpels; unbalanced seed distribution may result in malformation (Brault & de Oliveira 1995; Sheffield 2014). Repeated visitation, and visitation from different pollinator taxa, may help to ensure more comprehensive fertilisation (Stern, Eisikowitch & Dag 2001; Sapir *et al.* 2017).

The results of the insect pollinator apple blossom watches showed that differences in pollinator behaviour may make abundance a poor indicator of a taxa's contribution to pollination. During the crop watches many flies were observed on apple flowers, but were relatively rarely seen to come into contact with the reproductive structures at the centre of the flower. The chances of pollen transfer were therefore comparatively low for these species. Although they were less abundant than flies, honey bees came into contact with far more flower centres. The more active foraging behaviour of honey bees may explain why they accounted for a greater proportion of pollinators observed during the walked transects detailed in Chapter 4 (54% of individuals observed), when compared to the crop watches (20% of individuals observed). It may be that the higher rate of flower visitation made them more visible, leading to an overestimate of their relative numbers during transects. It is also possible that these differences were due to changes in abundance between the years as bee keepers may have moved their hives. Even coming into contact with the reproductive structures of the flowers does not necessarily indicate a pollination event. Honey bee visits have been shown to result in fewer pollen grains being deposited than visits from wild bees (Vicens & Bosch 2000a; Thomson & Goodell 2001; Martins, Gonzalez & Lechowicz 2015). This is in part because of honey bee foraging behaviour; honey bees often "side-work" flowers, where they perch on a petal and extend their tongues through the stamen filaments near their base to access the nectar. This behaviour lowers the chances of pollen grains on the anthers attaching to the bees or of pollen on the body of the insect coming into contact with the stigmas (Stern, Eisikowitch & Dag 2001; Thomson & Goodell 2001). Many of the honey bees which visited apple flowers during the crop-watches exhibited this behaviour, whilst solitary bees and bumblebees were often seen in close contact with both the stigmas and anthers as they sought out nectar and pollen. Hoverflies are also thought to be less efficient apple pollinators than wild bees (Garratt *et al.* 2016) and they, like the side-working honey bees, may be more focused on collecting nectar as much of their protein and nutrients demand are met during their predatory larval stages. As well as behaviour, the efficacy of a pollinator species is decided by their phenological and physical traits (Garibaldi *et al.* 2015; Blitzer *et al.* 2016). Many solitary bees collect pollen on scopa, specialised hairs covering much of the underside of their bodies, whilst bumble bees and honey bees store pollen in corbiculae, or pollen baskets, on their hind legs where it may be less likely to come into contact with stigmas. Solitary bees and bumble bees are thought to be particularly effective pollinators of a variety of crops, including apples, and these wild species are key for apple production in many areas (Ladurner *et al.* 2004; Monzon, Bosch & Retana 2004; Garibaldi *et al.* 2013; Sheffield 2014; Garratt *et al.* 2014a, 2016; Garantonakis *et al.* 2016; Sapir *et al.* 2017).

In some regions, apple pollination is almost completely reliant on honey bees (Marini *et al.* 2012), whilst in others it is performed by a diverse range of wild species (Blitzer *et al.* 2016; Földesi *et al.* 2016). This wide variation shows that whilst a crop may always be pollination dependent, pollination services vary between regions and pollination deficiencies may exist which require local assessment and remediation. Pollinator communities are in significant decline in some regions and are subject to a range of threats (Potts *et al.* 2016). It is important that this trend is reversed if the demand for pollination services are to meet (Klein *et al.* 2007; Garibaldi *et al.* 2011a). Growers who are currently paying to hire honey bee hives may find that supporting wild pollinators and enhancing pollination services from wild species reduces the need for this financial outlay, and wild pollinators have been shown to improve pollination services regardless of honey bee visits (Greenleaf & Kremen 2006; Garibaldi *et al.* 2011b, 2013). Efforts should be taken to reduce the harmful effects of intensive agriculture on pollinators and to increase the availability of floral resources and other habitat, as discussed in Chapter 4. Maintaining pollinator diversity is important as taxa can have synergistic effects on flower pollination (Brittain *et al.* 2013; Sapir *et al.* 2017) and are active under different weather conditions (Vicens & Bosch 2000b; Hansted *et al.* 2014). Climate change may affect both the areas in which crops can be grown and the diversity of potential pollinators (Imbach *et al.* 2017).

In this study the number of times a potential pollinator made contact with the structures at centre of the flower was recorded; it is recommend that for future studies wishing to quantify the contributions of different taxa that records should instead be taken of instances where contact was made with the anthers and stigmas alone, as this may give a more accurate indication of how much pollination is actually being performed. Knowing the identity of crop pollinators can help to improve management efforts to protect and enhance these beneficial species (Garratt *et al.* 2014b). For this reason, it is also recommended that future studies identify potential pollinators to greater taxonomic resolution, to species levels if possible.

In conclusion, the results of this study provide further evidence that the apple variety 'Gala', one of the most commercially important varieties in the UK and elsewhere (Garratt *et al.* 2014a), is pollination dependent; with 55.4% of yields depending on pollination. There was a strong trend showing lower rates of pollination resulting in lower production, and increased pollination resulting in improved production, at all scales. Evidence was also found for pollination deficits, with potential increases of yield to 167.3% of current production in orchards where the study was conducted. Variation in pollination treatment effect was seen at different spatial scales and it is recommend that, where possible, pollination dependence and deficit measurements are carried out at the plant scale, or for tree crops the more feasible branch scale. Seed set was also strongly affected by pollination treatment, and the number of seeds was shown to have a significant positive effect on fruit quality, therefore pollination dependence and pollination deficit were also found for fruit quality. It is advised that growers record seed set as part of their routine monitoring of fruit quality and development as this will give an indication of pollination levels in their orchards and may alert them to potential deficits. Whilst resource allocation and adaptive abscission may help to reduce the impact of poor pollination there is little that can be done to increase a year's production once low seed set and low fruit set occur. A diverse community of pollinators were observed during blossom, the majority of them wild species. The findings of this study highlight the importance of supporting wild pollinator communities and of enhancing pollination services for apple production.

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Chapter 6:

Discussion

(‘Oversown-clover’ alleyways, July 2016)

Chapter 6:

Discussion

6.1 Review of thesis aim and objectives

There are many threats to food security (Godfray *et al.* 2010), but if intensive agriculture is able to reduce its reliance on agrochemical inputs by adopting ecologically sound methods, a number of these threats may be reduced. The huge areas of land under agricultural management mean that changes and developments in agricultural practices have the potential to affect a significant proportion of the world's land surface. Even minor improvements could result in benefits to biodiversity conservation and food production alike. This highlights the importance of applied research which can be used to design agroecosystems and inform management.

The studies in this thesis help to address knowledge gaps regarding the implementation of ecologically intensive methods in UK apple orchards; showing that alleyway cover crops and mulching with cover crop cuttings can boost the numbers of important ecosystem service providers. Often studies of habitat management practices focus on a single species group or a single ecosystem service and may therefore overlook additional benefits of a practice (Seppelt *et al.* 2011; Liss *et al.* 2013). Agroecosystems are highly complicated and changes in management can affect different species and different ecosystem services in different ways; it is important, therefore, to study these systems as holistically as possible. Based on current knowledge gaps, this thesis aims to explore the multiple ecosystem service benefits that habitat creation and management can have in UK apple orchards. It demonstrates that alleyway cover cropping can have positive effects on a range of ecosystem service providers, both above ground (e.g. pollinators and natural enemies of crop pests) and below ground (e.g. earthworms). This kind of multiple benefit is particularly valuable because space is highly limited in intensive agricultural systems (Fiedler, Landis & Wratten 2008; Boreux *et al.* 2013).

Objective 1: Evaluate the effects of different mulches on soil fertility and soil-derived ecosystem services. It is hypothesised that the addition of mulch improves soil fertility and soil-derived ecosystem services.

It was demonstrated that mulching can provide a number of benefits to soil fertility: compost was shown to increase soil carbon and soil nitrogen, and the straw and 'legume-grass2' treatments increased soil moisture. Both of the higher-rate cover crop cutting treatments showed increased numbers of earthworms and the compost and ‘legume-grass2’ treatments resulted in increased leaf litter decomposition rates. Despite the variation in results, the study in Chapter 2 shows that both traditional and cover crop-derived mulches can help to reduce dependence on agrochemicals. The use of cover crop cuttings as mulch may be an effective, low-cost, way to integrate this once common practice in commercial apple production, with little change to management.

Many of the benefits of using mulch are highly relevant to food security. The results of this study and others show that mulches can: act as organic fertilisers, reducing the need for synthetic inputs (Sirrine *et al.* 2008; Mullinix & Granatstein 2011); reduce evaporation (Pinamonti 1998; Van Donk *et al.* 2012), thereby lowering the demand for water, a resource in increasing demand due to increased human populations and climate change; and help to smother weeds (Pullaro *et al.* 2006; Van Donk *et al.* 2012), potentially reducing the number of herbicide applications required. A reduction in herbicide use and increased addition of plant material onto the herbicide strip can also boost beneficial species abundance and improve soil biodiversity-derived ecosystem services, such as leaf litter decomposition (Hartley *et al.* 1996; Sullivan & Sullivan 2003; Jacometti, Wratten & Walter 2007; Andersen *et al.* 2013). Increased leaf litter decomposition can lower fungal pest abundance thereby reducing the need for fungicide sprays, this in turn reduces the negative effects on soil biota which these chemical controls can cause (Wang, Zhou & Cang 2009; Jacometti, Wratten & Walter 2010; Komárek *et al.* 2010). Mulching can also help to increase soil organic matter (Merwin *et al.* 1995; Pinamonti 1998). This soil property is important for both soil fertility and for reducing atmospheric carbon levels, but it has been declining in many intensively managed agricultural systems, including orchards (St. Laurent, Merwin & Thies 2008; Steenwerth & Belina 2008; Schmidt *et al.* 2011).

Objective 2: Determine the effects of alleyway cover crops on natural enemy abundance and the resulting implications for pest regulation services. Alleyway cover crops are predicted to provide a higher quality habitat than current management, leading to an increase in natural enemy abundance and an enhancement of pest regulation services.

Objective 3: Assess the value of alleyway cover crop habitat for pollinators and evaluate their impact on pollinator abundances and pollinator services. Pollinators are also hypothesised to benefit from the higher quality habitat provided by the cover crops, and increasing their abundance is predicted to improve pollination services.

The results showed similar responses for these two objectives and so are they discussed together. Overall, alleyway cover crops were shown to provide valuable habitat for above ground beneficial invertebrates, providing more floral resources and greater vegetation structure than current practice. Cover cropping led to significantly greater abundances of a number of natural enemies of crop pests and of all of the main apple pollinator taxa. However, neither pest regulation nor pollination services showed a detectable increase.

Pollinators were not found to be more abundant in the cover crops plots during blossom and so the lack of difference in pollination service was not surprising. However, the greater numbers seen during the summer months show that the cover crops could support more pollinators through much of their active periods. Supporting larger and more diverse populations through the summer months is likely to result in more apple pollinators being present during blossom in the following year. If alleyway cover cropping was conducted throughout an entire orchard, or an entire landscape, it is likely that populations of pollinators would increase, as long as they were not limited by a lack of other resources such as nesting or overwintering sites (Altieri & Letourneau 1982; Kells & Goulson 2003; Potts *et al.* 2005). Cover crops also take time to establish, and the populations of beneficial species can take several years to respond to the increase in resources (Blaauw & Isaacs 2014); it may be that if the study had run for longer there would have been detectable changes in services. Providing floral resources and improving habitat have been shown to improve both pollination and pest regulation services in a number of other studies (Altieri, Ponti & Nicholls 2005; Berndt, Wratten & Scarratt 2006; Fiedler, Landis & Wratten 2008; Blaauw & Isaacs 2014, 2015, Tschumi *et al.* 2015, 2016).

If the high demands for pest regulation and pollination services in intensively managed crops are to be met, it is likely the abundance of ecosystem service providers will need to be kept artificially high. Creating and managing habitat, such as cover crops, which has been designed specifically for these species may be the most effective way of boosting their numbers and enhancing services. Both pollination and pest regulation services are reduced with distance away from habitat and so having cover crops in close proximity to the trees is likely to be beneficial (Miliczky & Horton 2005; Carvalheiro *et al.* 2010; Bailey *et al.* 2014). Although it is possible that cover crops could also increase pest populations (Bone *et al.* 2009) no evidence was found for this in these studies.

Objective 4: Quantify the role of pollination in apple production and identify potential pollination deficits. Pollination dependence and pollination deficits are expected to be found, with pollination affecting both yield and fruit quality.

The study in Chapter 5 quantified ‘Gala’ apple pollination dependence and showed that increased pollination services could help to close both yield gaps and quality gaps. The study also helps to refine the methods used to detect pollination dependence and deficits. Some of the difficulties in measuring ecosystem service provision were also demonstrated: the crop-watch surveys showed that flower visitation is unlikely to give an accurate indication of a species’ contribution to pollination, and variations were seen in the extent of pollination dependence and deficits found when measured at different scales. If further yield gaps are to be identified, and the effectiveness of different management methods used to ecologically intensify production are to be accurately assessed, it is important that the measured variables are a good proxy of service provision. It is recommended therefore that future studies assessing pollination dependence and pollination deficits use entire-plant pollination treatments or branch scale treatments where this is not feasible.

6.2 Methodological constraints

A number of other studies have also reported that habitat management practices, such as introducing areas of flowering plants, have resulted in increased abundances of beneficial species, however fewer studies have quantified the effects on the actual ecosystem services provision, and fewer still have detected effects on production (Seppelt *et al.* 2011; Liss *et al.* 2013). This may be due to the complexity of ecological systems and the influence of intensive agricultural management, which is already focused on maximising production. It may also be because ecosystem services are only detectable in those years when pest pressure is high, or when adverse weather reduces the numbers of pollinators during blossom. The fact that the studies in this thesis were conducted in orchards managed with conventional pesticide regimes is likely to have affected the abundance of all of species monitored, either directly or indirectly, and to have masked treatment effects. However, conducting the experiments at these sites reduces concerns as to whether such treatments would work in commercial settings.

Varied cover crop establishment was found between blocks, despite the same ground preparation and sowing methods being used. It is likely that local conditions, particularly soil properties due to previous land use and weed abundance, were responsible for these differences. The local species pool of beneficial species will also have varied between sites. A similar situation would be encountered for cover crops established outside of an experimental setting and so the results are realistic; however these differences will have increased variation in species abundances and reduced the ability to statistically differentiate results between treatments.

A common limitation of the studies in this thesis, and the majority of those referenced, is that they cover only a short period in the lifespan of an orchard. Modern apple orchards are expected to be commercially viable for between 15-20 years (Robinson, Ferree & Warrington 2003), very few studies are able to cover this timespan. Other tree crops may last even longer; pear orchards can be commercially viable for over 60 years, for example, and fruit growers have a phrase that you: “plant pears for heirs”. Soil properties, below ground arthropod populations, and trees can all take years to respond to changes in management and it is possible that the treatment effects of mulching and cover cropping may have been more pronounced when considered over the lifespan of the orchard.

6.3 Management recommendations and potential issues

If methods for ecological intensification are to be adopted they must be able to successfully integrate into existing agricultural and horticultural systems: they must not cause excessive disruption to crop management, be unaffordable, or be technically unachievable (Wade, Gurr & Wratten 2008). The studies in this thesis show that alleyway cover cropping can be an effective method for increasing the abundance of beneficial species which can be successfully integrated into UK intensive apple orchard systems with little change to management.

Despite this, some potential issues were identified. Pruning is an important part of tree management and is used to shape trees, increase light interception, and remove diseased material. When trees are pruned, the removed branches are commonly left in the alleyways were they are then pulverised with tractor-mounted flails. Pulverising may damage the cover crops and reduce the abundance of floral resources, particularly if it is done during the summer months. Removing pruned material is a potential option; however it is not standard practice and will require the transportation of large quantities of material, which has associated costs. Another option is to sow cover crops in every other row; pruned branches can then be left in the 'empty' rows for pulverising. This method has worked effectively in UK orchards (M. McKerchar, personal communication). Although no differences were detected in the air temperatures between ground cover treatments, if orchards, or parts of orchards, are located in high frost risk areas the effect of alleyway vegetation on air movement should be considered (Barden & Neilsen 2003). High growing alleyway vegetation may also impede orchard workers who are moving through alleyways.

The species used for cover crops will depend on both the ecosystem services which are required and local context (soil, species pool, weather, etc.). If both pest regulation and pollination services are required it is recommended that a mixture of plant species with both open and long-corolla flowers are sown (Campbell *et al.* 2012). Lower growing species such as white clover may be better suited to orchard alleyways as they are less likely to be damaged by tractor traffic or impede orchard workers. White clover, the predominant species in the 'oversown-grass' mixture, produced more flowers than any other species and was shown to be attractive to a range of beneficial species. This plant would be a suitable base-species for a cover crop mixture. It is recommended that several white clover varieties are included to extend flowering times and improve the chances of good establishment. In addition to white clover, other species which grew and flowered well included birdsfoot trefoil, yarrow, musk mallow, and oxeye daisy. Lesser knapweed and cocksfoot grass may also be considered. Between them, these species provide a range of flower structures and are likely to be a suitable 'multi-service' mixture for orchards. All of these species are relatively long-lived, or have the ability to reseed, which should help to extend the duration of the cover crop. There is potential to tailor cover crops mixture to suit target species and ecosystem services; if pest regulation was the primary desired ecosystem service the proportion of plants with open, simple structured flowers, such as oxeye daisy or yarrow, should be increased as these flowers are more suitable for many natural enemies (Branquart *et al.* 2000; Wäckers & van Rijn 2012; Laubertie, Wratten & Hemptinne 2012; van Rijn, Kooijman & Wäckers 2013). If supporting pollinators was the main purpose of the cover crop a greater proportion of clovers and legumes may be beneficial (Carvell *et al.* 2007, 2011).

The 'oversown-grass' treatment attracted similar numbers of beneficial species as the more expensive 'red' treatment, despite consisting of only two species which were over sown into existing vegetation. Of the two species only white clover showed significant growth and flowering. In established orchards scarification of existing vegetation followed by over-sowing with white clover seeds, a reduction in mowing frequency, and an increase in cutting height may prove a low-cost option for ecologically intensifying orchards. Cover crops seeds could also be broadcast onto alleyways following ploughing and rolling to remove ruts, and if a cover crop was sown at the same time as a new orchard was being created ground preparation cost would be reduced. The cost per hectare for agricultural white clover is similar to that of grass and replacing a proportion of the grass seed commonly sown in alleyways during orchard establishment with white clover could increase floral abundance at little or no net cost. Sowing cover crops at the time of orchard establishment would also allow populations of beneficial species several years to build up, before the trees started to yield. Naturally occurring flowers such as common daisy, dandelion, and hawksbit species were also shown to grow and flower well in the alleyways, and such volunteer species can be valuable to ecosystem services providers (Danne *et al.* 2010; Walton & Isaacs 2011; García & Miñarro 2014).

Mowing management has been discussed in Chapters 4 and 5 and is summarised here. It is recommended that growers reduce mowing frequency and raise the height of cuts; currently alleyways are often mowed to 5cm, and cutting at this height would damage cover crop plants and provide little shelter for beneficial species. It is therefore recommended that mowing heights are increased to 10-15cm. The frequency and timing of cuts will depend on soil fertility and weed abundance as well as which species constitute the cover crop, but if mixtures are based on perennial legumes a single cut in mid-July or August is likely to be appropriate (Pywell & Nowakowski 2006; Pywell *et al.* 2011). This allows plants to flower throughout much of the beneficial species active periods and reduces alleyway vegetation prior to harvest, improving access for workers. Reducing mowing frequency is also likely to lower operational costs as even when mowing and spraying are combined the energy used to power and pull the mower will increase diesel usage and machinery maintenance. This change in mowing management is also likely allow naturally occurring plants to grow taller and produce more flowers (Noordijk *et al.* 2009). It is recommend that cuttings are removed from areas of wildflowers to reduce smothering and remove nutrients (Ash, Bennett & Scott 1992; Carvell *et al.* 2016), and so spreading cuttings onto the herbicide strip as mulch will benefit both the cover crops and the soil fertility of the herbicide strip. For this reason the adoption of side-discharging mowers is recommended irrespective of the main purpose of the cover crop.

If cover crops were managed primarily to produce mulch, densely growing legumes and grasses such as the species used in the 'legume-grass' mixture (predominantly lucerne and cocksfoot grass) are recommended. Cheaper options include white clover over-sown into the existing sward, similar to the 'oversown-grass' treatment. There will be trade-offs between mulch production and providing floral resources and habitat for above ground species; optimal mulch production is likely to require more frequent mowing than would otherwise be recommended, with up to 4 cuts per year between May and October (AHDB 2014). The nutrient content and timing of mulch application should be considered as mulch that is too high in nitrogen or that supplies large amounts of nitrogen at the wrong time of the year can result in a reduction in fruit quality and excessive leafy growth (Granatstein & Mullinix 2008; De Angelis, Sánchez & Tognetti 2011). However, given the relatively small volumes of material that are produced in the narrow orchard alleyways it is unlikely that excess nitrogen will be produced (Mullinix & Granatstein 2011). Although cover crop cuttings are unlikely to fulfil the trees entire nitrogen demands at current alleyway widths, they may reduce demand for expensive synthetic fertilisers (Sirrine *et al.* 2008; Mullinix & Granatstein 2011).

Local conditions should be taken into consideration when sowing cover crops as some species will be unsuitable for certain soil types and climatic conditions. Frost can damage or kill plants which are not cold-hardy, and many species will not survive in soils prone to drought. Orchard soils are likely to be more fertile than is ideal for many wildflowers, reducing their ability to compete with grasses; removing cuttings to the herbicide strip can help to reduce alleyway soil fertility and boost soil fertility underneath the trees. The pressure from weeds will vary between sites and even with proper ground preparation and sowing there may be issues with weed growth and cover crop establishment. Sowing mixtures of plant species, and varieties, will help to improve the chances of establishment success.

There is a tendency for growers to manage orchards so that they look 'neat and tidy', with alleyways and windbreaks which resemble lawns and uniform garden hedges. Unfortunately, like their domestic counterparts, these habitats can be relatively poor quality habitat for biodiversity (Dobbs & Potter 2014; Wastian, Unterweger & Betz 2016), including those beneficial species which are so valuable to crop production. Leaving corners of fields and the areas underneath windbreaks to develop denser vegetation will improve the availability of nesting and overwintering sites, and maintaining flower rich cover crops which are alive with beneficial species would be a more rewarding use of alleyway space.

6.4 Future research

There are a number of areas related to the management of alleyway cover crops which would benefit from further research. There are a range of potential cover crops management options including different cover crop species, sowing dates, sowing methods, and mowing regimes. This study goes some way to identify suitable and unsuitable species and the results suggest that specialised alleyway mixes will be needed for optimal effects. The use of cover crops and other habitat strips, both perennial and annual, appears to be a space-efficient way to boost the abundance of ecosystem services providers and it may be applicable for a wide variety of crops.

There is still a need to identify which natural enemies are the most valuable to pest regulation services in orchards, and which plant species and management methods are the most suitable for these species. More knowledge on natural enemy interactions, including competition and predation, could help to develop cover crop species mixtures which are more optimal. It may be that cover crop species mixtures and ground cover management can be modified to reduce inter-species competition, perhaps by sowing different mixtures in different rows to segregate natural enemy populations. Important and considerable knowledge gaps surround how pesticides affect natural enemies. This is also true for pollinators, and because most pollinators are only needed in apple orchards during blossom it may be best to create habitat for them elsewhere, if their exposure to pesticides in the alleyways is significant.

Long-term, large-scale studies which look at the effects of cover cropping on beneficial species populations at landscape scales would enable the population and community level effects of cover cropping to be investigated (Tscharntke *et al.* 2005). Long-term experiments are also likely to be needed to fully determine the effects of mulches on soils and trees, and such studies would be useful for producing accurate cost-benefit analyses of these management practices. It is possible that in the future alleyway cover cropping could be supported by agri-environment schemes.

It would be useful to investigate the soil fertility effects of cover crops in more detail and to improve understanding of how they affect soil moisture and nutrients in the alleyways as well as the herbicide strip. Further knowledge is also needed regarding how cover cropping effects nutrient leaching, as they may help to ‘catch’ nutrients and reduce fertiliser pollution (Steenwerth & Belina 2010; Atucha *et al.* 2011; Gabriel, Garrido & Quemada 2013). Cover crop plant may also affect tree root growth and could potentially increase mycorrhizal connections with tree roots (Baumgartner, Smith & Bettiga 2005). Mycorrhizal connections can improve access to nutrients and reduce the potential damage from drought, but they are negatively affected by intensive management (Yang *et al.* 2007; Meyer, Wooldridge & Dames 2015; Van Geel *et al.* 2015).

It would useful to improve knowledge on the optimal width of the herbicide strip and to quantify any competition between trees and alleyways vegetation. This could inform management on cover crops so that competition was minimised, perhaps by mowing cover crops during droughts to both reduce water usage and add water-conserving mulch to the herbicide strip. If herbicide strips were found to be unnecessarily wide this could allow a reduction in the use of herbicides. A narrower herbicide strip would also increase the area available to grow cover crops, and more cuttings could be produced to mulch a smaller area, thus amplifying their beneficial effects. Increased mulch production could also be achieved by increasing alleyway widths; wider alleyways are common in many cider orchards. Increasing the width of the alleyway vegetation would also provide more habitat and floral resources for natural enemies and pollinators. This practice is unlikely to increase yields but it could help to make production more sustainable and may be useful for developing low-input orchards (Simon *et al.* 2017). The effect of cover crop height could also be further investigated as the effect of cover crops on orchard microclimate and the consequences for frost damage and disease risk are still not fully known; It has been suggested that taller vegetation could help to suppress the movement of apple scab ascospores (Aylor 1998).

The further development of artificial nesting sites and refugia for beneficial species is likely to be beneficial for ecological intensification as a whole. These tools have the potential to improve both pollination (Torchio & Biology 1985; Vicens & Bosch 2000; Bosch & Kemp 2002; Maccagnani & Burgio 2007; Sheffield *et al.* 2014) and pest regulation services (Corbett & Rosenheim 1996; Suckling *et al.* 2006; Roubos, Rodriguez-Saona & Isaacs 2014). It is possible that beneficial species themselves may require considerable management if they are to be maintained at high enough abundances to fulfil the ecosystem services demands of intensive agricultural systems.

As well as identifying yield gaps which may be closed by enhancing ecosystem services, it is important that the constraints on ecosystem services are assessed. Once yield gaps and the relevant ecosystem service-providing species have been identified, the constraints on suboptimal service provision should be determined (Luck, Daily & Ehrlich 2003; Kremen & Ostfeld 2005; Luck *et al.* 2009). It is likely that the constraints will be due to low abundances of service providers, perhaps because of some of the issues previously discussed, including negative effects of pesticides, lack of floral resources, or lack of nesting and overwintering sites. However, other constraints may exist: pollinators may be being attracted to another crop (Grab *et al.* 2017), or inter-species competition may be disrupting pest regulation (Moerkens *et al.* 2009). Once the constraints have been identified, management practices can be employed to reduce them. If all of the constraints are not identified changing management may not be effective: increasing nesting sites may not increase service provider populations if they are also subsequently constrained by a lack of food resources. Developing research methods and ecosystem models which can identify these constraints could help to improve the effectiveness of ecological intensification.

The methods of cover cropping and mulching using alleyways cuttings have the potential to be used in a wide variety of perennial row-grown fruit and nut crops. The creation of perennial habitat strips may also be applicable for row-grown annual crops where a reservoir of beneficial species can be maintained adjacent to cultivated rows. Alleyway cover cropping may also be useful in tropical agroecosystems, and low-input or subsistence agriculture. The practices of using of crop waste for mulch may be beneficial for agroforestry systems; straw from alleyway grown wheat could be used to mulch trees for example. Alleyway cover cropping is likely to be particularly useful in organic fruit orchards where fertilisers options are more limited and there is demand for more effective weed control practices which can replace soil-damaging tillage (Granatstein & Sánchez 2009).

6.5 Final conclusions

Food security will be an increasingly significant issue in the future, as the global population continues to grow and the demands for food, fuel, and fibre increase (Tilman 2001; McKee *et al.* 2004; Godfray *et al.* 2010). Further loss of biodiversity, declines in finite resources, and the increasing impact of climate change will also impact on food security (Godfray *et al.* 2010; Godfray & Garnett 2014). To increase the sustainability of agricultural production it important we reduce reliance on agrochemical inputs and reduce environmental damage. Enhancing ecosystem services and supporting beneficial species is therefore vital. This will include limiting practices which have negative effects on beneficial species, such as the use of excessively harmful agrochemical and mechanical practices. It will also involve a cessation of the destruction of natural habitat (Fiedler, Landis & Wratten 2008; Garibaldi *et al.* 2011; Klein *et al.* 2012; Park *et al.* 2015).

If ecological intensification is to be successful more active methods will also be needed. The quality of remaining natural habitat will need to be improved (Goverde *et al.* 2002; Hendrickx *et al.* 2007; Bailey *et al.* 2010), and it is likely that targeted habitat creation will be needed near to crops (Landis, Wratten & Gurr 2000). The studies in this thesis highlight the potential benefits of biodiversity-derived ecosystem services to apple production and demonstrate that orchard alleyway cover crops are a multifunctional management practice which can be a valuable tool for ecological intensification.

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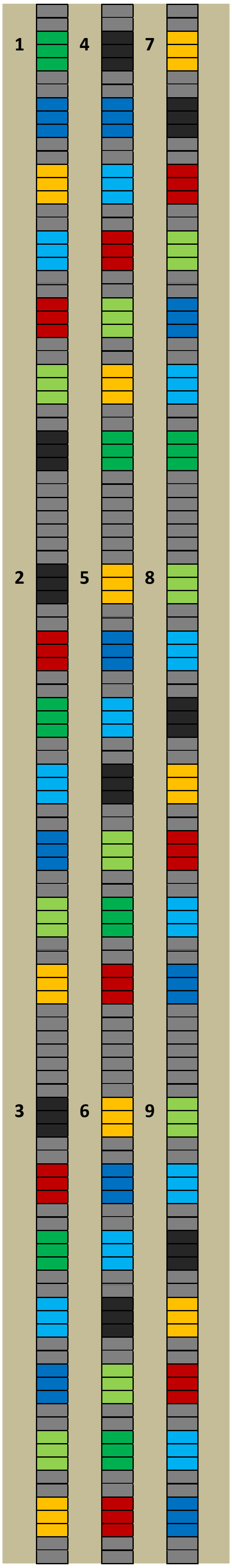
7.1 Appendix 1

Mulch trial experimental design and orchard management details:

The experimental area consisted of randomised complete block design with 9 blocks spread across three tree rows. Trees were spaced 3.5 m between rows and 1 m within the row. Crop trees were 'Gala' apples with polliniser trees (*Malus spp*.) planted between every 10th and 11th crop tree.

⬛ Control (bare soil)
🟨 Straw
🟥 Compost
🟦 Legume-grass cuttings
🟦 Legume-grass x 2 cuttings
🟩 Oversown-clover cuttings
🟩 Oversown-clover x 2 cuttings

🟫 Alleyway
▨ Spacer tree (no treatment)
1 Block number
___ Pollensier tree



Mulch trial plot design.

Mowing: Mowing of the orchard alleyways was carried out at a height of 5 cm once every 7-10 days between March and August.

Herbicide was applied to a 1 m wide strip either side of tree row (leaving 1.5 m of alleyway vegetation, predominately perennial ryegrass, *Lolium perenne*). Herbicide treatments for all plots were as follows:

May/June 2015:
Round Up Biactive (Glyphosate) - 4lts/Ha
Banlene Super (Mecoprop-p, Dicamba, MCPA) - 4lts/Ha

December/January 2015:
Rosate 36 (Glyphosate 36%) - 4lts/Ha
Banlene Super (Mecoprop-p, Dicamba, MCPA) - 4lts/Ha

May/June 2016:
Rodeo (Glyphosate 36%) - 2.4lts/Ha
Transfer (Mecoprop-p, Dicamba, MCPA) - 2.4lts/Ha

November/December 2016:
Spray Guard (Water Conditioner) - 0.4lts/Ha
Kyleo (Glyphosate 24% + 2,4-D 16%) 4lts/Ha

For pesticide and nutritional sprays see Farm 2, Appendix 2

Appendix 1

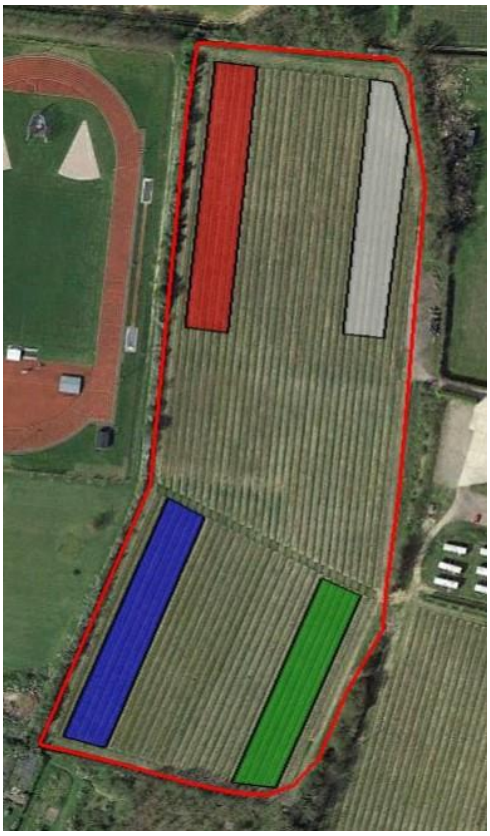
7.2 Appendix 2

Cover-crop trial experimental design and orchard management details:

This experiment consisted of a randomised complete block design using 6 orchard blocks across 2 farms (3 blocks per farm). Plots were at least 40 m away from each other and a minimum of 10 m from the orchard edge. Blocks were at least 200 m away from each other.

Mowing: Mowing of the control plot orchard alleyways was carried out on the dates of spray applications (see below), approximately once every 7-10 days between March and August, at a height of 5 cm.

Mowing of the Legume-grass, and Oversown-clover plots was carried out at a height of 15 cm in June, July, and August 2015, and July and August in 2016. The Floral-rich plots were mown in July and August 2015 and 2016, with only alternate alleyways mown in July.



An example of an orchard block with alleyway cover crop plots.

Herbicide treatment of the weed strips was as follows:

May/June 2015:
Round Up Biactive (Glyphosate) - 4lts/Ha
Banlene Super (Mecoprop-p, Dicamba, MCPA) - 4lts/Ha

December/January 2015:
Rosate 36 (Glyphosate 36%) - 4lts/Ha
Banlene Super (Mecoprop-p, Dicamba, MCPA) - 4lts/Ha

May/June 2016:
Rodeo (Glyphosate 36%) - 2.4lts/Ha
Transfer (Mecoprop-p, Dicamba, MCPA) - 2.4lts/Ha

November/December 2016:
Spray Guard (Water Conditioner) - 0.4lts/Ha
Kyleo (Glyphosate 24% + 2,4-D 16%) 4lts/Ha

Pesticides and nutritional sprays were as follows (spray application dates were on the date shown or within one day of this date):

Farm 1:

2015			**2016**		
Date	**Treatments**	**Rate per hectare**	**Date**	**Treatments**	**Rate per hectare**
28/03/2015	Headland Inorganic Liquid Copper	250 lt	24/03/2016	Headland Inorganic Liquid Copper	2.5 lt
07/04/2015	SYLLIT 400 SC	2.5 lt	02/04/2016	SYLLIT 400 SC	2.5 lt
	C-Tech Urea	2 kg	12/04/2016	Pyrus 400 SC (Pyrimethanil 40.0%)	0.75 lt
	Pyrus 400 SC (Pyrimethanil 40.0%)	0.75 lt		DITHIANON Flowable (Dithianon)	1.1 lt
15/04/2015	DITHIANON Flowable (Dithianon)	1.1 lt		C-Tech Urea	2 kg
	EQUITY	1 lt	22/04/2016	DITHIANON Flowable (Dithianon)	1.1 lt
	OPTE B	1 lt		KINDRED (Meptyldinocap)	0.6 lt
	ZINTRAC	1 lt		MAXICROP TRIPLE	2 lt
	C-Tech Urea	2 kg		ZINTRAC	1 lt

25/04/2015	KINDRED (Meptyldinocap)	0.6 kg
	PP Captan 80 Wg (Captan)	2 kg
	MAINMAN (Flonicamid)	0.14 kg
	HEADLAND PANDA	5 lt
04/05/2015	TOPENCO 100 EC (Penconazole)	0.5 lt
	PP Captan 80 Wg (Captan)	2 KG
	Pyrus 400 SC (Pyrimethanil 40.0%)	1 lt
	Bellis (Boscolid + Pyraclostrobin)	0.8 kg
	C-Tech Urea	3 kg
12/05/2015	TOPENCO 100 EC (Penconazole)	0.5 lt
	PP Captan 80 Wg (Captan)	2 kg
	MAXICROP TRIPLE	2 lt
	Difference (Difenoconazole 25%)	0.2 lt
	SENIPHOS	10 lt
22/05/2015	PP Captan 80 Wg (Captan)	2 kg
	Systhane 20 EW (Mycolbuntanil)	0.33 kg
	STROBY Wg (Kresomix-methyl)	0.2 lt
	CALYPSO (Thiacloprid)	0.375 lt
	Frutrel	5 lt
01/06/2015	PP Captan 80 Wg (Captan)	2 kg
	Systhane 20 EW (Mycolbuntanil)	0.33 kg
	STROBY Wg (Kresomix-methyl)	0.2 kg
	Frutrel	5 lt
	Difference (Difenoconazole 25%)	0.2 lt
11/06/2015	PP Captan 80 Wg (Captan)	2 kg
	Cosine (Cyflufenamid)	0.5 lt
	MAXICROP TRIPLE	2 lt
	HEADLAND PANDA	5 lt
21/06/2015	PP Captan 80 Wg (Captan)	2 kg
	NIMROD (Bupirimate)	0.6 lt
	RUNNER (Methoxyfenozide)	0.6 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
01/07/2015	PP Captan 80 Wg (Captan)	2 kg
	CORAGEN (Chlorantraniliprole)	0.175 lt
	Cosine (Cyflufenamid)	0.5 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
11/07/2015	PP Captan 80 Wg (Captan)	2 kg
	NIMROD (Bupirimate)	0.6 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
21/07/2015	TOPENCO 100 EC (Penconazole)	0.5 lt
	CORAGEN (Chlorantraniliprole)	0.175 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
31/07/2015	TOPENCO 100 EC (Penconazole)	0.5 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
10/08/2015	SENIPHOS	10 lt
	MAXICROP TRIPLE	2 lt

	OPTE B	1 lt
01/05/2016	Mix-Mate	0.25 lt
	PP Captan 80 Wg (Captan)	2 kg
	CALYPSO (Thiacloprid)	0.375 lt
	TOPAS (Penconazole)	0.5 lt
	MAXICROP TRIPLE	2 lt
	HEADLAND PANDA	5 lt
11/05/2016	Mix-Mate	0.25 lt
	PP Captan 80 Wg (Captan)	2 kg
	TOPAS (Penconazole)	0.5 lt
	MAXICROP TRIPLE	2 lt
	HEADLAND PANDA	5 lt
20/05/2016	Mix-Mate	0.45 lt
	Clayton Core (Captan 80.0%)	2 kg
	Geoxe (Fludioxonil)	0.45 kg
	Systhane 20 EW (Mycolbuntanil)	0.33 lt
	MAXICROP TRIPLE	2 lt
30/05/2016	Mix-Mate	0.25 lt
	PP Captan 80 Wg (Captan)	2 kg
	CALYPSO (Thiacloprid)	0.375 lt
	Systhane 20 EW (Mycolbuntanil)	0.33 lt
	MAXICROP TRIPLE	2 lt
	Frutrel	5 lt
09/06/2016	Mix-Mate	0.45 lt
	Clayton Core (Captan 80.0%)	2 kg
	Systhane 20 EW (Mycolbuntanil)	0.33 lt
	STROBY Wg (Kresomix-methyl)	0.2 kg
	Frutrel	2.5 lt
	MANTRAC 500	1 lt
	Difference (Difenoconazole 25%)	0.2 lt
17/06/2016	Mix-Mate	0.45 lt
	PP Captan 80 Wg (Captan)	2 kg
	STROBY Wg (Kresomix-methyl)	0.2 kg
	Systhane 20 EW (Mycolbuntanil)	0.33 lt
	RUNNER (Methoxyfenozide)	0.4 lt
	Difference (Difenoconazole 25%)	0.2 lt
25/06/2016	Mix-Mate	0.45 lt
	Clayton Core (Captan 80.0%)	2 kg
	Cosine (Cyflufenamid)	0.5 lt
	Difference (Difenoconazole 25%)	0.2 lt
	Frutrel	2.5 lt
	MANTRAC 500	0.5 lt
05/07/2016	Fontelis (Penthiopyrad 20.0%)	0.75 lt
	CORAGEN (Chlorantraniliprole)	0.175 lt
	Difference (Difenoconazole 25%)	0.2 lt
	MAXICROP TRIPLE	2 lt
	HEADLAND PANDA	5 lt
15/07/2016	Mix-Mate	0.45 lt
	PP Captan 80 Wg (Captan)	2 kg
	Cosine (Cyflufenamid)	0.5 lt
	Maxicrop Xtra-fol	3 lt

20/08/2015	Bellis (Boscolid + Pyraclostrobin)	0.8 kg	25/07/2016	Fontelis (Penthiopyrad 20.0%)	0.75 lt
	SENIPHOS	10 lt		CORAGEN (Chlorantraniliprole)	0.175 lt
	MAXICROP TRIPLE	2 lt		MAXICROP TRIPLE	2 lt
30/08/2015	Bellis (Boscolid + Pyraclostrobin)	0.8 kg		Potassium Nitrate G Grade	3 kg
	SENIPHOS	10 lt	04/08/2016	SENIPHOS	10 lt
	MAXICROP TRIPLE	2 lt		MAXICROP TRIPLE	2 lt
				C-Tech Urea	3 kg
				FERLEAF	1 lt
				Systhane 20 EW (Mycolbuntanil)	0.33 lt
			14/08/2016	Systhane 20 EW (Mycolbuntanil)	0.33 lt
				MAXICROP TRIPLE	2 lt
				Potassium Nitrate G Grade	3 kg
			24/08/2016	Bellis (Boscolid + Pyraclostrobin)	0.8 kg
				SENIPHOS	10 lt
			03/09/2016	Bellis (Boscolid + Pyraclostrobin)	0.8 kg
				SENIPHOS	10 lt

Farm 2:

2015			2016		
Date	**Treatments**	**Rate per hectare**	**Date**	**Treatments**	**Rate per hectare**
26/03/2015	Headland Inorganic Liquid Copper	2.5 lt	22/03/2016	Headland Inorganic Liquid Copper	2.5 lt
05/04/2015	SYLLIT 400 SC	2.5 lt	31/03/2016	SYLLIT 400 SC	2.5 lt
	C-Tech Urea	2 kg	10/04/2016	Pyrus 400 SC (Pyrimethanil 40.0%)	0.75 lt
	Pyrus 400 SC (Pyrimethanil 40.0%)	0.75 lt		DITHIANON Flowable (Dithianon)	1.1 lt
13/04/2015	DITHIANON Flowable (Dithianon)	1.1 lt		C-Tech Urea	2 kg
	EQUITY	1 lt	20/04/2016	DITHIANON Flowable (Dithianon)	1.1 lt
	OPTE B	1 lt		KINDRED (Meptyldinocap)	0.6 lt
	ZINTRAC	1 lt		MAXICROP TRIPLE	2 lt
	C-Tech Urea	2 kg		ZINTRAC	1 lt
23/04/2015	KINDRED (Meptyldinocap)	0.6 lt		OPTE B	1 lt
	PP Captan 80 Wg (Captan)	2 kg	29/04/2016	Mix-Mate	0.25 lt
	MAINMAN (Flonicamid)	0.14 kg		PP Captan 80 Wg (Captan)	2 kg
	HEADLAND PANDA	5 lt		CALYPSO (Thiacloprid)	0.375 lt
02/05/2015	TOPENCO 100 EC (Penconazole)	0.5 lt		TOPAS (Penconazole)	0.5 lt
	PP Captan 80 Wg (Captan)	2 kg		MAXICROP TRIPLE	2 lt
	Pyrus 400 SC (Pyrimethanil 40.0%)	1 lt		HEADLAND PANDA	5 lt
	Bellis (Boscolid + Pyraclostrobin)	0.8 kg	09/05/2016	Mix-Mate	0.25 lt
	C-Tech Urea	3 kg		PP Captan 80 Wg (Captan)	2 kg
10/05/2015	TOPENCO 100 EC (Penconazole)	0.5 lt		TOPAS (Penconazole)	0.5 lt
	PP Captan 80 Wg (Captan)	2 kg		MAXICROP TRIPLE	2 lt
	MAXICROP TRIPLE	2 lt		HEADLAND PANDA	5 lt
	Difference (Difenoconazole 25%)	0.2 lt	18/05/2016	Mix-Mate	0.45 lt
	SENIPHOS	10 lt		Clayton Core (Captan 80.0%)	2 kg
20/05/2015	PP Captan 80 Wg (Captan)	2 kg		Geoxe (Fludioxonil)	0.45 kg
	Systhane 20 EW (Mycolbuntanil)	0.33 lt		Systhane 20 EW (Mycolbuntanil)	0.33 lt
	STROBY Wg (Kresomix-methyl)	0.2 kg		MAXICROP TRIPLE	2 lt
	CALYPSO (Thiacloprid)	0.375 lt	28/05/2016	Mix-Mate	0.25 lt
	Frutrel	5 lt		PP Captan 80 Wg (Captan)	2 kg
30/05/2015	PP Captan 80 Wg (Captan)	2 kg		CALYPSO (Thiacloprid)	0.375 lt
	Systhane 20 EW (Mycolbuntanil)	0.33 lt		Systhane 20 EW (Mycolbuntanil)	0.33 lt
	STROBY Wg (Kresomix-methyl)	0.2 kg		MAXICROP TRIPLE	2 lt
	Frutrel	5 lt		Frutrel	5 lt

	Difference (Difenoconazole 25%)	0.2 lt
09/06/2015	PP Captan 80 Wg (Captan)	2 kg
	Cosine (Cyflufenamid)	0.5 lt
	MAXICROP TRIPLE	2 lt
	HEADLAND PANDA	5 lt
19/06/2015	PP Captan 80 Wg (Captan)	2 kg
	NIMROD (Bupirimate)	0.6 lt
	RUNNER (Methoxyfenozide)	0.6 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
29/06/2015	PP Captan 80 Wg (Captan)	2 kg
	CORAGEN (Chlorantraniliprole)	0.175 lt
	Cosine (Cyflufenamid)	0.5 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
09/07/2015	PP Captan 80 Wg (Captan)	2 kg
	NIMROD (Bupirimate)	0.6 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
19/07/2015	TOPENCO 100 EC (Penconazole)	0.5 lt
	CORAGEN (Chlorantraniliprole)	0.175 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
29/07/2015	TOPENCO 100 EC (Penconazole)	0.5 lt
	MAXICROP TRIPLE	2 lt
	STOPPIT	10 lt
08/08/2015	SENIPHOS	10 lt
	MAXICROP TRIPLE	2 lt
18/08/2015	Bellis (Boscolid + Pyraclostrobin)	0.8 kg
	SENIPHOS	10 lt
	MAXICROP TRIPLE	2 lt
28/08/2015	Bellis (Boscolid + Pyraclostrobin)	0.8 kg
	SENIPHOS	10 lt
	MAXICROP TRIPLE	2 lt
12/11/2015	OPTE B	2 lt
	ZINTRAC	1 lt
	BITTERSALZ	7 kg
	C-Tech Urea	3 kg
07/06/2016	Mix-Mate	0.45 lt
	Clayton Core (Captan 80.0%)	2 kg
	Systhane 20 EW (Mycolbuntanil)	0.33 lt
	STROBY Wg (Kresomix-methyl)	0.2 kg
	Frutrel	5 lt
15/06/2016	Mix-Mate	0.45 lt
	PP Captan 80 Wg (Captan)	2 kg
	STROBY Wg (Kresomix-methyl)	0.2 kg
	Systhane 20 EW (Mycolbuntanil)	0.33 lt
	Difference (Difenoconazole 25%)	0.2 lt
	RUNNER (Methoxyfenozide)	0.4 lt
	MAXICROP TRIPLE	2 lt
23/06/2016	Mix-Mate	0.45 lt
	Clayton Core (Captan 80.0%)	2 kg
	Cosine (Cyflufenamid)	0.5 lt
	Difference (Difenoconazole 25%)	0.2 lt
	MAXICROP TRIPLE	2 lt
	Frutrel	5 lt
03/07/2016	Fontelis (Penthiopyrad 20.0%)	0.75 lt
	CORAGEN (Chlorantraniliprole)	0.175 lt
	Difference (Difenoconazole 25%)	0.2 lt
	MAXICROP TRIPLE	2 lt
	HEADLAND PANDA	5 lt
13/07/2016	Mix-Mate	0.45 lt
	PP Captan 80 Wg (Captan)	2 kg
	Cosine (Cyflufenamid)	0.5 lt
	Maxicrop Xtra-fol	3 lt
23/07/2016	Fontelis (Penthiopyrad 20.0%)	0.75 lt
	CORAGEN (Chlorantraniliprole)	0.175 lt
	Difference (Difenoconazole 25%)	0.2 lt
	MAXICROP TRIPLE	2 lt
	Potassium Nitrate G Grade	3 kg
02/08/2016	Systhane 20 EW (Mycolbuntanil)	0.33 lts
	Delan Pro (12.50% Potassium phosphonate)	2.5 lt
	MAXICROP TRIPLE	2 lt
	Potassium Nitrate G Grade	3 kg
12/08/2016	Systhane 20 EW (Mycolbuntanil)	0.33 lt
	PP Captan 80 Wg (Captan)	2 kg
	MAXICROP TRIPLE	2 lt
	Potassium Nitrate G Grade	3 kg
22/08/2016	Bellis (Boscolid + Pyraclostrobin)	0.8 kg
	PP Captan 80 Wg (Captan)	2 kg
	SENIPHOS	10 lt
01/09/2016	Bellis (Boscolid + Pyraclostrobin)	0.8 kg
	SENIPHOS	10 lt
21/10/2016	PP Captan 80 Wg (Captan)	2 kg
	BITTERSALZ (EPSOTOP)	8 kg
	Maxicrop Xtra-fol	1 lt
	ZINTRAC 700	1 lt
	OPTE B	2 lt