Bespoke Field Margins Delivering Multiple Benefits to Fresh Produce

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> "Think where man's glory most begins and ends,
> And say my glory was I had such friends."
>
> W. B. Yates

For my Grandparents

Abstract

Conservation biological control has been proposed as a practice that can improve the sustainability of farming by reducing the need for synthetic chemical insecticide use and increasing resources for insect conservation. However, measures to help implement conservation biological control often do not result in increased delivery of pest control in crops and require land to be taken out of production. Carrot growers are keen to investigate how flower strips can be included in their fields to control insect pests and support pollinating insects but want to optimise the use of these strips. This thesis evaluates the efficacy of various annual seed mixes, tested over three seasons in attracting key ecosystem service providers and delivering pest regulation in the crop. Critically, the effects of the mixes on yield and quality variables at harvest were also assessed. Finally, the commercial implications of these flower strips are investigated.

Invertebrate sampling in trials across the plot, field and commercial-scale revealed that seed mix composition manipulated insect community composition, but cannot be linked with direct evidence for pest control delivery as assessed by pest aphid numbers, sentinel prey predation, and insect-damaged carrots. There was, however, indirect evidence for pest control delivery, suggesting that flowering strips can increase net carrot yield. However, these benefits were most apparent at the field edge and depended on spray regime and any existing edge effect strength. Furthermore, when the commercial implications of flower strips are subsequently analysed, flower strips incur significantly more cost in the field interior. The analysis of flower-insect visitor networks across all three trials shows that the Phacelia and Cornflower mixes best support flower visitors.

This thesis demonstrates that flower strip plant composition can be tailored for pest control and insect conservation aims, and their spatial placement can be optimised to minimise barriers to adoption from growers.

Declaration of original authorship

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

Hannah McGrath

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Chapter 1 - Introduction

"To point out that the world is a better place than it once was does not mean that, for many, existence is not still cruel and brutish: the FAO also estimates that 820 million people today face chronic food deprivation leaving 150 million children stunted".

p.41 - Henry Dimbleby, National Food Strategy, 2021

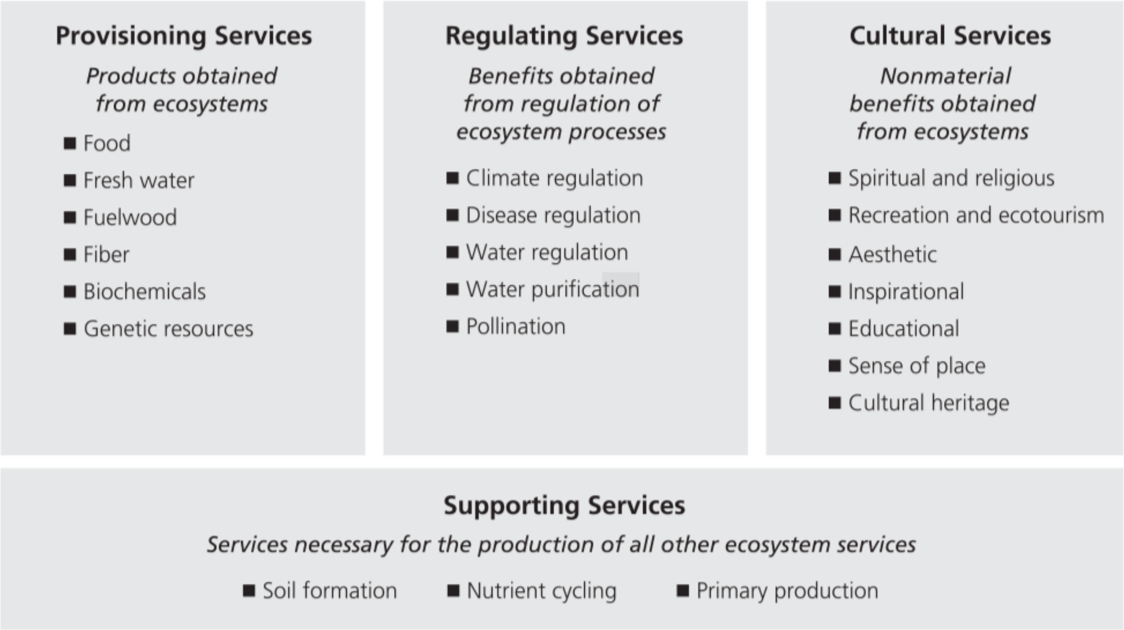
1.1 Global Environmental Crisis

Humanity's survival relies upon a functioning, healthy natural environment to provide fresh air, clean water, and sufficient food to eat. Anthropogenic impacts on the natural environment have precipitated a joint ecological and climate crisis, posing an existential threat to humanity (IPCC, 2021). Humans have successfully manipulated the natural environment for at least the last 10,000 years, domesticating crops and animals, thereby providing plentiful food for many (Brown et al., 2009). The United Nations estimates that the global population is growing by 1.1 per cent each year, with a predicted 9.8 billion people on Earth by 2050 (2017). Despite recent revisions to the required increase in food production to meet the population's food needs, agricultural production still needs to increase between 25% and 70% to meet demand (Hunter et al., 2017). Yet, these calls for increased production come amidst increasing awareness of anthropogenic agricultural activity's detrimental effects upon the natural environment. There are a wide range of drivers underlying such impacts, such as the consequences of land-use change (Gaston et al., 2003, Phalan et al., 2011), the feedback loops between agriculture and climate change (Godfray et al., 2010, Howden et al., 2007, Tilman et al., 2011), agricultural processes driving 'marine dead-zones (Beman et al., 2005), or the excess use of nitrogen fertilizers harming air, water or soil quality (Sutton et al., 2011).

The IPBES 2019 Global Assessment Report on Biodiversity and Ecosystem Services states that in the last 50 years, land-use change has had a significantly negative impact on nature. Whilst land-use change has many drivers, agricultural expansion is the most significant factor, with over one-third of the planet's terrestrial area used for cropping and livestock (IPBES, 2019). The IPBES report is clear that we can feed the world and do so in a sustainable manner. However, this will require widespread adoption of sustainable agricultural practices, such as multifunctional landscape planning, food waste reduction, and providing evidence and opportunities for supply chain transformation (IPBES, 2019). These global concerns are also echoed in the UK, particularly within the State of Nature Report published in 2019. This document also paints a similarly bleak picture of UK species, with a 13% decline in assessed indicator species abundance and a 5% decline in species distribution (Hayhow et al., 2019). Alarmingly, the International Union for the Conservation of Nature has assessed 8,431 UK species and found that 14% are threatened by extinction (IUCN, 2021). The State of Nature Report considered the increasing farm intensification and associated agricultural management as the most significant drivers of biodiversity loss (Hayhow et al., 2019). This widespread biodiversity decline is not unanticipated; the post-war intensification of UK farming since the 1940s has led to drastic land use changes (Green, 1990, King, 2011). So that by 1980, Britain had lost 97% of its species-rich grasslands and semi-natural wildflower meadows (King, 2011).

The conservation of species is vital not just for sentimental reasons or species' innate value but because humans rely upon species for effective ecosystem functioning. Following the first use of 'ecosystem services' (ES) in 1981 (Ehrlich and Ehrlich, 1981), the Millennium Ecosystem Assessment popularised the term E.S. and the underlying concept. ES are "the benefits people obtain from ecosystems" (p.53, (MEA, 2005). Within this broad definition, there is the further division into the four services, namely provisioning services, regulating services, cultural services, and supporting services, summarised in Figure 1.1.

With the rise in ES's popularity and use, the debate around the effectiveness of the concept has grown. The classification of an individual ES is not static and will depend on scale. For a beekeeper, the honey resulting from a single honey bee foraging is a provisioning service. In an apple orchard, the pollination provided by that bee is critical (Garratt et al., 2014) but can be thought of as a regulating service (Figure 1.1). Yet, that bee is redundant in a wheat field as the crop's yield is not reliant upon pollinators. The numerous and overlapping ES definitions may also have diluted the concept, and ES do not facilitate a standardized method for assigning economic value required for valid comparisons to GDP (Boyd and Banzhaf, 2007). However, given the continuing degradation of nature since the concept's inception, improvements in how ES are implemented are not enough for others with more fundamental criticisms of the approach (Melathopoulos and Stoner, 2015). Ultimately, given the urgent need to implement sustainable agriculture at scale, the Millennium Ecosystem Assessment provides a heuristic classification that can underpin the progression of sustainable approaches. So, generally, any processes in which humans benefit from natural ecosystem functioning as 'ecosystem services' (Seppelt et al., 2011).



Provisioning Services

Products obtained from ecosystems

- Food
- Fresh water
- Fuelwood
- Fiber
- Biochemicals
- Genetic resources

Regulating Services

Benefits obtained from regulation of ecosystem processes

- Climate regulation
- Disease regulation
- Water regulation
- Water purification
- Pollination

Cultural Services

Nonmaterial benefits obtained from ecosystems

- Spiritual and religious
- Recreation and ecotourism
- Aesthetic
- Inspirational
- Educational
- Sense of place
- Cultural heritage

Supporting Services

Services necessary for the production of all other ecosystem services

- Soil formation
- Nutrient cycling
- Primary production

Figure 1.1. Classes of Ecosystem Services taken from the Millennium Ecosystem Assessment

1.2 Insect Population Changes

There is particular concern about widely reported global insect declines and the consequences of these losses. Reductions are so significant that following the publication of work purporting to show 82% decline in insect biomass caught during mid-summer over 27-years (Hallmann et al., 2017), ‘Insect Armageddon’ was a term gathering considerable media attention (Carrington, 2017, The New York Times, 2017). Beyond the reported 45% mean abundance decline in 67% of all monitored invertebrate populations (Dirzo et al., 2014), there are also concerns about declines amongst moths and aphids (Bell et al., 2020), dragonflies and damselflies (Clausnitzer et al., 2009), pollinators (IPBES, 2016, Potts et al., 2010), aquatic taxa (Sánchez-Bayo and Wyckhuys, 2019) and large carabid beetles (Brooks et al., 2012). Additionally, these losses could be more profound in less developed countries with fewer data about historical populations (Sánchez-Bayo and Wyckhuys, 2019).

However, caution is required when interpreting these papers, as there have been instances when severe concerns regarding the methods used to draw conclusions have been raised (Didham et al., 2020, Montgomery et al., 2020). For example, the methods used to search for papers included within the Sánchez-Bayo and Wyckhuys (2019) meta-analysis has drawn criticism that it may lead to overstated conclusions (Mupepele et al., 2019). Additionally, doubt was cast over the findings drawn by Seibold et al. (2019), who found that declines in insect biomass, abundance and richness could be associated with the percentage of agricultural land surrounding sites. However, the conclusions drawn from Seibold et al.'s statistical analysis are profoundly different when the year was included as a random effect in the analysis (Daskalova et al., 2021). Therefore, by accounting for heterogeneity between years in the analysis, four of the five previously statistically significant arthropod declines were no longer significant (Daskalova et al., 2021).

The bleak picture of species losses is not ubiquitous, as insect declines are balanced by reports of stable populations in flying insects across three sites in Southern Britain (Shortall et al., 2009). Agricultural invertebrates also have variable responses over time, with some increases seen (Ewald et al., 2015). Whilst 54% of U.K. moth species were in decline, 22% of species, especially those with southern distributions, were found to be increasing (Conrad et al., 2004). Similarly, although 260 UK species of micro-moth declined significantly from 1970 to 2010, there were significant increases for 160 species (Fox et al., 2014). Across a wide range of species, terrestrial invertebrate biomass, abundance and richness have been assessed to be stable (Daskalova et al., 2021), and slight increases in freshwater insect abundance have been seen (Van Klink et al., 2020).

Despite the presence of media sensationalism when terms like 'insect Armageddon' are used and the valid criticisms of some methods used to paint a picture of stark declines, there are still worrying changes in insect populations (Wagner, 2020). The concern is warranted even if insect fluctuations are not at levels worthy of the 'Armageddon' adjective. A specific taxa's declines could be balanced with a limited number of increases from other taxa. However, such fluctuations are not thought to be insect populations rebounding due to targeted conservation schemes efforts. It would be an understatement to say that identifying a definitive list of drivers, and their interactions, of changing insect populations is a challenge. Nevertheless, many efforts have been made to investigate the drivers of these population fluctuations. Although criticised, Sánchez-Bayo and Wyckhuys's (2019) finding that habitat loss, driven by intensive agriculture and urbanization, is the leading cause of insect losses is not unsurprising given the body of work highlighting the importance of habitat for insects (Ekroos et al., 2010, Hunter, 2002, Saunders, 2016, Tscharntke et al., 2016). The impact of insecticides upon insect populations has also been stated as a leading cause of fluctuations (Wagner, 2020). Again, this is an anticipated finding given the increased use of these products since the ‘Green Revolution’ and widespread deployment of these chemicals over the last 60 years had been previously associated with insect declines (Ewald et al., 2015, Goulson et al., 2015, Pisa et al., 2015). However, insecticide impact may be mitigated depending on the amount of non-cropped land (Wagner, 2020). Another angle, addressing a posited diurnal bias, considers artificial light at night another source of anthropogenic disturbance that interferes with insect populations (Owens et al., 2020). This work on insect populations is supplemented by literature demonstrating the impact of extreme weather events and climate change (Ewald et al., 2015, Sánchez-Bayo and Wyckhuys, 2019), pests and pathogens (Goulson et al., 2015, Sánchez-Bayo and Wyckhuys, 2019), and insect mortality due to vehicles have impacted upon insect populations (Baxter-Gilbert et al., 2015).

There remains uncertainty in the human well-being impact resulting from insect changes, particularly pollinator declines (Dicks, 2021). However, a lack of evidence documenting the disastrous consequences of insect losses does not, unfortunately, mean the magnitude of declines will not be significant. Documented insect declines are worrisome as agriculture heavily relies upon beneficial insects to perform critical functions to food production. There are many insect dependent functions such as nutrient cycling, pollination, and natural pest control and these services can be assigned monetary value (Losey and Vaughan, 2006). For instance, in 2007, UK crop pollination was valued at £430 million (Smith et al., 2011). Specific sectors, such as the UK’s apple industry receive a £37 million service from pollination (Garratt et al., 2014). The global estimation of pollination’s value has been placed at $361 billion (Lautenbach et al., 2012), and reveals how around 75% of leading crops depend on pollination (IPBES, 2016, Klein et al., 2007). Like all methods of attributing a monetary value to a natural process, there is doubt about the methods used to estimate these figures (Hanley et al., 2015), but what remains irrefutable is the importance of pollination to maintaining global food security. The valuation of pest control services has also been attempted, despite methods facing similar limitations to estimations of pollination services. For instance, insect pest control was valued at $4.5 billion per year within the United States (Losey and Vaughan, 2006). More recently, by extending Letourneau et al.’s (2015) model to calculate pest control’s value (2015), Zhang et al. (2018) estimated that natural, non-augmented pest control could be worth up to £2.3 million to wheat farmers in the South-East of Britain.

1.3 Sustainable Agriculture

There have been numerous proposed methods of farming that could increase agriculture's sustainability, such as organic farming, agroecological crop protection, regenerative agriculture, Ecological Intensification, Sustainable Intensification, and Integrated Pest Management (IPM). IPM is one of the most notable examples of proposed farming improvements. IPM can be thought of as various tools deployed by farmers to mitigate their impact, such as designing robust and diverse cropping systems, improving pest monitoring, and understanding how resistance arises (Barzman et al., 2015). IPM is not simply reducing pesticide applications, although this is often the most explicit aim (Barzman et al., 2015). However, as IPM is a popular approach, it is a victim of its success, namely that with its proliferation, there have been many definitions of IPM given (Deguine et al., 2017). There is concern that these nebulous definitions have caused a dilution of IPM's principles and that it may simply be used as a buzzword to justify the application of pesticides (Deguine et al., 2017).

A more recent suggestion to further agriculture's sustainability is Sustainable Intensification (SI) (Tilman et al., 2011). SI prioritizes increasing yields on the current land farmed to ensure that harm is concentrated on pre-existing land rather than incurring the negative environmental impacts of land conversion (Garnett et al., 2013). SI is not prescriptive about what methods should be adopted; only those methods to increase sustainability should be viewed with the same respect as efforts to increase productivity (Garnett et al., 2013). However, the notable concern regarding SI is the gap between academic interest in SI and its implementation in farming (Kleijn et al., 2019). To overcome this, Kleijn et al. (2019) propose that more work is required in commercially relevant settings with outputs of interest to farmers, such as implementation costs.

Common across many proposed solutions to help enact sustainable farming is reducing farmer reliance on synthetic solutions to pests and pathogens. The use of pesticides – be those insecticides, herbicides or fungicides – has been highly successful in increasing food production, doubling crop yields at harvest from 1965 to 1990 (Popp et al., 2013). However, these bountiful harvests have not come without environmental costs. Beyond the adverse effects of insecticides upon pollinator populations documented above (Dicks, 2021), there have been fatal consequences of pesticide pollution in rivers and streams on the freshwater invertebrates in those habitats (Let et al., 2021) and concerns over the persistence of commonly used products in soil and the subsequent impacts for soil fauna (Jones et al., 2014).

Given the nature of the resulting impacts from the use of pesticides, both on the environment and consumer awareness, there have been significant policy decisions made. These policy decisions cover two key areas; the regulation of products currently available for use and the regulatory pathway that potential products must pursue to enter the market. In the case of the former, there have been notable moves to restrict the most harmful pesticides. One of the most widely known restrictions was the ban of the use of DDT, an organochloride that has been seen to persist in the environment decades after its last use in the 1980s (Kurek et al., 2019). More recently, there has been prominent public debate over the risk of neonicotinoid seed coatings on oilseed rape using clothianidin, imidacloprid, and thiamethoxam. At the root of this discussion was the sub-lethal effects of these seed coatings upon pollinators and particularly wild bee populations, which have seen declines (Woodcock et al., 2016), as well as insectivorous birds (Hallmann et al., 2014) and aquatic invertebrates (Beketov et al., 2013). A two-year restriction placed on the seed coatings by the European Commission in 2013 has since continued, and farmers now face severe challenges to control cabbage stem flea beetles (*Psylliodes chrysocephala*) as a result (Pickering et al., 2020). New plant protection products seeking approval and entering the market face considerable barriers, especially in the European Union. Products must undergo toxicity tests in the approval process and account for any uncertainty in their safety if used widely (Schafer et al., 2019). Approval can often require expensive, complex laboratory and field tests to demonstrate that products do not breach safe concentrations or to show that any breaches will not have severe impacts (Schafer et al., 2019). There has been a marginally 75% reduction in products applying for approval from 2001 to 2009 (Chapman, 2014). Suggesting this is evidence of the scale of the challenge, even for major crops that occupy a significant market share. For minor crops, with smaller and less attractive market shares for agrochemical companies, this reduction in new products along the product development pipeline may be disastrous (Chapman, 2014).

Insecticide resistance, however, may pose an even more fundamental challenge to farmer reliance upon plant protection products. For instance, *Myzus persicae* is the most economically important aphid pest globally (Bass et al., 2015). As a polyphagous pest, it has a wide range of plant hosts and causes crop damage through direct feeding and producing honeydew, but primarily as a vector for viruses (Edwards et al., 2008). As a result of *M. persicae's* polyphagous nature, it is exposed to a range of plant protection products, and at least seven resistance mechanisms have been documented within the species (Bass et al., 2014). These proposed resistance mechanisms are all thought to be constitutive, whereby the pathway through which resistance is conferred is always ‘on’, which can incur fitness costs to the aphid (Bass et al., 2014). Whilst there is debate about the scale of fitness costs to individual aphids (ffrench-Constant and Bass, 2017), it is reasonable to expect that if resistance requires the constant production of a metabolic enzyme, then this could come at an energetic cost for the aphid (Kliot and Ghanim, 2012). Fitness costs matter as it has been found that these resistance mechanisms can affect aphid alarm pheromones, which could have broader implications for pest management (Foster et al., 1999). It has recently been proposed that like pests, NE can also evolve resistance to insecticides (Bielza, 2016). Such NE resistance should not be used as a justification for the continued prophylactic use of insecticides, as in a similar manner to *M. persicae*; there could be associated fitness costs for NE. Furthermore, resistance to insecticides is not just molecular, as behavioural changes in dispersal have been seen when *M. persicae* is exposed to insecticide-treated plants (Fray et al., 2014).

Recently the model of combating insect pests has relied upon waiting for a new product to enter the market. A solution that is typically based upon a similar method to its predecessors. However, through the tripartite interaction between regulatory restrictions, a lack of emerging products, and bourgeoning pest resistance, the foundations of this paradigm are being shaken.

1.4 Conservation Biological Control

Given the purported negative impacts of synthetic pesticides on insect populations (Dicks, 2021) combined with target insects' resistance to insecticidal applications (Barzman et al., 2015, Gould et al., 2018), alternative approaches to pest control are now necessary. Using natural pest control, harnessing the power of predatory insects that can ‘biologically control’ populations is a popular alternative. Conservation biological control (CBC) is an approach that could help to sustainably intensify agriculture (Begg et al., 2017, Gurr et al., 2017, Landis et al., 2000), reduce over-reliance upon plant protection products (Wratten and Gurr, 2000), and help to conserve insect populations (Fiedler et al., 2008). Although many have defined CBC, generally, there is consensus that CBC is thought of as habitat manipulation that helps to encourage, enhance, conserve, protect or support beneficial natural enemy populations which control pests, thereby reducing crop losses (Begg et al., 2017, DeBach and Rosen, 1991, Eilenberg et al., 2001, Gurr et al., 2017, Landis et al., 2000).

CBC has been further split into two considerations that can be made: those regarding the ‘conservation’ of natural enemies and then the subsequent ‘biological control’ of pests (Begg et al., 2017). Evidently, like many conceptual frameworks, distinctions between these categories are blurred. However, this split allows us to assess CBC efforts from activities attempting to increase NE population or help support insect conservation and those that measure pest control delivery in an area. A unifying principle underlies the practice of CBC, i.e., increasing resources to increase natural enemy abundance and survival. The often-used mnemonic, ‘SNAP’, is a useful way of categorising the shelter, nectar, alternative food and pollen resources required for CBC (Gurr et al., 2017). An alternative categorisation of support for NE splits resources into trophic and structural resources, with trophic resources including pests, alternative prey, or non-pest food such as pollen, nectar, or fungi (Iuliano and Gratton, 2020). Structural resources are the habitat structures that facilitate NE sheltering from weather, predation and disturbance or may be features that enable reproduction and overwintering (Iuliano and Gratton, 2020). These resources are typically provided by some form of habitat manipulation, such as banker plants, insectary plants, secondary plants, areas of flowering vegetative strips or grassy beetle banks. At the field scale, a fundamental commonality between these approaches is the inclusion of non-crop plants used to attract beneficial insects.

Given the urgent need to implement sustainable agricultural practices, it is necessary to assess whether CBC effectively controls insect pests. Vegetative strips are a common method used in field level CBC. Strips typically contain a combination of grass and flowering plants, which generally increase insect abundance and diversity compared with cropped areas (Haaland et al., 2011). For a more comprehensive review of CBC and flower strips, readers should consult Haaland et al. (2011), Holland et al. (2017) and Johnson et al. (2021). A successful example of vegetative strips are efforts to support ground beetles. Tussock grasses increase general and predatory beetle abundance and richness compared with fine grasses (Woodcock et al., 2005, Woodcock et al., 2008). Beetle communities also benefit from the creation of grassy ridges, or ‘beetle banks’, which have been demonstrated to favourably increase predatory beetle densities compared with conventional hedge boundaries (Collins et al., 2003, Collins et al., 2002).

Flower strips have been used to alter aerial insect communities to favour aphid predators successfully. However, there remains considerable variability in the effectiveness of flower strips at attracting key ecosystem service providers (Haaland et al., 2011). For instance, in wheat crops in Belgium, there was a significant reduction in aphid number between in-field wildflower strips, thought to be associated with high adult hoverfly abundance (Hatt et al., 2017). However, some individual syrphid species, like *Episyrphus balteatus*, have been associated with field margins rather than in-field wildflower patches (Sutherland et al., 2001). Despite numerous examples of successful insect community manipulations, knowledge gaps remain, as vegetable, root crops, and perennial systems remain understudied (Holland et al., 2017).

There is a well-documented failure within CBC papers to quantify the impact of these upon pest numbers, crop yield or quality and even fewer examples demonstrating the impact upon economic considerations (Holland et al., 2017, Johnson et al., 2021, Griffiths et al., 2008, Haaland et al., 2011). This is an arguably frustrating barrier, as when the trophic cascades from these insects onto ecosystem service delivery are considered, there are examples of success. Across a five-year rotation on a 900 hectare farm, there were increases in the total per unit productivity following the creation of diverse mix of habitats on 8% of land (Pywell et al., 2015). By considering the entire rotation, there was no loss to the farmer's gross yield or nutritional value as yield increases in beans could offset yield losses in wheat (Pywell et al., 2015). From a grower perspective, this is perhaps counter-intuitive point, whereby taking land out of production can result in comparable or even higher yields.

Although, caution is warranted as increasing the amount of non-cropped surrounding fields does not automatically result in increased pest suppression (Karp et al., 2018). It is possible that these CBC attempts are unsuccessful for a variety of well-discussed reasons (Tscharntke et al., 2016). Consequently, it is critical for growers and resulting wider environmental and societal impacts that the ecosystem service delivery from these flower strips to be quantified.

1.5 UK Carrot Production

The UK is almost self-sufficient in the supply of carrots (BCGA, 2018). Unlike arable agriculture, where there are thousands of farmers, carrot production is controlled by a handful of growers on around 10,000 hectares (BCGA, 2018). One of the largest growers is Huntapac Produce, a fourth-generation family-run business, farming around 1500 hectares across the UK, with typically 1000 hectares dedicated to carrot production. Huntpac supply carrots various retailers throughout the year, with production in their packaging facility only halting on Christmas Day. Consequently, Huntapac are under pressure to ensure a constant supply of carrots ready to be harvested throughout the year. Any disruption in this supply to supermarkets could endanger the likelihood that the business can secure future contracts with retailers.

Like all crops, there are a range of pests and pathogens that cause damage to carrots and impact production and profits. A notably challenging period for Huntapac is the carrot crops sown around the middle of March, the 'second-early' crop of carrots. These second-early crops are susceptible to colonization by *Cavariella aegopodii* (willow-carrot aphid). Typically around the beginning of May, *C. aegopodii* migration from their winter hosts begins, with aphids moving from willow trees (*Salix* spp.) to their summer hosts, carrots and other apiaceous plants (Dunn, 1965). Whilst there are some yield losses associated with the direct feeding from aphids (AHDB, 2015), significant harm is caused by aphid-vectored viruses. As the aphids migrate into the crop, *C. aegopodii* and *Myzus persicae* can vector a suite of viruses which that infect carrots (Adams et al., 2014). Carrots are particularly vulnerable as the virus infection often happens at the plants' cotyledon stage, subsequently causing complete plant death (AHDB, 2015). Carrot viruses can also cause internal necrosis within the root, but with no externally visible symptoms (Adams et al., 2014), this can lead to difficulties removing the infected carrots during sorting. If the infected crop is not removed, subsequent customer complaints can have obvious commercial implications (*B Madarasi, personal communication*).

Predictably, there are serious business repercussions from aphid damage. In 2015, there was a notably high pest pressure from *C. aegopodii,* and the associated virus transmissions caused yield declines of 13-14% in Huntapac's fields (*B Madarasi, personal communication*). More widely, growers saw yield declines of up to 15% across the country, which caused £20 million worth of losses to the carrot industry (Hinds, 2016). Consequently, it is of critical importance to businesses like Huntapac to control aphid pests. Currently, some of the typical products used to control *C.*

aegopodii are Movento (Active Ingredient: Spirotetramat), Biscaya (Thiacloprid) and Pyrethroids (Cypermethrin, Deltamethrin, & Lambda-cyhalothrin) (Collier, 2017, Hinds, 2016). Following concern in the industry for some time, resistance to lambda-cyhalothrin has recently been discovered within *C. aegopodii* (Foster, 2018). This, along with the lack of new products in the pipeline (Hinds, 2016), has driven the industry to alternative methods of control.

IPM methods have been explored, particularly monitoring, to help increase the amount of information available to aid the timings of chemical applications (Barzman et al., 2015, Ehler, 2006). For example, the Rothamsted Insect Survey's network of suction traps is used alongside a day-degree model to forecast the timing of aphid migration each spring (Collier, 2018). Additionally, growers like Huntapac also use yellow water traps alongside traditional crop walking to monitor the migration of aphids into the crop. However, whilst forecasting systems and pest monitoring are useful components of IPM (Barzman et al., 2015), resistance to insecticides, unfortunately, negates any potential avenue to target some chemical control applications. Another proposed aphid control solution could be to delay sowing, as this form of cultural control could reduce the crop's susceptibility to aphid colonization. However, this would impact growers' ability to meet supermarket demands for carrots all year round. Therefore, this option is not seen favourably by growers as a realistic aphid control tactic (*B Madarasi, personal communication*). Moreover, IPM is often successful as it relies upon thresholds (Ramsden et al., 2017). For instance, within arable systems, pest populations such as *Psylloides chrysocephala* (Cabbage stem flea beetle) can build up, but until the number of beetle larvae reaches 2 per plant, the oilseed rape plants can compensate for the injury and yield losses are not seen (Ramsden et al., 2017, Walters et al., 2001). Whereas, within horticulture, as supermarkets and consumers have high crop quality standards, the need for high-quality crops can often supersede demand for high yielding crops. As carrot crops cannot compensate for the damage caused by viral infections, growers feel they cannot use thresholds (*B Madarasi, personal communication*).

Evidently, there are few methods to control aphid infestations in carrot crops that do not rely on chemistry. Huntapac are aware of the cases where CBC has successful controlled pests and would like to explore CBC as an alternative aphid control method. However, there are some significant agronomic differences between these published approaches and carrot production. Unlike widely used perennial flower strips, CBC in carrots requires annual vegetation strip as crops are grown in rented fields as part of a five-year rotation. Nevertheless, despite these differences there is still a demand for alternative control methods.

Social responsibility is an additional driver from Huntapac to have multifunctional margins to supporting wider insect biodiversity. The business is aware that their activities can have negative impacts upon wildlife and are keen to ensure they are supporting pollinators, farmland birds and other insect biodiversity where possible.

1.6 Aims

This thesis, therefore, set out to identify the best seed mix for pest control and insect conservation within carrot fields. This began with work to identify the key Ecosystem Service Providers (ESP) for *Cavariella aegopodii* pest control using small plots and sentinel plants, before building into commercial field trials in carrots. This first commercial trial assessed the integration of flower strips and developed methods to assess the direct and indirect evidence for Ecosystem Service Delivery (ESD) from strips. This was followed by a larger-scale trial across multiple fields considering the ESP supported by seed mixes, subsequent ESD into the crop and then a holistic consideration of flower strip impacts. Then, to address each seed mixes' support for insect conservation, flower visitors across all trials are investigated. Finally, this thesis concludes by synthesising the findings from all years to discuss the 'best' performing mix and the success of CBC and flower strips.

1.6.1 Specific Research Questions

1) Can the key ESPs that control *Cavariella aegopodii* be identified? *Chapter 2.*

2) What is the 'best' seed mix for supporting these key ESPs? *Chapters 2 & 4.*

3) When the impacts of flower strips upon carrot production, economics, and environmental factors are quantified, does this demonstrate CBC success? *Chapters 3 & 4.*

4) By assessing seed mixes for their support for insect visitors, is there a mix that performs well? *Chapter 5.*

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Chapter 2 – Assessing seed mixes for their ability to support ecosystem service providers and deliver pest control on a plot scale.

2.1 Abstract

With increasing pressure to farm sustainably, Conservation Biological Control is purported to allow farmers to reduce their reliance upon synthetic pest control products. Whilst many have studied the associations between sown vegetative areas and key ecosystem service providers, there is a lack of evidence quantifying the impact these species have in terms of delivery of pest regulation. There is little information available regarding the fundamental ecology of natural enemies of carrot pests, which is critical for growers seeking to reduce their environmental impacts. Consequently, a replicated manipulative plot trial was implemented on an experimental farm with the aim of assessing the efficacy of a range of commercially available annual plant species at attracting key ecosystem service providers. Alongside these mixes, sentinel carrot plants with exclusion cages were used to identify the different effects between foliar and epigeal natural enemies. Due to extreme weather conditions in the record-breaking summer of 2018, the sentinel plants element of the trial failed. The foliar and epigeal invertebrate communities sampled from the sown mixes differed, suggesting these communities could be manipulated via the species composition of mixtures. However, there was no measurable improvement in ecosystem service delivery, here measured by predation on sentinel prey cards and barley yield. These results suggest that further work is required to elucidate the impact of differing insect communities upon carrot pests.

2.2 Introduction

Agricultural intensification has been associated with reducing landscape features known to support natural enemies (NE) (Bianchi et al., 2006, King, 2011, Green, 1990). A reduction in NE abundance and particularly diversity is concerning as this could lead to increased pest outbreaks (Wilby and Thomas, 2002). In addition, parasitoid populations in monoculture crops finding pollen and nectar sources is challenging (Wackers, 2004). To counter this, Conservation Biological Control (CBC) is a posited solution. CBC is deployed at varying scales from the plot, field, farm, regional, or landscape level, with a vast amount of ecological knowledge required for a successful habitat manipulation (Begg et al., 2017, Gonzalez-Chang et al., 2019, Landis et al., 2000).

Arable crops are a particular focus of CBC efforts, with one quarter of papers focusing on wheat crops (Holland et al., 2017). By comparison, only 9% of papers considered barley crops, and even fewer considered vegetables and root crops (Holland et al., 2017). Of these papers, the vast majority focus on Ecosystem Service Providers (ESP) and NE communities, with little focus on the delivery of pest control services (Haaland et al., 2011, Holland et al., 2017, Johnson et al., 2021). Contrary to this consistent theme, Holland et al. (2008) reveals it is possible to go beyond considering the insects present at a mix and investigate the impact of natural enemy guilds on predation. In this instance, flying and epigeal NE access to sentinel wheat plants was restricted using exclusion cages (Holland et al., 2008). Through this manipulative experiment, the aerial NE were more effective at controlling *Sitobion avenae* aphids than epigeal predators (Holland et al., 2008). Efforts like this, helping to elucidate the underlying mechanisms by which NE control pests, are critical to designing effective CBC efforts.

2.2.1 Methods used in plant mix selection

The use of plant traits, particularly flower corolla length, to assess the impact of increasing functional diversity upon abundance and diversity of NE populations has been widely considered (Balzan et al., 2014, Campbell et al., 2012, Fontaine et al., 2006, Hatt et al., 2017). However, this work has mixed findings with a varying trend in increasing plant functional diversity and resultant insect abundance and diversity changes. In a controlled scenario, complementary pollinators, bumble bees and syrphid flies respond positively to plots of open and tubular flower shapes compared to plots containing only one flower type (Fontaine et al., 2006). However, this response to increasing plant functional diversity does not appear to be ubiquitous, especially in field trials. Hatt et al. (2017) found that some increases in NE abundance and richness were seen with increasing plant functional diversity, as assessed by a plant functional diversity index. However, these NE population increases did not peak with maximal levels of plant functional diversity. This effect on the NE population is perhaps not unsurprising as Campbell et al. (2012) found that interactions and competition between different NE guilds at higher plant functional diversity resulted in lower parasitoid flower visitation. These differential responses of NE guilds were also found by Balzan et al. (2014). Here, ground predators did not benefit from increased sown plant functional diversity plots in the same manner that aerial NE did (Balzan et al., 2014). Whilst traits like corolla length, colour or UV reflectance can assist the design of flowering mixes, it is not a guarantee of success.

Another effective approach to designing flowering mixes has been the targeted attraction of certain groups of NE, which is of interest to this study given the purported success of Syrphinae larvae and Braconid wasps Aphidiinae at controlling aphid pests (Ramsden et al., 2017). The method relies on extensive research investigating the responses of NE guilds or NE species to individual flowers. The attraction of parasitoid species to flowers has spawned the development of the 'nectar provision hypothesis' (Heimpel and Jervis, 2005), which presents an attractive solution to help parasitoids overcome any nectar or pollen limitation (Heimpel, 2019). There are numerous examples of the success of this method (Balzan and Wackers, 2013, Dively et al., 2020, Ribeiro and Gontijo, 2017, Sivinski et al., 2011, Wackers, 2004); reviewed by Russell (2015). There are sources of conflict within the literature, such as the effectiveness of *Daucus carota* in attracting parasitoids (Key, 2013, Sivinski et al., 2011, Wackers, 2004). However, there are documented instances of *Fagopyrum esculentum, Anethum graveolens* and *Lobularia maritima* positively affecting parasitoid populations. Such as the increase in the mass of the parasitoid *Trybliographa rapae*, useful in the control of *Delia radicum* (cabbage root fly) (Nilsson et al., 2011).

Pollen analysis of the gut of aphidophagous hoverflies, *Euphodes corollae*, *Episyrphus balteatus* and *Sphaerophoria rueppellii* revealed *Coriandrum sativum* pollen following the plant's inclusion within sweet pepper glasshouses (Pineda and Marcos-Garcia, 2008). However, such feeding on coriander pollen was not seen to increase subsequent *S. rueppellii* offspring fitness (Amoros-Jimenez et al., 2014). Other work has focused on *Episyrphus balteatus* fitness after feeding at six common flowering plant species finding that individual plant species differentially influence hoverfly populations under laboratory conditions (Laubertie et al., 2012). For instance, while

Phacelia tanacetifolia significantly increases *E. balteatus* oviposition and lifetime fecundity, *Fagopyrum esculentum* had the highest *E. balteatus* lifespan thereby increasing egg laying duration (Laubertie et al., 2012). Additionally, research into aphidophagous coccinellids has revealed that *Coriandrum sativum* can help sustain populations of *Cycloneda sanguinea* (spotless ladybird) as they control aphids on tomatoes (Togni et al., 2016). This example highlights that CBC is not simply about nectar or pollen provision, as whilst *C. sanguinea* female survival increases after feeding on *C. sativum* flowers', *C. sativum* is mainly used by *C. sanguinea* as an oviposition site (Togni et al., 2016).

Encouraging individual NE guilds or species is helpful if a great deal is known about the target pest or growing conditions can be controlled, such as protected horticultural production. However, in the context of field crops, especially minor horticultural crops such as carrots, supporting a rich and diverse range of NE and functional traits may be advisable (Snyder, 2019, Greenop et al., 2018). First, it may be that there is insufficient information about a specific pest and NE dynamic. Second, it is possible that species complementarity could be enhanced, whereby a rich NE assemblage can occupy various ecological niches with high functional redundancy and therefore increase ecosystem functioning (Tilman, 2000, Feit et al., 2019). Finally, it may also be wise to consider targeting an individual species approach in the context of threats from widespread biodiversity loss and climate change with associated species shifting their range, particularly as decreasing NE species number may result in herbivore population outbreaks (Wilby and Thomas, 2002). However, there are inconsistent effects of increasing NE richness so this is not a guarantee of success (Finke and Snyder, 2010). Rather than simply considering NE taxonomic diversity, there is growing support for considering the diversity of NE functional traits (Greenop et al., 2018).

Therefore, this chapter seeks to address a knowledge gap regarding the sowing of different plant mixes to control carrot pests through a more mechanistic manipulation of NE populations. This can be investigated via sentinel plants and exclusion cages which are a useful tool for the elucidation of importance of different NE guild at controlling aphid pests.

2.2.2 Research Questions

Ecosystem service providers

1) Do sown seed mixes attract more ecosystem service providers (ESP) than control plots? Will there by more aerial ESP in flowering mixes than control and grass plots? Or more epigeal ESP invertebrates present in grass plots than sown flower mixes?
2) Are there distinct differences in the invertebrate communities present in different sown mixes, especially when compared with control plots?

Ecosystem service delivery

3) Are there measurable improvements in pest control as assessed via sentinel plants adjacent to flower mix plots? Does pest control delivery happen predominantly via foliar or epigeal natural enemies?
4) Are there measurable improvements in pest control as assessed via sentinel aphid cards and barley harvest yield?

2.3 Materials and Methods

2.3.1 Study Site – Rothamsted Research Experimental Farm

In the 2018 and 2019 field seasons, a replicated plot experiment was conducted across five fields on Rothamsted Farm in Harpenden, Hertfordshire, UK. In 2018, six treatments (five flower mix treatments and one barley control) were sown in six replicates over four fields. In 2019, one field was replaced, and eight treatments (seven flower mix treatments and a barley control) were sown. The soil across the fields varied but generally soil was a clay loam containing variable, but often large amounts of flint. All selected fields were sown to winter cereal crops (wheat, barley, or oats). To standardise for crop interior, experimental seed mixes were sown as separate plots within a 12 m wide buffer strip of spring barley sown at the crop edge. Replicates were positioned as near as practically possible on a north/south aspect to avoid directional influence between replicates. To accommodate sufficient spacing of at least 30m between experimental plots, each replicate was split into 2 subsets, with half of the plots in the 'north-facing' side of the field and the remaining treatments in the 'southern-facing' side of the field (Figure 2.2)f. The experiment was designed as an incomplete block design, ensuring each flower mix treatment occurred equally on northern and southern sides, at which point treatments were randomly allocated to plots within each replicate.

The spring barley buffer strips were established each year in early February by ploughing up an area 12m wide x 150m (min) long from the established cereal crop along the northern and southern edge of the field. These areas were then drilled with barley (KWS Irina at 350 seeds/m^2) on 26/2/2018 and 25/2/2019 in the respective years. Areas for the individual seed mix treatment plots (6m x 10m) were created within the spring barley buffer strip by spraying off the barley with herbicide (Glyphosate) on 19/4/2018 and 25/3/2019. Around five days after the application of glyphosate, it was apparent that the herbicide had killed off the barley and the seed mixes for each treatment were hand-broadcast over the 6m x 10m treatment plots (23/4/2018). Seeds with a low 1000 seed weight were mixed with sand to facilitate hand-broadcasting. To ensure even coverage of the margin plots, the total seed to be sown was split and broadcast from two different directions. Seed mixes were sown on 23/4/2018 and 4/4/2019 and following broadcasting, plots were rolled to ensure good seed-soil contact to encourage germination.

Despite efforts in 2018 to collect experimental data, due to challenges with the record-breaking temperatures during the summer (McCarthy et al., 2019), these efforts mostly failed. Subsequently, this chapter mostly focuses upon data collected in 2019.

2.3.2 Sentinel Carrots

In 2018, an experiment using potted carrot plants and exclusion cages was designed to test the efficacy of foliar and epigeal natural enemies and the interaction between the two, upon pest control of *Cavariella aegopodii* aphids upon sentinel carrot plants. More detail about the design of this experiment can be found in Appendix 2.7.1.

To reduce the challenges with keeping sentinel plants in pots alive, in 2019, alongside each flower strip carrot seeds were sown directly at the same time as seed mixes were broadcast. Sentinel aphids were going to be placed upon these plants, and the exclusion cages from 2018 applied. However, it became apparent that there was an accidental application of herbicide which affected these strips of sentinel carrot plants. Unfortunately, this had an unequal effect between replicates and treatments and would have resulted in poor quality data, so this element of the trial was not pursued.

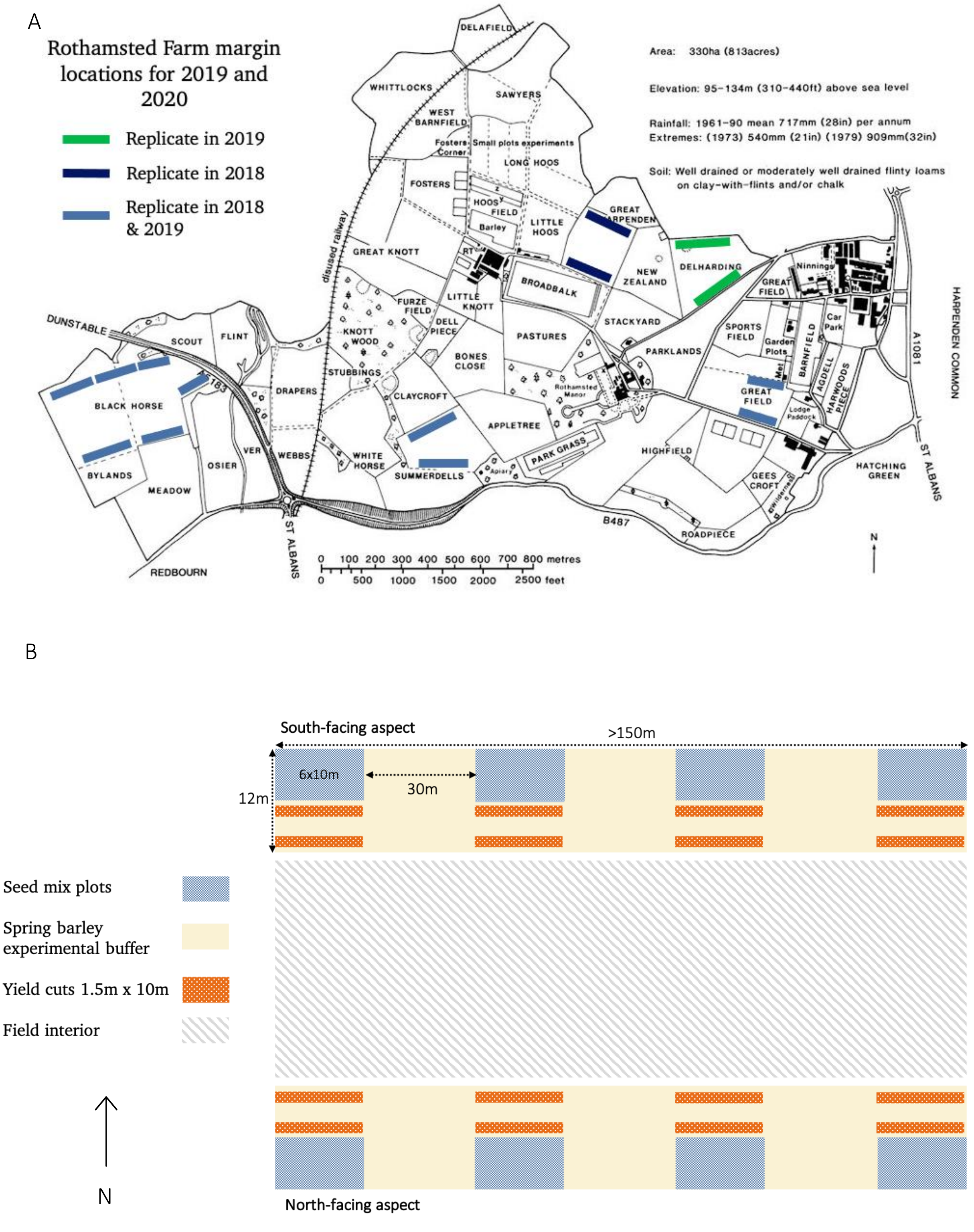


Figure 2.2. **A** Rothamsted Farm map showing the locations of replicate strips of different flower mixtures tested in 2018 and 2019. **B** Experimental layout of plots of flower mixture treatments within the spring barley buffer within each replicate strip. Field interior was always sown to cereal crops.

2.3.3 Seed Mix Composition

The selection of candidate species for mixes through plant traits and attracting key ecosystem service providers have been instrumental in the design of flower mixes used in this study (Table 2.1). Although, due to the time constraints upon the project, a combined method has been used to select flower species tested in this experiment. As the land used here is being taken out of production in some of the country's most productive and expensive agricultural land, growers must be confident that plants reliably establish, support NE and deliver pest control services. Also, especially in the context of carrot production, these mixes must perform well across a range of landscape compositions, qualities and configurations, which have well-documented influences upon NE and pest assemblages (Karp et al., 2018, Plecas et al., 2014, Tscharntke et al., 2016, Woltz et al., 2012). Furthermore, given the desire to eventually use any successful mixes on a national wide commercial scale, the availability of seed and the willingness of landowners and farmers to sow these mixes on their land had to be considered. These practical realities resulted in certain species being removed from contention due to concerns over their potential to become established weeds, such as borage volunteers in following OSR crops (Allison, 2014). Finally, the project's close collaboration with carrot growers who only have access and control to their fields on an annual rented basis heavily restricted the use of perennial species, especially *Lobularia maritima*.

Campbell et al. (2012) make a valuable suggestion to separate flower plots to quantify the effect and response of individual insect taxonomic units to a flower species. However, given the time constraints, a compromise was found whereby mixes sown comprised of three plant species, with eight mixes tested in this chapter.

To help ensure mixes established well, species that were thought to be dominant, specifically *Phacelia tanacetifolia, Centaurea cyanus* and *Fagopyrum esculentum* were separated into different mixes (Marek Nowakowski, personal communication). Given this project's carrot focus, an 'Apiaceae' mix was created to investigate whether this mix was a potential reservoir for pests or viruses, or if any specific NE had co-evolved alongside Apiaceous flowers. A grass mix was created following the documented success of beetle banks upon ground predators and farmland birds (Woodcock et al., 2005). Finally, two mixes designed on their purported early or late flowering phenology were selected (Ian Wilkinson, Cotswold Seeds, personal communication).

Table 2.1. Composition of seed mixes planted in on Rothamsted Farm in 2018 & 2019.

		2018	2019
Mix Name	Species names	Density per plot (g/m^2)	Density per plot (g/m^2)
Apiaceae	Dill – *Anethum graveolens*	0.67	0.60
	Coriander – Coriandrum sativum	0.67	0.73
	Wild carrot – *Daucus carota*	0.25	0.50
Phacelia	Coriander – *Coriandrum sativum*	0.67	0.73
	Phacelia tanacetifolia	1.05	0.98
	White mustard – *Sinapsis alba*	2.15	2.05
Cornflower	Field poppy – *Papaver rhoeas*	0.05	0.15
	Cornflower – *Centaurea cyanus*	0.13	0.13
	Crimson clover – *Trifolium incarnatum*	1.67	1.67
Buckwheat	Buckwheat – *Fagopyrum esculentum*	5.00	4.50
	Corn chamomile – *Anthemis arvensis*	0.05	0.05
	Corn cockle – *Agrostemma githago*	0.13	0.40
Grass	Westerwold ryegrass – *Lolium multiflorum* west.	4.05	3.03
	Italian ryegrass – *Lolium multiforum*	3.75	2.82
	White millet – Panicum miliaceu	2.68	3.35
'Early' Flowering	Corn marigold – *Chrysanthemum segetum*	-	2.50
	Persian clover – *Trifolium respinatum*	-	1.12
	White mustard – *Sinapsis alba*	-	2.05
'Late' flowering	Linseed – *Linum usitatissimum*	-	5.00
	Forage rape – *Brassica napus*	-	1.12
	Birdsfoot trefoil – *Lotus corniculatus*	-	0.83

2.3.2 Sampling

2.3.2.1 Seed Mix Establishment

In each seed mix plot, the vegetation present was surveyed on 1st August 2019. Three 0.5m2 quadrats, covering 1.5% of the total plot area, were placed within the margins. The three quadrat locations were chosen so that the vegetation sampled was representative of the entire margin plot. Each individual plant species was visually identified (Rose et al., 2006). The percentage cover of each species along with any bare ground present was then scored using the Domin scale (Kent, 2011). After assessing the area, if vegetation overlapped, the plant material was moved aside so that any species below could be visually assessed. Consequently, the total Domin score for plots can exceed 100% vegetation coverage. In the grass mix, it was not possible to distinguish between the two rye-grass species so their Domin scores' have been collated. Each week, during mid-summer, to try to capture the flowering phenology of the mixes, visual assessments of the percentage cover of the plots were made.

2.3.2.2 Yellow water traps

To sample the insect community present at each seed mix plot, yellow water traps were used (diameter: 15cm; Ringot, Nickerson Bros., UK) at a height of between 75cm and 1m, with one trap per replicate. Traps were filled with a solution of water and general-purpose detergent to break the surface tension. Traps were run over two periods (21/06/2019 - 26/06/2019 and 06/07/2019 - 10/07/2019). At the end of each trapping period, the insects caught were sieved and stored in 70% ethanol for later identification.

2.3.2.3 Pitfall traps

Pitfall traps were white plastic cups with a diameter of 7cm and depth of 7.5cm. Traps were placed in holes dug into the ground and set flush with the soil surface. Pitfall traps positioned in the approximate centre of each plot, at least two weeks prior to trapping to ensure there was minimal disturbance prior to sampling. Pitfalls were filled with approximately 100ml of a 50% ethylene glycol and 50% water mix. Sampling occurred on two separate trapping periods (from 21/06/2019 until 26/06/2019 and from 06/07/2019 to 10/07/2019). All invertebrates were identified by professional entomologists at Rothamsted to the highest possible resolution required for a functional analysis to be completed with adult carabids identified to species level.

2.3.2.4 Sentinel Aphid Cards

Sentinel aphid cards comprised five adult aphids (*Myzus persicae*) glued to thin, white plastic plant labels. The strips were sprayed with adhesive (3M SprayMount) and five adult aphids were transferred alive to the centre of the plastic strips using a fine paintbrush. Aphid cards were placed out in the field as soon as possible after production, always on the same day. In each replicate, one card was placed on the soil surface with aphids facing up and were secured with a metal pin. Cards were also attached to a barley leaf using a small wire tag approximately 60cm above ground. Cards were set out on 28/07/2019 and collected after 24 hours, and any remaining aphids were counted.

2.3.2.5 Crop Harvest

To assess the impact of the seed mix upon crop yield, between 02/09/2019 - 07/09/2019), two 1.5mx 10m wide strips were combined at a distance of 2m and 5m from the flower mix plots into the buffer of spring barley (Figure 2.2). The total fresh weight of grain collected by the combine was recorded. The barley samples were then weighed before they were dried at 105°C until a constant mass was reached, and samples reweighed. This allowed for the percentage dry mass of each sample (%GDM) to be calculated:

$$\%GDM = \frac{grain\ sample\ dry\ mass}{grain\ sample\ fresh\ mass} \times 100$$

Following this, the standardised grain yield in tonnes per hectare, at 85% dry matter, for each 1.5m x 10m yield cut was calculated:

$$Grain\ yield\ (t/h) = fresh\ mass\ (kg)\ \times \frac{\%GDM}{85} \times \frac{10}{area}$$

2.3.4 Statistical Analyses

2.3.4.1 Yellow Water Traps

Samples with incomplete labels or damaged samples have been removed from the analysis with four samples removed across both trapping periods, leaving 46 traps in Run 1 and 46 in Run 2. Run 1 and Run 2 were analysed separately using R Studio 1.1.456 (R Core Team, 2020).

To analyse the overall invertebrate community for each trapping period, the following metrices were used as response variables: species richness, Shannon's Index, Simpson's Index and total abundance as calculated by the *vegan* package v2.5-7 (Oksanen et al., 2020). To assess the effect of seed mix on these four response variables, Linear Mixed Models (LMMs) were created using *lme4* and *lmertest* (Bates et al., 2011, Kuznetsova et al., 2015). The fixed effect specified was seed mix treatment and the random effects were defined as replicate crossed with aspect (North or South-facing), nested within this was plot. Least Significant Differences were calculated using the *predictmeans* package (Luo et al., 2018). Residual plots were assessed to ensure each response variable met the assumptions of LMMs.

The invertebrates recorded were assigned to three different functional groups (NE, herbivore, and the 'other') using information from published literature (Appendix Table 2.7.3). To assess the effect of seed mix on the abundance of NE, herbivore, and the 'other' category, LMMs were created and analysed with the same fixed and random effects structure as the community diversity metrics.

In addition to this, four further insect taxonomic units were created: total aphid abundance, total Oedemeridae (false blister beetles) abundance, Chalcididae wasp abundance, and total wasp abundance, and these were analysed as above. Four additional response variables – total Ichneumonidae abundance, total *Andrena* sp. abundance, all bee abundance, and all bee richness – could not be adequately transformed for analysis using parametric LMMs. Therefore, Generalised Linear Mixed Models (GLMMs) with poisson distribution (log-link) function were fitted using the same fixed and random effects as above. All GLMMs were compared to null models to identify significant seed mix effects.

The effect of seed mix upon the community assemblage of each trapping period was investigated via the use of partial Redundancy Analysis (pRDA) and the '*vegan*' package v2.5-7 (Oksanen et al., 2020). A pRDA analysis was selected as response data were compositional and had a gradient 2.3 SD units long, so a linear method was preferred. To reduce the bias that infrequent species may have on the pRDA, species were excluded from the analysis if they were found in less than 3 traps across each run.

To identify the variation explained by seed mix, field, and aspect, three partial RDAs was conducted using *vegan* (Oksanen et al., 2020). In each case, an environmental factor (e.g. seed mix) was specified as the factor of interest and the remaining two factors (e.g. aspect and field) were ‘partialled out’ thereby allowing the effect of each single environmental factor upon the community assemblage to be quantified. Following this, each factor’s significance was analysed via a Permutation test for RDA under reduced model, with Permutation set to ‘free’ and 999 permutations ran. This approach was selected as the various invertebrate data were collected from a designed, blocked experiment and therefore it was necessary to account for the influence of these factors upon community assemblage. Biplots presented for each run are for RDAs with seed mix, field and aspect specified.

2.3.4.2 Pitfall Traps

Samples with incomplete labels or damaged samples were removed from the analysis; five samples were removed across both trapping periods, leaving 47 traps in Run 1 and 44 in Run 2. The following analyses were each completed on Run 1 and Run 2 separately. The community wide metrics for pitfall invertebrates were calculated and analysed as per the yellow trap data. To relate the observed invertebrate communities to ecosystem service provision, the invertebrates sampled were assigned to five different functional groups based on the literature (Appendix Table 2.7.2). To investigate the effect of seed mix upon the abundance of NE, pest, herbivore, pollinator, and the ‘other’ category, LMMs were created and analysed with the same fixed and random effects structure as stated before in section 2.3.4.1. Four further insect taxonomic units have been identified or created to investigate the response of key ecosystem service providers. Namely the total abundance of Parasitica, all spiders, all carabids, all solitary bees, and the abundance of three common carabid species *Pterostichus cupreus*, *P. madidus* and *P. melanarius* have been analysed as above.

The carabids captured in the pitfall traps were allocated to the groups proposed by Cole et al (2002) to give further insight into ESP functional composition (Appendix Table 2.7.3). These groups were analysed using LMMs with the previously stated fixed and random effects structure. Residual plots were assessed to ensure each response variable met to the assumptions of LMMs, and the variables transformed are documented in Table 2.2. However, transformation was not sufficient for five proposed beetle groups which had low numbers, so these groups were not analysed.

Partial Redundancy Analysis was again used to investigate the effect of seed mix on the community assemblage of each Run of i) all captured invertebrates, ii) all adult carabid species and iii) spider taxonomic units as above (section 2.3.4.1). To identify the variation explained by mix, field and aspect, three partial RDAs were conducted for i) all pitfall invertebrates, ii) all adult carabid species and iii) spider taxonomic units. These data were then analysed and presented as above for yellow water trap data.

2.3.4.3 Sentinel Aphid Cards

To test for the effect of seed mix treatment on sentinel prey predation GLMMs were fitted to the number of aphids remaining on cards after 24 hours. Using lme4 and lmertest (Bates et al., 2011, Kuznetsova et al., 2015). A Poisson distribution (log-link) function was fitted, with fixed effects as seed mix treatment and random effects as replicate crossed with aspect, and nested within this was plot. Analysis was ran separately for aphid cards on the ground and in foliage. To ascertain the significance of the effect seed mix had upon sentinel pest predation, an ANOVA was used to compare GLMMs with null models.

2.3.4.4 Harvest

Due to a complication when harvesting, there are 4 missing values, and one further yield value was removed from the analysis as the barley yield plot was unintentionally affected by a graminicide herbicide which resulted in reduced barley yield. To assess the effect of seed mix upon on three harvest response variables i.e., fresh grain yield, adjusted grain yield (at 85%) and grain dry matter, LMMs were used as before. The fixed effect was flower mix treatment and the distance the cut was made from the treatment plot (either 2m or 5m) and the random effects was defined as replicate crossed with aspect, nested within this was plot. Residual plots were assessed to ensure each response variable met to the assumptions of LMMs and no transformations were required.

2.3 Results

2.3.1 Margin Establishment

The margin mixes established well in 2018 and some small adjustments to the mixes based on their performance (Appendix 2.7.1). The flowering phenology of the mixes sown in 2019 is displayed in Figure 2.3.

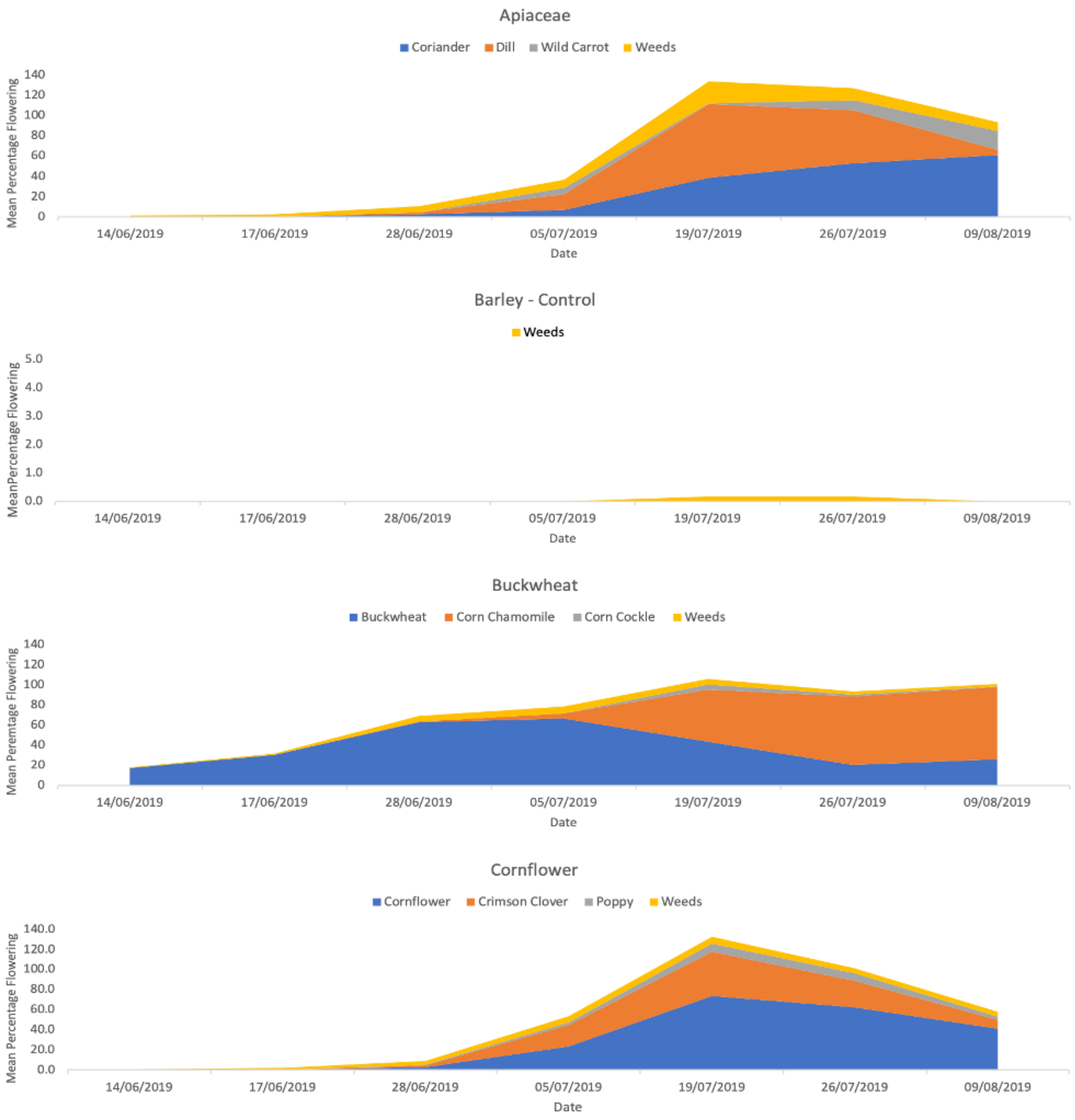


Figure 2.3. Flowering phenology of species present in different flower mix treatments on Rothamsted Farm 2019). Mean percentage flowering across all plots shown.

A – Apiaceae seed mix,

B – Control – barley,

C – Buckwheat seed mix,

D – Cornflower seed mix.

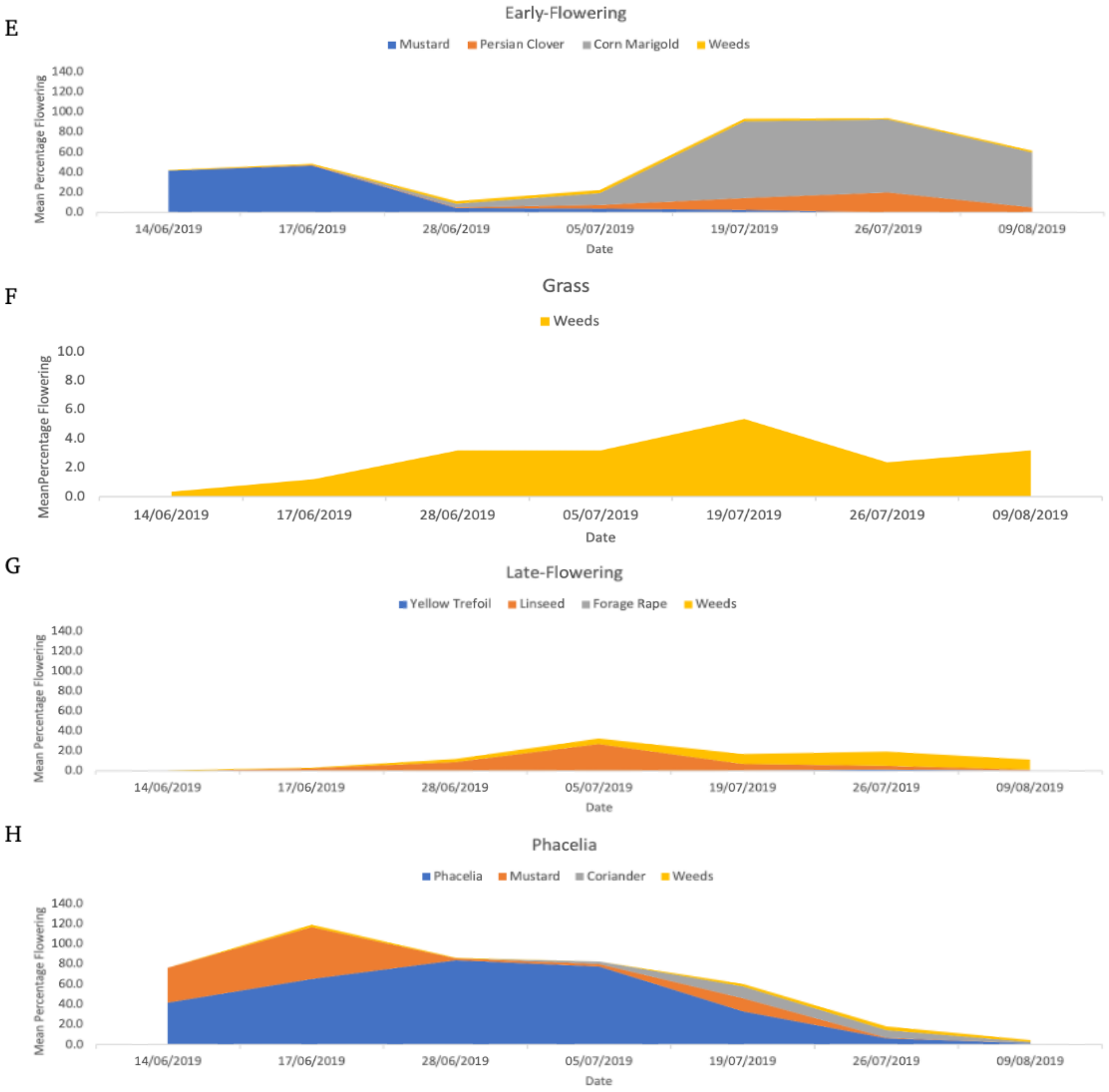


Figure 2.3 continued.

Mean percentage flowering across all plots shown.

E – Early-Flower mix,

F – Grass mix,

G – Late-Flowering mix,

H – Phacelia seed mix.

2.3.2 Yellow Water Trapping

Across both trapping periods in 2019, 10,100 invertebrates were recorded and identified across 86 different taxonomic units. The most abundant ten taxonomic units were Diptera (2,957 individuals), aphids (1,232 individuals), *Brassicogethes* spp. (1,184 individuals), Thysanoptera (1,002 individuals), Nitidulidae (527 individuals), Oedemeridae (484 individuals), Chalcididae (383 individuals), Ichneumonidae (199 individuals), *Andrena* spp. (189 individuals), and Hybotidae (179 individuals).

2.3.2.1 Community Wide Diversity Metrics

Seed mix did not significantly affect Species Richness, Shannon's index, or the total invertebrate abundance for both trapping periods of yellow water trap sampling (Table 2.2). However, the Simpson's index of invertebrates was significantly affected by seed mix in the second sampling run (Table 2.2). The Control, Grass, Apiaceae and Early-flowering mixes had significantly lower diversity compared with the Late-flowering, Buckwheat, Cornflower, Phacelia mixes (Table 2.2) (Figure 2.4).

Table 2.2. Output from LMMs on yellow water trap community metrics. Significant or marginally significant results highlighted in bold, with significance levels indicated by: ** = $p<0.01$, "." = $p<0.1$.

Response Variable	Run	Trans.	NumDF	DenDF	F value	Pr(>F)	Sig	Aveg. LSD
Species Richness	1	-	7	31.5	0.90	0.518	NS	3.98
	2	-	7	30.2	1.61	0.170	NS	4.65
Shannon's Index	1	-	7	38.0	1.30	0.278	NS	0.25
	2	-	7	31.3	2.21	0.061	.	0.46
Simpson's Index	1	-	7	38.0	1.55	0.180	NS	0.06
	2	-	7	34.0	3.52	0.006	******	0.13
Total Abundance	1	-	7	32.2	0.41	0.886	NS	61.3
	2	-	7	31.3	0.77	0.616	NS	58.9

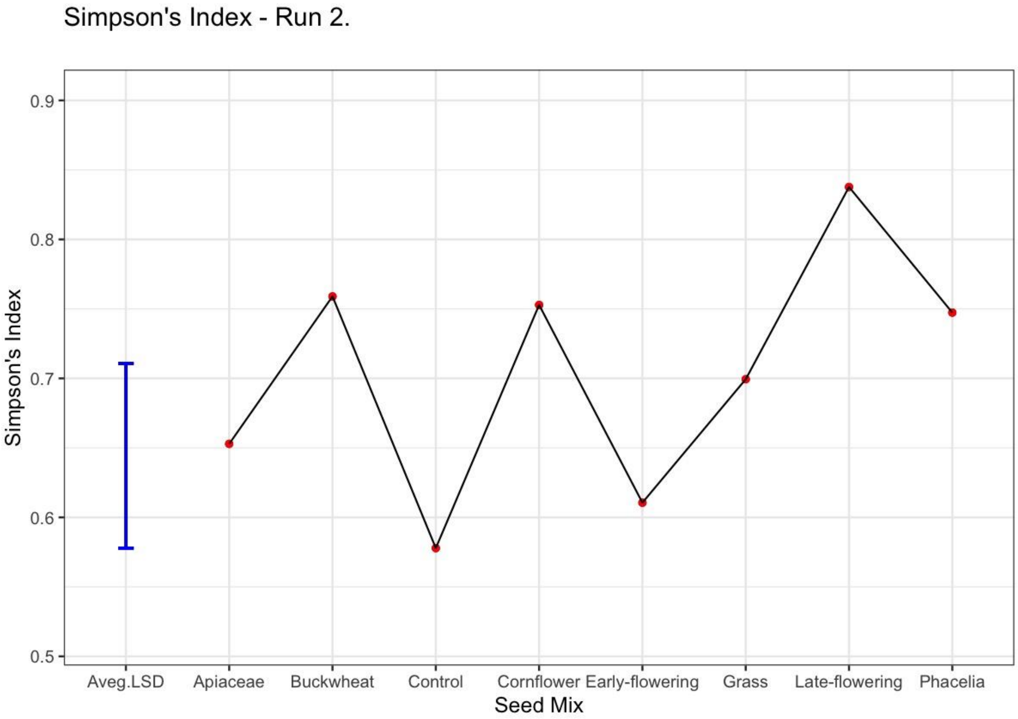


Figure 2.4. Predicted means for the Simpson's Index for arthropods captured in yellow water traps placed in different seed mix treatments on Rothamsted Farm between 06/07/2019 to 10/07/2019 fitted using an LMM (Average LSD=0.13).

2.3.2.2 Functional Groups

The Grass mix plots had significantly higher abundance of NE compared with all other seed mixes in Run 2 of yellow water trapping (Table 2.3). Seed mix did not significantly affect NE abundance in run 2, nor NE richness, herbivore abundance, richness, the invertebrates in the 'other' category in either trapping period (Table 2.3).

Table 2.3. Output from LMMs on functional groups of insects sampled in yellow water traps. Significant or marginally significant results highlighted in bold, with significance levels indicated by: * = $p<0.05$, "." = $p<0.1$.

Response Variable	Run	Trans.	NumDF	DenDF	F value	Pr(>F)	Sig	Aveg. LSD
NE	1	log(x+1)	7	32.3	1.42	0.23	NS	0.72
abundance	2	log(x+1)	7	32.6	2.36	0.045	*	0.77
NE	1	-	7	30.6	0.69	0.678	NS	2.26
richness	2	-	7	33.3	1.97	0.089	.	1.95
Herbivore	1	-	7	31.7	0.75	0.634	NS	37.5
abundance	2	-	7	31.2	1.30	0.281	NS	11.5
Herbivore	1	-	7	32.3	0.72	0.653	NS	1.62
richness	2	-	7	32.8	0.94	0.492	NS	1.80
'Other'	1	-	7	30.7	0.44	0.872	NS	29.5
abundance	2	log(x+0.1)	7	32.4	1.35	0.259	NS	1.38
'Other'	1	log(x+0.1)	7	33.7	1.02	0.434	NS	1.94
Richness	2	log(x+0.1)	7	32.4	1.35	0.259	NS	1.38

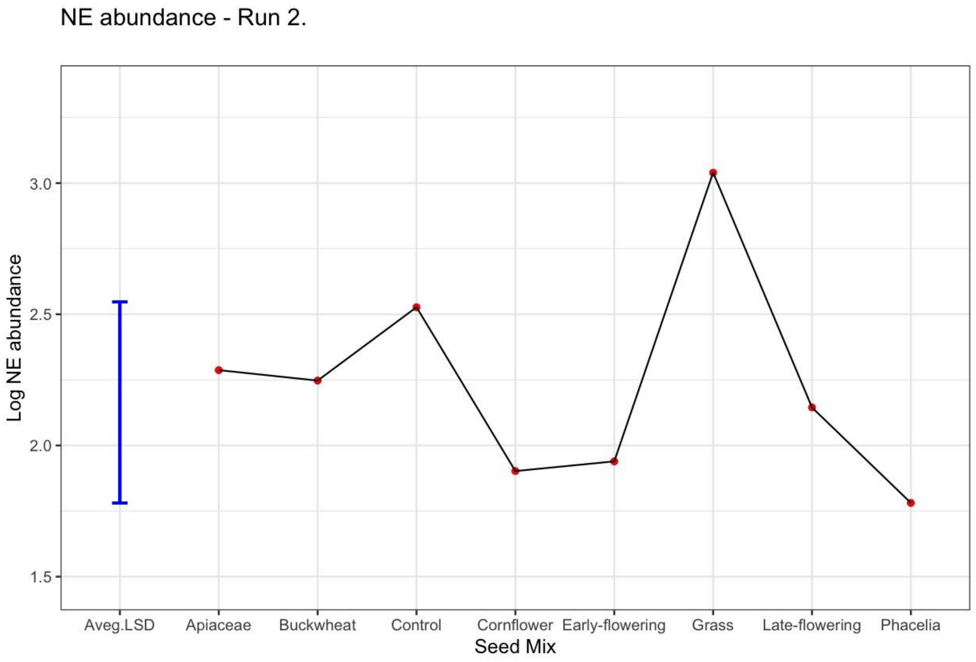


Figure 2.5. Predicted means for the natural enemy abundance captured in yellow water traps placed in different seed mix treatments on Rothamsted Farm between 06/07/2019 to 10/07/2019, fitted using an LMM (Average LSD=0.77).

2.3.2.3 Key Ecosystem Providers

In at least one trapping, total aphid abundance, Chalcididae wasp, Oedemeridae abundance and total wasp abundance were found to be significantly different between treatments (Table 2.4). Across both runs of trapping, the early-flowering mix had significantly lower aphid abundance than the Buckwheat, Cornflower, Grass, Apiaceae and Late-flowering mixes (Figure 2.6). The Control and Phacelia mixes consistently had aphid abundances between these two significantly different groups (Figure 2.6). For all wasp taxonomic units, during Run 1 there were significantly more wasps in the Control plots, a finding which appears to be driven by the high abundance of Chalcidid wasps (Figure 2.7,Table 2.4). Looking now at the key ESPs analysed via GLMMs (Table 2.5), in the first run, the Control plots had significantly fewer solitary bees than the other mixes (Z=-2.25, *p*=0.024). Also in the first run, the Phacelia plots had significantly higher Ichneumonidae wasp abundance (z=2.06, *p*=0.039). In the second trapping period, the differences in Ichneumonidae abundance become more pronounced as the Buckwheat (z=-2.25, *p*=0.024), Control (z=-2.34, *p*=0.0.018), Cornflower (z=-3.44, *p*=0.0006), and Late-flowering (z=-2.74, *p*=0.006) mixes all had significantly lower abundances than the other mixes.

Table 2.4. Output from LMMs on Key Ecosystem Providers abundances sampled in yellow water traps. Significant or marginally significant results highlighted in bold, with significance levels indicated by: ** = p<0.01, * = p<0.05, "." = p<0.1.

Response Variable		Trans.	NumDF	DenDF	F value	Pr(>F)	Sig	Aveg. LSD
Aphid	1	log(x+1)	7	37.0	3.19	0.010	**	0.88
abundance	2	log(x+1)	7	39.0	2.41	0.038	*	1.71
Chalcididae	1	log(x+1)	7	35.1	2.65	0.026	*	0.94
abundance	2	log(x+1)	7	30.7	0.53	0.805	NS	0.69
Oedemeridae	1	log(x+1)	7	33.1	1.12	0.373	NS	0.77
abundance	2	log(x+0.01)	7	32.0	2.51	0.036	*	2.92
Wasp	1	log(x+1)	7	33.3	2.60	0.030	*	0.30
abundance	2	log(x+1)	7	31.5	1.86	0.111	NS	0.74

Table 2.5. Output from GLMMs on Key Ecosystem Providers abundances sampled in yellow water traps. Significant or marginally significant results highlighted in bold, with significance levels indicated by: *** = p<0.001, ** = p<0.01, * = p<0.05.

Response variable	Run	Chisq	Df	Pr(>Chisq)	Sig.
Andrena	1	4.66	7	0.701	NS
abundance	2	17.7	7	0.013	*
Ichneumonidae	1	17.7	7	0.013	*
abundance	2	18.8	7	0.009	**
All bee richness	1	5.62	7	0.585	NS
	2	2.30	7	0.942	NS

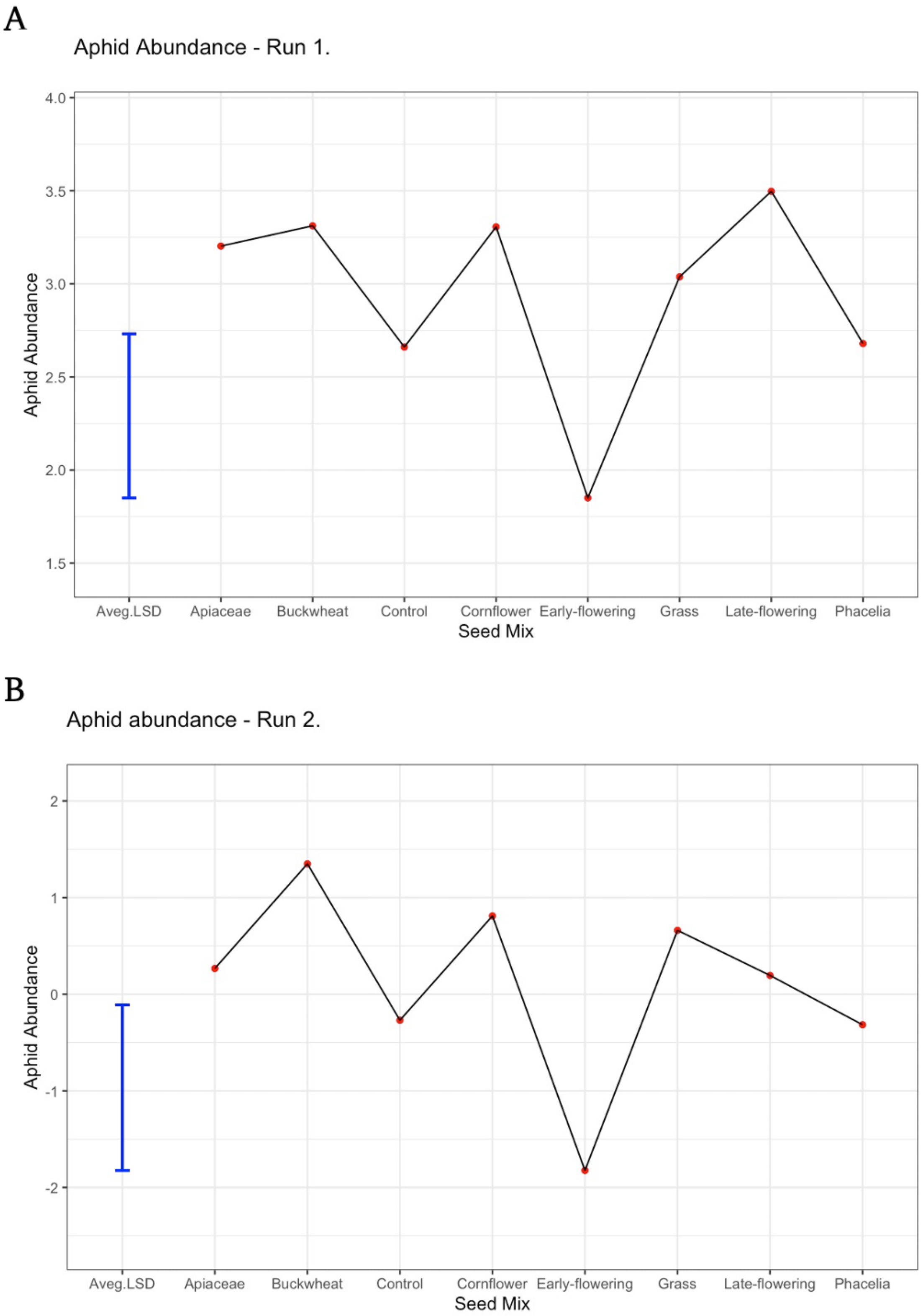


Figure 2.6. Predicted means for all aphid abundances for each seed mix treatment from yellow water traps on Rothamsted Farm, fitted using an LMM. **A** – predicted means for aphid abundance between 21/06/2019 to 26/06/2019 (Average LSD=0.88). **B** – predicted means for aphid abundance between 06/07/2019 to 10/07/2019 (Average LSD=1.71).

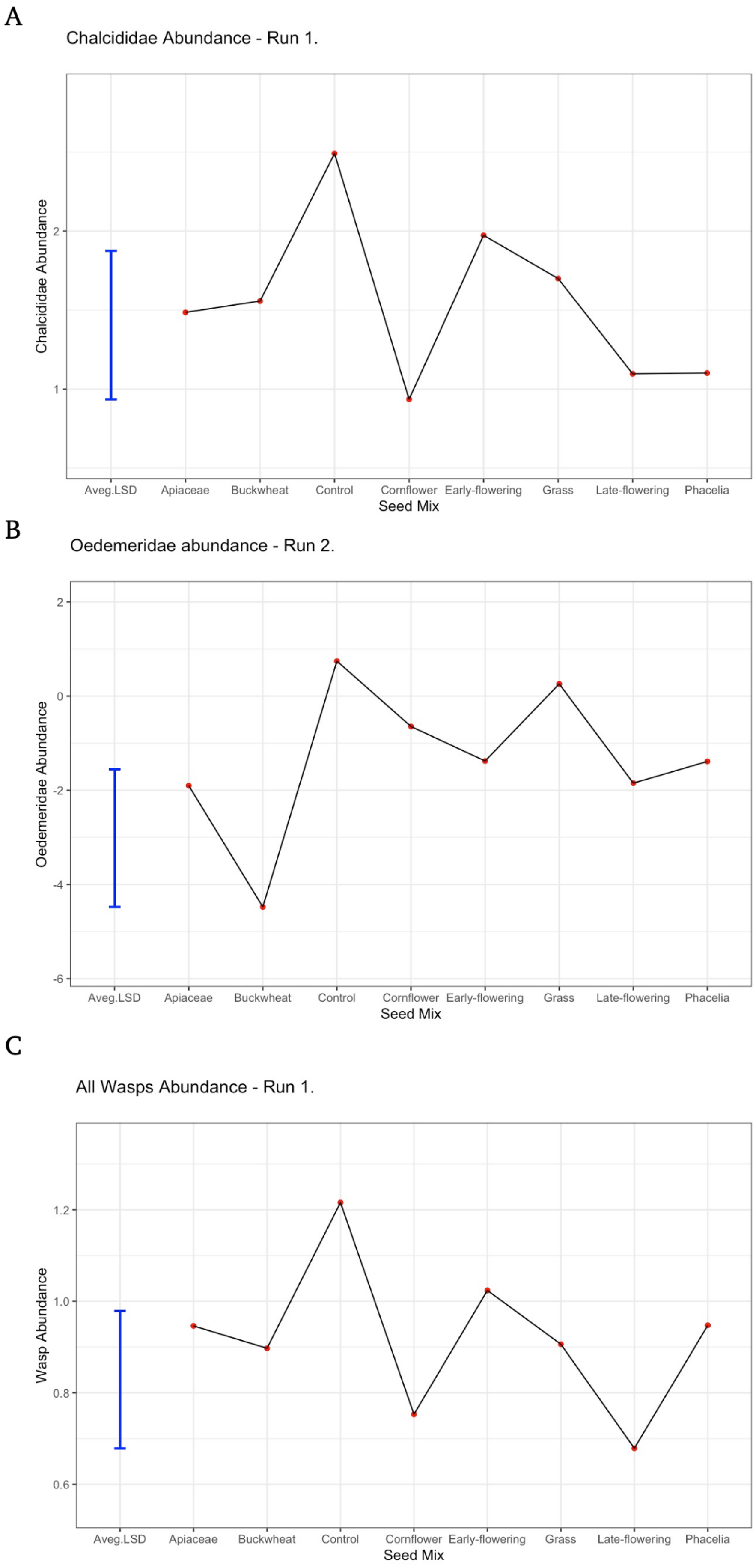


Figure 2.7. Predicted means for three ecosystem service provider abundances for each seed mix from yellow water trapping on Rothamsted Farm fitted using an LMM.

A – predicted mean Chalcididae abundance between 21/06/2019 to 26/06/2019 (Average LSD=0.94).

B – predicted mean Oedemeridae abundance between 06/07/2019 to 10/07/2019 (Average LSD=2.92).

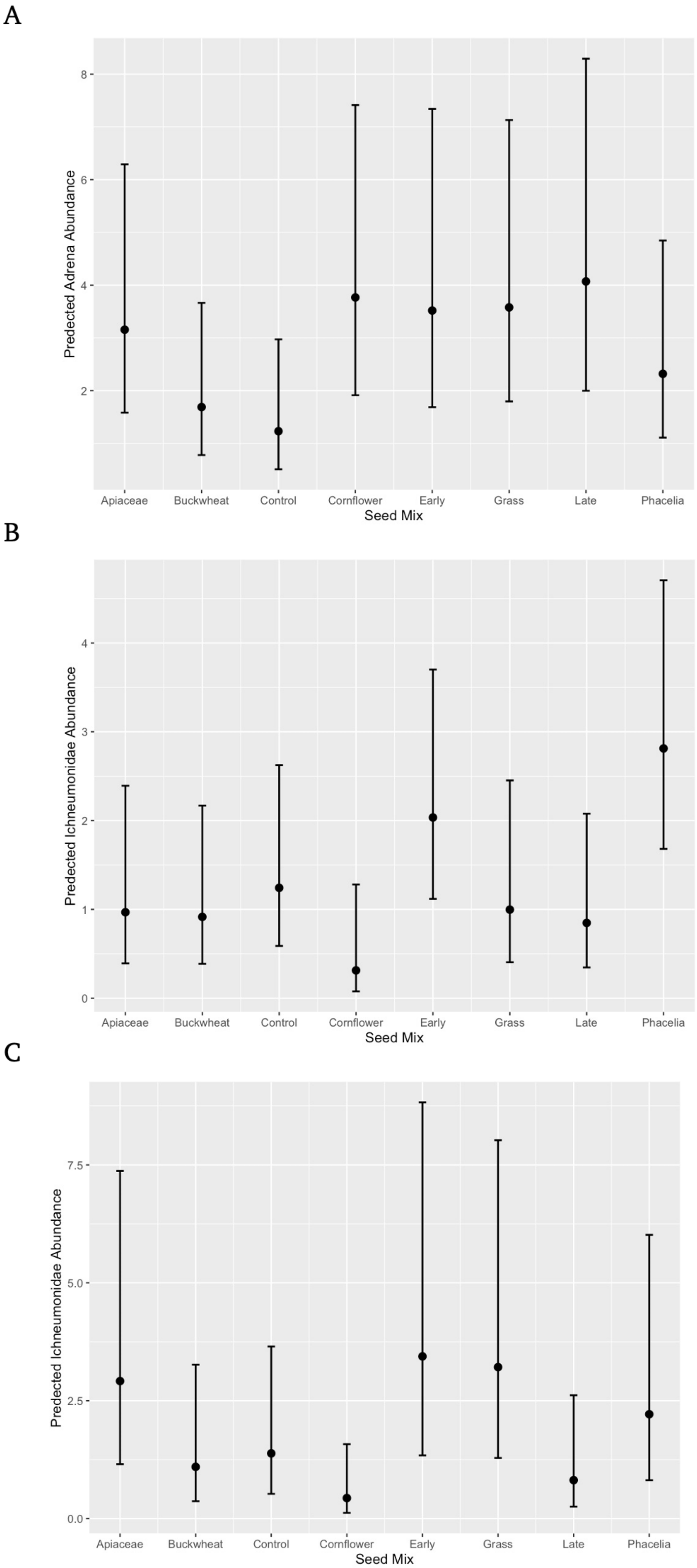
C – predicted mean total abundance of all wasps between 21/06/2019 to 26/06/2019 (Average LSD=0.30).

Figure 2.8. Predicted means for three ecosystem service provider abundances for each seed mix from yellow trapping on Rothamsted Farm fitted using an GLMM.

A – predicted mean Adrena abundance between 06/07/2019 to 10/07/2019, with 95% confidence intervals shown.

B – predicted mean Ichneumonidae abundance between 21/06/2019 to 26/06/2019, with 95% confidence intervals shown.

C – predicted mean for Ichneumonidae abundance between 06/07/2019 to 10/07/2019, with 95% confidence intervals shown.



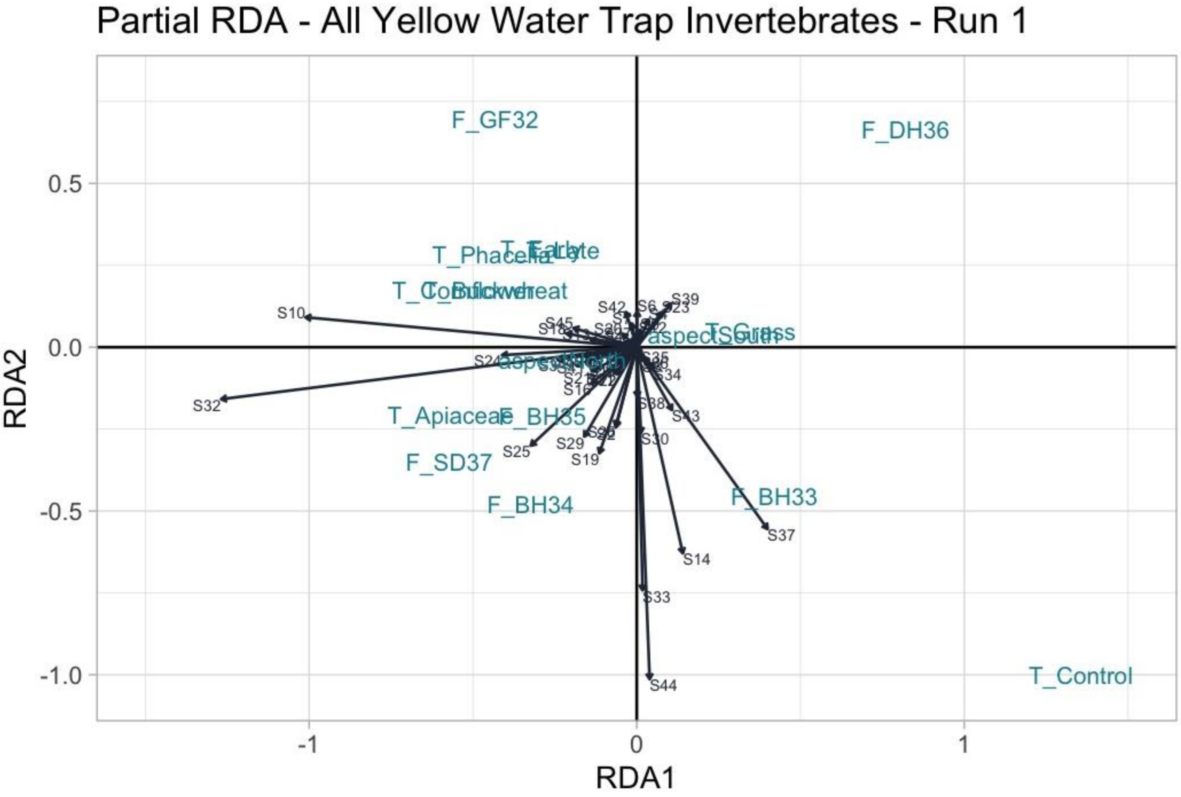


Figure 2.9. Redundancy analysis ordination of all invertebrates caught in yellow water traps in Run 1, with axes constrained by field (F_), seed mix (T_), and aspect. Invertebrates present denoted by: S1: Aleocharinae, S2: *Aphidius* spp., S3: Aphids, S4: Apidae, S5*: Athalia rosae,* S6: Bibionidae, S7: *Bombus* spp., S8: *Bombus terrestris*, S9: Braconidae, S10: *Brassicogethes* spp., S11: *Bruchus* spp., S12: Cantharidae, S13: Cerambycidae, S14: Chalcididae, S15: Cicadellidae, S16: Cicadidae, S17: Curculionoidea, S18: Curculionidae, S19: Diptera, S20: Drosophilidae, S21: Figitidae, S22: *Harmonia axyridis*, S23: *Hoplia philanthus*, S24: Hybotidae, S25: Ichneumonidae, S26: Latridiidae, S27: Lauxaniidae, S28: Lepidoptera, S29: Linyphiidae, S30: *Malachius bipustulatus*, S31: Miridae, S32: Nitidulidae, S33: Oedemeridae, S34: Opomyzidae, S35: *Orius* spp., S36: Pentatomidae, S37: Phoridae, S38: Platygastridae, S39: Psylloidea, S40: Staphylinidae, S41: *Tachyporus* spp., S42: Tenthredinidae, S43: *Tenthredo* spp., S44: Thrips, S45: Tipulidae.

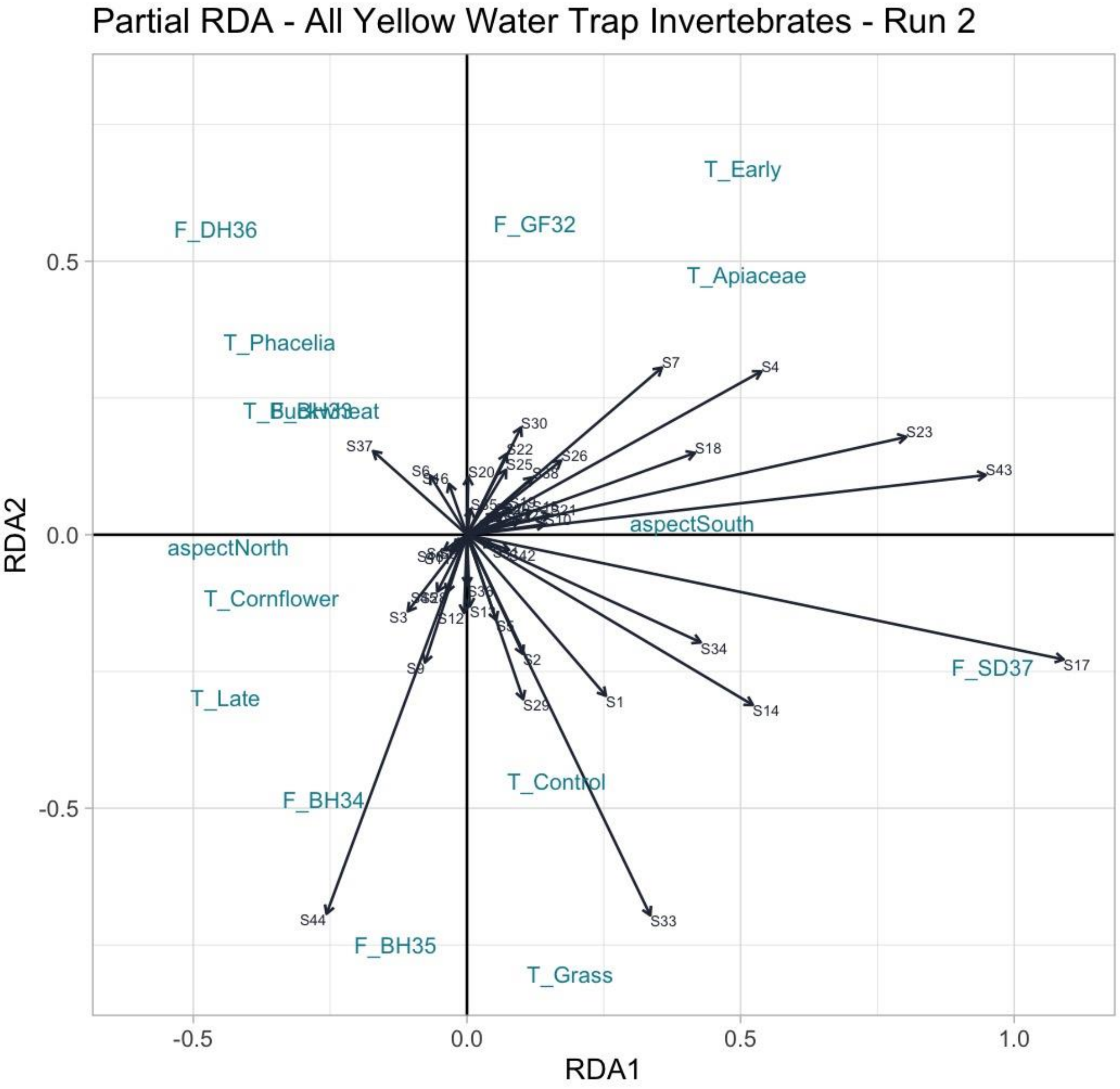


Figure 2.10. Redundancy analysis ordination of all invertebrates caught in yellow water traps in Run 2, with axes constrained by (F_), seed mix (T_), and aspect. Invertebrates present denoted by: S1: *Andrena* spp., S2: *Aphidius* spp., S3: Aphids, S4: Apidae, S5: *Apis melifera*, S6: *Apis* spp., S7: Bibionidae, S8: *Bombus* spp., S9: Braconidae, S10: *Brassicogethes* spp., S11: Cantharidae, S12: Cerambycidae, S13: Ceratina spp., S14: Chalcididae, S15: Cicadellidae, S16: Curculionidae, S17: Diptera, S18: Drosophilidae, S19: *Episyrphus balteatus*, S20: Evanioidea, S21: Figitidae, S22: Hybotidae, S23: Ichneumonidae, S24: Latridiidae, S25: Lauxaniidae, S26: Lepidoptera, S27: Linyphiidae, S28: Lycosidae, S29: *Malachius bipustulatus*, S30: Miridae, S31: Neuroptera, S32: Nitidulidae, S33: Oedemeridae, S34: Phoridae, S35: Platygastridae, S36: Psylloidea, S37: Pteromalidae, S38: *Rhagonycha fulva*, S39: Syrphidae, S40: Staphylinidae, S41: *Tachyporus* sp., S42: *Tenthredo* sp., S43: Thrips, S44: Tineidae, S45: Tipulidae, S46: *Vespula vulgaris*.

2.3.2.4 Invertebrate Community Assemblage

Across both trapping periods, the variation in the invertebrate community sampled in yellow water traps were significantly explained by both seed mix and field (Table 2.6). Although, the amount of variation in the sampled communities explained by seed mix was always greater than the variation explained by field (Table 2.6). At no point, in either run, did field or the aspect of the plots explain a significant amount of variation in the communities of all invertebrates, although in the second run, aspect was marginally significant (Table 2.6).

In the first trapping period, the Control plots were outliers to all other seed mixes, and were favoured by Phoridae flies, Chalcididae wasps and Thrips (Figure 2.9). Conversely, the Buckwheat, Phacelia, Early-Flowering, Late-flowering, and Cornflower seed mixes all appear have relatively similar community assemblages (Figure 2.9). The significant difference in assemblage between different fields is evident here, with some prominent outliers (Figure 2.9).

Whilst the variation explained by seed mix during the second trapping period (17.6%) is similar to that of the Run 1 (18.5%), the variation within community assemblages is much more apparent, with strong species association with seed mix and field present (Figure 2.10). The Apiaceae and Early-flowering mix appeared to have similar assemblages with both mixes favoured by Bibionidae flies and Apidae bees. With almost opposite community assemblage to the Apiaceae and Early-flowering mix, the Control and Grass mix were both associated with Oedemeridae beetles. The Cornflower and Late-flowering mixes appeared to have relatively similar community assemblages, with both mixes favoured by aphids and Braconidae wasps. Finally, the Buckwheat and Phacelia mixes were mixes favoured by Pteromalidae wasps. Notably, there are significant differences between each community assemblage between fields, even for Blackhorse which contained three replicates (BH33, BH34, BH35) (Figure 2.10).

Table 2.6. Table showing the percentage variance explained by seed mix, field and plot aspect in the partial Redundancy analysis on all invertebrates sampled in yellow water traps as well as the subsequent ANOVA analysis of the partial Redundancy analysis model. Permutation test for partial RDA under reduced model. Permutation: free, number of permutations: 999. Significance codes: $p<0.01$ = '**', $p<0.05$ = '*', $p<0.1$ = '.'.

Run	Fixed effect	Df	F	Pr(>F)	Sig	adjusted R^2	Variance explained (%)
1	Seed Mix	7	1.48	0.007	**	0.080	18.5%
	Field	5	1.90	0.002	**	0.124	17.5%
	Aspect	1	0.79	0.703	NS	0.005	1.44%
2	Seed Mix	7	1.36	0.02	*	0.061	17.6%
	Field	5	1.38	0.03	*	0.061	14.6%
	Aspect	1	1.69	0.056	.	0.019	3.4%

2.3.3 Pitfall Trapping

Across the two trapping periods, 6414 invertebrates were recorded after traps with incomplete or damaged samples were removed. These invertebrates spanned 72 different taxonomic units, with the most abundant ten taxonomic units being Diptera (1139 individuals), Parasitica (824 individuals), Formicidae (701 individuals), Aranaea that were not able to be identified further (513), Solitary bees (356 individuals), *Erigone* sp. (320 individuals), *Pterostichus madidus* (305 individuals), *Phyllotreta* sp. (193 individuals), *Poecilus cupreus* (180 individuals) and *Oedothorax* sp. (174 individuals). Also of note was *P. melanarius* (153 individuals).

2.3.3.1 Community Wide Diversity Metrics

There were no statistically significant effects of seed mix on species richness, Shannon's index, Simpson's Index or total invertebrate abundance for Run 1 of sampling (Table 2.7). For Run 2, whilst there was no significant effect of seed mix upon species richness, there was a marginally significant effect of seed mix upon Shannon's index, Simpson's Index, and total invertebrate abundance (Table 2.7).

2.3.3.2 Functional Groups

Seed mix did not appear to have any effect on the abundance of key functional groups of NE, pests, herbivores, pollinators, or other arthropods found in pitfalls from both trapping periods (Table 2.7). Interestingly, although overall there was no apparent effect of seed mix on the herbivores, there were some notable differences. For instance, the back transformed predicted mean abundance of herbivores present in the control in Run 1, of 1.23, was marginally significantly higher than the cornflower mix's value of 0.224 (Average LSD=1.44) (Figure 2.12).

2.3.3.3 Key Ecosystem Providers

There was no apparent effect of seed mix upon the abundance of Parasitica, all spiders, all carabids, solitary bees*, Pterostichus madidus* or *P. melanarius* across both Runs (Table 2.7). The abundance of *P. cupreus* was, however, significantly different between seed mix treatments in Run 1 ($F_{7,33.0}$=2.67, $p<0.05$) and Run 2 ($F_{7,30.4}$=2.56, $p<0.05$) (Table 2.7). However, there was no seed mix that appeared to be associated with universally high or low *P. cupreus* abundance (Figure 2.13).

Table 2.7. Output from LMMs on pitfall traps. Significant or marginally significant results highlighted in bold, with significance levels indicated by: ** = p<0.01, * = p<0.05, "." = p<0.1.

	Response Variable	Trapping Period	Trans.	NumDF	DenDF	F value	Pr(>F)	Sig.	Aveg. LSD
Community Metrics	Species Richness	1	-	7	33.1	0.89	0.524	NS	4.69
		2	-	7	31.3	1.39	0.245	NS	3.64
	Shannon's Index	1	-	7	33.0	0.38	0.905	NS	0.47
		2	-	7	31.1	1.96	0.093	.	0.53
	Simpson's Index	1	-	7	39.0	0.43	0.877	NS	0.12
		2	-	7	30.8	1.97	0.092	.	0.18
	Abundance	1	-	7	32.4	1.27	0.297	NS	29.8
		2	-	7	28.8	2.01	0.088	.	58.1
Functional Groups	NE	1	-	7	34.1	1.24	0.309	NS	24.2
		2	-	7	31.6	0.65	0.710	NS	27.9
	Pests	1	-	7	32.4	1.09	0.392	NS	7.62
		2	-	7	29.6	0.9	0.518	NS	5.11
	Herbivore	1	log(x+0.1)	7	38.2	1.55	0.179	NS	1.44
		2	log(x+0.1)	7	31.1	1.05	0.418	NS	1.34
	Other	1	log(x+0.1)	7	33.2	0.21	0.979	NS	0.88
		2	log(x+0.1)	7	28.9	1.68	0.153	NS	1.00
	Pollinators	1	log(x+0.1)	7	33.3	1.88	0.105	NS	1.93
		2	log(x+0.1)	7	30.9	1.44	0.225	NS	2.31

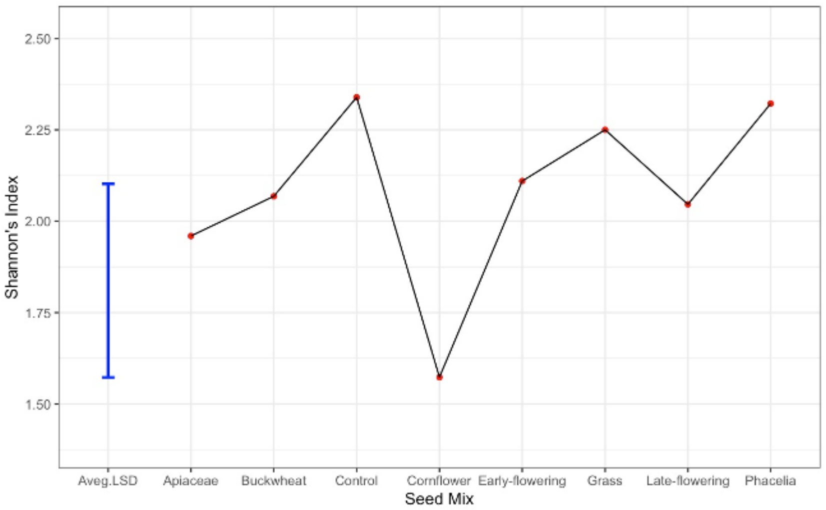
Table 2.7 cont. Table of results from LMMs on pitfall traps. Results in bold are significant, or marginally significant, with significance levels indicated by: ** = *p*<0.01, * = *p*<0.05, "." = *p*<0.1.

	Response Variable	Run	Trans.	NumDF	DenDF	F value	Pr(>F)	Sig.	Aveg. LSD
Key ESPs	Parasitica	1	log(x+0.1)	7	38.0	0.53	0.809	NS	1.57
		2	log(x+0.1)	7	30.9	1.44	0.225	NS	2.31
	All spiders	1	-	7	33.4	1.34	0.264	NS	12.50
		2	-	7	30.3	0.51	0.820	NS	22.30
	All carabids	1	log(x+0.1)	7	34.1	1.07	0.401	NS	1.45
		2	log(x+0.1)	7	31.4	0.31	0.943	NS	1.75
	Solitary Bee	1	log(x+0.1)	7	33.3	1.41	0.236	NS	2.07
		2	log(x+0.1)	7	30.9	0.96	0.476	NS	2.07
	P. cupreus	1	log(x+0.1)	7	33.0	2.67	0.027	*	2.40
		2	log(x+0.1)	7	30.4	2.56	0.034	*	1.40
	P. madidus	1	log(x+0.1)	7	32.7	0.88	0.531	NS	2.00
		2	log(x+0.1)	7	30.7	0.47	0.846	NS	2.25
	P. melanarius	1	log(x+0.1)	7	33.1	1.19	0.334	NS	1.53
		2	log(x+0.1)	7	33.1	1.19	0.334	NS	1.53
	All carabid richness	1	-	7	33.0	1.33	0.268	NS	0.22
		2	-	7	30.3	0.99	0.453	NS	1.55

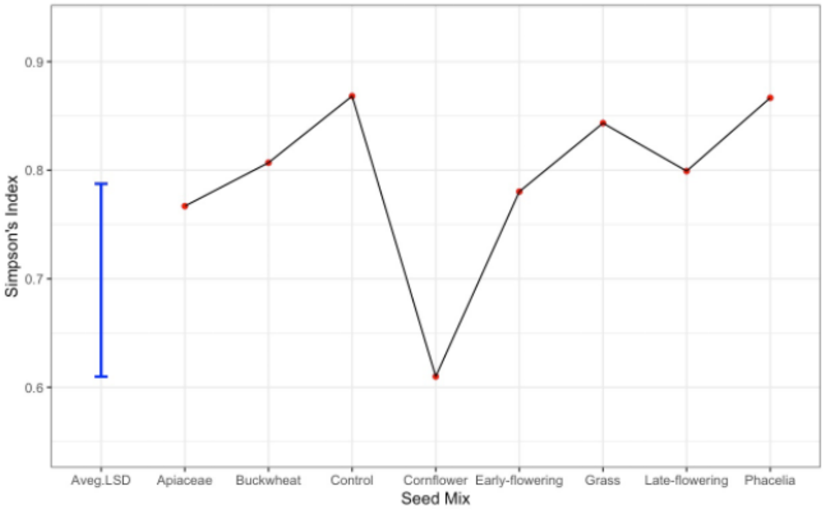
Table 2.7 cont. Table of results from LMMs on pitfall traps. Results in bold are significant, or marginally significant. with significance levels indicated by: ** = $p<0.01$, * = $p<0.05$, "." = $p<0.1$.

	Response Variable	Run	Transformation	NumDF	DenDF	F value	Pr(>F)	Sig.	Aveg. LSD
Cole et al. 2002 - Beetle groups	Group 1	1	log(x+0.1)	7	35	1.04	0.422	NS	1.49
		2	log(x+0.1)	7	31.4	0.52	0.816	NS	2.01
	Group 2	1	TI	-	-	-	-	-	-
		2	TI	-	-	-	-	-	-
	Group 3	1	TI	-	-	-	-	-	-
		2	TI	-	-	-	-	-	-
	Group 4	1	log(x+0.1)	7	35	1.05	0.414	NS	1.29
		2	log(x+0.1)	7	35	0.45	0.863	NS	1.37
	Group 5	1	log(x+0.1)	7	39	0.68	0.685	NS	1.51
		2	log(x+0.1)	7	30.4	1.66	0.157	NS	1.58
	Group 6	1	TI	-	-	-	-	-	-
		2	TI	-	-	-	-	-	-
	Group 7	1	log(x+0.1)	7	34	0.34	0.93	NS	1.52
		2	log(x+0.1)	7	31.5	0.28	0.958	NS	1.55

A Shannon's Index - all pitfall invertebrates - Run 2.



B Simpson's Index - all pitfall invertebrates - Run 2.



C Abundance - all pitfall invertebrates - Run 2.

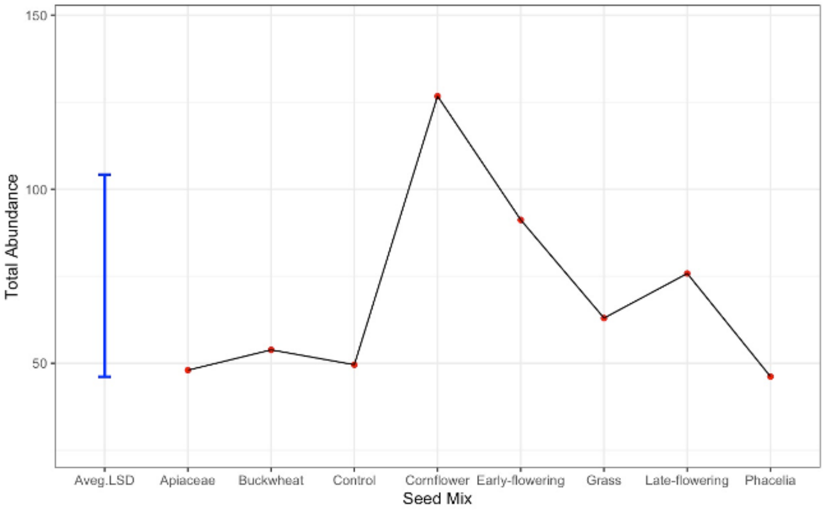


Figure 2.11. Community wide species diversity metrics – Legend continues overleaf.

Figure 2.11. Predicted mean log transformed abundance for three community wide species diversity metrics for invertebrates caught in pitfall traps placed in seed mix treatments on Rothamsted Farm between 06/07/2019 to 10/07/2019, fitted using an LMM and identified to the maximum taxonomic resolution possible. **A** –Shannon’s diversity index (Average LSD=0.53). **B** –Simpson’s diversity index (Average LSD=0.18). **C** – Total abundance of all invertebrates (Average LSD=58.1).

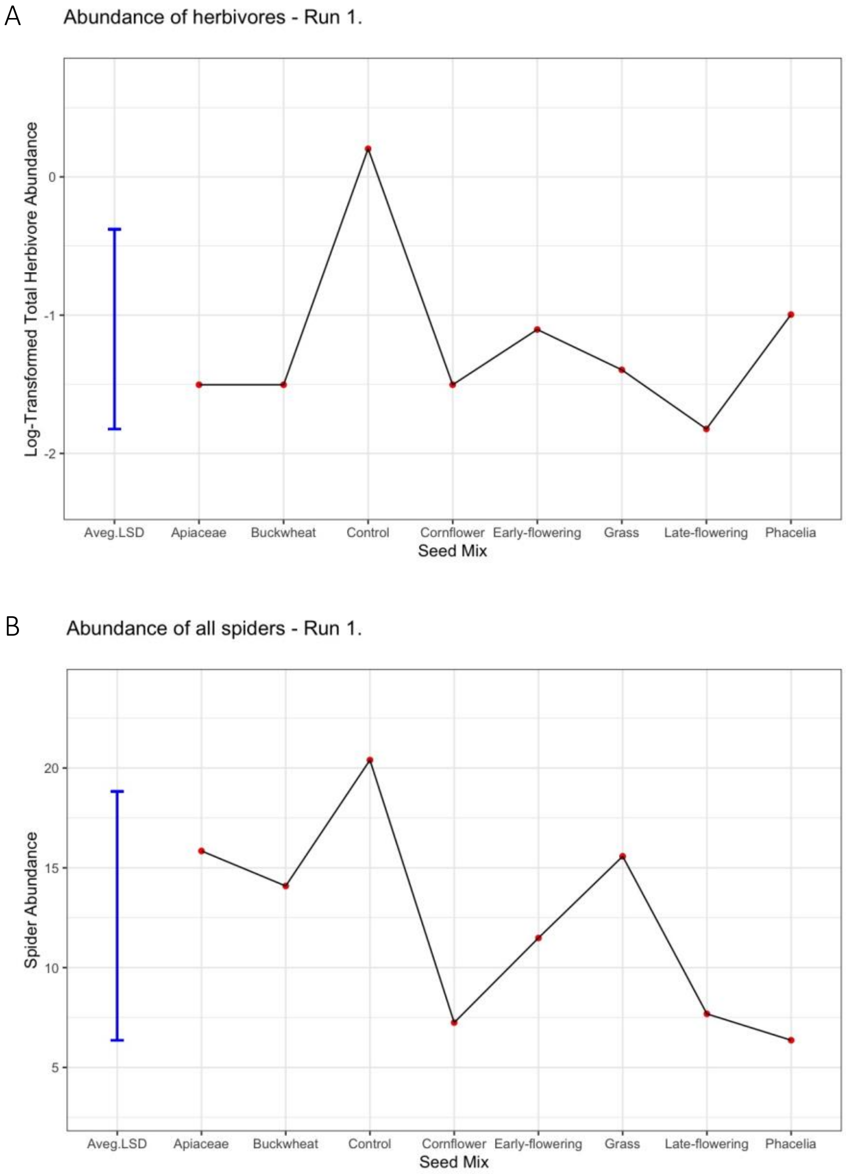


Figure 2.12. Predicted means of two invertebrate groups from pitfall trapping run in plots of different seed mixtures, Rothamsted Farm between 21/06/2019 to 26/06/2019, fitted using an LMM. **A** – predicted means for log transformed herbivore abundance (Average LSD=1.44). **B** – predicted means for spider abundance (Average LSD=12.5).

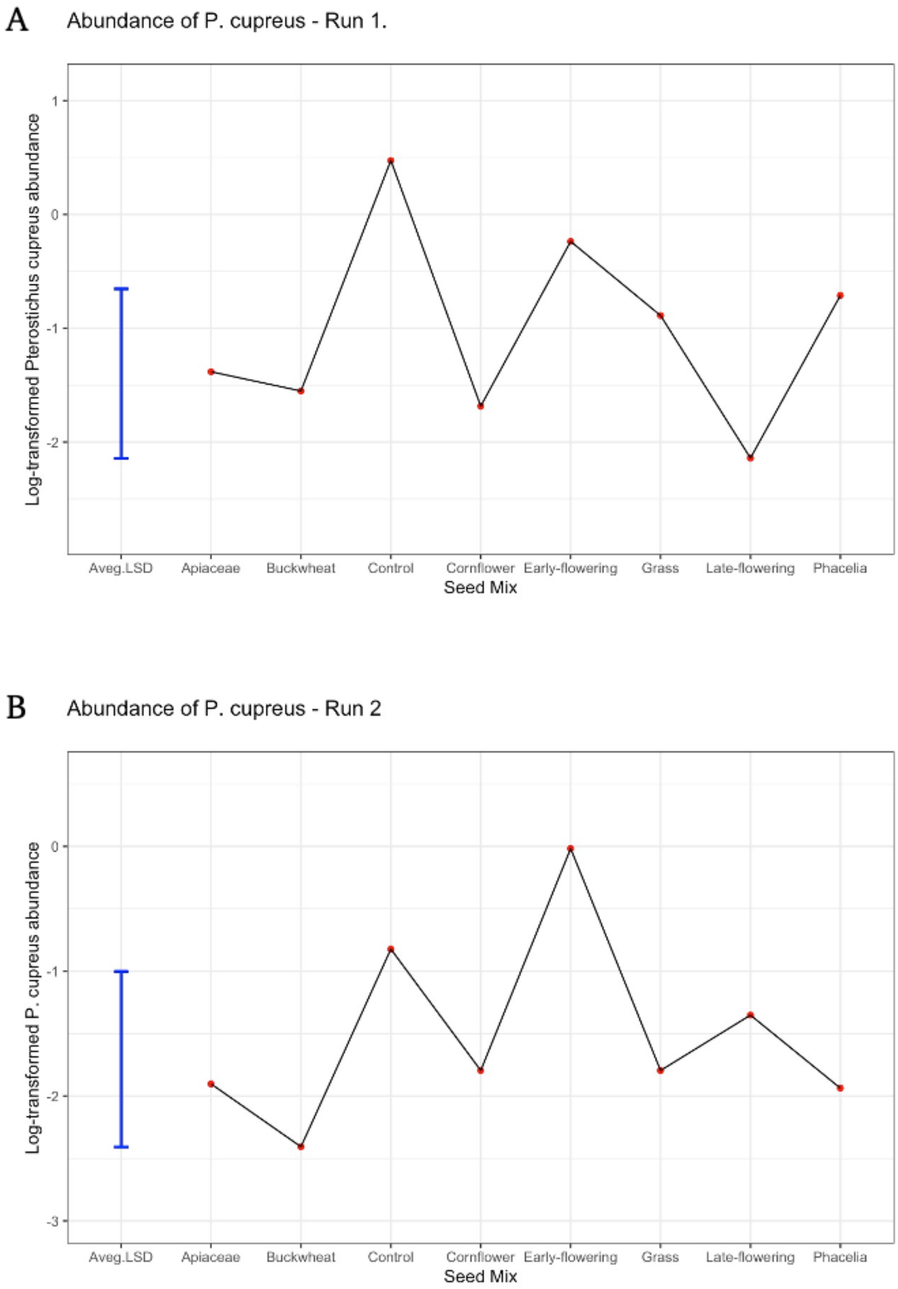


Figure 2.13. Log-transformed predicted mean *Pterostichus cupreus* abundance from pitfall trapping in seed mix plots on Rothamsted Farm, fitted using an LMM. **A** – predicted means for log transformed *P. cupreus* abundance from 21/06/2019 to 26/06/2019 (Average LSD=2.41). **B** – predicted means for log transformed *P. cupreus* abundance from 06/07/2019 to 10/07/2019 (Average LSD=1.40).

2.3.3.4 Invertebrate Community Assemblage

The variation in both the entire invertebrate community assemblage and adult carabid assemblage were significantly explained by seed mix, whereas this did not significantly explain the variation in the spider community (Table 2.8). At no point, in either run, did field or the aspect of the plots explain a significant amount of variation in the communities of all invertebrates, all carabid adults or all spiders (Table 2.8)(Table 2.7).

During the first trapping period, seed mix explained 23.7% of the variation in the entire community of pitfall invertebrates (F=1.76, *p*=0.001). There were notable species associations between *Pterostichus madidus*, *P. melanarius*, other Araneae and *Philonthus* spp. beetles with the control plots. The Late-flowering and Buckwheat mixes also were noteworthy with distinct communities (Figure 2.14). In the second trapping period, seed mix explained 23.2% of variation in the pitfall invertebrate community (F=1.59, *p*=0.004). Here, the Phacelia, Late-flowering and Cornflower mixes had distinctly different community assemblages, with solitary bees strongly associated with the Phacelia mix and Formicidae with the Cornflower mix.

Table 2.8. Percentage variance explained by seed mix treatment, field, and plot aspect in the partial Redundancy analysis on all invertebrates and adult carabid species from pitfall traps, as well as the subsequent ANOVA analysis of the partial Redundancy analysis model. Permutation test for partial RDA under reduced model. Permutation: free, number of permutations: 999. Significance codes: *p*<0.001 = '***', *p*<0.01 = '**', *p*<0.05 = '*'.

	Run	Fixed effect	Df	F	Pr(>F)	Sig	adjusted R^2	Variance explained (%)
All pitfall	1	Seed Mix	7	1.76	0.001	***	0.135	23.7
		Field	5	0.96	0.555	NS	-0.005	9.05
		Aspect	1	0.71	0.811	NS	-0.008	1.36
	2	Seed Mix	7	1.59	0.004	**	0.116	23.2
		Field	5	1.09	0.335	NS	0.014	11.6
		Aspect	1	0.88	0.559	NS	-0.004	1.82
Carabids	1	Seed Mix	7	2.91	0.001	***	0.286	21.3
		Field	5	0.68	0.886	NS	-0.039	5.49
		Aspect	1	0.75	0.586	NS	-0.006	1.27
	2	Seed Mix	7	1.87	0.012	*	0.167	26.8
		Field	5	1.07	0.395	NS	0.011	11.5
		Aspect	1	1.58	0.164	NS	0.016	3.22

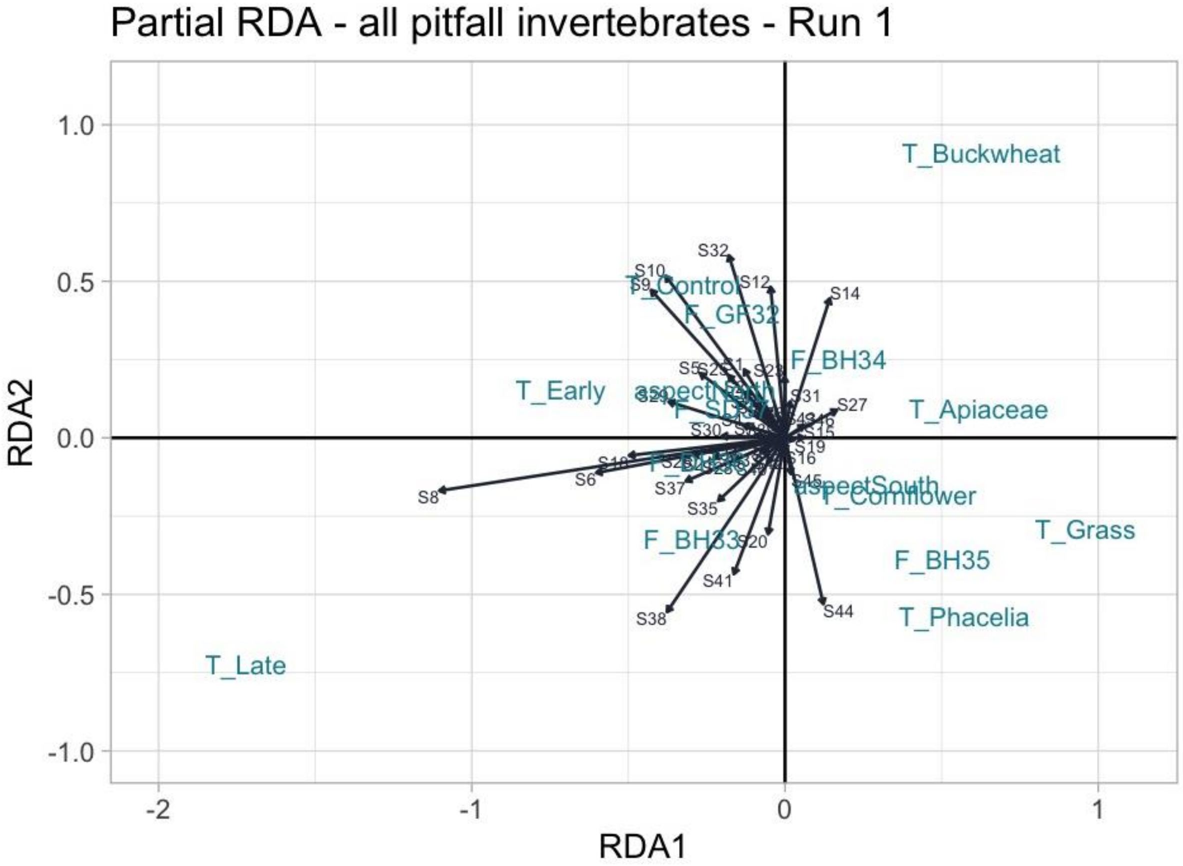


Figure 2.14. Redundancy analysis ordination of all pitfall invertebrates in Run 1, with axes constrained by (F_), seed mix (T_), and aspect. Invertebrates identified to the maximum required resolution and denoted by: S1: *Agonum dorsale*, S2: *Amara similata*, S3: *Bembidion lampros* SW, S4: *Brachinus crepitans*, S5: *Harpalus affinis*, S6: *Harpalus rufipes*, S7: *Nebria brevicollis*, S8: *Pterostichus cupreus*, S9: *Pterostichus madidus*, S10: *Pterostichus melanarius*, S11: *Trechus quadristriatus*, S12: *Philonthus* sp., S13: Tachyporinae , S14: Other Staphylindae, S15: Elateridae, S16: adult Coccinellid, S17: Coccinellid larvae, S18: adult *Brassicogethes* spp S19: *Brassicogethes* spp larvae, S20: *Oederma* sp., S21: *Phyllotreta* sp., S22: Curculionidae, S23: other Coleoptera, S24: other Coleopteran larvae, S25: *Erigone* sp., S26: Oedothorax sp., S27: *Lepthyphantes tenuis*, S28: Linyphidae other, S29: *Pardosa* sp., S30: other Lycosidae, S31: *Pachygnatha degeeri*, S32: other Araneae, S33: Syrphid, S34: Empid, S35: other Diptera, S36: Millipede, S37: Oniscidea, S38: Solitary bee, S39: *Apis mellifera*, S40: Bumble bee, S41: Aphidoidea, S42: Lepidopteran larvae, S43: Parasitica, S44: Formicidae, S45: Heteroptera, S46: Auchenorhyncha.

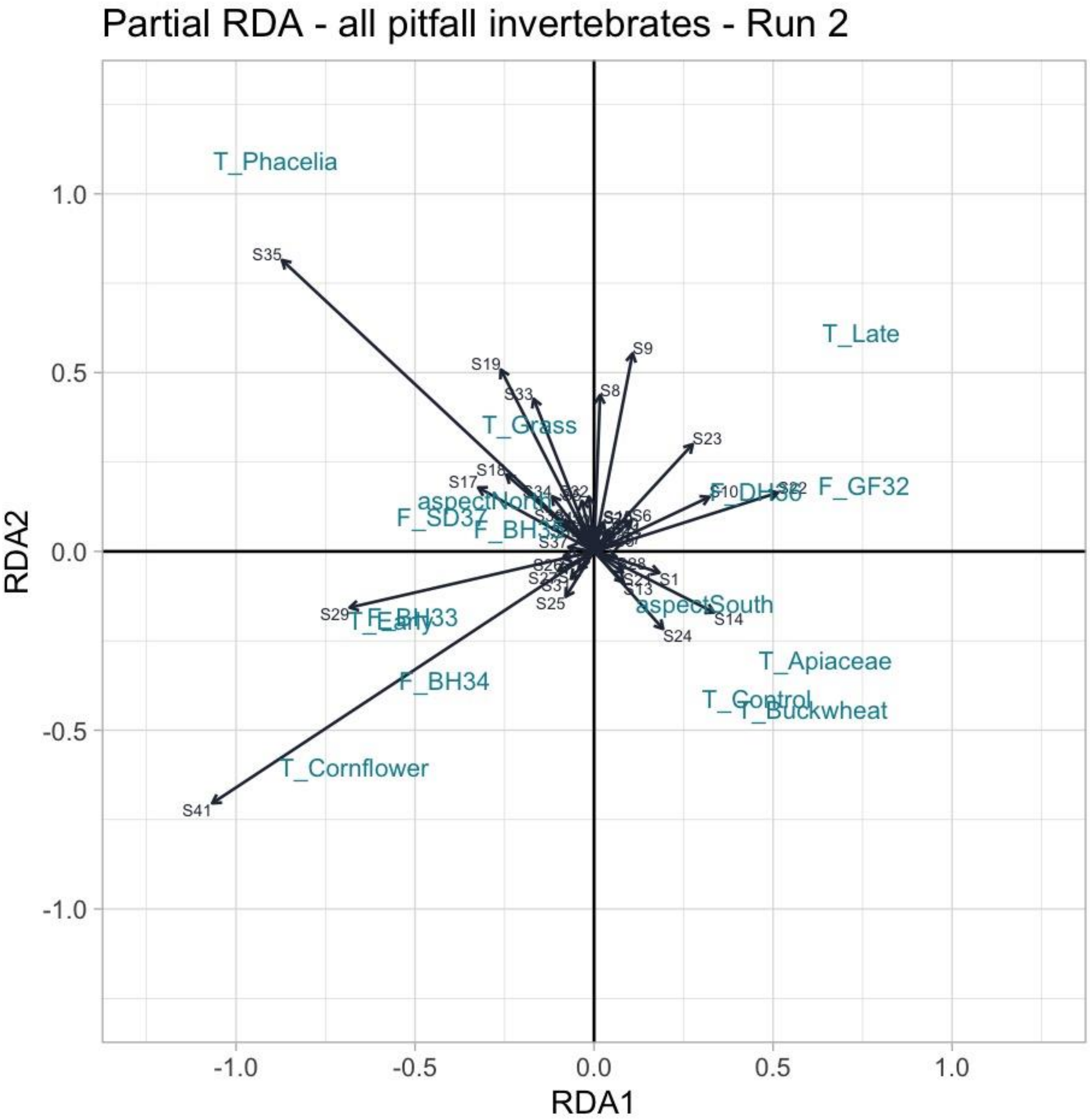


Figure 2.15. Redundancy analysis ordination of all pitfall invertebrates in Run 2, with axes constrained by (F_), seed mix (T_), and aspect. Invertebrates identified to the maximum required resolution and denoted by: S1: *Agonum dorsale*, S2: *Amara similata*, S3: *Bembidion lampros* SW, S4: *Carabus violaceus*, S5: *Harpalus affinis*, S6: *Harpalus rufipes*, S7: *Loricera pilicornis*, S8: *Pterostichus cupreus*, S9: *Pterostichus madidus*, S10: *Pterostichus melanarius*, S11: other Carabid larvae , S12: *Loricera pilicornis* larvae, S13: Tachyporinae , S14: other Staphylindae, S15: Cantharidae, S16: Coccinellid larvae, S17: adult *Brassicogethes* spp, S18: *Oederma* sp., S19: *Phyllotreta* sp., S20: Curculionidae, S21: other Coleoptera, S22: *Erigone* sp., S23: *Oedothorax* sp., S24: *Lepthyphantes tenuis*, S25: Linyphidae other, S26: *Pardosa* sp., S27: Lycosidae other, S28: *Pachygnatha degeeri*, S29: other Araneae, S30: Opiliones, S31: Syrphid, S32: Empid, S33: other Diptera, S34: Oniscidea, S35: Solitary bee, S36: *Apis mellifera*, S37: Bumble bee, S38: Aphidoidea, S39: Lepidopteran larvae, S40: Parasitica, S41: Formicidae, S42: Heteroptera, S43: Auchenorhyncha.

The species level taxonomic resolution in the adult carabid community has allowed the significant effect of seed mix upon carabids to be identified (Table 2.8). In the first run, seed mix explained 21.3% of the carabid community variation (F= 2.91, *p*=0.001). Both *Pterostichus madidus* and *P. melanarius* had a strong association with the Apiaceae mix (Figure 2.16). *P. cupreus* showed an association with the Phacelia mix and *Harpalus rufipes* with the Late-flowering mix. Additionally, whilst the control mix had a distinctly different community, there was no apparent strong species association (Figure 2.16).

During the second run, seed mix explained 26.8% of the carabid community variation (F= 1.87, *p*=0.012). For the first time, the grass mix was particularly distinct, although with no strong species association. The Apiaceae, and to a lesser extent, the Phacelia mix were associated with *Agonum dorsale* (Figure 2.17).

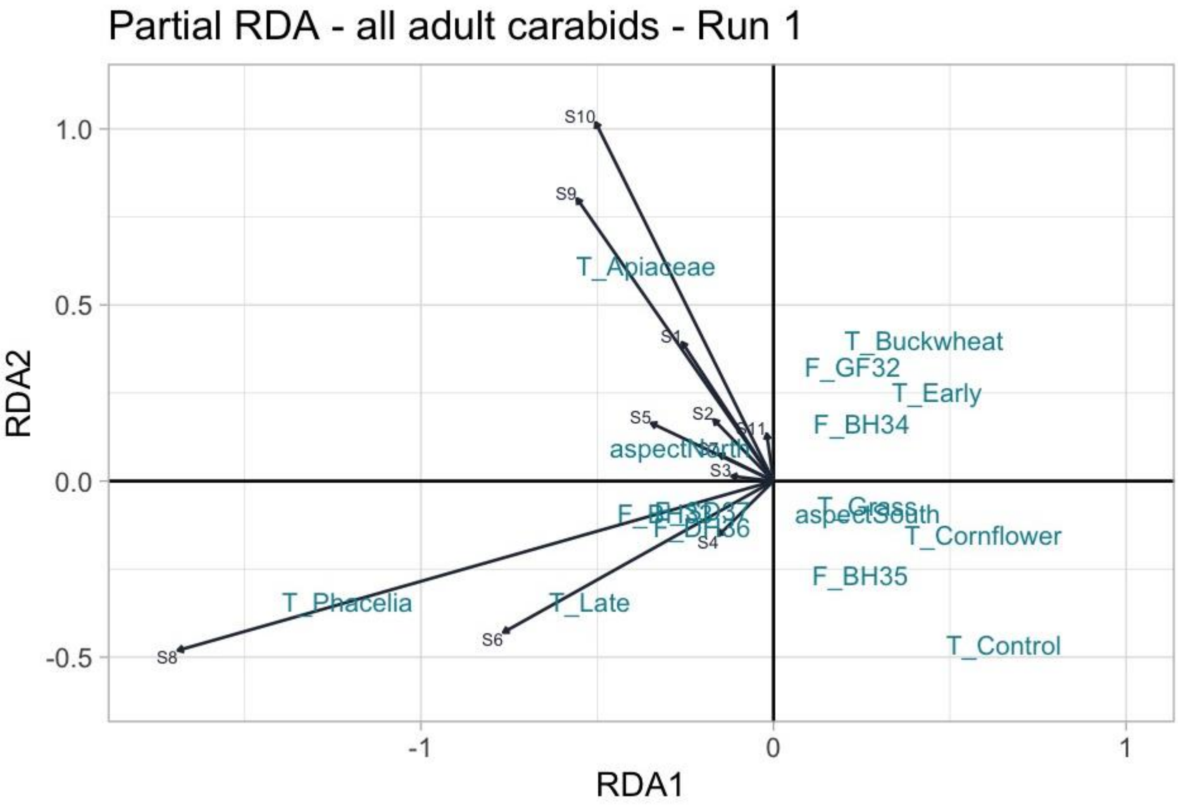


Figure 2.16. Redundancy analysis ordination of all Carabid species in pitfall trapping Run 1, with axes constrained by (F_), seed mix (T_), and aspect. Invertebrates identified to the maximum required resolution and denoted by: S1: *Agonum dorsale*, S2: *Amara similata*, S3: *Bembidion lampros* SW, S4: *Brachinus crepitans*, S5: *Harpalus affinis*, S6: *Harpalus rufipes*, S7: *Nebria brevicollis*, S8: *Pterostichus cupreus*, S9: *Pterostichus madidus*, S10: *Pterostichus melanarius*, S11: *Trechus quadristriatus*.

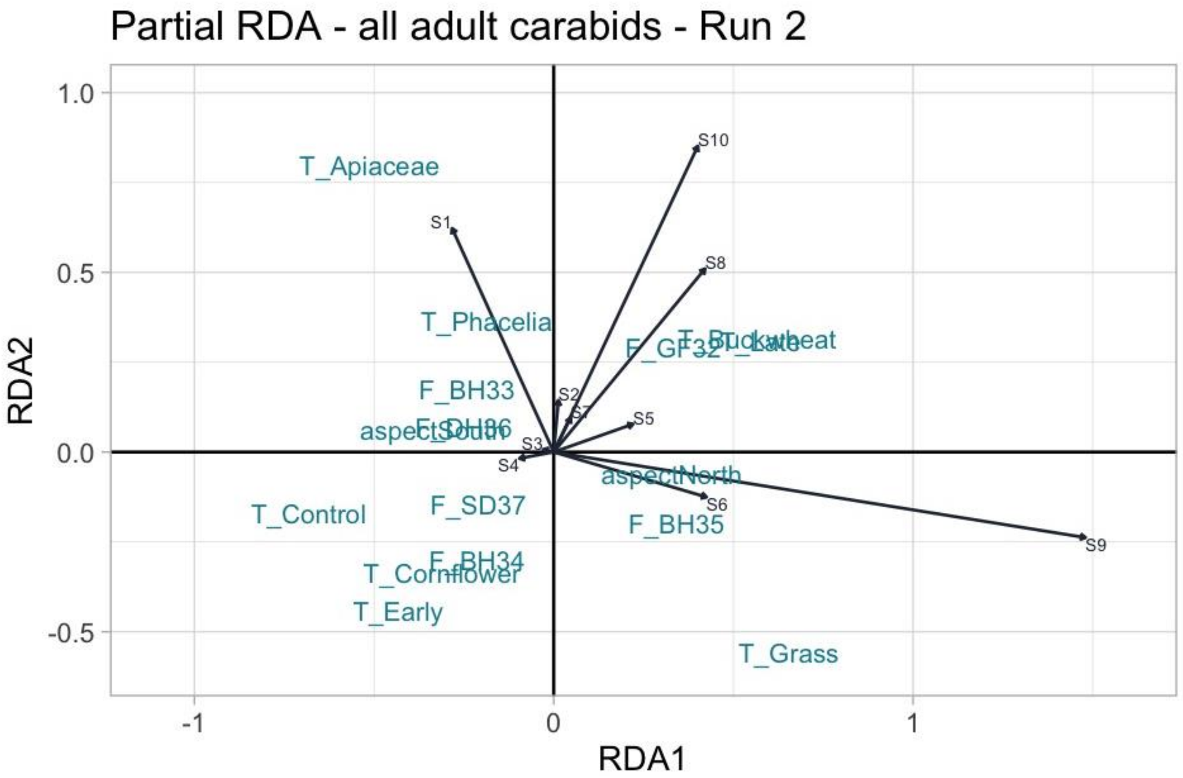


Figure 2.17. Redundancy analysis ordination of all Carabid species in pitfall trapping Run 2, with axes constrained by (F_), seed mix (T_), and aspect. Invertebrates identified to the maximum required resolution and denoted by: S1: *Agonum dorsale*, S2: *Amara similata*, S3: *Bembidion lampros* SW, S4: *Carabus violaceus*, S5: *Harpalus affinis*, S6: *Harpalus rufipes*, S7: *Loricera pilicornis*, S8: *Pterostichus cupreus*, S9: *Pterostichus madidus*, S10: *Pterostichus melanarius*.

2.3.4 Sentinel Aphid Cards

There was no significant effect of seed mix treatment upon the count of aphids remaining on sentinel cards placed on the ground (χ = 10.0, df = 7, *p*=0.1885), nor did the predation of sentinel aphids vary by seed mix on cards attached to barley foliage (χ = 10.0, df = 7, *p*=0.1885). The predation rate of sentinel aphids from cards placed on the ground does look to be higher, although due to low sample size this has not been statistically tested (Table 2.9).

2.3.5 Barley Yield

There were no statistically significant differences in the fresh mass of grain harvested, the adjusted grain yield (at 85% moisture) or the percentage of grain dry matter associated with seed mix, or the distance the cut was made from the seed mix plots, nor a significant interaction between mix and cut (Table 2.10). The only notable outcome was a marginally a significant difference in the grain dry matter percentage with the distance the grain was cut (Table 2.10).

Table 2.9. Mean number of aphids remaining on sentinel cards on cards placed on the ground and on barley foliage for each seed mix.

	Ground		Foliage	
Seed Mix	Mean aphids remaining	Standard Deviation	Mean aphids remaining	Standard Deviation
Apiaceae	3.67	0.82	4.67	1.97
Buckwheat	3.67	0.00	5.00	1.86
Control	3.17	0.41	4.83	1.83
Cornflower	3.00	0.41	4.83	2.28
Early-flowering	4.83	0.00	5.00	0.41
Grass	4.17	0.41	4.83	1.60
Late-flowering	1.83	1.22	4.50	1.83
Phacelia	3.67	0.00	5.00	1.21

Table 2.10. Results of barley grain harvest analysis from linear mixed effects models.

Response Variable	Main effect	Num DF	Den DF	F value	Pr(>F)	Significance
Fresh yield mass	Seed mix	7	28	0.46	0.852	NS
	Cut distance	1	36	1.26	0.269	NS
	Seed mix * cut distance	7	36	0.61	0.747	NS
Adjusted yield at 85% moisture (t/ha)	Seed mix	7	27	0.45	0.862	NS
	Cut distance	1	35	1.58	0.217	NS
	Seed mix * cut distance	7	35	0.50	0.832	NS
Grain dry matter	Seed mix	7	27	0.60	0.747	NS
	Cut distance	1	35	3.86	0.058	.
	Seed mix * cut distance	7	35	0.59	0.757	NS

2.4 Discussion

As anticipated, seed mixes do support *different* insect communities, but perhaps not *more* key ESPs. However, there were variable responses between seed mixes and different invertebrate groups, and these are ephemeral associations fluctuating across the season. Connecting these different invertebrate communities with increased delivery of pest control remains elusive. The failure of these efforts to identify whether foliar or epigeal NE are more effective at controlling aphid pests as monitored via sentinel plants was a setback for this thesis.

Whilst it was expected that sown seed mixes would attract more of the key ESPs compared with controls (Haaland et al., 2011), there was limited evidence to support this hypothesis from pitfall and water trapping data collected in this study. Moreover, when there were higher abundances of key ESPs, this was not always associated with dicot flowering mixes producing nectar. For instance, yellow water trapping revealed more NE and Ichneumonid wasps in grass plots, and more Chalcidid wasps and all wasps in the control plots, the two monocot mixes. Interestingly, these plots also had high, if not the highest aphid abundances. Perhaps, the high ESP abundances seen here was simply CBC in action, with NE attracted to plants harbouring pests. It has been demonstrated that increasing proportions of semi-natural habitat in the area around a location can result in increased predation and reduced pest outbreaks, potentially due to high visitation rates or a quicker arrival (McHugh et al., 2020, Le Gal et al., 2020). Potentially, the Chalcidid wasps and other NE are still using structural or trophic resources provided by the flowering plots. After adulting feeding, NE may move into control plots to complete a stage of their lifecycle, such as egg-laying in a pest aphid. These findings demonstrate the challenge of using a relatively small, even if hopefully well targeted, sampling effort capturing a snapshot of a season (Iuliano and Gratton, 2020).

Given that CBC is not simply all about increasing pest control but also aims to improve insect conservation (Begg et al., 2017), metrics like species richness are critical for community resilience and response to change (Wilby and Thomas, 2002, Jones et al., 2009). The inability to identify all species present in the total species pool may have masked true treatment differences. However, when it was possible to identify insects down to a species level, such as adult carabid richness, seed mix differences were still not apparent.

Seed mix was a significant factor explaining variation in both aerial and epigeal communities. However, for the assemblage of these aerial communities, field was also a significant influence. For aerial insects, this is not a surprising finding given the aforementioned numerous studies documenting the impact of landscape upon insects. However, the lack of influence field had on the ground-active community assessed by pitfall trapping, in particular carabid beetle communities, is a surprise given the work showing the farm-scale impact landscape features can have upon carabids (Jowett et al., 2019, Eyre et al., 2013). Although, the finding that species richness did not vary by seed mix here does fit with previous work demonstrating landscape structure does not influence carabid richness (Vanbergen et al., 2010). Perhaps species responses to local habitat features vary between landscapes.

The community assemblage analysis of the second period of water trapping adds strength to the findings around NE attraction to pest outbreaks, with the Cornflower and Late-flowering mixes both having strong associations with both aphids and Braconidae wasps. For the first time, it is revealed that Buckwheat has a strong association with a NE and in particular, a parasitic wasp with the association with Pteromalid wasps in the second period of yellow water trapping. Given the previous link between Pteromalidae and their potential role in pest control of Asparagus Miner (Diptera: Agromyzidae), this may be a positive association (Morrison et al., 2014). However, there are concerns around the limited impact of parasitoids have upon pest control in part due to trophic interactions hyperparasitoids (Mackauer and Volkl, 1993, Feng et al., 1992). Therefore, from a grower perspective, reliance upon parasitoids may appear to be a risky proposition.

Regarding ES delivery, there were no conclusive results suggesting pest control delivery was enhanced next to the sown seed mixes, nor any differentiation within mixes. The failure of work using sentinel carrot plants in 2018 and 2019 was disappointing. This work would have been valuable to reveal a more mechanistic understanding and provide enhanced ecological knowledge regarding the efficacy of different NE at regulating populations of *C. aegopodii*. Assessing the delivery of pest control ecosystem services through barley yield measurements at harvest was possible in 2019, although this did not reveal any relevant treatment differences. While this trial was replicated with controls, it would be challenging to identify this ESD at the farm scale (Tscharntke et al., 2016). Additionally, if barley was the sole focus of this experiment, it would have required more intensive barley sampling, such as monitoring the crop’s pests and parasitism rates, a feature that is lacking across CBC efforts (Holland et al., 2017).

As sentinel systems offer the opportunity to overcome low and unequal pest numbers between treatments, they are vital to successful work on CBC (McHugh et al., 2020, Greenop et al., 2019). The finding that there were no significant differences in pest control measures upon sentinel cards between seed mixes may reflect biological reality or may be an artefact of the sampling method. In the former, pest control delivery has notable instances of inconsistent responses to landscape composition and habitat manipulation (Karp et al., 2018, Tscharntke et al., 2016). Perhaps this work is simply another example of unsuccessful CBC. In the case of the latter, the impact of any volatile chemical compounds from the glue used here upon predators cannot be ruled out (Birkhofer et al., 2017). Additionally, whilst sentinel prey can be sensitive enough to detect pest control differences at this local level, as only one type of sentinel prey was used, this may be insufficient if different NE guilds are active (McHugh et al., 2020, Greenop et al., 2019). Further work would also benefit from greater temporal replication to provide greater power to distinguish if there were differences in the predation rates of foliar and ground sentinel cards. Whilst foliar cards do not fully reflect predation by aerial predators, a comparison between foliar and ground predation would be worthwhile to investigate if there are functional differences in invertebrate communities associated with seed mixes. Finally, as the water and pitfall trapping revealed, there are changeable insect communities throughout the summer, suggesting more temporal replications of the sentinel cards are likely required to draw robust conclusions.

From an applied or growers' perspective, as the first trapping period coincided with around the time that aphids would be migrating into carrot crops, it appears tailoring plant flowering and insect phenology with this period will be a challenge. However, a few weeks later, by the second trapping period, there were more distinctly different insect communities, particularly the aerial communities, thus giving more credence to the idea that pest control services can be tailored. Although, the few statistically significant differences in the abundance of key ESPs might be worrying. Whilst one could argue that maybe the sampling duration was not long enough, the differences in *P. cupreus* abundance does suggest that if there are large differences, they can be found.

As anticipated, different seed mixes do support different insect communities supporting the approach that flower margins could be tailored to support ESPs relevant to CBC in certain crops. However, this varies for different insect groups and associating these insects with increased delivery of pest control remains elusive.

Acknowledgements

I would like to thank Todd Jenkins and Matt Skellern for their respective yellow water trap and pitfall trap invertebrate taxonomic expertise.

2.6 Supplementary Materials

2.6.1 Description of 2018 Sentinel Carrot Experiment

In mid-March, over 144 pots of carrots (cv. Natuna) were sown, using plant pots sized 22cm x 22cm x 33cm containing a Rothamsted prescription compost mix with added water-retaining crystals to aid with water retention. Despite the water-retaining crystals, at the height of summer, these carrots were being watered up to 6 times a day to ensure plants did not wilt.

Approximately 30cm away from each margin plot, four holes were dug 25cm x 25cm x 30cm into the ground for placement of the sentinel carrot pots. It was planned that four pots of carrot plants would be randomly assigned to one of four treatments – ‘open’ control pots, ‘closed’ control pots, foliar restricted pots, and epigeal restricted pots. To exclude foliar predators, exclusion cages were constructed. These were built around of a frame 20cm x 20cm x 50xm covered with a white polyethylene mesh with gauge size of 1.5mm (LBS Horticultural Supplies), which then covered the upper portion of the carrot foliage. This was designed to exclude most flying natural enemies. Epigeal predators were excluded via a plastic barrier secured around the base of the plant pot (15cm above pot surface). ‘Closed’ control pots would have both foliar and epigeal predators excluded. Conversely, ‘open’ controls were pots that were placed into the holes without anything restricting natural enemy access.

However, due to the extreme heat conditions during the hottest summer on record for England (McCarthy et al., 2019), completing the digging of all 144 holes required for this experiment was not possible. Consequently, the experiment was altered to focus on ‘closed’ and ‘open’ controls instead. On 18/7/2018, 5 apterous *C. aegopodii* aphids were transferred onto the same area of foliage and secured with a clip cage to ensure aphids did not immediately move away. The inoculated leaf was labelled with white twine. After inoculation, plants were left for 24 hours before being moved into place next to margin plots. At this point, the clip cages were removed. If there were not 5 aphids present on leaf area that had been caged, additional aphids were transferred onto the plants to bring the total to 5. The exclusion cage frames, and plastic barriers were then attached. At this point the experiment began, on 19/7/2018 and the plants were left until 23/7/2018. Although we tried to the time of the use of sentinel plants when it was cooler, the temperature across these days peaked at 30.6°C. Despite moving 500 litres of water around the farm to water the plants and the inclusion of water retaining crystals, the plants struggled in the heat.

On 23/7/2018, the area of carrot foliage marked with white twine was checked for any aphids. In case the aphids had moved around the plants, the remaining carrot foliage was also sampled, placed into labelled, sealed plastic bags and stored at -18°C; foliage was later checked for aphid presence. Of the 36 ‘open’ treatments, only one plot had 1 aphid present. In the 36 ‘closed’ treatments, there were only four plots found with 1 aphid on the leaf inoculated with aphids. These numbers were too small to warrant analysis.

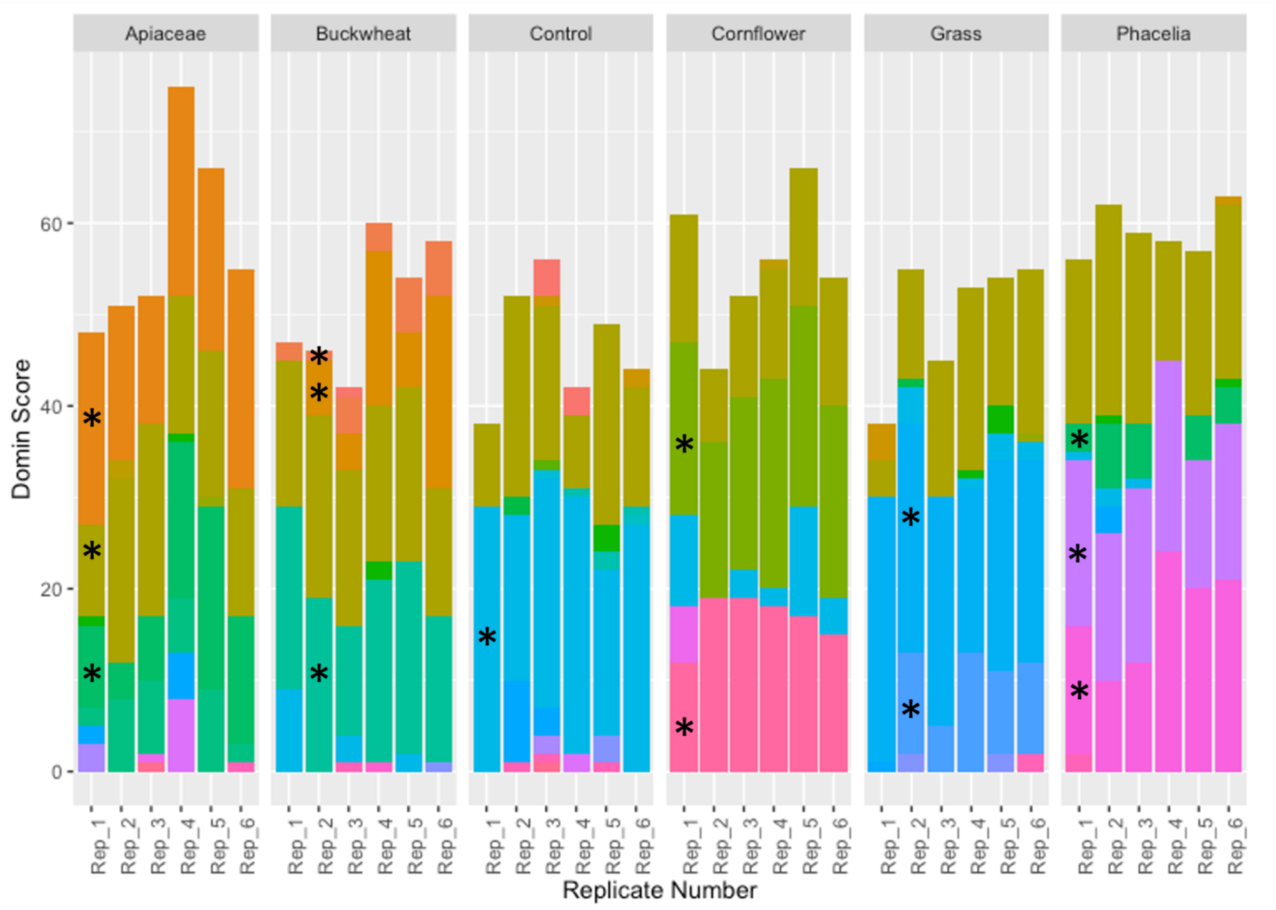


Figure S.2.18. Domin scores from vegetation surveys of flower mix treatments across all treatments and replicates on Rothamsted Farm in 2018. The sections of the bars marked with an asterisk indicate bars where the species was sown as part of the treatment. Replicates 1, 2 and 3 were separate fields, replicates 4, 5 and 6 were in the same field.

2.6.2 Table showing arthropods identified from yellow water traps placed in sown flower mix treatments and thieir assigned functional group/s

Arthropod Taxonomic Unit	Functional group	Reference
Acari	Other/various	
Aleocharinae	NE/ predator	(Staton et al., 2021)
Andrena spp.	Bee	
Anthicidae	Other/various	(Staton et al., 2021)
Aphidius spp.	NE (& wasp)	(Holland and Oakley, 2007)
Aphids	Herbivore	(Staton et al., 2021)
Apidae	Bee	
Apis melifera	Bee	
Apis spp.	Bee	
Athalia rosae	Herbivore	(Staton et al., 2021)
Bibionidae	Herbivore	(Staton et al., 2021)
Bombus spp.	Bee	
Bombus terrestris/lucorum	Bee	
Brachinus nigricornis	Other/various	
Braconidae	NE (& wasp)	(Staton et al., 2021)
Brassicogethes spp.	Herbivore	(Staton et al., 2021)
Bruchus spp.	Herbivore	(Staton et al., 2021)
Cantharidae	NE/predator	(Staton et al., 2021)
Cerambycidae	Herbivore	(Staton et al., 2021)
Ceratina spp.	Other/various	
Chalcididae	NE (& wasp)	(Staton et al., 2021)
Chloromyia formosa	Other/various	
Cicadellidae	Herbivore	(Staton et al., 2021)
Cicadidae	Other/various	
Coccinella septempunctata	NE / predator	(Staton et al., 2021)
Coleoptera	Other/various	(Staton et al., 2021)
Coreidae	Other/various	
Crabronidae	Other/various	
Chrysomelidae	Herbivore	(Staton et al., 2021)
Curculionoidea	Herbivore	(Staton et al., 2021)
Curculionidae	Herbivore	(Staton et al., 2021)
Diptera	Other/various	(Staton et al., 2021)
Drosophilidae	Other/various	(Staton et al., 2021)
Ectopsocus spp.	Other/various	
Elateridae	Herbivore	(Staton et al., 2021)
Episyphus balteatus	NE	(Staton et al., 2021)
Evanioidea	Other/various	
Figitidae	NE	(Holland and Oakley, 2007)
Formicidae	Other/various	(Staton et al., 2021)

Harmonia axyridis	NE	(Staton et al., 2021)
Hemiptera	Other/various	(Staton et al., 2021)
Hoplia philanthus	Other/various	
Homoeolabus analis	Other/various	
Homoptera	Other/various	
Hybotidae	NE	(Holland and Oakley, 2007)
Ichneumonidae	NE (& wasp)	(Staton et al., 2021)
Latridiidae	Other/various	(Staton et al., 2021)
Lauxaniidae	Other/various	(Staton et al., 2021)
Lepidoptera	Herbivore	(Staton et al., 2021)
Linyphiidae	NE	(Staton et al., 2021)
Lycosidae	NE	(Staton et al., 2021)
Melyridae	NE	(Staton et al., 2021)
Melanostoma	NE	(Staton et al., 2021)
Malachius bipustulatus	NE	(Staton et al., 2021)
Miridae	Other/various	(Holland and Oakley, 2007)
Neuroptera	NE	(Staton et al., 2021)
Nitidulidae	Other/various	(Staton et al., 2021)
Oedemeridae	Other/various	(Staton et al., 2021)
Opomyzidae	Herbivore	(Staton et al., 2021)
Orius spp.	Other/various	
Pentatomidae	Herbivore	(Staton et al., 2021)
Phalacridae	Other/various	
Philonthus spp.	NE	(Staton et al., 2021)
Phoridae	Other/various	(Staton et al., 2021)
Phyllotreta spp.	Herbivore	(Holland and Oakley, 2007)
Platygastridae	NE (& wasp)	(Holland and Oakley, 2007)
Praon volucre	NE (& wasp)	(Holland and Oakley, 2007)
Psylloidea	Herbivore	(Staton et al., 2021)
Pteromalidae	NE (& wasp)	(Holland and Oakley, 2007)
Rhagonycha fulva	Other/various	
Scarabaeidae	Other/various	
Sphecodes spp.	Bee	
Syrphidae	NE	(Staton et al., 2021)
Staphylinidae	Other/various	(Staton et al., 2021)
Tachyporinae	NE	(Staton et al., 2021)
Tachyporus spp.	NE	(Staton et al., 2021)
Tenthredinidae	Other/various	
Tenthredo spp.	Other/various	
Thomisidae	NE	(Staton et al., 2021)
Thrips	Herbivore	(Staton et al., 2021)
Tineidae	Other/various	
Tipulidae	Other/various	(Staton et al., 2021)

Triozidae	Herbivore	(Staton et al., 2021)
Tytthaspis sedecimpunctata	Other/various	(Staton et al., 2021)
Vespidae	NE (& wasp)	(Staton et al., 2021)
Vespula vulgaris	NE (& wasp)	(Staton et al., 2021)

2.6.3 Table showing invertebrates identified from pitfall traps placed in sown seed mixes and their assigned functional groups

Insect taxonomic unit	Functional Group	Author
Carabidae		
Abax parallelepipedus	Not assigned	All Carabid groups assigned as per Cole et al. (2002)
Agonum dorsale	5	
Agonum muelleri	6	
A. aulica	Not assigned	
A. lunicollis	3	
A. similata	Not assigned	
Asaphidion flavipes	4	
Bembidion lampros FW	4	
B. lampros SW	4	
Brachinus crepitans	Not assigned	
Calathus fuscipes	1	
Carabus violaceus	2	
Harpalus affinis	7	
H. rufipes	1	
Harpalus sp. (?sabulicola)	Not assigned	
H.rufibarbis	Not assigned	
Loricera pilicornis	4	
Microlestes maurus	Not assigned	
Nebria brevicollis	1	
Nebria salina	1	
Notiophilus biguttatus	4	
Pterostichus cupreus	Not assigned	
P. madidus	1	
P. melanarius	1	
P. niger	1	
Trechus quadristriatus	3	
Carabid larvae (other)	Not assigned	
Carabide larve (*L. pilicornis*)	Not assigned	

Staphylinidae			
	Philonthus sp	NE	(Staton et al., 2021)
	Tachyporinae	NE	(Staton et al., 2021)
	Stenus sp	NE	(Staton et al., 2021)
	Quedius sp	Other/various	
	Other Staphylindae	Other/various	(Staton et al., 2021)
Other Coleoptera			
	Cantharidae	NE	(Staton et al., 2021)
	Elateridae	Other/various	
	Coccinellid adult	NE	(Holland and Oakley, 2007 & Staton et al., 2021)
	Coccinellid larvae	NE	(Holland and Oakley, 2007 & Staton et al., 2021)
	Pollen beetle - Meligethes adults	Pest	(Holland and Oakley, 2007)
	Pollen beetle - Meligethes larvae.	Pest	(Holland and Oakley, 2007)
	Psylliodes chrysocephala	Pest	(Holland and Oakley, 2007)
	Oederma sp.	Other/various	(Staton et al., 2021)
	Phyllotreta sp.	Pest	(Holland and Oakley, 2007)
	Curclionidae	Herbivore	(Staton et al., 2021)
	Other coleoptera	Other/various	(Staton et al., 2021)
	Other coleopteran larvae	Other/various	(Staton et al., 2021)
	Collembola	Other/various	(Staton et al., 2021)
Araneae			
	Erigone sp. (Linyphiidae)	NE	(Staton et al., 2021)
	Oedothorax sp. (Linyphiidae)	NE	(Staton et al., 2021)
	Lepthyphantes tenuis (Linyphiidae)	NE	(Staton et al., 2021)
	Other Linyphidae	NE	(Staton et al., 2021)
	Pardosa sp. (Lycosid)	NE	(Staton et al., 2021)
	other Lycosidae	NE	(Staton et al., 2021)
	Pachygnatha degeeri	NE	(Staton et al., 2021)
	Pachygnatha clercki	NE	(Staton et al., 2021)
	other Araneae + immatures	NE	(Staton et al., 2021)
	Opiliones	NE	(Staton et al., 2021)
Diptera			
	Syrphid	NE	(Staton et al., 2021)
	Empid	NE	(Staton et al., 2021)

	Dolichopod	NE	(Staton et al., 2021)
	other Diptera	Other/various	
Misc. others			
	Millipede	Herbivore	(Staton et al., 2021)
	Centipede	Other/various	
	Woodlouse	Other/various	
	Solitary bee	Pollinator	
	Honeybee	Pollinator	
	Bumble bee	Pollinator	
	Aphid	Pest	
	Lepidopteran adult	Herbivore	(Staton et al., 2021)
	Lepidopteran larv.	Herbivore	(Staton et al., 2021)
	Sawfly larv.	Herbivore	(Staton et al., 2021)
	Parasitica	NE	(Staton et al., 2021)
	other wasp	NE	(Staton et al., 2021)
	Ant	Other/various	(Staton et al., 2021)
	Heteroptera	Other/various	(Staton et al., 2021)
	Auchenorhyncha	Other/various	
	Psyllid	Pest	

The groups assigned to sampled beetles here originate from Cole et al.’s 2002 work classifying farmland carabid beetle species. Cole et al. (2002) used multivariate fuzzy clustering to objective assign beetles to groups based on ten ecological traits. These traits reflected ecological differences between the groups such as size, breeding season, wing morphology and diet (Cole et al., 2002). The use of non-taxonomic traits for classification provides some indication of ecological functioning beyond purely considering the presence and relative abundance of a few common carabid species (Cole et al., 2002).

2.6.4 Spider Partial Redundancy Analysis

Table 2.11. Table showing the percentage variance explained by seed mix, field and plot aspect in the partial Redundancy analysis spider taxonomic units, as well as the subsequent ANOVA analysis of the partial Redundancy analysis model. Permutation test for partial RDA under reduced model. Permutation: free, number of permutations: 999. Significance codes: $p<0.001$ = '***', $p<0.01$ = '**', $p<0.05$ = '*'.

Response Variable	Run	Fixed effect	Df	F	Pr(>F)	Sig	adjusted R^2	Variance explained (%)
Spiders	1	Seed Mix	7	1.34	0.112	NS	0.064	19.2
		Field	5	1.18	0.258	NS	0.027	12.0
		Aspect	1	0.44	0.875	NS	-0.016	0.90
	2	Seed Mix	7	1.44	0.11	NS	0.094	22.8
		Field	5	0.97	0.47	NS	-0.004	11.0
		Aspect	1	0.79	0.553	NS	-0.006	1.75

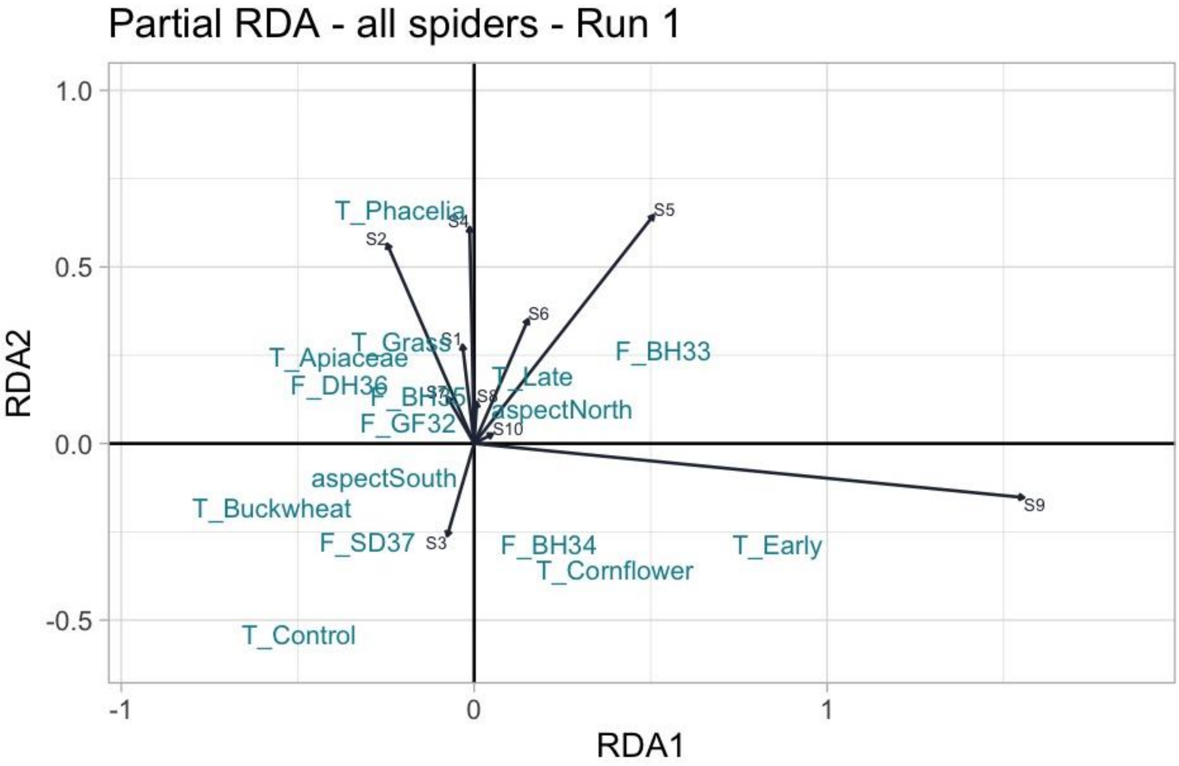


Figure S.2.19. Redundancy analysis ordination of all spiders in Run 1, with axes constrained by (F_), seed mix (T_), and aspect. Invertebrates identified to the maximum required resolution and denoted by: S1: *Erigone* sp., S2: *Oedothorax* sp., S3: *Lepthyphantes tenuis*, S4: other Linyphiidae, S5: *Pardosa* sp., S6: other Lycosidae, S7*: Pachygnatha degeeri*, S8: *Pachygnatha clercki*, S9: other Araneae, S10: Opiliones.

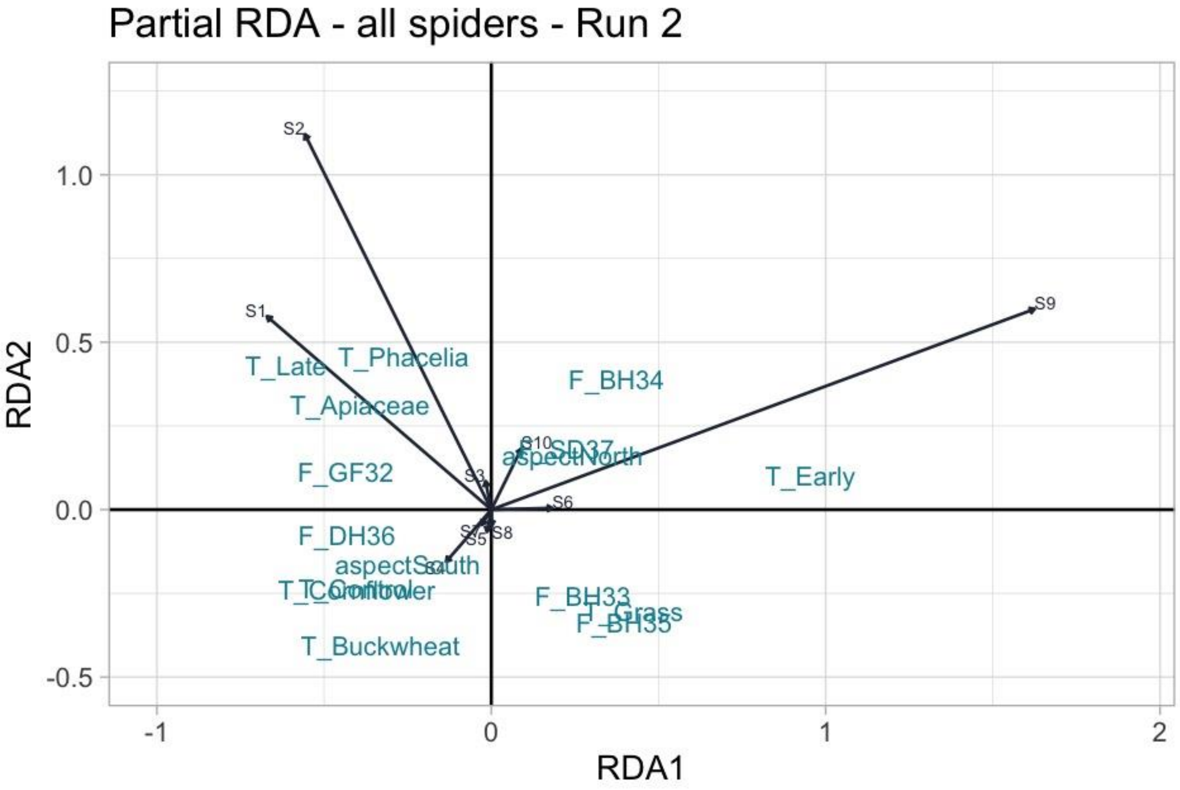


Figure S.2.20. Redundancy analysis ordination of all spiders in Run 2, with axes constrained by (F_), seed mix (T_), and aspect. Invertebrates identified to the maximum required resolution and denoted by: S1: *Erigone* sp., S2: *Oedothorax* sp., S3: *Lepthyphantes tenuis*, S4: other Linyphiidae, S5: *Pardosa* sp., S6: other Lycosidae, S7*: Pachygnatha degeeri*, S8: *Pachygnatha clercki*, S9: other Araneae, S10: Opiliones.

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Chapter 3 – Establishing flower strips in commercial carrot fields and developing methods to assess the delivery of pest control services.

3.1 Abstract

This chapter investigates the potential of in-field flower strips to support Conservation Biological Control and enhance pest control in a commercial carrot field. There is a need to investigate alternatives to synthetic insecticide control of insect pests in carrots, and growers are keen to optimise flower strips for ecosystem service delivery. There are some documented instances where in-field strips increased the abundance and richness of key ecosystem service providers in other crops. But there is little known about delivery of these services in terms of impact on pest regulation and previous work on delivery of pest control in carrot crops is especially sparse. Therefore, in-field strips were sown in a commercial carrot crop, and sampling efforts focused on developing and assessing the direct evidence for pest control delivery via direct aphid abundance counts, sentinel prey card predation and insect-damaged carrots. There were no instances whereby these direct measurements of pest control differed next to sown seed mixes. There was also no measurable increase indirect ecosystem service delivery found, as assessed by harvest metrics such as net yield. However, commercially used grading methods were used on samples of harvested carrots and where defects were present this revealed statistically and biologically relevant differences, although not related to pest control delivery. This trial showed the potential of flower strips to affect yield variables in the adjacent crop and the need for future work to investigate and quantify the role of natural enemies in pest regulation of aphids in carrots. This future work would be necessary to identify any optimal seed mix for growers.

3.2 Introduction

3.2.1 Scientific Background: Insect Pests of Carrots

Aphid-vectored viruses of carrots present a significant challenge to individual growers and, subsequently, national carrot production (Hinds, 2016). Therefore, efforts to reduce virus damage in the field typically focus on the control of pest aphids. *Cavariella aegopodii* and *Myzus persicae* are longstanding, well-known key vectors for viruses (Murant et al., 1976, Watson and Serjeant, 1964).

Understanding the causal agent of yield loss, the suite of viruses that aphids transmit to carrots, is critical. Unfortunately for carrot growers, carrot viruses are complex. Within the UK, the predominant viruses are Parsnip yellow fleck virus (PYFV) and Carrot Motley Dwarf complex (CMD) (Adams et al., 2014). CMD is thought to be caused by a suite of viruses, including Carrot red leaf virus (CtRLV), Carrot mottle virus (CMoV) and Carrot red leaf associated RNA (CtRLVaRNA) (Adams et al., 2014, Yoshida, 2020). CtRLV association with red foliage and PYFV's role in carrot seedling dieback has been known for over 30 years (Vandijk and Bos, 1989). More recently, CtRLVaRNA in carrots was first found in the UK in 2002, isolated from Huntapac's carrot fields in Lancashire (Fox and Mumford, 2017, Morton et al., 2003). Then, harnessing the development of next-generation sequencing, four novel viruses were discovered in carrots in Yorkshire in 2014 (Adams et al., 2014). Further work on a new virus, Carrot torrado virus (CaTV), has found the virus in foliage samples from Norfolk and Yorkshire, demonstrating CaTV's widespread distribution across the UK (Rozado-Aguirre et al., 2016). Beyond considering which viruses are present, Rozado-Aguirre et al.'s work also revealed that *Myzus persicae* were more prolific virus vectors than *Cavariella aegopodii* (2016). However, given the small scale and controlled environment nature of, Rozado-Aguirre et al.'s (2016) experiment, these findings may not be reflected in the field. Their work also detailed how aphids become infected with viruses and the potential role infected plants surrounding fields play in virus transmission to crops (Rozado-Aguirre et al., 2016). This work builds upon the established conclusion that not all aphids are carrot virus vectors and highlights subtleties even within carrot virus vectors.

Carrot fly, *Psila rosae*, is also an economically important insect pest in carrots. This phytophagous fly is a voracious carrot pest. After adults lay eggs and larvae mine the carrot roots, the tracks left by the larvae on the root surface render the carrot unsellable (Vincent, 1999). The wild host plants of *P. rosae* are common features of the agricultural landscape and include *Anrhriscus* *sylvestris* (cow parsley), *Heracleuin sphondylium* (hogweed) and *Conium maculatum* hemlock (Hardman et al., 1990). Both virus and insect-damaged crops have distinctive markings that indicate the levels and nature of damage in the crop (Collier et al., 2016), with examples of such damage indicated in Figure 3.1.

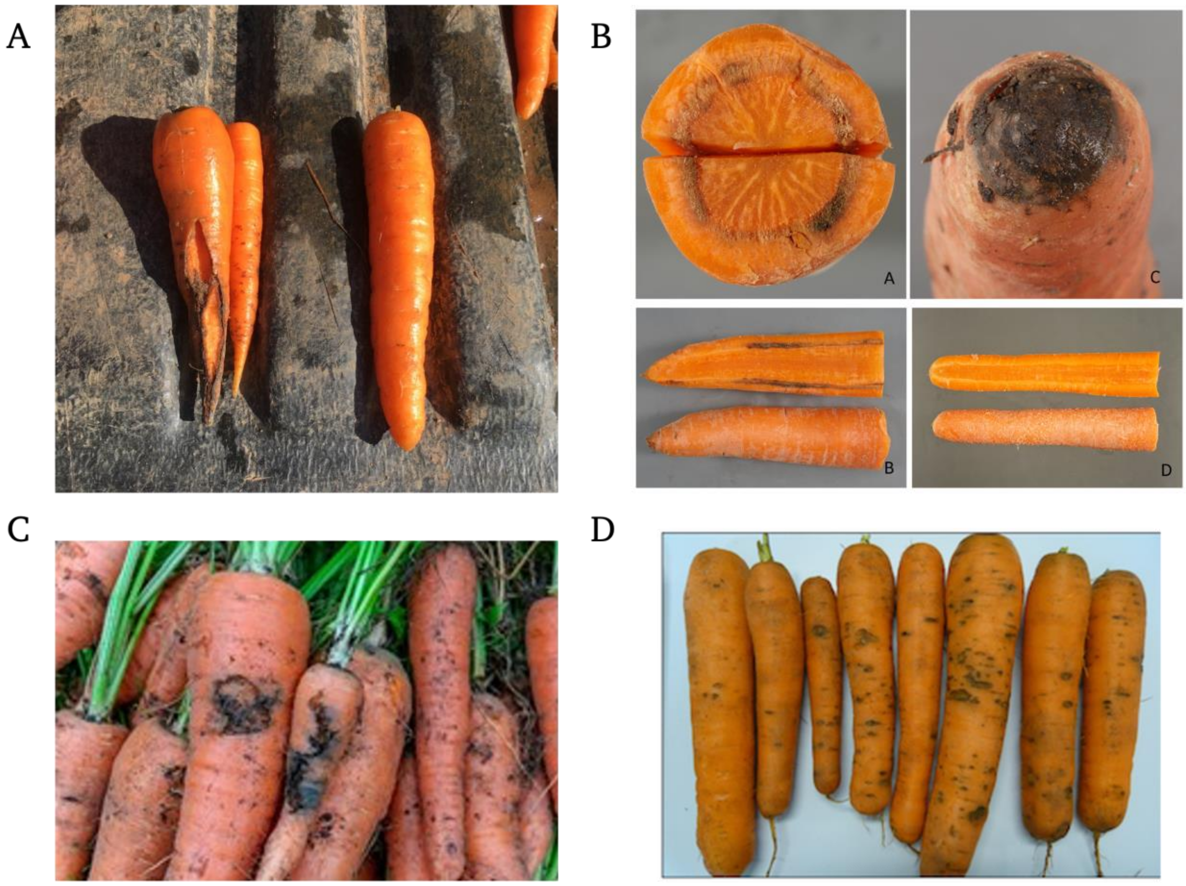

Figure 3.1. Representative images of pest and pathogen damage on carrots. **A** – On the left typical necrosis and splits in roots which growers refer to as virus damage, compared with a typical carrot on the right. **B** – Internal Necrosis thought to result from Carrot yellow leaf virus (CYLV) – image from Adams et al. (2014). **C** – Carrot fly larvae damage – image from AHDB (2015). **D** – Cavity spot damage – from John Clarkson (University of Warwick).

3.2.2 Commercial Context: Implications of Insect Pests

Like much of horticulture, carrots have high aesthetic standards that produce must meet to gain the highest possible revenue. The route to market with the highest value for a carrot grower like Huntapac will be to supermarket retailers, followed by the wholesale market and then other avenues such as processed products and animal feed. However, the sale of carrots to these additional routes will typically not yield a profit; it is more about covering production costs. For the more profitable markets, the 'ideal' carrot has a crown diameter of around 35mm; smaller carrots are still valuable, although no retailer will accept carrots with a crown diameter below 20mm. Likewise, carrots with a crown diameter up to 45mm can still be sold to retailers in some product lines, but those over 45mm tend to be too big for consumers at supermarkets.

Across all retailers, no carrot can be sold with visible insect pest damage, although there are tolerances for some minor defects. For instance, cavity spot (*Pythium violae*) is a common soil-borne fungal pathogen of carrots that causes lesions on the root surface (AHDB, 2015, Sapkota and Nicolaisen, 2018). Cavity spot damage is acceptable at low levels. The abundance of cavity spot damaged carrots, and the extent of damage that a retailer will tolerate in a bag of carrots will vary. These specifications will depend on individual retailers and even between separate product lines within a supermarket. These specifications are also confidential documents that Huntapac cannot share. However, Huntapac's agronomy and quality assurance teams have developed a crop monitoring system. After harvesting a 1m^2 area, carrots can be washed and graded, and any defect present can be identified. This system helps monitor the crop for any diseases present and helps predict the crop's yield and inform when carrot harvest should occur. Such sampling allows the packhouse where the carrots are washed, graded, and packaged to be forewarned if additional staff will be required to grade carrots and remove those which are substandard. When these small plots are harvested, the crop is assessed to the highest possible quality standards, even if an individual damaged carrot may still be sold to a retailer. This sampling protocol has direct commercial relevance and was used in my study to facilitate statistical investigations to identify any treatment differences.

The price of producing carrots will vary throughout the year. For instance, carrots harvested in winter are protected from frost by being covered in straw, which might cost thousands of pounds a hectare, and will often require additional fungicide applications in inclement weather. Alternatively, carrots sown in early spring are covered in polythene to insulate the seedlings from cold temperatures, and these crops may then be susceptible to aphids that migrate in spring and require insecticide applications to prevent economic damage. Capturing these costs for a business, and indeed an academic project is challenging. However, from an agronomic perspective, Huntapac will often use representative values to assist with production. Given these differing costs, and the challenges in ensuring year-round crop production, the price that carrots are sold for varies. Although, typically contracts with retailers will be set a representative value throughout the year. Predictably, the price at which carrots are sold to retailers is confidential. However, it is possible to access Defra statistics about the cost of 1kg of washed, topped carrots throughout the year. For 2018, the mean price of carrots sold at four markets around the UK was £0.48/kg in 2019, £0.45/kg and in 2020, this rose to £0.52/kg (Department of Environment Food and Rural Affairs, 2021).

Currently, both *C. aegopodii* and *P. rosae* are controlled by foliar applications of plant protection products. *P. rosae* control is reportedly good, although there are some industry concerns regarding resistance. Control relies on pyrethroids: lambda-cyhalothrin and deltamethrin (Collier et al., 2016). Whilst lambda-cyhalothrin is used for *C. aegopodii* control; there is reported resistance within the aphid (Foster, 2018). In an attempt to optimise the timings of these applications, pest forecasting and monitoring is run from the Wellesbourne Campus of the University of Warwick (Collier, 2017).

Whilst, not the most expensive horticultural product, carrots still present a valuable opportunity to study the efficacy of CBC to control insect pests. First, the impact of an individual pest can often be identified on a carrot, and therefore, the efficacy of a control method can be estimated. Second, the high value that crops are sold for may result in growers' willingness to investigate more novel control methods. For instance, organic carrot growers might spend up to £3,000/acre on hand weeding (J. Rolfe, *personal communication*).

3.2.3 Flower Strips in Commercial Crops

Flower strips are cultivated to help increase the delivery of CBC into crops, with variable impacts upon crop yield as well as providing shelter to natural enemies and floral resources for pollinators (Gontijo, 2019, Ganser et al., 2019, Tschumi et al., 2016b, Balzan et al., 2014, Quinn et al., 2017, Meena et al., 2019, Buhk et al., 2018, Amy et al., 2018, Tschumi et al., 2015, Denys and Tscharntke, 2002, Juventia et al., 2021). However, the delivery of services from flower strips is not consistent, with notable variability in the measured impact of strips upon yield (Albrecht et al., 2020). The inconsistency in flower strip performance might reflect the role landscape may have upon supporting ecosystem service delivery (Karp et al., 2018, Tscharntke et al., 2016), whilst others note concerns that flower strips may act use as a reservoir for pests (Ganser et al., 2019). Flower strips typically are under 3m wide, run around the field exterior and will generally run the length of a field (Gontijo, 2019), although there is increasing interest in placing these strips in the field interior. Flower strips are often comprised of perennial plants (Hatt et al., 2019),(Tschumi et al., 2016a). However, annual strips can also be successful, for instance those planted in wheat crops increased the abundance of natural enemies resulting in reduced pest infestation and crop damage (Tschumi et al., 2015), as well as driving increased biological control of aphids in potato crops (Tschumi et al., 2016b).

With the desire to reduce reliance upon insecticides and keeping the increased support for pollinators in mind, this chapter investigates CBC and the potential of flower strips to increase the delivery of pest control into carrots. To assess pest control service delivery, the direct evidence for ecosystem service delivery will be considered through sentinel prey cards, aphid counts and assessments of damaged carrots. Indirect pest control will be considered via broader metrics for carrot yield and quality. This trial will also demonstrate to growers that flower strips can be sown in a fully commercial carrot crop with operational practicalities in mind.

3.2.4 Research questions

Direct Evidence for Ecosystem Service Delivery

Are there measurable increases from sown seed mixes in the direct evidence for pest control delivery to carrots as measured by pest aphid numbers, sentinel prey predation and insect damaged carrots?

Indirect Evidence for Ecosystem Service Delivery

Are there measurable increases from sown seed mixes in the indirect evidence for pest control delivery to carrots as measured by broader carrot quality and yield metrics?

Developing sampling techniques

Can commercial sampling protocols for carrot harvest be repurposed to identify treatment differences between seed mixes and control plots for the delivery of pest control?

3.3 Materials and Methods

3.3.1 Study Site

In 2019, a field experiment was conducted in one commercial, spring-sown carrot field in Shropshire, with the aim of testing the hypothesis that sown seed mixes increase the delivery of pest control into the commercial carrot crop.

The study field was located at 52° 37' 36.588'' N, 2° 18' 53.3052'' W, on sandy soil. The field's previous crop was barley. Two flower strips were sown, each 2m wide, running the length of the field (Figure 3.2). The width of the flower strip was based on machinery widths used commercially. The placement of strips mid- field, rather than along the edge, as a field margin, allowed the crop on both sides of the strip to be sampled. This doubled the potential study area to measure the delivery of pest control whilst minimising the area taken out of commercial production. Given that the immediate landscape feature adjacent to a field margin can influence the natural enemy community (e.g. (Jowett et al., 2019), using in-field strips also helped to minimise and standardise the influence of any surrounding landscape feature on the performance of the seed mix. The first strip was placed 44m from the plough line into the crop (Figure 3.2). This distance was chosen based on machinery width to help minimise any disruption to Huntapac operations. Then another 44m was sown with carrots as normal before the second bed was sown. The composition of the seed mixes sown in this field was standardised with Rothamsted Farm in 2019 in Chapter 2 (Table 3.1). Control plots were sown with carrots (cv. Nairobi) using a commercial drill at a rate of 170 seeds/m^2.

The experimental design included four replicates of five seed mix treatments sown with at least one carrot control per replicate. Each seed mix plot was 30m x 2m. To ensure the strips ran the length of the field, two additional carrot control plots were sown in Strip 2. The allocation of these plots within each replicate was determined via a neighbour balanced design to try to mitigate any 'spill over' effects from one seed mix plot onto another plot. With treatments allocated to location based on the criteria that treatments can't occur at the beginning or end of a margin strip more than once, treatments can't occur directly next to the same treatment (e.g., grass next to grass) and treatments can't occur next to another treatment more than twice (e.g., grass next to cornflower more than twice).

The field was prepared with the standard commercial preparation of subsoiling, ploughing, de-stoning, bed-forming and then drilling. Once the beds had been formed, each plot was marked out and the seed mixes were broadcast by hand on the 24/04/2019. To improve seed-soil contact, encourage germination and ensure seeds were distributed evenly, plots were raked immediately after seed broadcasting. The two beds of carrots immediately adjacent to the flower strip were unsprayed with any pesticide applications (Figure 3.2).

Table 3.1. Sown seed mix composition

Mix Name	Species names	Density per plot (g/m^2)
Apiaceae	Dill – *Anethum graveolens*	0.60
	Coriander – *Coriandrum sativum*	0.73
	Wild carrot – *Daucus carota*	0.50
Phacelia	Coriander – *Coriandrum sativum*	0.73
	Phacelia tanacetifolia	0.98
	Mustard – *Sinapsis alba*	2.05
Cornflower	Field poppy – *Papaver rhoeas*	0.15
	Cornflower – *Centaurea cyanus*	0.13
	Crimson clover – *Trifolium incarnatum*	1.67
Buckwheat	Buckwheat – *Fagopyrum esculentum*	4.50
	Corn chamomile – *Anthemis arvensis*	0.05
	Corn cockle – *Agrostemma githago*	0.40
Grass	Westerwold ryegrass – *Lolium multiflorum* west.	3.03
	Italian ryegrass – *Lolium multiforum*	2.82
	White millet – *Panicum miliaceu*	3.35

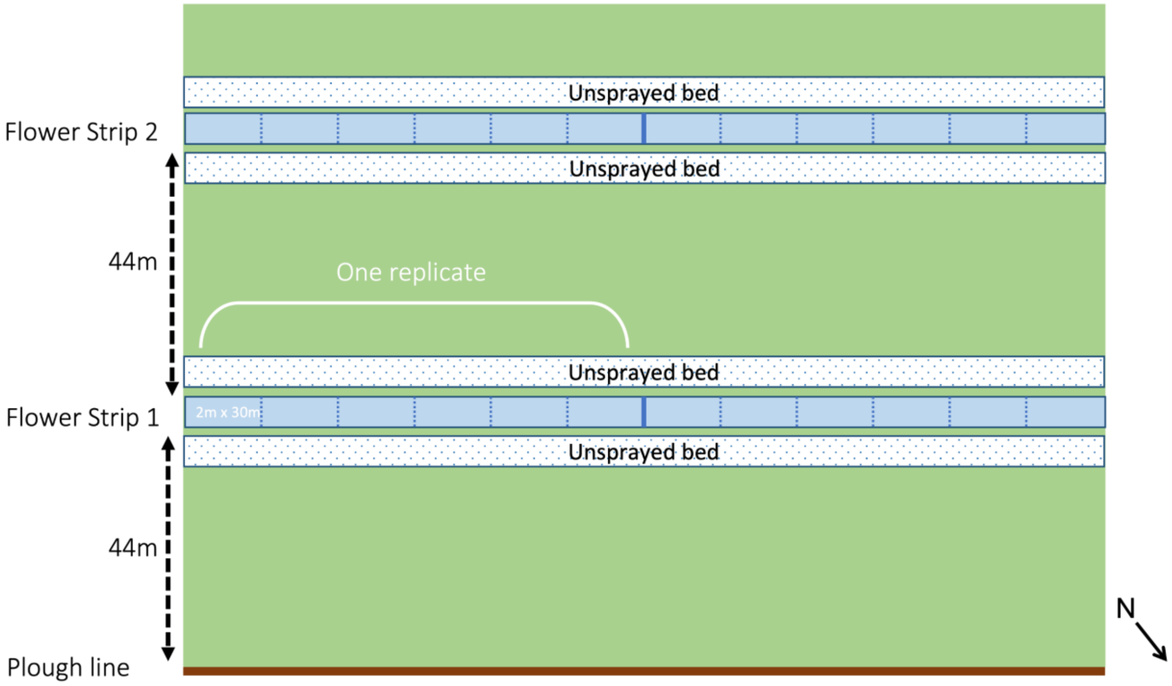

Figure 3.2 Field experiment layout.

Figure 3.3. Images of the 2019 study site. **A** – Close up of one flower strip, showing the different treatments along the length of the strip and with the two carrot beds immediately adjacent to the plots which were unsprayed and sampled at harvest. **B** – Drone image of both flower strips in the study field, the hedge used as the plough line is also marked. **C** – Satellite image of the study field, placement of the two flower strips shown in green; the surrounding ~1km area around the field site is visible, and comprised rrelatively simple landscape immediately encompassing the field.

3.3.2 Sampling

3.3.2.1 Flower Strip Vegetation

A visual assessment of the flower strip's vegetation was conducted on 26/06/2019. In each plot, four 0.25m^2 quadrats were sampled with each chosen so that the vegetation sampled was representative of the entire margin plot. All plant species present were identified to species level (Rose et al., 2006). The cover of each species along with any bare ground present was then scored using the Domin scale (Kent, 2011) (Table 3.2). After assessing the area, if vegetation overlapped, the plant foliage was moved aside so that any species below could be visually assessed. Consequently, the total Domin score for plots can exceed 100% vegetation coverage. In the grass mix, it was not possible to distinguish between the two rye-grass species so their Domin scores' have been summed.

Table 3.12. Domin score and the corresponding percentage cover for each score.

Cover	Domin Score
91–100%	10
76–90%	9
51–75%	8
34–50%	7
26–33%	6
11–25%	5
4–10%	4
<4% (many individuals)	3
<4% (several individuals)	2
<4% (few individuals)	1

When this vegetation survey was conducted, it became apparent that the flower strips had received an application of herbicide with burnt leaf tips apparent. On the 14/06/2019 the flower strips had accidentally been sprayed with Defy (active ingredient: prosulfocarb) and Frizbee (active ingredient: metribuzin). To assess the impact of these products upon the plots, an herbicide impact score was created, and each plot was assessed (Table 3.13).

Table 3.13. Herbicide impact scores to estimate effects of accidental herbicide application on plots of different sown seed mixtures.

Impact Score	Herbicide tolerance
0	No vegetation
1	Sparse, mostly dead
2	Frequent dead plants
3	Mostly alive with frequent dead plants
4	Some damage but majority of plants healthy
5	Largely unaffected healthy plot

3.3.2.2 Aphid Abundance Counts

A 0.1m^2 area of carrot foliage was destructively sampled by cutting the foliage from the bed and placing leaves into a sealed plastic bag. Samples were stored, as soon as possible after sampling, at -20°c thus stopping any clonal reproduction of aphids. To count and identify any insects present on the carrot foliage, samples were placed into water and all insects present were picked out and stored in 95% ethanol before identification. Samples were taken on 14/06/2019, 20/06/2019 and 03/07/2019.

4.3.2.3 Sentinel Aphid Cards

To assess any difference in pest predation pressure between seed mixes, sentinel aphid cards were used. Aphid cards comprised five adult aphids glued to thin, white plastic plant labels. The strips were sprayed with adhesive (3M SprayMount) and five adult *Cavariella aegopodii* were transferred alive to the centre of the plastic strips using a fine paintbrush. The impact of any chemical volatile compounds upon predators cannot be ruled out (Birkhofer et al., 2017). Aphid cards were placed out in the field as soon as possible after production, always on the same day. Cards were placed on the soil surface with aphids facing up and were secured with a metal pin.

Adjacent to each flower plot, one aphid card was placed directly onto the soil surface 1m away from the strip into the neighbouring carrot bed on the southern side of the field. Cards were set out on 04/09/2019 and collected after 24 hours, with any remaining aphids were counted. Weather during this period was poor with significant rainfall.

3.3.2.4 Crop Harvest

Carrots were sampled to identify any difference in yield and quality when grown adjacent to the different seed mix treatments. Within the central 10m of each plot within the strip, four 0.5m^2 areas were dug, in total therefore 2m^2 was harvested next to each plot. The foliage was removed from these carrots, and they were washed to remove excess soil. Carrots were then graded to commercial specifications, with each carrot assessed to see if it had any defect which would render it unsellable; if so the cause of the defect was recorded (AHDB, 2015) & R. Gallimore, *personal communication*). Carrots that did not possess any visual damage were then sorted based on their crown diameter into four categories, under 20mm, 20-35mm, 35-45mm and over 45m. Carrots under 20mm and over 45mm are unsellable due to their size. After grading, each sample of washed, topped carrots was weighed. Carrots were stored at 4 degrees for, at most, 3 days prior to grading. A subsample of approximately 30% of each sample was analysed further, with each carrot's mass, crown diameter and length recorded.

3.3.3 Derivation of harvest variables and basic economic impacts

Following the grading assessment at harvest, the percentage of each sample that contained sellable carrots, the 'pack out', was calculated as per Equation 1. This is a metric of quality and reflects the number of carrots that could be packed out into supermarket retailer bags.

$$\text{percentage sellable carrots} = \frac{number\ of\ sellable\ carrots}{total\ number\ of\ carrots} \times 100 \tag{1}$$

Using the percentage of sellable carrots multiplied with the gross yield figure taken from the mass of washed, topped carrots, it is then possible to calculate the net yield of carrots. Net yield takes both the yield and quality of the sample into consideration (Equation 2).

$$net\ yield\ (t/ha) = gross\ yield\ (t/ha) \times\ \%\ sellable\ carrots \tag{2}$$

Retailer contracts for carrot price vary between supermarkets, depend on the product line and change seasonally and with demand. However, for ease a representative figure of 40p/kg of washed, topped (carrots with the green tops removed) carrots has been used in this analysis. Given this value, the income or turnover (£/ha) from carrots can be calculated (Equation 3).

$$turnover\ (£/ha) = net\ yield\ (t/ha) \times\ 400(£/tonne) \tag{3}$$

Mean carrot mass is a metric that may reflect that there are effects of abiotic and biotic stress that do not result in visual cosmetic damage to a carrot, but that do result in a lower mass of carrot. This has been calculated for all carrots sampled.

$$mean\ carrot\ mass = \frac{sample\ mass}{total\ number\ of\ carrots} \tag{4}$$

Given this focus on conservation biological control and the focus on aphid control, it is also possible to identify carrots with symptoms of virus damage, characterised by splitting of the root and necrosis at the root tip (Figure 3.1). These carrots cannot be sold due to supermarket quality requirements. The identification of these virus damaged carrots can be used to calculate the percentage of virus damaged carrots (Equation 5).

$$virus\ carrots\ (\%) = \frac{count\ of\ virus\ damaged\ carrots}{total\ number\ of\ carrots} \times\ 100 \tag{5}$$

Carrot fly larvae also leave characteristic damage on the carrot root, rending it unsellable (Figure 3.1). Together with the count of virus damage carrots, it is possible to calculate the percentage of a sample that has is damaged by insects (Equation 6).

$$\begin{aligned} &insect\ damaged\ carrots\ (idc)\ (\%) \\ &\qquad = \frac{virus\ damaged\ carrots + carrot\ fly\ carrots}{total\ number\ of\ carrots} \times\ 100 \end{aligned} \tag{6}$$

With the total mass of the sample and the percentage of insect damaged carrots, the insect damaged (id) mass can be calculated (Equation 7).

$$id\ mass\ (t/ha) = insect\ damaged\ carrots\ \% \times\ gross\ yield\ (t/ha) \tag{7}$$

Using the percentage of insect damaged carrots and the gross yield of a sample, it is possible to calculate the mass of insect damaged carrots. Then using the representative price of £400/tonne, it is possible to calculate the income lost from these insect damaged carrots had they not have incurred damage (Equation 8).

$$idc\ income\ lost\ (£/ha)\ = idc\ mass\ (t/ha)\ \times\ 400\ (£/\text{tonne}) \tag{8}$$

3.3.4 Statistical Analyses

3.3.4.1 Aphid Abundance Counts and Sentinel Aphid Cards

To test for the effect of seed mix treatment on aphid abundance linear mixed effects models (LMMs) were fitted to the total number of aphids, the count of both *C. aegopodii* and *M. persicae* aphids identified, and the total number of natural enemies, using the *lme4* package (Bates et al., 2011), *lmertest* (Kuznetsova et al., 2015), and R version 1.1.456 (R Core Team, 2020). The fixed effects were specified as seed mix treatment and random effects as replicate crossed by aspect nested within strip. All three response variables were log(x+0.5) transformed after the residual plots were checked to ensure each response variable met to the assumptions of LMMs.

This was repeated for sentinel aphid predation data i.e., on the number of aphids remaining on cards after 24 hours, with the random effect structure as replicate nested within strip. The residual plot was assessed to ensure the response variable met to the assumptions of LMMs and a transformation was not required.

3.3.4.2 Grower Relevant Harvest Metrics

To test for the effect of seed mix upon on twelve harvest response variables, LMMs were created as above. The fixed effect specified was seed mix treatment and the random effects as replicate crossed by aspect nested within strip. Residual plots were assessed to ensure each response variable met to the assumptions of LMMs and the response variables which required transformations are detailed in Table 3.17.

To identify if there were any treatment effects due to seed mixes in the distribution of carrots based on their crown diameter, a Chi-squared test was performed using R version 1.1.456 (R Core Team, 2020). The evenness of the distribution of carrots in key crown diameter size categories (under 20mm, between 20-35mm, 35-45mm and over 45mm) was analysed for effects due to adjacent seed mix. To account for the unequal number of samples given the increased number of control treatments, the observed carrot counts for each seed mix in each size category divided by the number of plots. The carrots in these four size categories did not contain any defects.

3.3.4.2 Harvest Sub-sample Carrot Continuous Measurements

In the sub-sampled carrots, to investigate the effect of seed mix on continuous variables, crown diameter, carrot mass and length, LMM of carrots in adjacent beds were analysed as above. The fixed effect structure was seed mix and the random effects were specified as plot nested within replicate crossed by aspect nested within strip. Plot was added to account for pseudo-replication following the collection of multiple measurements in each sample.

As above, the evenness of the distribution between seed mixes of carrots between crown size categories were analysed using a Chi-squared test. Due to the low number of carrots in the over 45mm crown diameter category, this was summed with the 35-45mm category, creating an over 35mm category. Carrots were assigned to these three size categories based on their measured crown diameter, and as such, the carrots in these three size categories contained carrots that had defects.

3.3 Results

3.3.1 Flower Strip Vegetation

The impact of the herbicides upon each plot is shown in Table 3.14. The seed mix that appeared to be most affected by the herbicide application were the Buckwheat plots. The Grass mix tolerated the application well, as did the Cornflower mix although there was some variability within the Apiaceae mix and the Phacelia mix. Despite this accidental application, the plots, in particular the Cornflower and Apiaceae mix rebounded well.

Table 3.14. Impact of herbicides upon flower strip plots.

Seed mix	Strip	Replicate	Impact Score	Herbicide Impact
Apiaceae	2	A	4	Some damage but majority of plants healthy
Apiaceae	2	B	5	Largely unaffected healthy plot
Apiaceae	1	D	1	Mostly dead
Apiaceae	1	D	4	Some damage but majority of plants healthy
Buckwheat	2	A	2	Frequent dead plants
Buckwheat	2	B	2	Frequent dead plants
Buckwheat	1	C	3	Mostly alive with frequent dead plants
Buckwheat	1	D	2	Frequent dead plants
Cornflower	2	A	4	Some damage but majority of plants healthy
Cornflower	2	B	4	Some damage but majority of plants healthy
Cornflower	1	C	5	Largely unaffected healthy plot
Cornflower	1	D	3	Mostly alive with frequent dead plants
Grass	2	A	4	Some damage but majority of plants healthy
Grass	2	B	4	Some damage but majority of plants healthy
Grass	1	C	4	Some damage but majority of plants healthy
Grass	1	D	4	Some damage but majority of plants healthy
Phacelia	2	A	3	Mostly alive with frequent dead plants
Phacelia	2	B	3	Mostly alive with frequent dead plants
Phacelia	1	C	2	Frequent dead plants
Phacelia	1	D	3	Mostly alive with frequent dead plants
Control	2	A	-	
Control	2	A	-	
Control	2	B	-	
Control	2	B	-	
Control	1	C	-	
Control	1	D	-	

3.3.2 Aphid Abundance Counts and Sentinel Aphid Predation

Of the 91 samples of carrot foliage taken on the three sampling dates, 14/06/2019, 20/06/2019 and 03/07/2019, 412 aphids were identified in total. Of these, 264 aphids were *Cavariella aegopodii* or *Myzus persicae*, both known virus vectors to carrots. Alongside these, 32 different natural enemies were recorded. There was no significant effect of seed mix on alate aphid abundance, pest aphid abundance or NE abundance (Table 3.15). Seed mix did not have a significant effect on the predation of sentinel aphids ($F_{5,17.0}$=2.12, p=0.113) (Table 3.16).

Table 3.15. Output from LMMs of the effect of seed mixtures on aphid alate, pest aphid and natural enemy (NE) abundance from samples adjacent to flower strips.

Response variable	Transformation	NumDF	DenDF	F value	Pr(>F)	Sig	Average LSD
Alate abundance	Log(x+0.5)	5	69.5	0.96	0.448	NS	0.47
Pest aphid abundance	Log(x+0.5)	5	69.1	1.22	0.311	NS	0.52
NE abundance	Log(x+0.5)	5	70.9	0.83	0.528	NS	0.37

Table 3.16. Mean number of aphids remaining on sentinel cards on cards in the carrot crop at 1m from the flower strip.

Seed Mix	Mean remaining aphids	Standard Deviation
Apiaceae	0.50	0.58
Buckwheat	0.50	0.58
Control	1.20	1.64
Cornflower	1.33	1.15
Grass	1.50	1.29
Phacelia	2.75	2.22

3.3.3 Grower Relevant Crop Harvest Metrics

Table 3.17 shows the varying effect of seed mixes on crop metrics following harvest. Whilst the majority of the analysed harvest metrics were not significantly affected by seed mix, the percentage of sellable carrots was significantly different between treatments (Figure 3.4). The percentage of sellable carrots, a measure of quality, shows that the Grass, Apiaceae and Buckwheat mixes have significantly higher quality carrots in the beds adjacent to the strips, compared with the Control, Phacelia and Cornflower plots ($F_{5,42.3}=4.95$, $p<0.01$). Given the lack of significant differences between metrics such as percentage insect damage, it appears that the sown mixes are not measurably improving pest control and thus driving differences in carrot quality (Table 3.17). However, it does appear that percentage of cavity spot damaged carrots is responsible for these quality differences (Figure 3.5). Here, significantly more cavity spot damage was recorded in Control, Phacelia and Cornflower plots than other mixes ($F_{5,43.8}=2.47$, $p<0.05$).

Table 3.17. Output from LMM of the effect of seed mix upon various carrot harvest metrics following harvest. Significant results highlighted in bold, with significance levels indicated by: ** = p<0.01, * = p<0.05, "." = p<0.1.

Harvest metric	Transformation	NumDF	DenDF	F value	Pr(>F)	Sig	Average LSD
Total carrots	-	5	43.1	0.91	0.481	NS	23.7
Mean carrot mass (g)	-	5	42.0	2.43	0.051	.	10.0
Gross yield (t/ha)	-	5	38.6	2.00	0.100	NS	9.52
Percentage of sellable carrots	**-**	**5**	**42.3**	**4.95**	**0.001**	**	**0.11**
Net yield (t/ha)	-	5	42.8	1.91	0.113	NS	12.9
Turnover (£/ha)	-	5	42.8	1.91	0.113	NS	5168
Percentage of cavity spot	**log(x+0.2)**	**5**	**43.8**	**2.47**	**0.047**	*	**1.16**
Percentage of virus	-	5	43.5	1.47	0.220	NS	1.00
Percentage of insect damaged carrots	log(x+0.6)	5	44.1	0.89	0.494	NS	0.57
Mass of insect damaged carrots (t/ha)	log(x+0.5)	5	42.9	1.16	0.344	NS	0.58
Income lost from insect damaged carrots (£/ha)	log(x+200)	5	42.9	1.16	0.344	NS	0.58

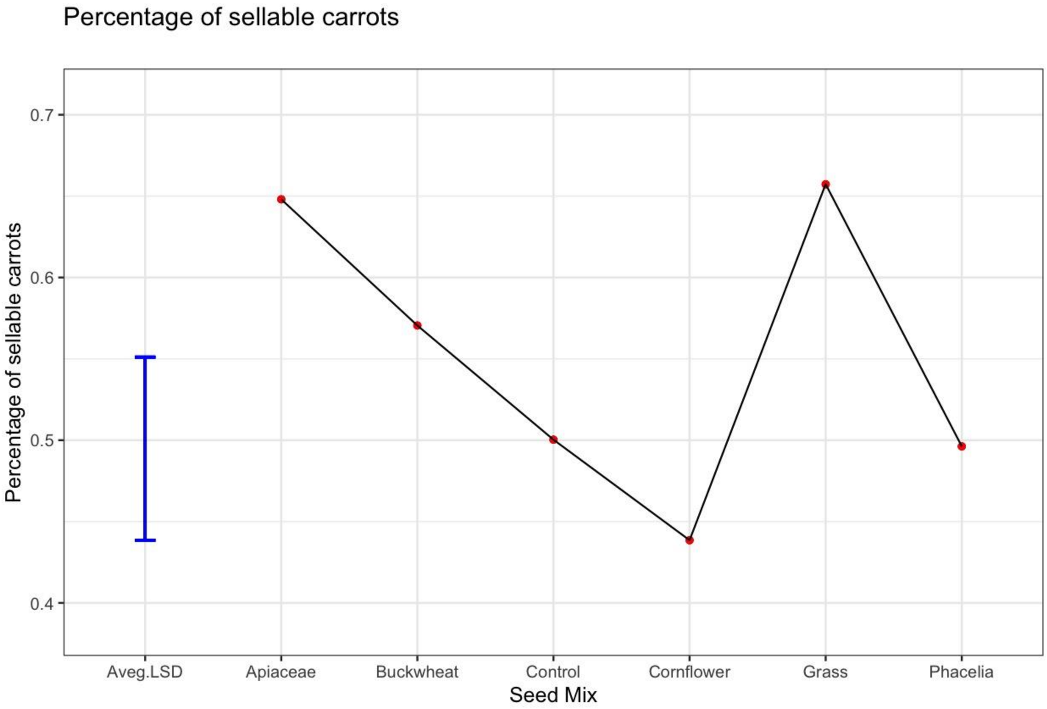

Figure 3.4. Predicted means for the proportion of sellable carrots sampled next to sown mixes in flower strips, fitted using an LMM. Average LSD=0.11.

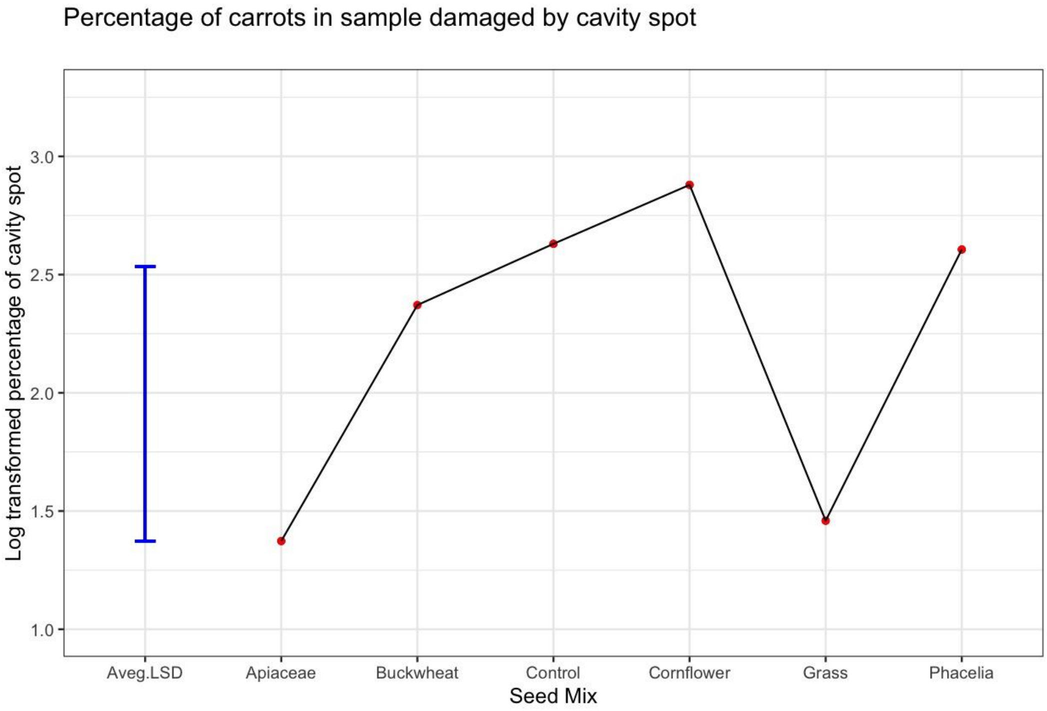

Figure 3.5. Predicted means for the log-transformed percentage of cavity spot damaged carrots sampled next to sown mixes in flower strips, fitted using an LMM. Average LSD=1.16.

3.3.3 Harvest Sub-sample Carrot Continuous Measurements

Across all plots, in the harvest sub-sample 3,940 carrots were measured, weighing 317kg. Seed mix had a significant effect upon carrot crown diameter ($F_{5,39.4}$=3.82, p<0.01), with the crown diameter of the Control and Cornflower treatments significantly bigger than Apiaceae and Grass plots (Figure 3.6). However, seed mix does not significantly affect carrot length or mass between treatments, although carrot mass is marginally significantly different between seed mixes (Table 3.18). The variability of crown diameter, length and mass did not significantly differ between seed mixes (Table 3.18, Figure 3.7, Figure 3.8, Figure 3.9).

Table 3.18. Output from LMM of the effect of seed mix upon various continuous carrot measurements and each measurement's variance. Significant or marginally significant results highlighted in bold, with significance levels indicated by: ** = *p*<0.01 and "." = *p*<0.1.

	NumDF	DenDF	F value	Pr(>F)	Significance	Average LSD
Crown diameter	**5**	**39.4**	**3.82**	**0.006**	**	**1.44**
Diameter variance	5	40.3	1.55	0.197	NS	11.2
Carrot length	5	40.1	0.84	0.529	NS	5.44
Length variance	5	42.1	0.64	0.668	NS	281
Carrot mass	**5**	**39.7**	**2.32**	**0.061**	**.**	**9.86**
Mass variance	5	40.3	1.27	0.295	NS	874

The linear regression of carrot crown diameter against carrot length and then mass reveals a strong positive relationship between these variables for each seed mix (Figure 3.10, Figure 3.11). These correlations reveal that crown diameter is a robust indicator of carrot length and mass, across all seed mixes.

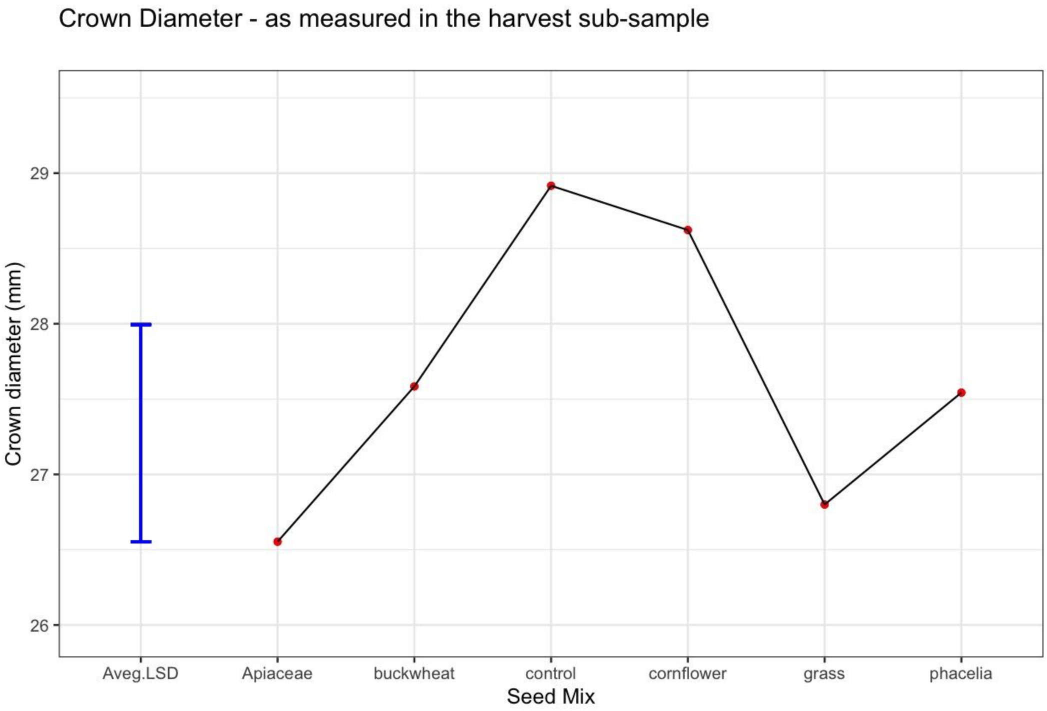

Figure 3.6. Predicted means for the crown diameter of carrots grown in beds adjacent to each seed mix treatments from the sub-sampled continuous measurements from harvested carrots, fitted using an LMM. Average LSD=1.44.

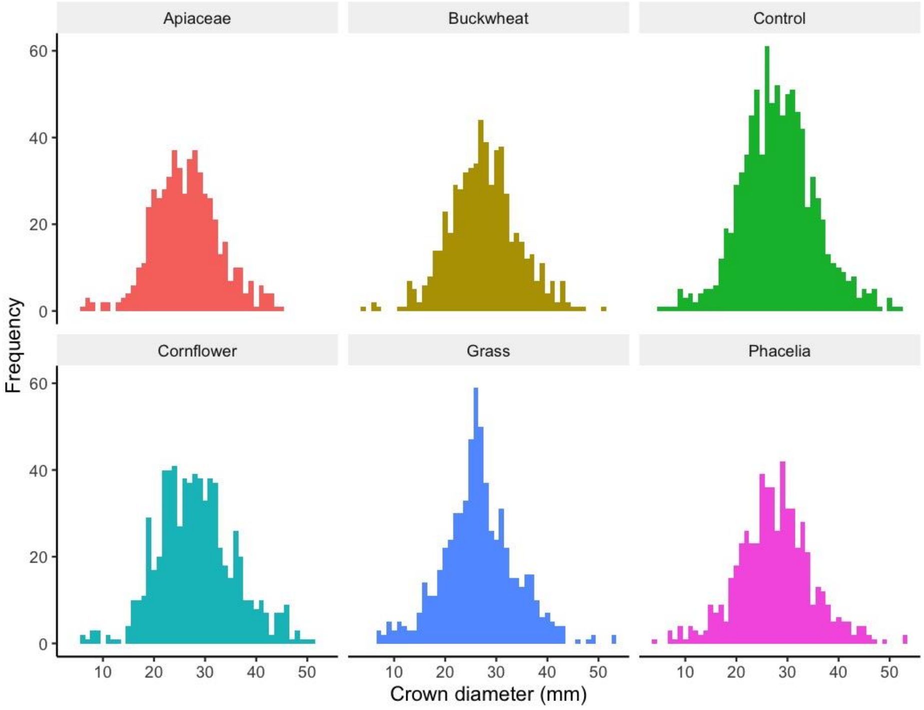

Figure 3.7. Histograms for each seed mix of the frequency distribution of carrot crown diameter from sub-sample plots.

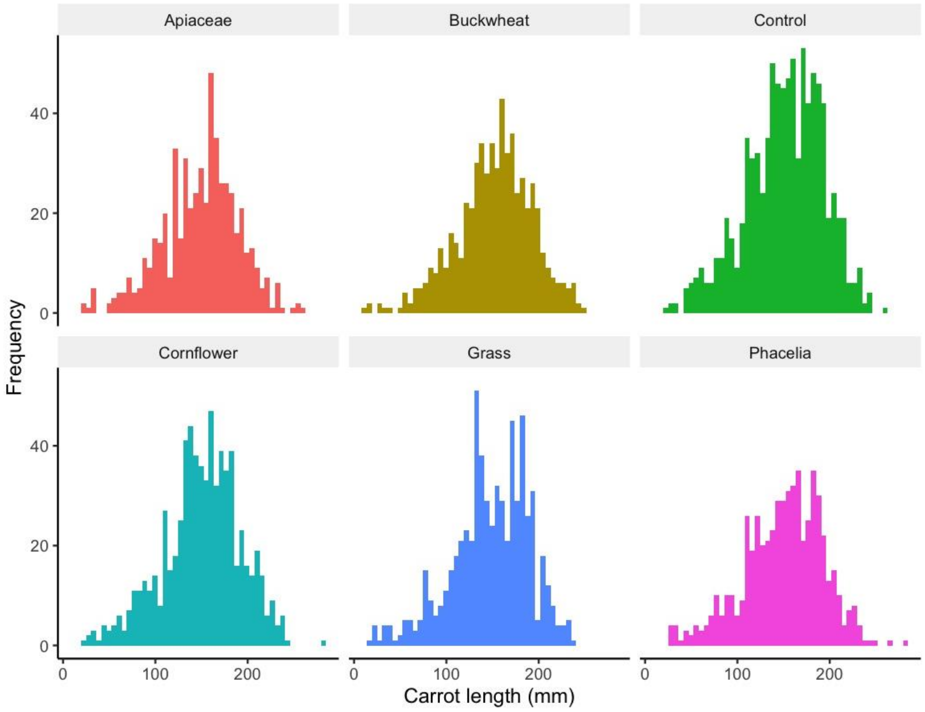

Figure 3.8. Histograms for each seed mix of the frequency distribution of carrot length diameter from sub-sample plots.

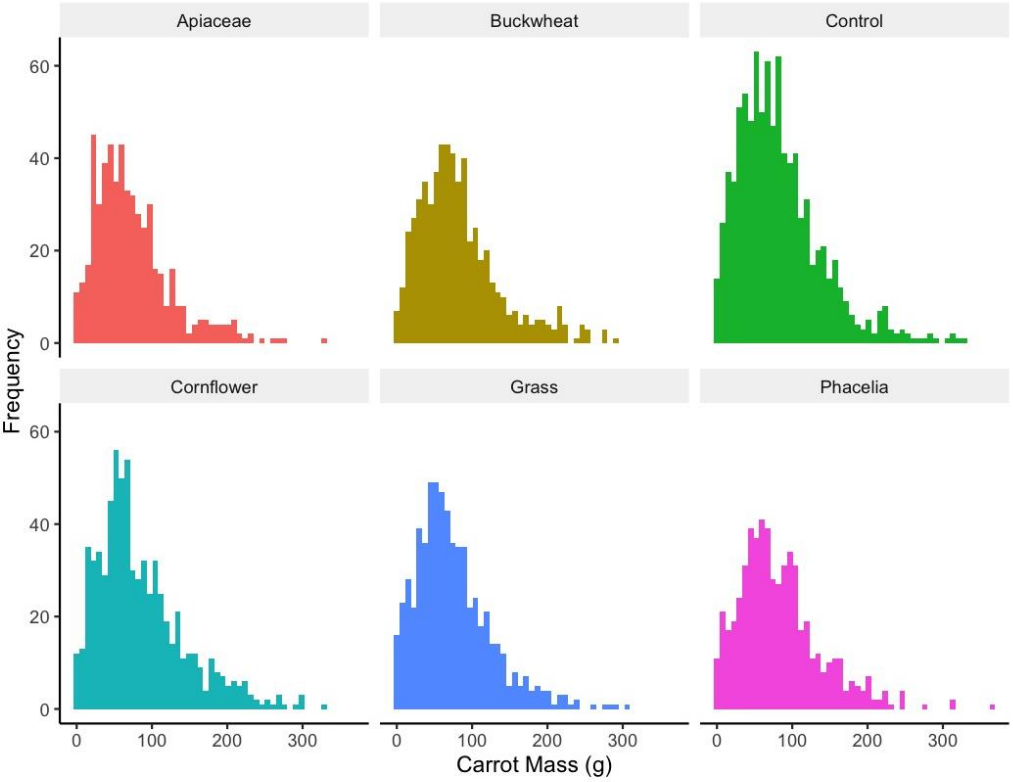

Figure 3.9. Histograms for each seed mix of the frequency distribution of carrot mass diameter from sub-sample plots.

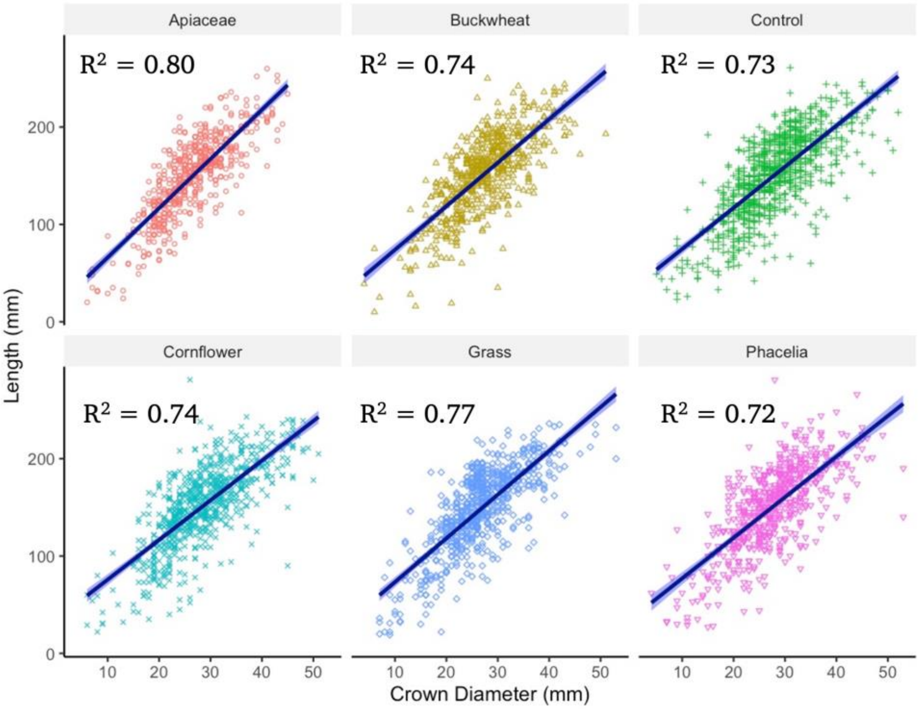

Figure 3.10. Linear regression of crown diameter by carrot length for each seed mix, with blue shaded areas showing the 95% confidence interval around the fitted line.

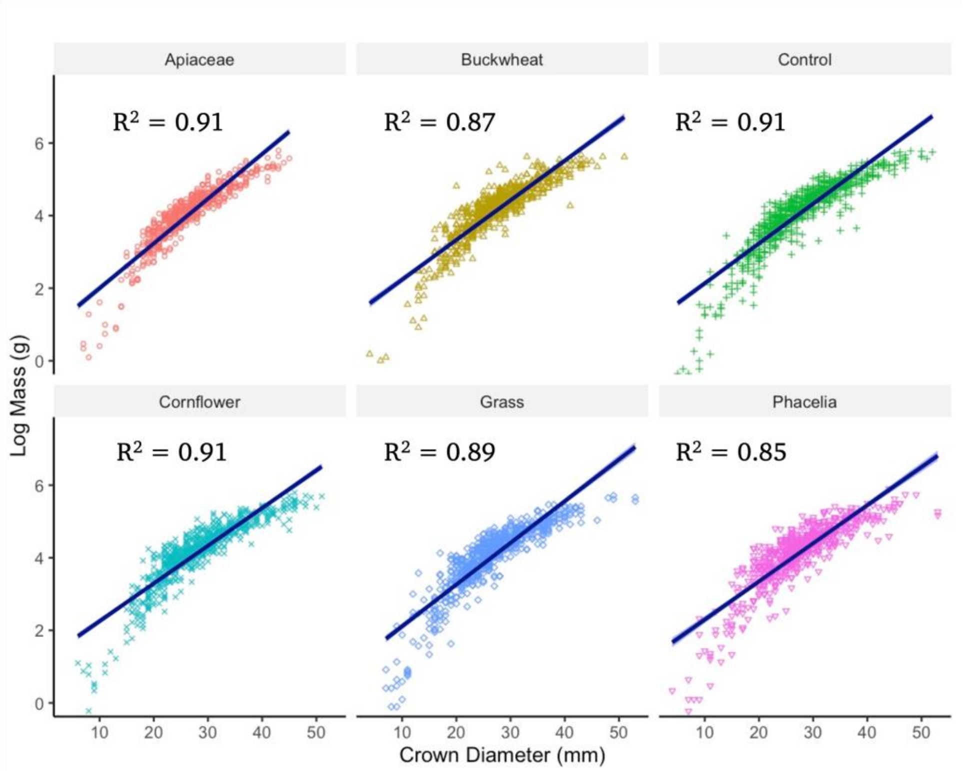

Figure 3.1121. Linear regression of crown diameter by log carrot mass for each seed mix, with blue shaded areas showing the 95% confidence interval around the fitted line.

3.3.4 Comparison Between Graded Carrots and Continuous Measurements

Table 3. shows that next to the Apiaceae and Control plots there were significantly more of the under 20mm sized carrots than other seed mixes and there were much fewer small carrots next to the Phacelia mix. This finding has been drawn from the full data set. However, in the sub-sampled data containing continuous measurements, which were assigned to relevant size categories is considered, there were no significant differences in the number of small carrots between seed mixes (Table 3.19). Similarly, there were significant more carrots between 35-45mm next to the Buckwheat and Grass mixes than the other seen mixes, (Table 3.). This was not found in the sub-sample of carrots (Table 3.19).

Table 3.9. Chi-squared test outcome for the number of carrots in each of the four size categories for each seed mix. Data from whole sample categorised based on size. Significant results highlighted in bold, with significance indicated by: *** = p<0.001, ** = p<0.01, "." = p<0.1.

		Seed Mix						Test result			
	Crown diameter	Apiaceae	Buckwheat	Control	Cornflower	Grass	Phacelia	$\chi 2$	df	*p*-value	Sig.
Observed – expected	under 20mm	32.3	-3.67	38.3	-22.7	-10.7	-33.7	34.7	5	0.000	***
	20-35mm	21.8	-2.33	-2.46	-18.0	10.4	-9.46	10.1	5	0.073	.
	35-45mm	-2.22	11.3	-5.64	-10.0	8.91	-2.35	16.8	5	0.005	**
	Over 45mm	0.50	0.25	-0.75	0.13	0.75	-0.88	1.11	5	0.953	NS

Table 3.19. Chi-squared test outcome for the number of carrots in each of the four size categories for each seed mix. Data from sub-sample measurements which were then put into three relevant crown diameters based on their measured size.

		Seed Mix						Test result			
	Crown diameter	Apiaceae	Buckwheat	Control	Cornflower	Grass	Phacelia	$\chi 2$	df	*p*-value	Sig.
Observed - expected	under 20mm	0.45	-0.99	-1.64	0.07	3.02	-0.88	1.44	5	0.919	NS
	20-35mm	-0.33857	-2.69	0.54	1.59	6.80	-5.91	1.50	5	0.913	NS
	over 35mm	-4	-1.44	2.82	5.75	-1.43	-1.67	5.32	5	0.378	NS

3.4 Discussion

It was not possible to identify direct or indirect evidence for the delivery of pest control in this field from this year. However, as the significant difference in carrot quality observed between seed mixes demonstrates, this harvest protocol can identify when there are statistically significant differences in carrot harvest metrics between plots, albeit driven by cavity spot damage. These metrics have then successfully been advanced here with simple economic metrics, such as turnover, furthering the commercial relevance of the sampling protocol. From a grower perspective, these flower strips can be integrated into their operational requirements despite the unintentional herbicide application. Whilst this herbicide application was not ideal, through this, the commercial resilience of the mixes has been assessed. From a pragmatic perspective, herbicide drift or direct application is ideally avoided, but there will be instances where it does occur. The Apiaceae, Cornflower and Phacelia mixes tolerated the application and rebounded well, suggesting these mixes would fit well into operations. Additionally, fitting the flower strips into the 2m wide beds for carrot production can assist with the commercial practicality of the strips without diminishing ecosystem service delivery (Holland et al., 2008).

From the perspective of direct evidence for pest control, if there are no aphids present, then differences cannot be detected in pest abundance between treatments. A lack of aphids in field experiments is not uncommon (e.g. Bruce et al., 2015) and can be overcome by using sentinel prey cards that allow a standardised pest control assessment between treatments (McHugh et al., 2020). However, drawing conclusions about pest control via sentinel prey predation levels here was severely limited by the lack of temporal replication. Future work must include more replication of this to be effective.

To correctly identify direct evidence of insect pest control, the method used here relies upon accurately identifying the cause of an imperfect carrot. With multiple observers with varying levels of expertise, it can be relatively straightforward to identify when a carrot does not meet standards. However, identifying why the carrot would not be sellable is challenging. While the Carrot Crop Walking Guide is an excellent reference document (AHDB, 2015), carrot viruses complex and additive nature may incorrectly skew these simple assessments of visual damage. Moreover, there is little material accessible for growers to bring them up to date with the implications of more recent virus research.

The control and Apiaceae mixes have more small carrots than other mixes (Table 3.), which may reflect some indirect impact from viruses or other pests or pathogens (Collier et al., 2016). As carrots with a crown diameter under 20mm are too small to be sold to supermarket retailers, a sown seed mix that can reduce the number of small carrots is advantageous. However, to conclude about the efficacy of these seed mixes driving increased pest control of aphids and any subsequent changes in virus levels, further work is required to establish, at a molecular level, virus levels in the foliage next to flower strips. This challenge of correctly identify virus damage strengthens the need to consider the indirect evidence for pest control delivery (Collier et al., 2016). Here, net yield and the percentage of sellable carrots are variables that may act as a proxy for virus damage. These indirect methods could be pursued further in the future, but the high prevalence of cavity spot damage in this trial may restrict their efficacy.

This trial's variable and high presence of cavity spot damage does not have any pronounced or explainable link with the seed mix—instead, previous crop and soil type are drivers of cavity spot infections (Sapkota and Nicolaisen, 2018). Consequently, future trials would benefit from being spread over multiple fields, thereby reducing these soil effects. Moreover, as the beds immediately adjacent to flower strips did not receive any pesticide applications, it is hard to assess the indirect impacts of CBC amid the impact from incidental pests and pathogens. Further work would benefit from an additional focus on how these strips perform alongside high insecticide applications. This would also have implications for growers given the threat and risk that insecticide resistance poses (Foster, 2018), allowing the efficacy of these strips to be evaluated for future aphid control without insecticides.

The continuous measurements derived from the sub-sampled carrots appear to have significant flaws from a method development perspective. First, for some categories, such as carrots between 20-35mm crown diameter with a high abundance within a sample, it is possible to sample 30%. However, if only two carrots have virus symptoms within a sample, it is impossible to measure a 30% subsample. The obvious solution would be to measure each carrot’s crown diameter, mass, and length rather than selecting a sub-sample. However, during the time it would take to measure each of the 11,353 carrots harvested in this small field trial, there is the potential for post-harvest pathogens and rots negatively impacting the harvested sample and subsequent conclusions. Second, recording the cause of any defect on each carrot when measured was trialled; this was prohibitively time-consuming. Finally, whilst others have effectively deployed scoring assessments for disease severity (Sapkota and Nicolaisen, 2018), arguably, this could take time away from increasing a future trial's scale. These flaws likely are the driver behind the inability of the continuous measurements to identify treatment differences that were present when the grower-used sampling protocol did reveal differences between seed mix treatments. Additionally, the strong relationships between crown diameter and carrot length and mass demonstrate it is possible to streamline sampling by considering only crown diameter.

Whilst there was no apparent direct or indirect evidence for the successful delivery of pest control from flower strips, this trial successfully included flower strips into commercial carrot crops. Moreover, whilst applying an herbicide onto the strips was not intentional, it has identified resilient seed mixes that will tolerate commercial conditions to be taken forward. Additionally, the grower developed methods of assessing crop quality and yield have successfully demonstrated that statistically and commercial relevant differences can be detected if present.

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Chapter 4 – Evidence for Ecosystem Service Delivery from flower strips in carrot fields.

4.1 Abstract

Previous chapters have revealed that the seed mixes used in this thesis are associated with different insect communities. Work also demonstrated in-field strips can be successfully included in commercial carrot fields. However, how these strips function on a larger scale, and under different pesticide spray conditions is unknown, as is the commercial impacts of these factors. Similarly, the optimal placement of flower strips for pest control delivery has not been widely addressed. Without this understanding, there may be barriers to the successful deployment of flower strips at scale. This trial therefore included flower strips across multiple fields, with a contrasting pesticide spray regime and novel trial design. The aerial insects sampled at the seed mixes suggest the Cornflower mix effectively supported pest control ecosystem service providers and insect conservation more widely. Assessments via sentinel prey card predation, direct counts of aphid abundance, and insect-damaged carrots did not reveal any direct evidence for increased pest control delivery associated with a particular sown flower mix.

The indirect evidence of ecosystem service delivery from in-field flower strips considered predominantly net yield and found that net carrot yield of samples harvested adjacent to flower strips was significantly higher than control samples. However, the benefit of the flowering strips was only observed at the field edge and on carrots grown in close proximity (1m) to the flowering strips. The inclusion of flower strips at the field interior, over 100m into the field, did not increase net yield of adjacent carrots; indeed, total productivity losses were recorded, and the combined costs of flower strip inclusion were significantly higher. The successful integration of flower strips into carrot cropping systems may be possible and commercially viable but will rely on any 'edge effect' factors, which require further investigation, and which will likely vary across fields and years.

4.2 Introduction

Land managers seek to address a simultaneous ecological and climate emergency whilst feeding a growing population, with increasing and often conflicting demands within a landscape. The proliferation of sustainable agricultural practices relies upon farmer behaviour change, requiring detailed evidence of how efforts can deliver environmental and business outcomes at a scale relevant to growers (Kleijn et al., 2019). This need is further enhanced in horticultural crops, where it is critical to quantify the economic consequences of a change in management due to tight financial margins.

Within the field of Conservation Biological Control (CBC), there is a well-documented and longstanding failure to address the impacts of CBC on ecosystem service delivery (ESD), crop yield and further socio-economic factors (Griffiths et al., 2008). Thirteen years ago, Griffiths et al. posited that the costs resulting from CBC efforts are "difficult to characterize and quantify" (p.205, 2008); however, perhaps an increasing awareness and capture of data within farming businesses has made this economic data collection simpler. More recently, Johnson et al.'s review highlighted that only one-third of CBC papers reported on yield and only six papers out of a pool of 150 reported on economic factors (2021). Of these six papers considering economics, unsurprisingly, none documented CBC in carrots. From the six attempts at measuring the economic benefits of sustainable intensification, predictably, there was a mixed picture of results with no consistent yield or economic benefit across different countries, crops, pests and CBC methods (Aguilar-Fenollosa et al., 2011, Belay and Foster, 2010, Bowers et al., 2020, Gurr et al., 2016, Horgan et al., 2017, Wan et al., 2018).

Despite this variability in success, capturing economic data is essential as a traditional perspective argues that if there is no positive impact on a farm business's profits, implementation in a field is unlikely (Hayden et al., 2021, Naranjo et al., 2015). More recently, the challenges farmers face has expanded to include the holistic 'bundles' of challenges that solutions must address (Barrett et al., 2020). These challenges involve considering an effort's impact upon the environment, food production, a business's financial perspective and broader socio-economic considerations.

For optimal outcomes for nature, land must be actively managed, which can come at a monetary cost. There are multiple routes to finance these endeavours, with varying degrees of success. Historically, within European agriculture, Agri-Environment Schemes (AES) have been deployed. AES efforts were born out of the need to help address environmental degradation and species declines following the post-war intensification of farming and help address the failure of financial markets to capture environmental benefits (Clements et al., 2021). There have been multiple iterations of AES within the UK, with both the original and new Countryside Stewardship schemes prominent examples (Clements et al., 2021). The success of these schemes can be debated, and the resulting environmental outcomes are mixed (Albrecht et al., 2007, Alison et al., 2017, Batary et al., 2015, Kleijn et al., 2006, Mason et al., 2019). Drivers of uptake of these AES are naturally diverse and include economic factors such as farm diversification, reliance upon family for labour, farm size and farmer experience in AES (Lastra-Bravo et al., 2015). However, AES's notable criticisms include the prescriptive nature of scheme requirements (Arnott et al., 2019). AES sits under the broader umbrella of ecological economics, alongside concepts like Payment for Ecosystem Services (PES) and Markets for Ecosystem Services (MES), which also seek to economically value environmental externalities to incentivise their protection (Gomez-Baggethun et al., 2010). PES provides a route whereby the costs felt or perceived by land managers can be overcome to encourage the adoption of desirable behaviours and result in positive environmental outcomes (Bell et al., 2018). An MES is commonly used in climate mitigation activities and the valuation of carbon credits can influence sustainable land management outcomes (Warren-Thomas et al., 2018, Gomez-Baggethun et al., 2010). With the growing world of so-called ‘green finance’ and consumer awareness, the area of ecological economics is likely to receive increasing focus.

Beyond these well-established traditional perspectives, private incentivisation is receiving growing attention. At the landscape level, McHugh *et al*. (2020) have demonstrated that cereal aphid infestation reduces with the increasing proportion of semi-natural habitat in 1km radii around sites. These findings may not be ubiquitous across landscapes (Karp et al., 2018). Nevertheless, they supplement additional work demonstrating that habitat creation can maintain and even increase yields will no loss to total productivity on a 900-hectare commercial farm (Pywell et al., 2015). Therefore, it is thought that through these sustainable intensification mechanisms, growers may be able to take land out of production and increase yields and potentially even profits. However, it is equally important to document when these wildlife-friendly farming practices such as habitat creation and increasing rotation length do not increase yield as this can allow the trade-offs between these services to be quantified (Field et al., 2016). Given the logistical challenges of these studies, it may be attractive only to consider yield implications. However, farmers are 'system thinkers' and must consider the holistic 'bundles' of challenges that solutions must address (Barrett et al., 2020). Therefore, this chapter will assess whether the inclusion of flower strips can positively support ESPs, measurably increase ESD, and quantify the economic and productivity implications of their inclusion. How these factors are influenced by the pesticide spray regime can also be considered.

4.2.1 Research Questions

Ecosystem service providers

Do sown flower strips increase functional ESPs compared with controls? Do sown flower strips support insect conservation compared with controls? Are there any differences in community assemblage between sown seed mixes?

Evidence for direct ecosystem service delivery from flower strips

Do sown flower strips increase ESD in the field? Specifically, do foliage virus diversity and abundance, aphid abundance, sentinel prey predation, and in-field harvest metrics differ between flowering seed mixes and controls. How do these factors vary with increasing distance into the crop and how does a contrasting pesticide spray regime influence pest control delivery?

Evidence for indirect ecosystem service delivery from flower strips

How does a suite of carrot harvest metrics perform for each seed mix with increasing distance into the field and under different spray conditions? Do these metrics reveal associations between flower strips and increased carrot yield or quality?

Derived economic metrics from harvest

Do the economic impacts from key harvest metrics, turnover and income lost from insect damaged carrots, vary for seed mixes? Are there differences between flowering seed mixes and carrot controls?

Commercial implications of flower strips

What are the impacts from flower strips upon economic costs, greenhouse gas emissions and total productivity between flowering mixes? How do flower strips compare with 'business as usual'?

4.3 Materials and Methods

4.3.1 Study Site and Experiment Design

In 2020, a field experiment was conducted in four commercial, spring-sown carrot fields in Shropshire on sandy soil, testing the efficacy of the sown flowering seed mixes, sown as in-field strips, at increasing the delivery of pest control into the carrot crop. Due to the constraints placed upon experimental design by commercial fields, it was not possible to standardise the placement of strips along a compass direction, although the four fields did have a generally East-West strip direction.

To fit with operational and machinery requirements, mixes were sown after the preparation of 2m beds for carrot drilling. Following preparation, seed mixes were hand broadcast. Following broadcasting, plots were hand raked to ensure adequate coverage of the plots as well as good seed-soil contact to encourage germination. The first two fields were sown on the 17/04/2020, the third on the 22/05/2020 and the final field on 07/05/2020. The four fields used were located at, in order of drilling date, 52° 35' 31.6428'' N, 2° 22' 33.9852'' W; 52° 35' 25.3536'' N, 2° 22' 28.2684'' W; 52° 39' 42.3936'' N, 2° 20' 2.1372'' W; 52° 37' 52.7808'' N, 2° 19' 22.8216'' W, with each field containing one flower strip and two replicates. In total there were eight replicates, each containing three seed mix plots plus a carrot control (Table 2.1), with plot dimensions of 35m x 2m. The lengths of the fields necessitated the use of ‘carrot buffers’ in the field centre where the field length was longer than the total distance of the experimental treatments (Figure 4.2).

The trial was designed to investigate how ESD changes with increasing distance into the field, thereby allowing the inclusion of distance from the field edge as a fixed effect, therefore seed mixes were allocated to plots such that each seed mix would occur in each distance into the field twice. However, during the set-up of the trial there was an error in drilling the location of one control plot and therefore the trial design is not balanced. To investigate how ESD varies with a sprayed/unsprayed contrast, on either side of the flower strip the three beds immediately adjacent were designated either ‘sprayed’ or ‘unsprayed’. The sprayed side received a full commercial spray regime, as did the rest of the field. On the unsprayed side, it was requested that no pesticide applications were made. Ideally, the side of the mix which would have received sprays would have been randomly allocated to each plot, however, due to the error regarding previous herbicide applications to the strips (Chapter 3), it was pragmatic to make a small concession, so the unsprayed side ran along the same length of each strip. Unsprayed plots also did not receive fungicide or herbicide applications. Ideally these would have been made, however, as tank mixes are common in carrots, it was practical to reduce confusion and restrict all pesticide applications.

Figure 4.1. Locations of four fields sites in 2020 and location of the 2019 trial site. 2020 sites outlined blue, 2019 sites outlined in purple.

Table 4.20. Composition of seed mixes planted carrot fields in Shropshire in 2020.

Mix Name	Species names	Amount per plot (g/m^2)
	Dill – Anethum graveolens	0.7
Apiaceae	Coriander – Coriandrum sativum	0.85
	Wild carrot – *Daucus carota*	0.58
	Coriander – Coriandrum sativum	1.15
Phacelia	Phacelia tanacetifolia	-
	Mustard – Sinapsis alba	0.18
	Field poppy – *Papaver rhoeas*	0.15
Cornflower	Cornflower - Centaurea cyanus	1.93
	Crimson clover – Trifolium incarnatum	1.67

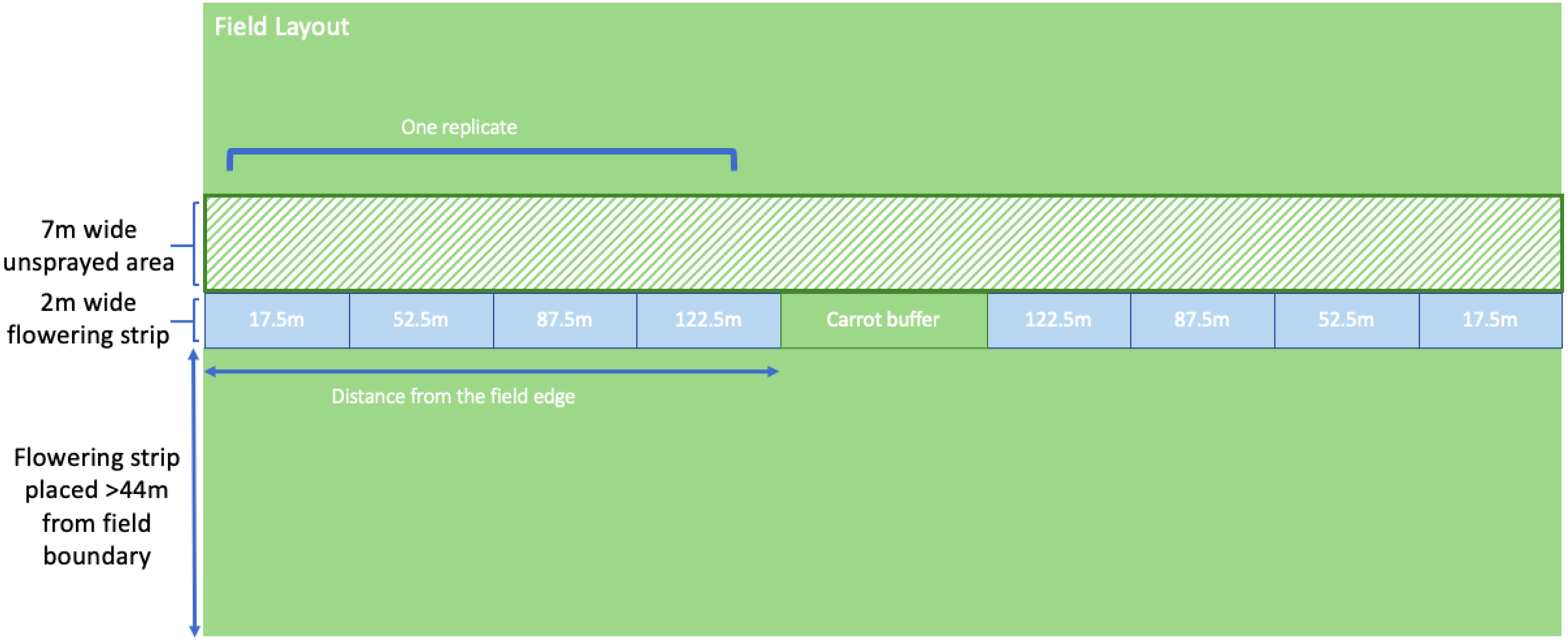

Figure 4.222. Layout of carrot field 2020 plot design, showing two replicates in each field.

4.3.2 Sampling

4.3.2.1 Yellow Water Traps

Yellow water traps were used as per the protocol laid out in Chapter 2. In this trial, yellow water traps were placed in the centre of each plot, and traps ran from 21/06/2020 to 26/06/2020 and then 04/08/2020 to 06/08/2020. Only the second time point has been included here due to time demands.

4.3.2.2 Aphid Abundance Counts and Plant Virus Levels

To assess aphid abundance and identify pest differences between seed mixes, a $0.1m^2$ area of carrot foliage was destructively sampled as previously described in Chapter 3. Here, carrot foliage samples were taken at 1m, 3m and 7m away from the flower strips on 3/7/2020 and 6/8/2020. Following removal of any insects present, foliage was then removed from the water, allowed to drain and returned to -20°c as soon as possible. Samples were sent to collaborators at the University of Newcastle and FERA, thereby allowing further work to identify viral RNA present in plant samples to be identified. This would allow a molecular understanding of whether any seed mix impacts the aphid-vectored virus. Unfortunately, due to delays resulting from Covid-19 restrictions, timely access to laboratories has not been possible and these samples have not yet been fully analysed.

4.3.2.2 Sentinel Aphid Cards

As in previous years, sentinel aphid cards were used to identify differences in predation potential next to flower strips. Cards comprised five adult *Cavariella aegopodii* aphids glued to thin, white plastic plant labels. The strips were sprayed with adhesive (3M SprayMount) and aphids were transferred alive to the centre of the plastic strips using a fine paintbrush. Aphid cards were placed out in the field later that day. In each plot, one card was placed on the soil surface with aphids facing up and were secured with a metal pin.

At each plot, one aphid card was placed at 1m away and 7m away from the flower strips, on both sprayed and unsprayed sides of the strips. Cards were set on the 12/08/2020 and collected after 24 hours, with any remaining aphids counted.

4.3.2.3 Crop harvest

To identify in-field ESD delivery and identify any differences due to the seed mixes, the central 10m of each seed mix plot within the strip, four 0.5m^2 areas were dug (Figure 4.3). In total 1m^2 was harvested next to each plot. The foliage was removed from these carrots, and they were washed to remove excess soil. Carrots were then graded to commercial specifications, with each carrot assessed to see if it had any defect which would render it unsellable, if so the cause of the defect was recorded (AHDB, 2015, R. Gallimore, personal communication). Carrots that did not possess any visual damage were then sorted based on their crown diameter into four categories, under 20mm, 20-35mm, 35-45mm and over 45m, using the same protocol as Chapter 3. After grading, each sample of washed, topped carrots was weighed. Carrots were stored at 4 degrees for, at most, 3 days prior to grading. Samples were taken at 1m and 7m away from the flowering strip on both the sprayed and unsprayed side.

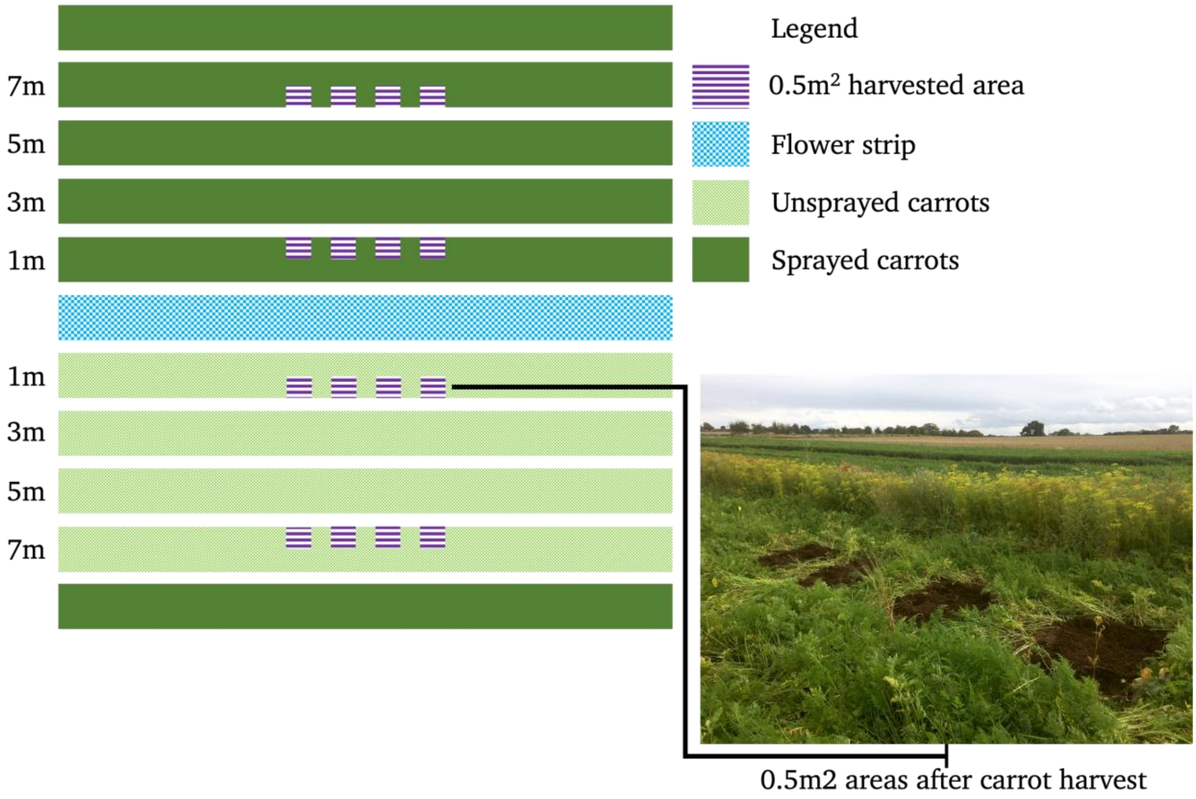

Figure 4.3. Layout of locations of plots harvested in carrot fields.

4.3.3 Derivation of downstream economic and supply chain variables

The percentage of sellable carrots, net yield, turnover, mean carrot mass, percentage of virus damaged carrots and percentage of insect damaged carrots and the income lost from insect damage was calculated as per Chapter 3.

It is also possible to quantify further economic considerations for this trial to build a fuller picture of the financial implications of these flower strips and spray regimes. In the commercial fields sampled , there were between seven and nine insecticide applications each growing season. The costs of these products clearly varies but working with Huntapac, representative prices of £475/ha for these applications including the price of the plant protection products and £15/ha for tractor and labour costs for each application have been estimated. This representative value of £475 can be added to the plots under the sprayed regime.

Following harvest, all carrots are transported to Preston to be washed and graded prior to sale, including those carrots with visible insect damage, which will go on to be discarded. Therefore, if one treatment combination has a higher mass of damaged carrots this will incur higher costs to transport these to the packhouse. Huntapac's representative price of transporting one trailer containing 27 tonnes of carrots is £1.80 for one mile. Therefore, as the average distance from these trial fields to Preston is 95 miles, the price of transporting a tonne of carrots this distance is estimated to cost £6.33.

There are also two easily measurable financial implications of taking land out of production to be put into these flower strips; the seed mix costs, and the income forgone from the land the strips are sown on. The seed costs are relatively low, for each 70m^2 plot the Apiaceae mix cost £4.30, the Phacelia mix cost £1.63, and the Cornflower mix cost £7.76 (Table S.4.28).

Next, the income forgone from the land taken out of production for the strips was calculated. As is established by the statistical analysis of the net yield results, the value of land varies within the field. It is therefore possible to calculate the income forgone using these variable figures therefore reflecting the presence of an edge effect. This helps to give a more realistic estimation of costs incurred by growers. See Table S.4.27 and

Figure 4. for the figures used for this calculation. It has been assumed that a 200m^2 area has been removed from production here.

It is possible to combine all these five economic factors into one economic assessment of income of the combined costs associated with the implementation of flowering strips (Equation 1).

$$\begin{aligned} combined\ costs\ (\pounds\ /\ ha) \\ &= insect\ damaged\ carrots\ income\ lost\ (\pounds/ha) \\ &+ \text{spraying costs at } \pounds 475/\text{ha} \\ &+ insect\ damged\ carrot\ mass\ \times \pounds 6.33 \\ &+ seed\ cost/\ \pounds \\ &+ income\ forgone\ from\ land\ (\pounds/ha) \end{aligned} \tag{1}$$

Following the application of insecticide protection products to crops, it is possible to estimate the CO_{2eq}/ha emissions per insecticide application. Evidently, each product applied will have different greenhouse gases (GHG) associated with production, but this is confidential information. Moreover, as this trial was split over four fields, information about emissions whilst traveling between fields has not been recorded. As such, a single representative figure of 20.5 kg CO_{2eq}/ha is used by the Cool Farm Tool, a commonly used carbon foot-printing tool used in agriculture (Cool Farm Alliance, 2020). An average of 8 insecticide applications has been assumed for these four fields. For the transportation of insect damaged carrots, an articulated lorry weighing 33 tonnes that is 100% laden is estimated to emit 1.76537 kg CO_{2eq} per mile (Department for Business, 2019). In a 33-tonne articulated lorry, there will be around 27 tonnes of carrots transported. Therefore, for the transport of one tonne of carrots travelling 95 miles from Shropshire to the packhouse, there are an estimated 6.21149 kg CO_{2eq} emitted. Treatments with more insect damaged carrots will incur more CO2eq emissions.

These two GHG emission considers can therefore be summed to give a value for combined emissions associated with the trial (Equation 2).

$$\begin{aligned} Emissions\ (\text{kg CO2eq/ha}) \\ &= number\ of\ insecticide\ applications \times 20.5\text{kg CO2eq} \\ &+\ insect\ damage\ mass\ \times\ 6.21149\text{kg CO2eq} \end{aligned} \tag{2}$$

4.3.4 Statistical Analyses

4.3.4.1 Aerial invertebrates

The invertebrates recorded from yellow water traps were assigned to three different functional groups based on published literature (Table S.4.27). To assess the effect of seed mix upon community wide metrics, functional groups, and key ESPs, Linear mixed effects models (LMMs) were created and analysed, as per previous chapters, with seed mix treatment was selected fixed effects within the model. Replicate was specified as the random effects, thereby taking any potential variation between and within replicates within fields into account.

As in Chapter 2, the effect of seed mix upon the community assemblage of each trapping period was investigated via the use of partial Redundancy Analysis (pRDA). To reduce the bias that infrequent species may have on the pRDA, species were excluded from the analysis if they were found in less than 1 trap across each run.

To identify the variation explained by seed mix and field, two partial RDAs were conducted. In each case, an environmental factor (e.g. seed mix) was specified as the factor of interest and the remaining factors was 'partialled out' thereby allowing the effect of each single environmental factor upon the community assemblage to be quantified. Following this, each factor's significance was analysed via a Permutation test for RDA under reduced model, with Permutation set to 'free' and 999 permutations ran. This approach was selected as the various invertebrate data were collected from a designed, blocked experiment and therefore it was necessary to account for the influence of these factors upon community assemblage. Biplots presented for each run are for RDAs with seed mix, field and aspect specified.

4.3.4.2 Aphid Abundance Counts and Sentinel Prey Cards

Of the 144 carrot foliage samples taken on the 3/7/2020, 117 samples contained no aphids. From the remaining 27 samples, in total 33 alate aphids were identified. Only 2 were *Myzus persicae,* known virus vectors. To overcome any statistical challenges with low pest aphid numbers and any misidentification of species, the total number of alates found was used.

To test for the effect of seed mix treatment upon aphid abundance linear mixed effects models (LMMs) was fitted to the total number of aphids and NE as per Chapter 3. The fixed effects were specified as seed mix treatment, spray regime (sprayed or unsprayed), distance from the field edge (17.5m, 52.5m, 87.5m, 122.5m), and distance from the flower strip (1m, 3m, 7m) and random effects as plot nested within replicate.

This was repeated for the sentinel aphid aphids on the number of aphids remaining on cards after 24 hours. The residual plot was assessed to ensure the response variable met to the assumptions of LMMs and a transformation was not required.

4.3.4.3 Carrot harvest data

Linear mixed effects models were used to investigate seed mix treatment effects on harvest factors and further downstream variables. Seed mix treatment, spray regime and the distance of the plot from the edge of the field, as well as the interaction between these, were fixed effects within the model. Plot ID was nested within replicate were the random effects, thereby taking any potential variation within replicates within fields into account. The data set has been split into two, to allow samples taken 1m and 7m away from the flowering strip to be analysed separately. The Least Significant Difference (LSD) has been used to compare estimates of the means of two response variables that is expected to be exceeded by chance in 5% in a repetition of this work; here all LSD reported are mean $\text{LSD}_{.05}$.

To investigate the differences between harvest metrics in sprayed and unsprayed samples further, the relative benefit of spraying has been calculated based upon the predicted means. The predicted mean values for unsprayed samples have been taken away from sprayed samples. Thereby indicating that when values are positive there was a benefit of spraying and when values are negative, there was no observable benefit of sprays.

To identify if there were any treatment effects due to seed mixes in the distribution of carrots based on their size, a Chi-squared test was performed using R version 1.1.456 (R Core Team, 2020). The evenness of the distribution of carrots in key crown diameter size categories (under 20mm, between 20-35mm, 35-45mm and over 45mm) was analysed for effects due to adjacent seed mix. To account for the unequal number of samples due to missing values, the observed carrot counts for each seed mix in each size category have divided by the number of plots. The carrots in these four size categories did not contain any defects.

4.4 Results

4.4.1 Aerial invertebrates in yellow water traps – ecosystem service providers

4.4.1.1 Community wide metrics, functional groups, and key ESPs

The insect communities present in the sown seed mixes were significantly different across community wide metrics, functional groups, and key ESPs (Table 4.21). The Cornflower performed well across all community wide metrics, with the highest species richness, Shannon's Index, Simpson's Index, and total insect abundance, thereby indicating the mix had a both diverse and abundant insect community present over the sampling period (Table 4.22). The Phacelia mix followed, with high species richness, Shannon's Index and Simpson's Index, however, the Phacelia mix had a significantly lower total insect abundance compared with the Cornflower mix ($F_{2, 25}$=5.41, $p<0.01$) (Table 4.21 & Table 4.22). The Apiaceae and Control carrots had a significantly lower performance suggesting a less diverse and abundant insect community was present during this sampling (Table 4.21 & Table 4.22).

A more mixed picture of the Cornflower is built when the functional groups of insects are considered. There were positives from a biological control perspective as Cornflower plots had the significantly highest NE abundance, and higher NE richness than the Apiaceae and Control plots (Table 4.21 & Table 4.22). However, Cornflower plots also had significantly high herbivore richness and abundance. At this point, ideally, this would be investigated further considering the pest potential of these herbivores. However as only 11 aphids were identified across the 24 traps running in this trapping period and therefore this was insufficient to analyse. The Phacelia, Apiaceae and Control mix all had lower NE abundance and richness as well as herbivore abundance (Table 4.21 & Table 4.22).

The cornflower mix continues to perform well when key ESPs are considered, with plots having significantly higher total wasp abundance, richness and Ichneumonidae abundance when compared with the Apiaceae, Phacelia and Carrot Controls (Table 4.21 & Table 4.22).

Table 4.21. Output from LMMs on yellow water trap invertebrate community. Significant results highlighted in bold, with significance levels indicated by: *** = *p*<0.001, ** = *p*<0.01, * = *p*<0.05, "." = *p*<0.1.

	Response variable	Trans.	NumDF	DenDF	F value	Pr(>F)	SIG	Average LSD
Community wide metrics	Species richness	-	3	25	6.17	0.003	**	4.46
	Total abundance	-	3	25	5.41	0.005	**	29.5
	Shannon's Index	-	3	17.6	3.42	0.040	*	0.42
	Simpson's Index	-	3	17.6	4.47	0.017	*	0.09
Functional groups	NE abundance	log(x+1)	3	25	5.92	0.003	**	0.81
	NE richness	-	3	25	6.26	0.003	**	1.50
	Herbivore abundance	log(x+1)	3	25	2.77	0.063	.	0.76
	Herbivore richness	-	3	25	5.14	0.007	**	1.34
Key ESPs	All Bee abundance	log(x+0.1)	3	28	4.42	0.012	*	1.53
	Total wasp abundance	log(x+1)	3	25	5.19	0.006	**	0.73
	Wasp richness	-	3	25	4.27	0.014	*	1.50
	Diptera abundance	log(x+1)	3	25	3.02	0.048	*	0.29
	Diptera richness	-	3	25	1.96	0.146	NS	2.56
	Ichneumonidae abundance	log(x+1)	3	25	5.12	0.007	**	0.77

Table 4.22. Predicted mean values from the LMMs run on yellow water trap invertebrate data for the variables which yielded significant differences for each seed mix from the first trapping period. Group indicates the statistically significant differences between seed mixes based upon the pairwise LSD comparisons and the standard error of the predicted mean. Response variables marked with "*" have been transformed and therefore the predicted mean presented has not been back transformed.

Response variable	Seed mix	Predicted Mean	Group	SE
Species Richness	Cornflower	17.5	A	2.408
	Phacelia	14.3	AB	2.408
	Apiaceae	10.5	BC	2.408
	Carrots	9.38	C	2.408
Shannon's Index	Cornflower	2.28	A	0.155
	Phacelia	2.20	AB	0.155
	Carrots	1.86	BC	0.155
	Apiaceae	1.75	C	0.155
Simpson's Index	Cornflower	0.85	A	0.029
	Phacelia	0.85	A	0.031
	Carrots	0.80	AB	0.031
	Apiaceae	0.74	B	0.029
Total abundance	Cornflower	79.9	A	13.958
	Phacelia	41.4	B	13.958
	Apiaceae	33.6	B	13.958
	Carrots	30.0	B	13.958
NE abundance *	Cornflower	2.32	A	0.307
	Phacelia	1.16	B	0.307
	Apiaceae	1.11	B	0.307
	Carrots	0.77	B	0.307
NE richness	Cornflower	4.00	A	0.609
	Phacelia	2.88	AB	0.609
	Apiaceae	1.88	BC	0.609
	Carrots	1.00	C	0.609
Herbivore abundance *	Cornflower	2.84	A	0.477
	Phacelia	2.63	AB	0.477
	Carrots	2.17	AB	0.477
	Apiaceae	1.89	B	0.477
Herbivore richness	Phacelia	4.50	A	0.754
	Cornflower	4.00	AB	0.754
	Carrots	3.13	BC	0.754
	Apiaceae	2.13	C	0.754

Table 4.22. Continued predicted means from LMMs.

Response variable	Seed mix	Predicted Mean	Group	SE
Total bee abundance *	Cornflower	0.52	A	0.526
	Phacelia	-0.34	AB	0.526
	Carrots	-1.70	B	0.526
	Apiaceae	-1.75	B	0.526
All wasp abundance *	Cornflower	2.06	A	0.278
	Phacelia	1.11	B	0.278
	Apiaceae	0.98	B	0.278
	Carrots	0.76	B	0.278
Wasp richness	Cornflower	3.38	A	0.632
	Phacelia	2.38	AB	0.632
	Apiaceae	1.63	B	0.632
	Carrots	0.88	B	0.632
Diptera abundance *	Cornflower	4.12	A	0.121
	Phacelia	3.91	AB	0.121
	Apiaceae	3.79	B	0.121
	Carrots	3.73	B	0.121
Diptera richness	Cornflower	8.50	A	1.043
	Phacelia	7.00	AB	1.043
	Apiaceae	6.75	AB	1.043
	Carrots	5.50	B	1.043
Ichneumonidae abundance *	Cornflower	1.44	A	0.278
	Apiaceae	0.31	B	0.278
	Phacelia	0.22	B	0.278
	Carrots	0.20	B	0.278

4.4.1.1 Invertebrate Community Assemblage

Both seed mix and field explain a significant amount of variation in the community assemblage of yellow water invertebrates in the first trapping period (Table 4.23). In the first trapping period, seed mix explained 13.7% of the variation in entire community of yellow water trap invertebrates (F=1.58, *p*=0.01). There were notable species associations between Ichneumonidae wasps and *Apis mellifera* with the Cornflower mix, along with Chloropidae and Empidoidea flies, and *Anthocoris* pirate bugs. Field explained 7.92% of the variation within this invertebrate community, with a notable association between Muscidae flies, *Athalia rosae*, and Fanniidae flies and Field 11.

Table 4.23. Table showing the percentage variance explained by seed mix and field, and plot aspect in the partial Redundancy analysis on all yellow water trap invertebrates, as well as the subsequent ANOVA analysis of the partial Redundancy analysis model. Permutation test for partial RDA under reduced model. Permutation: free, number of permutations: 999. Significance codes: *p*<0.01 = '**'.

Run	Fixed effect	Df	F-value	Pr(>F)	Sig	adjusted R2	Variance explained
1	Seed Mix	3	1.58	0.01	**	0.052	13.7%
	Field	1	2.73	0.005	**	0.056	7.92%

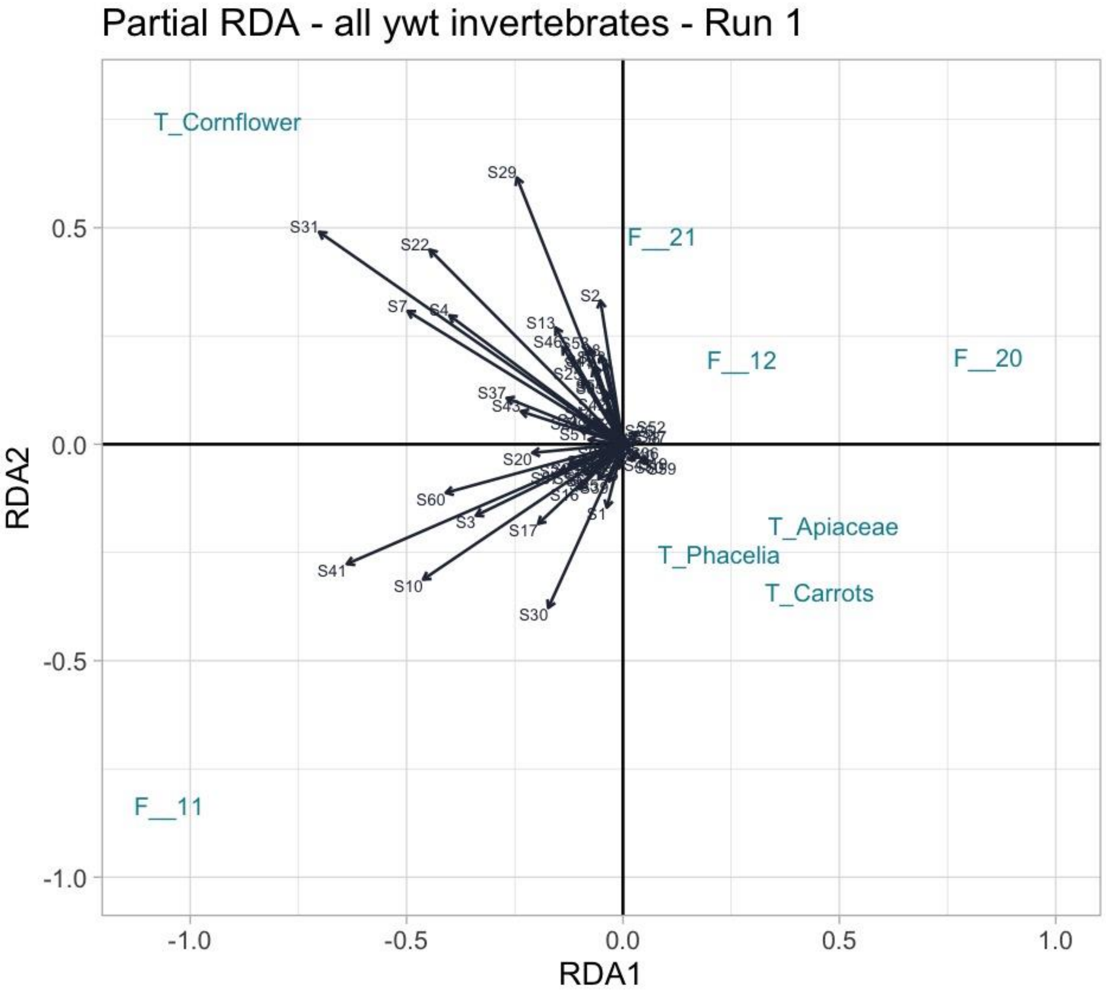

Figure 4.423. Redundancy analysis ordination of all yellow water trap invertebrates in Run 1, with axes constrained by (F_), seed mix (T_). Invertebrates identified to the maximum possible resolution and denoted by; S1: Acalyptrate, S2: Agromyzidae, S3: Anthomyiidae, S4: *Anthocoris* spp., S5: *Aphidius* spp., S6: Aphids, S7: *Apis melifera*, S8: Apocrita, S9: Asteiidae, S10: *Athalia rosae*, S11: *Bombus lapidarius*, S12: Brachycera, S13: Braconidae, S14: *Brassicogethese aenus*, S15: Calliphoridae, S16: Camillidae, S17: Carnidae, S18: *Cavariella* spp., S19: Cecidomyiidae, S20: Ceraphronidae, S21: Chalcididae, S22: Chloropidae, S23: Coleoptera, S24: Cynipoidea, S25: Diapriidae, S26: Diastatidae, S27: Dolichopodidae, S28: Ephydridae, S29: Empidoidea, S30: Fanniidae, S31: Ichneumonidae, S32: Lepidoptera, S33: Liopteridae, S34: Lonchaeidae, S35: Lonchopteridae, S36: *Lucilia* spp., S37: *Lygus pratensis*, S38: *Lygus spp.*, S39: Megaspilidae, S40: Miridae, S41: Muscidae, S42: Mymaridae, S43: Nematocera, S44: Odiniidae, S45: Pallopteridae, S46: Phoridae, S47: Phylini, S48: Platygastridae, S49: Proctotrupidae, S50: Psocoptera, S51: Pteromalidae, S52: *Rhagio tringarius*, S53: *Sarcophaga spp.*, S54: Sarcophagidae, S55: Sciaridae, S56: *Scathophaga stercoraria*, S57: Sepsidae, S58: *Sitonia* spp., S59: Staphylinidae, S60: Thysanoptera.

4.4.2 Aphid Abundance Counts & Sentinel Prey Cards – direct evidence for ecosystem service delivery

There were no significant main effects or interactions between seed mix ($F_{3,9.78}$=1.60, *p*=0.251), spray regime ($F_{1,57.3}$=0.03, *p*=0.956), distance from the field edge ($F_{3,9.85}$=0.36, *p*=0.779), and distance from the flower strip ($F_{2,52.4}$=0.03, *p*=0.601) on the log transformed total alates counts.

From the 144 foliage samples taken on taken on the 3/7/2020, 52 natural enemies were found and identified, including 13 parasitic wasps and 35 aphid mummies. Again, seed mix ($F_{3,\ 57.0}$=0.42, *p*=0.740), spray regime ($F_{1,\ 57.0}$=1.08, *p*=0.303), distance from the field edge ($F_{3,58.76}$=0.11, *p*=0.953), and distance from the flower strip ($F_{2,55.6}$=2.09, *p*=0.133) had no significant main effects or interactions between on the log transformed natural enemies counts.

As samples of carrot foliage taken on the 6/8/2020 did not contain any aphids or natural enemies and only contained 3 Diptera, no statistical analysis on these data was performed. Seed mix did not have a significant effect on the predation of sentinel aphids ($F_{3,53.2}$=0.37, *p*=0.682).

4.4.3 Carrot harvest metrics – direct evidence for ecosystem service delivery

Over a three-week period, across all fields and treatments, 40,462 carrots were harvested and graded by hand, which weighed 2.358 tonnes when washed.

4.4.1.1 Percentage of virus damaged carrots in a sample

At 1m away from the flowering strips, there were not significantly more virus damaged carrots on the unsprayed side of the flower strip than on the sprayed side. This could suggest that sprays have not obvious universal measurable effect across the field at reducing virus damage. There was, however, a significant interaction between seed mix, spray regime and distance from the edge of the field upon virus damaged carrots ($F_{9,\ 90.75}$= 2.13, $p<0.05$) (Figure 4.5). However, this seems to be as a result of considerable variation within the samples and there is no apparent trend. At 1m, on the sprayed side control plots did not have significant changes in the percentage of a sample displaying virus symptoms. However, on the unsprayed side, there is significant variation between 52.5m and 87.5m (Average LSD= 0.15) (Figure 4.5). As such there is also an inconsistent response of the benefit of spraying for control plots with increasing distance into the field (Figure 4.5).

At 7m away from the flowering strips, there was no significant difference between the log transformed percentage of a sample that contains virus damaged carrots in sprayed and unsprayed areas, again suggesting that sprays have no measurable effect at reducing virus damage (Figure 4.5). There were no other significant differences or significant interactions in the percentage of virus damaged carrots in a sample at 7m from the flowering strips, and no seed mix reductions in virus damage apparent.

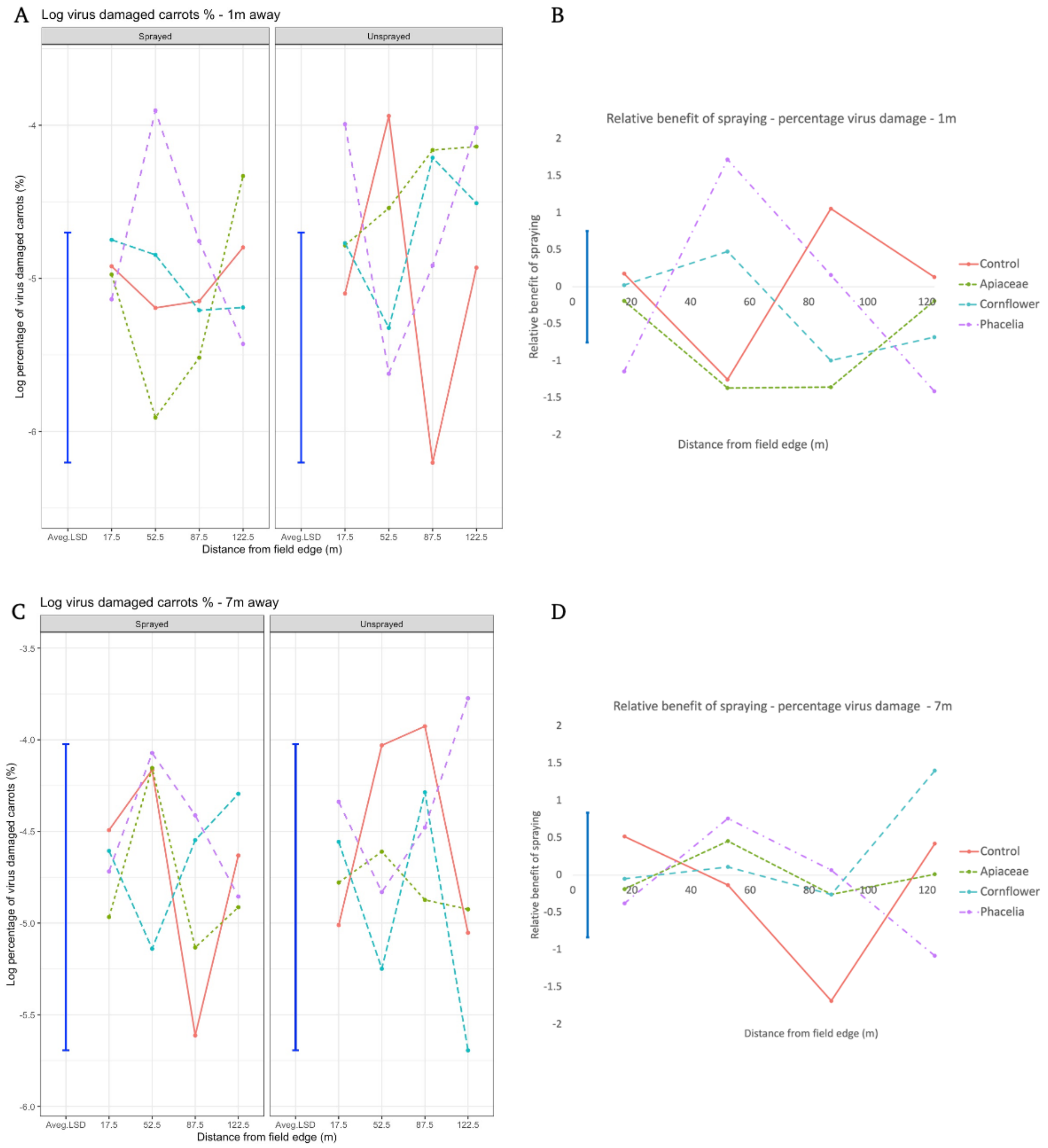

Figure 4.5. Predicted means and relative spraying benefit for the percentage of virus sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the percentage of virus sampled at 1m. Blue reference bar denotes the Average LSD= 1.50. **B** – Relative spraying benefit for percentage of virus sampled at 1m. Blue reference bar denotes the Average LSD= 1.50. **C** - Predicted means for the percentage of virus sampled 7m. Blue reference bar denotes the Average LSD= 1.67. **D** - Relative spraying benefit for the percentage of virus sampled at 1m. Blue reference bar denotes the Average LSD=1.67. Seed mix: Control= ━●━ , Apiaceae= ╍●╍ , Cornflower= ━●━ , and Phacelia= ━● .

4.4.1.2 Percentage of insect damaged carrots in a sample

At 1m away from the flowering strips, sprays have not obvious universal measurable effect across the field at reducing virus damage. There was not a significant difference between the log transformed percentage of a sample that contains insect damaged carrots in sprayed and unsprayed areas (Figure 4.6). There was, however, a significant interaction between seed mix, spray regime and distance from the edge of the field upon insect damaged carrots ($F_{9, 90.8}$=2.02, p<0.05) (Figure 4.6). Again though, this appears to reflect variability in the dataset rather than a trend or consistent seed mix reduction. Notably, there was high variability in control plots on the unsprayed side compared with the sprayed side, which also leads to no universal trends around the benefit of spraying for control carrots (Figure 4.6). There is also variability within the seed mix treatment, such as the significant increase in insect damage in the sprayed Phacelia plots (Average LSD=0.16) (Figure 4.6.A)

At 7m away from the flowering strips, there was no significant difference between the log transformed percentage of a sample that contains insect damaged carrots in sprayed and unsprayed areas. Unlike, 1m away, there were no other significant differences or significant interactions in the percentage of virus damaged carrots in a sample at 7m from the flowering strips. Together, these data from both sample distances suggest that sprays have no measurable effect at reducing insect damage (Figure 4.6). There was large within and between mix variability in insect damage, although a consistent increase in the benefit of spraying was seen for unsprayed cornflower plots 7m away from the flowering strips (Figure 4.6.D).

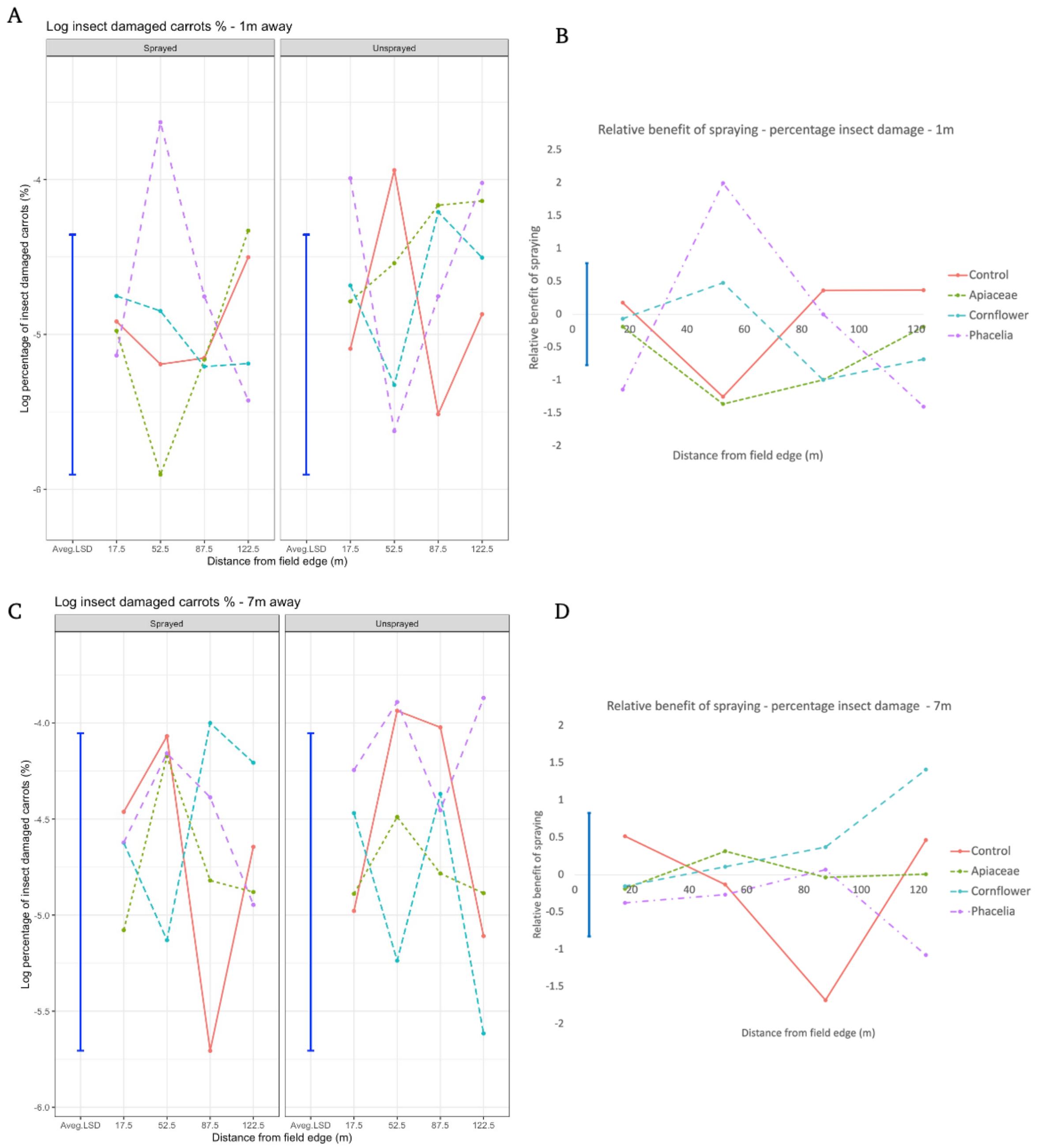

Figure 4.6. Predicted means and relative spraying benefit for the percentage of insect-damaged carrots sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the percentage of insect sampled at 1m. Blue reference bar denotes the Average LSD= 0.16. **B** – Relative spraying benefit for percentage of insect sampled at 1m. Blue reference bar denotes the Average LSD= 0.16. **C** - Predicted means for the percentage of insect sampled 7m. Blue reference bar denotes the Average LSD= 0.17. **D** - Relative spraying benefit for the percentage of insect damage sampled at 1m. Blue reference bar denotes the Average LSD=0.17. Seed mix: Control= ━●━ , Apiaceae= ╍●╍ , Cornflower= ╍●╍ , and Phacelia= ━● .

4.4.4 Carrot harvest metrics – indirect evidence for ecosystem service delivery

4.4.4.1 Total number of carrots

There were significantly more carrots in the samples taken on the unsprayed side of the flower strips compared with the sprayed side ($F_{1,74.6}$=9.14, p<0.01). The predicted mean number of carrots in the unsprayed side was 171 compared with 159 on the sprayed side (Average LSD=8.02). There was also a significant three-way interaction between seed mix, spray regime and distance from the edge of the field ($F_{9,74.5}$=2.80, p<0.01) (Figure 4.7.A). Whilst there is considerable variation within these data, there is no apparent trend nor clear indications of seed mix differences, although the trend seen in the sprayed Phacelia plots is reversed in unsprayed plots (Figure 4.7).

Unlike 1m away, there was not a significant effect of spray regime on the total number of carrots sampled 7m away from the flowering strip. There however, also a significant three-way interaction between seed mix, spray regime and distance from the edge of the field ($F_{9,82.9}$=2.72, p<0.01) (Figure 4.7.C). At 7m away from the strips, there is a trend within the control plots whereby the number of carrots rises with increasing distance into the crop interior, however this does not exceed the Average LSD=37.2. There is also notable variation within and between seed mixes seen in the unsprayed plots, such as the large variation in the benefit of spraying seen in Apiaceae plots (Figure 4.7.D).

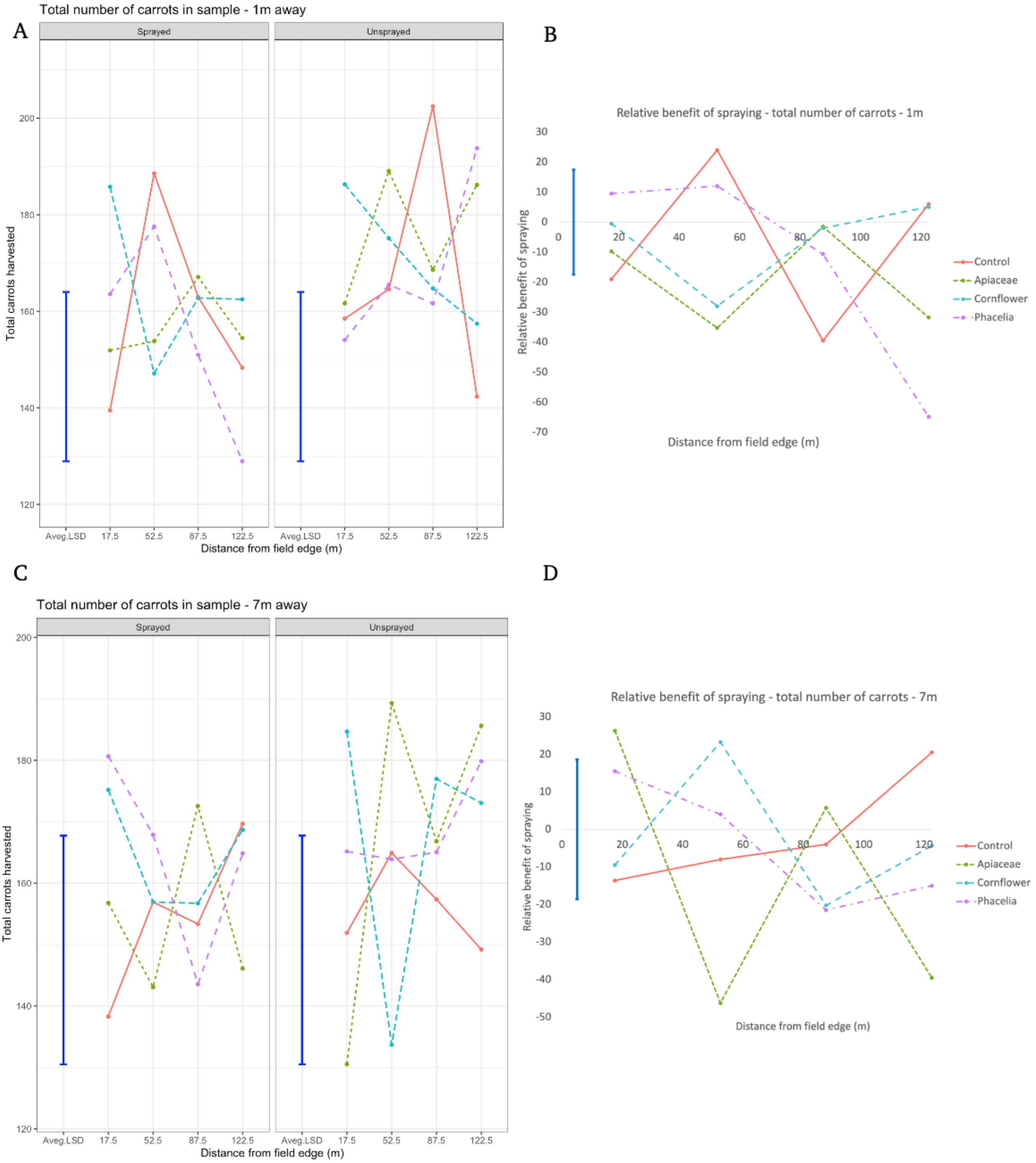

Figure 4.7. Predicted means and relative spraying benefit for the total number of carrots sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. A - Predicted means for the total number of carrots sampled at 1m. Blue reference bar denotes the Average LSD=35.0. B – Relative spraying benefit for total number of carrots sampled at 1m. Blue reference bar denotes the Average LSD=35.0. C - Predicted means for the total number of carrots sampled at 7m. Blue reference bar denotes the Average LSD=37.2. D - Relative spraying benefit for total number of carrots sampled at 1m. Blue reference bar denotes the Average LSD=37.2. Seed mix: Control= —●—, Apiaceae= -●-, Cornflower= -●-, and Phacelia= —●—.

4.4.4.2 Mean Carrot Mass

Across all distances into the field, sprayed carrots were significantly heavier than unsprayed carrots ($F_{1, 87.2}$= 39.3, $p<0.001$). For carrots sampled 1m from the flower strip, sprayed carrots had a predicted mean log transformed value of 4.34g compared with unsprayed carrots predicted mean log transformed value of 4.19g (Average LSD= 0.049) (Figure 4.8). In real terms, the predicted back transformed mass of carrots in sprayed areas was 76.7 g and 65.8 g in unsprayed areas. There is also a significant effect of distance into the field on transformed mean carrot mass ($F_{3, 88.1}$= 3.15, $p<0.05$) (Figure 4.8.A), whereby sprayed carrot's see a rise in mean carrot mass with increasing distance into the field. This is no consistent for all seed mixes however, as there was a significant interaction of seed mix and distance into the field ($F_{9, 90.6}$= 2.34, $p<0.05$) (Figure 4.8.A). Finally, there was a significant three-way interaction between seed mix, spray regime and distance into the field ($F_{9, 87.1}$= 5.92, $p<0.001$) (Figure 4.8.B).

For carrots sampled 7m away from the flowering strip, there were far fewer significant effects and interactions for mean carrot mass (Figure 4.8). Sprayed carrots were however again significantly heavier than unsprayed carrots ($F_{1, 81.9}$= 6.76, $p<0.05$), with sprayed carrots having a predicted mean log transformed value of 4.36 compared with unsprayed carrots predicted mean log transformed value of 4.29 (Average LSD= 0.053) (Figure 4.8.C). In real terms, the predicted back transformed mass of carrots in sprayed areas was 78.5 grams and 73.3 grams in unsprayed areas. Notably, the effect size of the relative benefit of spraying at 7m away from the flowering strip was approximately half of the relative benefit at 1m. This may suggest that there are reducing benefits away from the flowering strip.

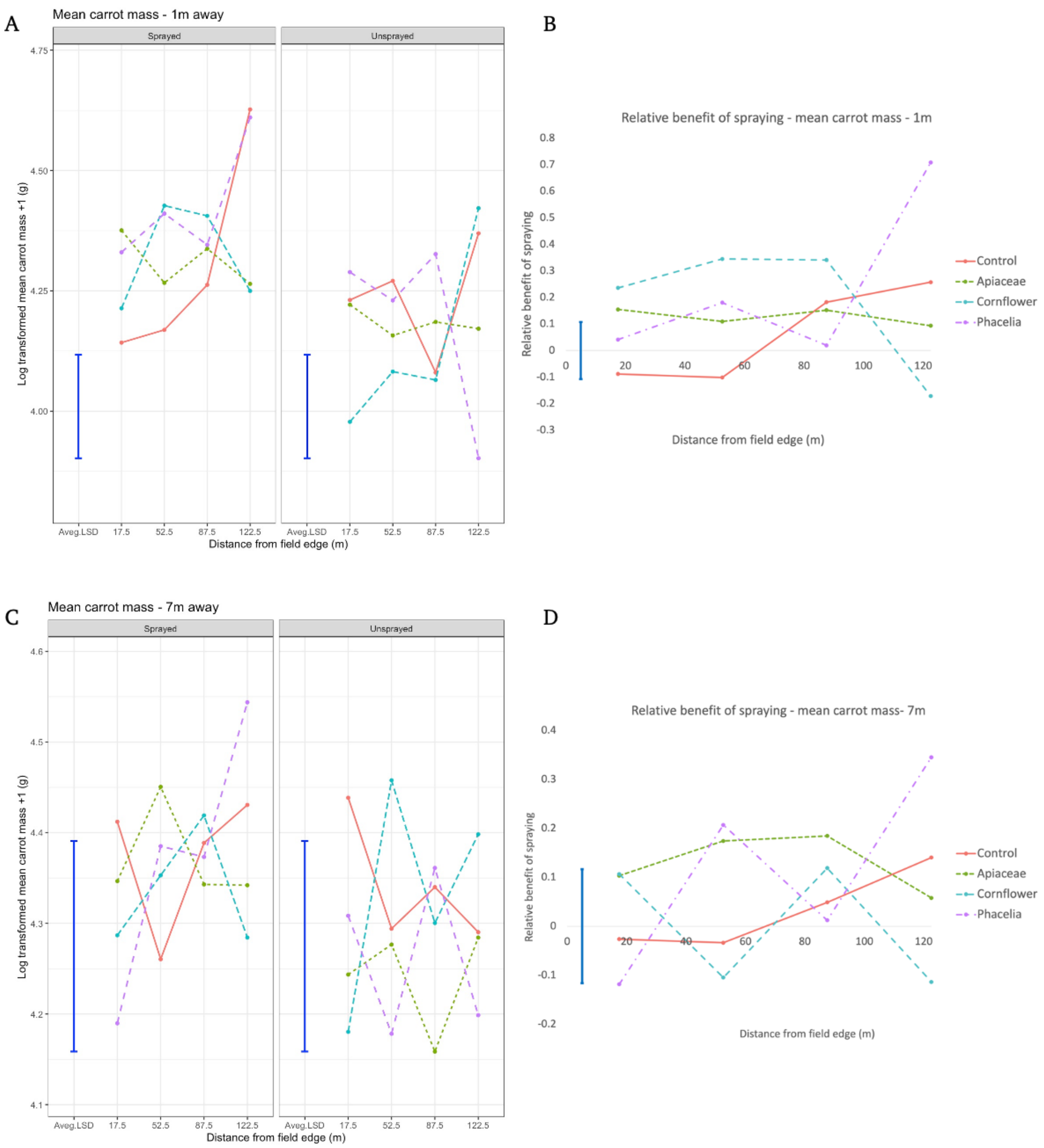

Figure 4.8. Predicted means and relative spraying benefit for the log mean carrot mass sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the log mean carrot mass sampled at 1m. Blue reference bar denotes the Average LSD=0.26. **B** – Relative spraying benefit for log mean carrot mass sampled at 1m. Blue reference bar denotes the Average LSD=0.26. **C** - Predicted means for the log mean carrot mass sampled at 7m. Blue reference bar denotes the Average LSD=0.38. **D** - Relative spraying benefit for log mean carrot mass sampled at 1m. Blue reference bar denotes the Average LSD=0.38. Seed mix: Control= —●—, Apiaceae= -●-, Cornflower= -●-, and Phacelia= —●.

4.4.4.3 Gross yield

Similarly, at 1m, sprayed carrots had a higher gross yield than samples from unsprayed areas ($F_{1, 80.1}$= 18.2, *p*<0.001), with sprayed carrots having a predicted mean gross yield of 95.7 t/ha compared with unsprayed carrots predicted mean gross yield of 86.2 t/ha (Average LSD=4.43) (Figure 4.9). There was also a significant interaction between seed mix and distance from the field edge (F(9,18.0)= 3.20, *p*<0.05) and a significant interaction between seed mix, spray regime and distance from the field edge (F(9, 79.9)= 6.72, *p*<0.001)(Figure 4.9.A). On the sprayed side, there was a notable 'edge effect' for control plots, whereby the predicted mean gross yield increased from 69.6t/ha at 17.5m to 124.0t/ha at 122.5m (Average LSD=23.0). There was a clear increasing relative benefit of spraying control plots with increasing distance into the field also apparent (Figure 4.9.A). This edge effect was not seen for the sown seed mixes, all of which had significantly lower gross yields at the furthest sample point into the field compared with the sprayed control plots (Figure 4.9.A). Conversely, on the unsprayed side, there was not noticeable edge effect for control plots. On the unsprayed side, there was no universal edge effect or trend where seed mixes performed differently as there was an increase in gross yield with increasing distance into the field for cornflower plots but a decrease in gross yield for Phacelia plots.

In samples harvested 7m from the flowering strips, sprayed carrots had a significantly higher predicted mean gross yield of 99.0t/ha compared with unsprayed carrots predicted mean gross yield of 92.9t/ha ($F_{1, 74.0}$= 4.42, *p*<0.05, Average LSD= 5.76) (Figure 4.9.C). There was also significant interaction between seed mix, spray regime and distance from the field edge (F(9, 73.9)= 6.3906, *p*<0.001)(Figure 4.9). Like 1m away, there is a notable edge effect and significant increase in the benefit of spraying with increasing distance into the field for control plots (Figure 4.9.D). In terms of seed mixes, as seen at 1m, there was also a high degree of variability although on the unsprayed side, the Cornflower plot's predicted gross yield peaks at 118.8t/ha, significantly higher than the control plots predicted gross yield of 81.6t/ha (Average LSD= 26.1).

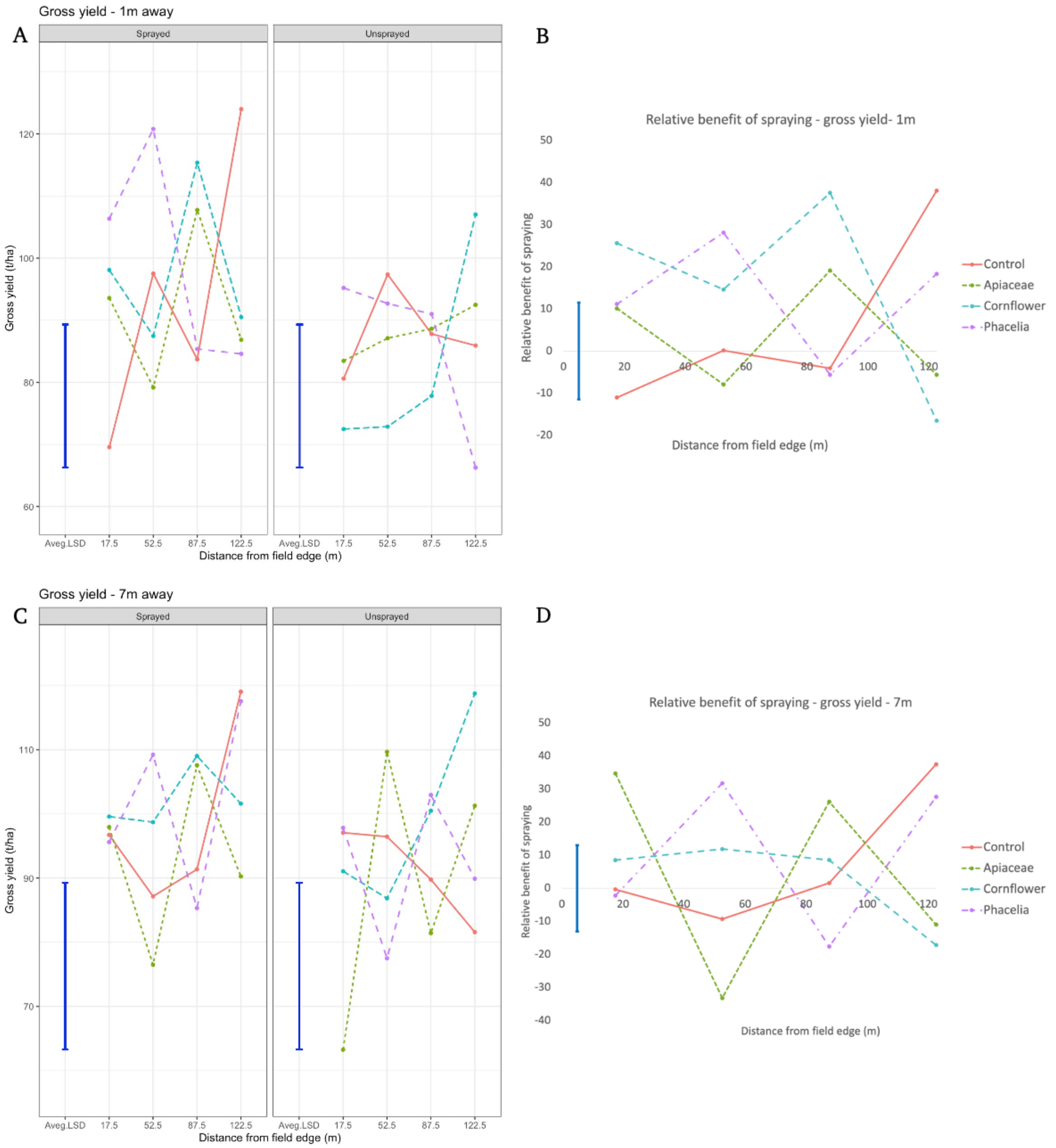

Figure 4.9. Predicted means and relative spraying benefit for the gross yield sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the gross yield sampled at 1m. Blue reference bar denotes the Average LSD=23.0. **B** – Relative spraying benefit for gross yield sampled at 1m. Blue reference bar denotes the Average LSD=23.0. **C** - Predicted means for the gross yield sampled at 7m. Blue reference bar denotes the Average LSD=26.1. **D** - Relative spraying benefit gross yield sampled at 1m. Blue reference bar denotes the Average LSD=26.1. Seed mix: Control= ━●━, Apiaceae= ╍●╍, Cornflower= ╍●╍, and Phacelia= ━●━.

4.4.4.4 Percentage of sellable carrots in a sample

Surprisingly, at 1m away from the flowering strip, the quality of carrots did not significantly vary in sprayed and unsprayed samples, nor was there a three-way interaction between seed mix, spray regime and distance into the field. However, a significant interaction between seed mix and distance into the field was present ($F_{9,85.0}$=2.06, p<0.05) (Figure 4.10), where on both sprayed and unsprayed control plots, there was increasing quality with increasing distance into the fields (Figure 4.10). Although this trend is not universally seen across seed mixes. For instance, in the Apiaceae plots, on the sprayed side there as a decline in quality with increasing distance into the field although this did not quite exceed the Average LSD of 12.8. On the unsprayed side, there was a significant amount of variation within seed mixes and between seed mixes with increasing distance into the field (Figure 4.10).

At 7m away from the flowering strip this picture was not mirrored. Here, sprayed carrots were higher quality than unsprayed samples ($F_{1,72.0}$=5.66 , p<0.05) (Figure 4.10). In real terms, the predicted mean percentage of sellable carrots was 60.6%, significantly higher than unsprayed mean of 56.5% (Average LSD=3.48). On the unsprayed side there is an edge effect (Figure 4.10). Whereby the significant increase in the control plots percentage sellable carrots indicated that unsprayed carrots' quality improved with increasing distance into the field. At 17.5m into the field, the unsprayed control carrots had around 20% fewer sellable carrots in the sample This trend is not seen as strongly on the sprayed side.

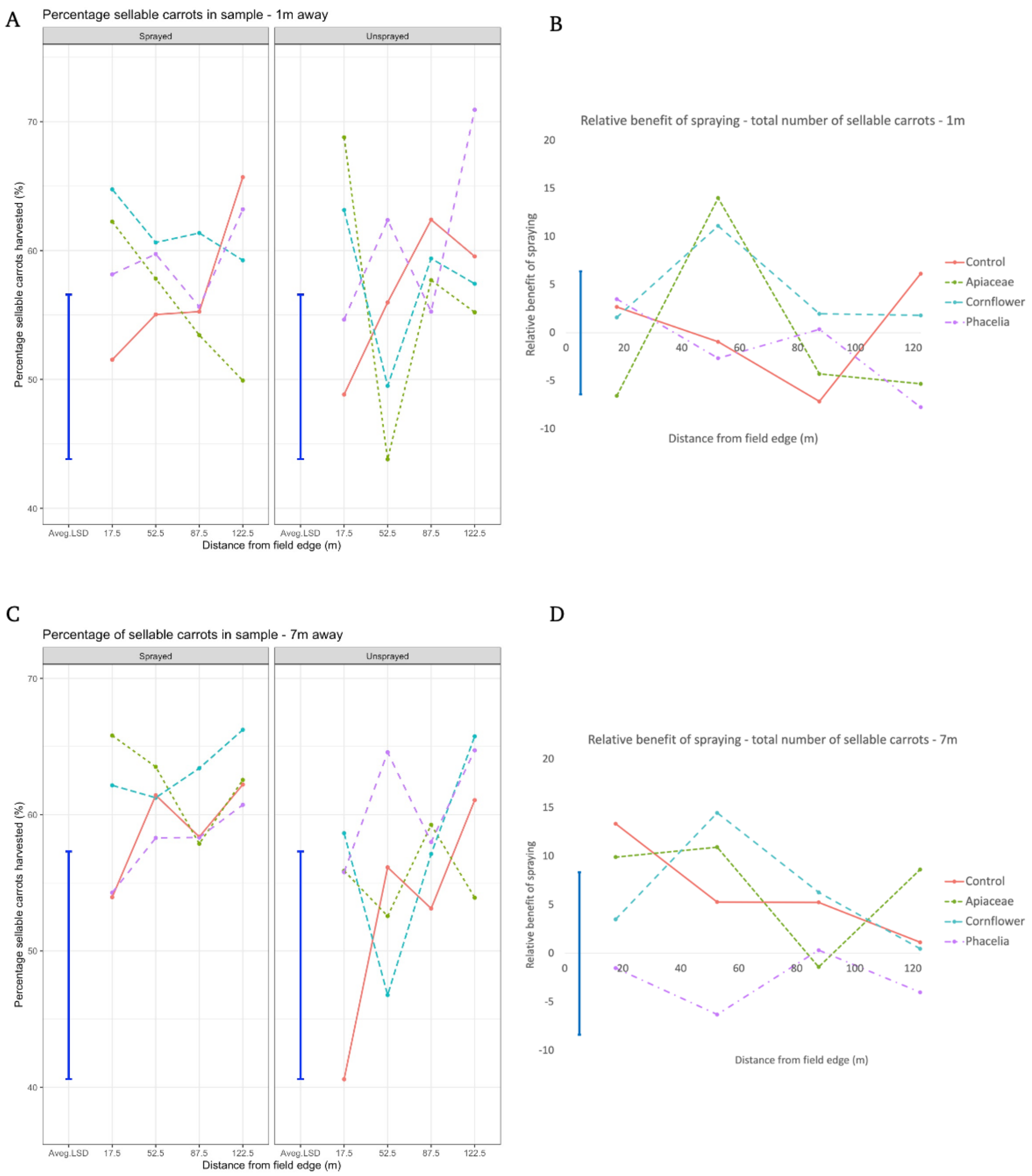

Figure 4.10. Predicted means and relative spraying benefit for the percentage of sellable carrots sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the total number of carrots sampled at 1m. Blue reference bar denotes the Average LSD=12.8. **B** – Relative spraying benefit for total number of carrots sampled at 1m. Blue reference bar denotes the Average LSD=12.8. **C** - Predicted means for the total number of carrots sampled 7m. Blue reference bar denotes the Average LSD=16.7. **D** - Relative spraying benefit for total number of carrots sampled at 1m. Blue reference bar denotes the Average LSD=16.7. Seed mix: Control= —●—, Apiaceae= - -●- -, Cornflower= - -●- -, and Phacelia= —●—.

4.4.4.5 Net yield

At 1m away from the flowering strip, samples that received sprays had a significantly higher net yield than unsprayed samples ($F_{1,80.9}$= 9.20, p<0.01). The predicted mean net yield of sprayed plots was 56.2 t/ha and therefore over 6t/ha higher than the predicted net yield of unsprayed plots at 49.9t/ha (Average LSD= 4.23) (Figure 4.11). There was also a significant interaction between seed mix, spray regime and distance from the edge of the field (F(9, 80.0) = 3.4412, p<0.01) (Figure 4.11). On the sprayed side, there was a clear 'edge effect' whereby the predicted mean net yield of control plots increased with increasing distance into the crop interior (Figure 4.11.A). Meanwhile, the predicted net yield of plots next to seed mixes at 17.5m, at the edge of the field, all had significantly higher net yields than the control plot (Average LSD=20.5). However, there was no apparent improvement in net yield for plots next to seed mixes with increasing distance into the field (Figure 4.11.A). Moreover, there was a clear relative benefit of spraying with increasing distance into the field for control plots which was not seen for plots next to seed mixes (Figure 4.11.B). Any 'edge effects' were less apparent on the unsprayed side where there were fewer trends, although there is a positive increase in the predict mean net yield for cornflower plots, rising from 40.4 t/ha at 52.5m to 63.0 t/ha at 122.5m (Average LSD=20.5).

At 7m away from the flowering strip, for the first time, there was marginally a significant effect of seed mix upon net yield ($F_{3,82.7}$=5.65, p=0.071). This was driven by all Cornflower plots having a predicted net yield of 61.6 t/ha which is marginally significantly higher than all controls plots predicted net yield of 54.0 t/ha (Average LSD=7.89). There was also a significant effect of spray regime upon net yield, whereby the predicted net yield of sprayed plots was 60.0 t/ha and therefore higher than the predicted net yield of unsprayed plots at 54.3 t/ha ($F_{1, 81.6}$= 5.64, p<0.05, Average LSD=4.80) (Figure 4.11).There was also a significant edge effect across all plots, whereby predicted net yield rose with increasing distance into the crop interior ($F_{9, 83.0}$= 3.00, p<0.05) (Figure 4.11).There was also a significant interaction between seed mix, spray regime and distance from the edge of the field ($F_{9, 73.3}$= 2.04, p<0.05) (Figure 4.11).The common trend of an edge effect for sprayed control plots was seen here at 7m, although the jump from the net yield 48.4t/ha at 87.5m to 74.9 t/ha at 122.5m was not significant (Average LSD= 21.1) (Figure 4.11). Here at 7m, on both sprayed and unsprayed sides, there was considerable variation between and within seed mixes, although at 122.5m the Cornflower plots had a significantly higher net yield than other seed mixes (Figure 4.11).

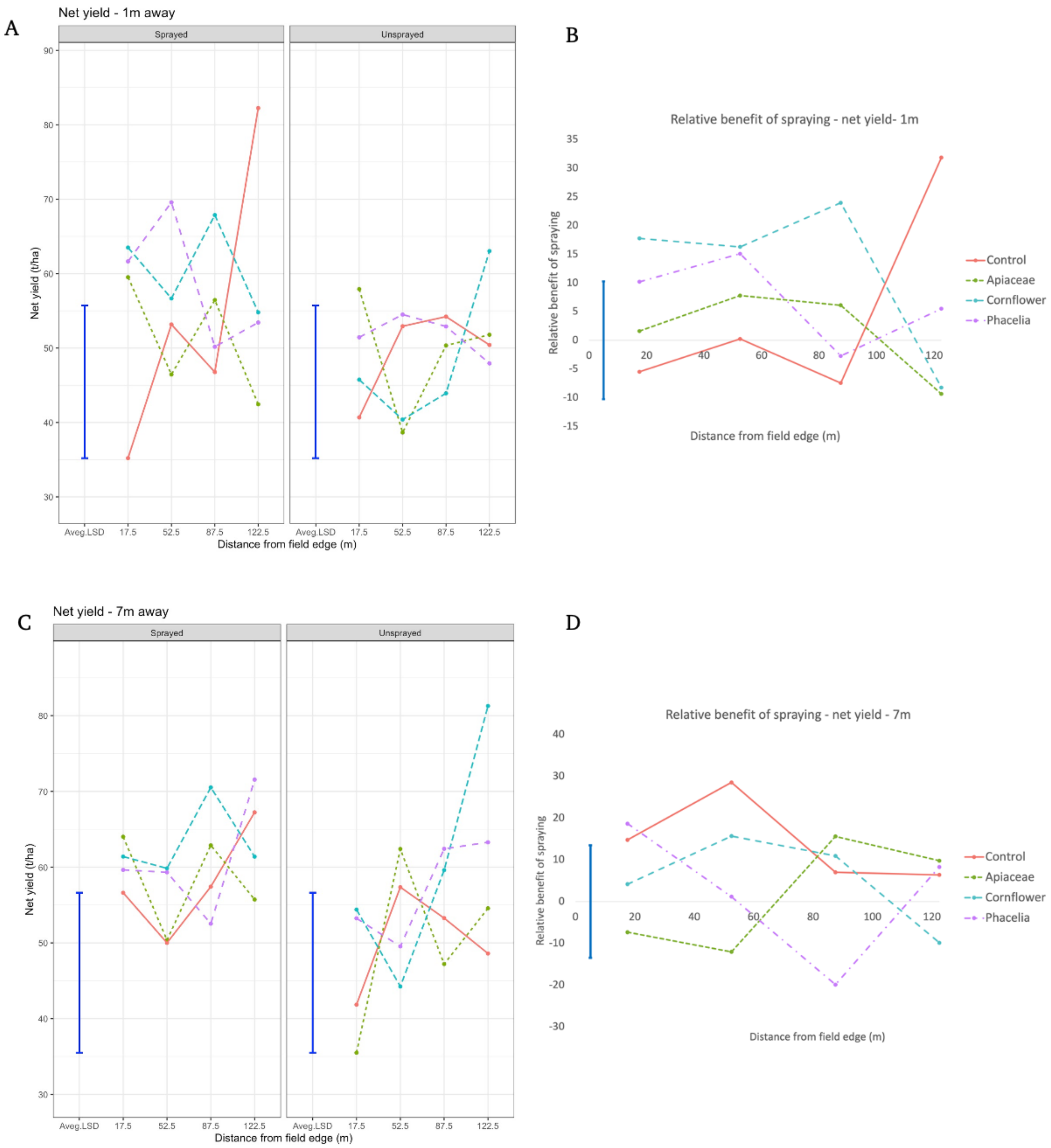

Figure 4.11. Predicted means and relative spraying benefit for the net yield sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the net yield sampled at 1m. Blue reference bar denotes the Average LSD=20.5. **B** – Relative spraying benefit for net yield sampled at 1m. Blue reference bar denotes the Average LSD=20.5. **C** - Predicted means for the net yield sampled 7m. Blue reference bar denotes the Average LSD=21.1. **D** - Relative spraying benefit for net yield sampled at 1m. Blue reference bar denotes the Average LSD=21.1. Seed mix: Control= ●, Apiaceae= ●, Cornflower= ●, and Phacelia= ●.

4.4.4.6 Distribution of carrots between size categories

Seed mix did not significantly affect the distribution of carrots between the four key crown diameter size categories (Table 4.24).

Table 4.24. Chi-squared test outcome for the number of carrots in each of the four size categories for each seed mix.

		Seed Mix				Test result			
	Crown diameter	Apiaceae	Control	Cornflower	Phacelia	χ2	df	*p*-value	Sig.
Observed – expected	under 20mm	0.13	0.96	-0.85	-0.25	0.08	3	0.994	NS
	20-35mm	1.46	-7.08	3.47	2.14	0.79	3	0.852	NS
	35-45mm	-1.95	2.92	0.43	-1.39	1.66	3	0.646	NS
	Over 45mm	-0.26	0.36	0.21	-0.33	0.33	3	0.954	NS

4.4.5 Derived economic metrics from harvest

4.4.5.1 Income lost from insect damaged carrots in a sample

In samples taken from 1m away from the flowering strips, there was no difference in the transformed predicted mean income lost from insect damaged carrots in a sample between sprayed and unsprayed plots. This could suggest that there was no universal measurable economic benefit in reduced visible insect damage seen for an intensive spray regime. This point is also illustrated as for control plots, there is only a benefit of spraying at the furthest distance into the field (Figure 4.12.B).

There was a significant interaction between seed mix, spray regime and distance from the edge of the field upon the income lost from insect damaged carrots ($F_{9,89.8}=2.15$, $p<0.05$)(Figure 4.12). However, there is no apparent trend present with spray regime, increasing distance into the field nor seed mix differences. In sprayed control plots, there was no significant change in the income lost from insect damage, whereas on the unsprayed side, there was significant income variability (Figure 4.12.A). In real terms, the back transformed predicted mean income lost from insect damage was £1367/ha at 52.5m before dropping to £262/ha at 87.5m.

At 7m away from the flowering strips, there are no significant effects or interactions present. This is reflected in the large variability present in the log predicted mean values for the income lost from insect damaged carrots (Figure 4.12.C).

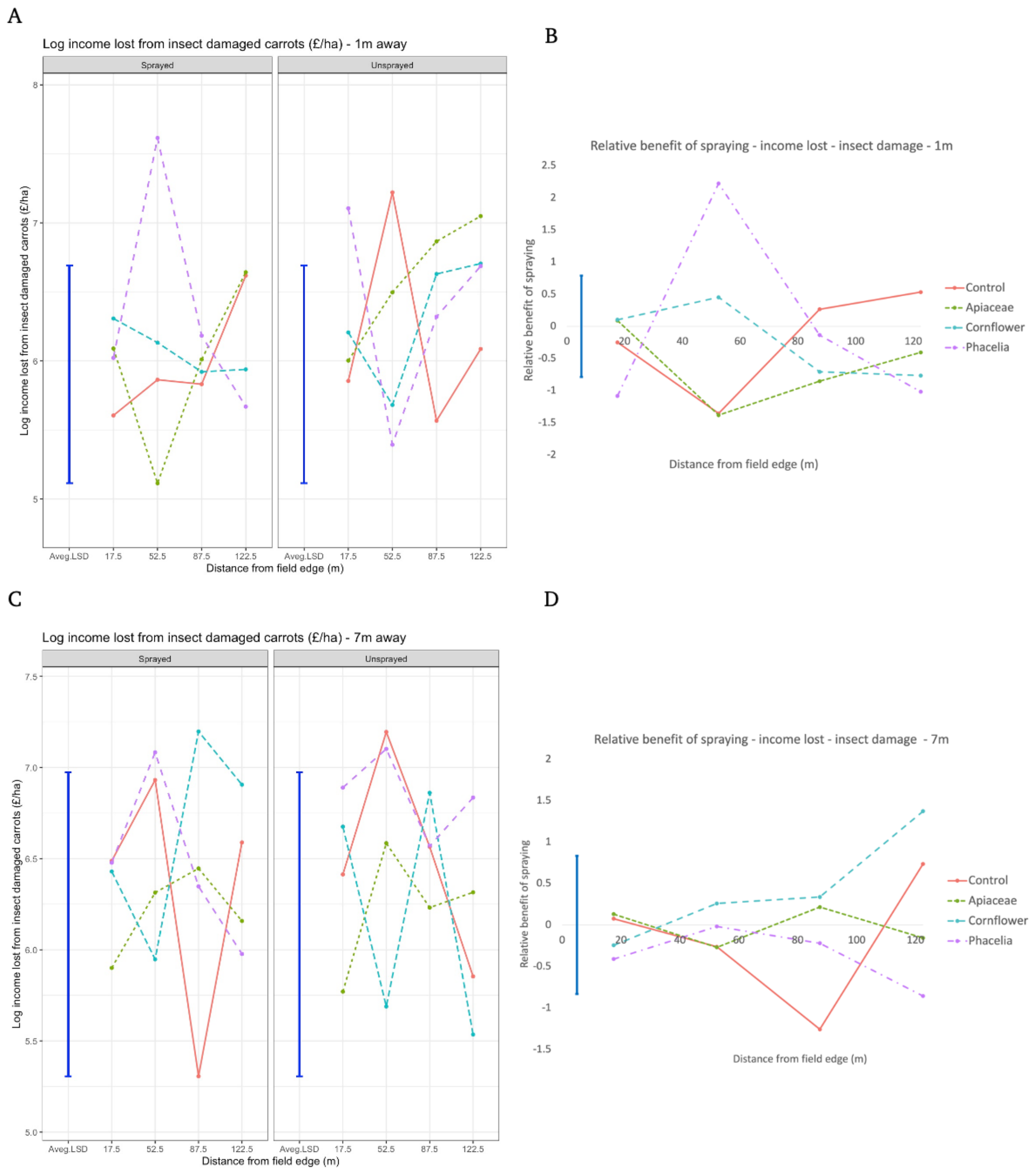

Figure 4.12. Predicted means and relative spraying benefit for the log transformed income lost from insect damage sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the income lost from insect damage sampled at 1m. Blue reference bar denotes the Average LSD= 1.56. **B** – Relative spraying benefit for percentage of insect sampled at 1m. Blue reference bar denotes the Average LSD= 1.56. **C** - Predicted means for the income lost from insect damage sampled 7m. Blue reference bar denotes the Average LSD= 1.67. **D** - Relative spraying benefit for the income lost from insect damage sampled at 1m. Blue reference bar denotes the Average LSD=1.67. Seed mix: Control= ●, Apiaceae= ●, Cornflower= ●, and Phacelia= ●.

4.4.5.2 Turnover

As turnover is the net yield multiplied by the constant price of 40p/kg of washed topped carrots, these predicted mean values shown in Figure 4.11, therefore, mirror the pattern shown in net yield figures.

At 1m away from the flowering strip, the predicted mean turnover of sprayed plots was £22,496/ha and therefore significantly higher than the predicted net yield of unsprayed plots at £19,919/ha ($F_{1,80.9}$= 9.20, $p<0.01$, Average LSD= 1691) (Figure 4.13). There was also a significant interaction between seed mix, spray regime and distance from the edge of the field upon turnover ($F_{9, 80.0}$= 3.44, $p<0.01$) (Figure 4.13). Again, there was the notable edge effect for sprayed control plots, which was not seen for unsprayed plots and the higher turnover at the first distance into the field of the plots next to flowering mixes compared with control plots (Figure 4.13).

At 7m away from the flowering strip, mirroring net yield, there was marginally a significant effect of seed mix upon turnover ($F_{3,82.7}$=5.65, p=0.071). There was also a significant effect of spray regime upon turnover, whereby the predicted turnover of sprayed plots was £24,011/ha and therefore higher than the predicted turnover of unsprayed plots at £21,718/ha ($F_{1,81.6}$= 5.64, $p<0.05$, Average LSD=1922) (Figure 4.13). There was also a significant edge effect across all plots, whereby predicted turnover rises with increasing distance into the crop interior ($F_{9,83.0}$= 3.00, $p<0.05$) (Figure 4.13). There was also a significant interaction between seed mix, spray regime and distance from the edge of the field ($F_{9,73.3}$= 2.04, $p<0.05$) (Figure 4.13). The common trend of an edge effect for sprayed control plots was seen here at 7m, although the jump from the turnover £22,969/ha at 87.5m to £26,894/ha at 122.5m wasn’t significant (Average LSD=8447) (Figure 4.13). Here at 7m, on both sprayed and unsprayed sides there was considerable variation between and within seed mixes, notably at 122.5m the Cornflower plots had a significantly higher turnover than other seed mixes (Figure 4.13).

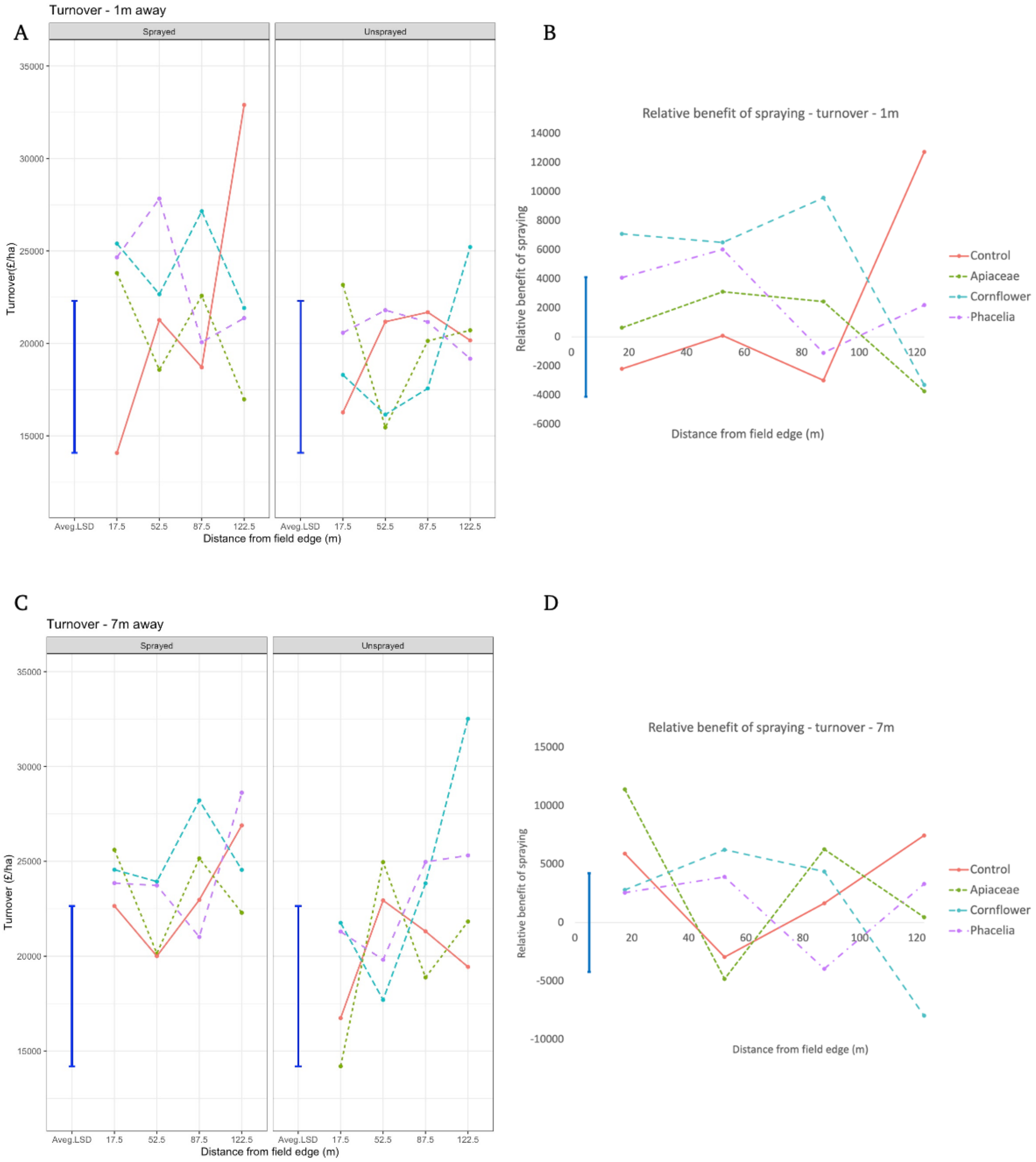

Figure 4.13. Predicted means and relative spraying benefit for the turnover sampled at 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the turnover sampled at 1m. Blue reference bar denotes the Average LSD=8218. **B** – Relative spraying benefit for turnover sampled at 1m. Blue reference bar denotes the Average LSD=8218. **C** - Predicted means for the turnover sampled 7m. Blue reference bar denotes the Average LSD=8447. **D** - Relative spraying benefit for turnover sampled at 1m. Blue reference bar denotes the Average LSD=8447. Seed mix: Control= ●, Apiaceae= ● , Cornflower= ●, and Phacelia= ● .

4.4.6 Commercial implications of flower strips

4.4.5.3 Combined costs from income lost from and transportation of insect damaged carrots, insecticide applications, flowering mix seed costs and income forgone from the area taken out

For the first time, at 1m from the flowering strips, the log transformed predicted means for the combine costs differed between seed mixes ($F_{3,93}$= 3.39, $p<0.05$) (Figure 4.14). The driver behind significant differences between seed mixes is the unsprayed control plots, which obviously incur reduced costs through income forgone through the land take out of production and no insecticide application costs. There was also a significant effect of spray regime upon combined costs ($F_{1,93}$= 34.0, $p<0.001$), where in real terms, the back transformed predicted costs of sprayed plots were £943/ha, significantly higher than the unsprayed plots at £437/ha (Figure 4.14).

Finally, with increasing distance into the field, the combined costs of the strips increased significantly from £937/ha at the field edge (17.5m) to £1550/ha at the field interior (122.5m) ($F_{1,93}$= 9.99, $p<0.001$) (Figure 4.14). Notably, given the relatively small value of income forgone due to land taken out, the sprayed control plots did not incur significantly lower costs than the sprayed seed mix alternatives.

At 7m from the flowering strips, was no significant effect of seed mix or distance from the field edge upon log transformed predicted mean combined costs, although the significant effect of spray regime remained. At 7m away, the back transformed combined costs of sprayed plots were significantly higher at £1044/ha compared with the unsprayed plots at £689/ha ($F_{1,74.1}$= 37.2, $p<0.001$) (Figure 4.14).

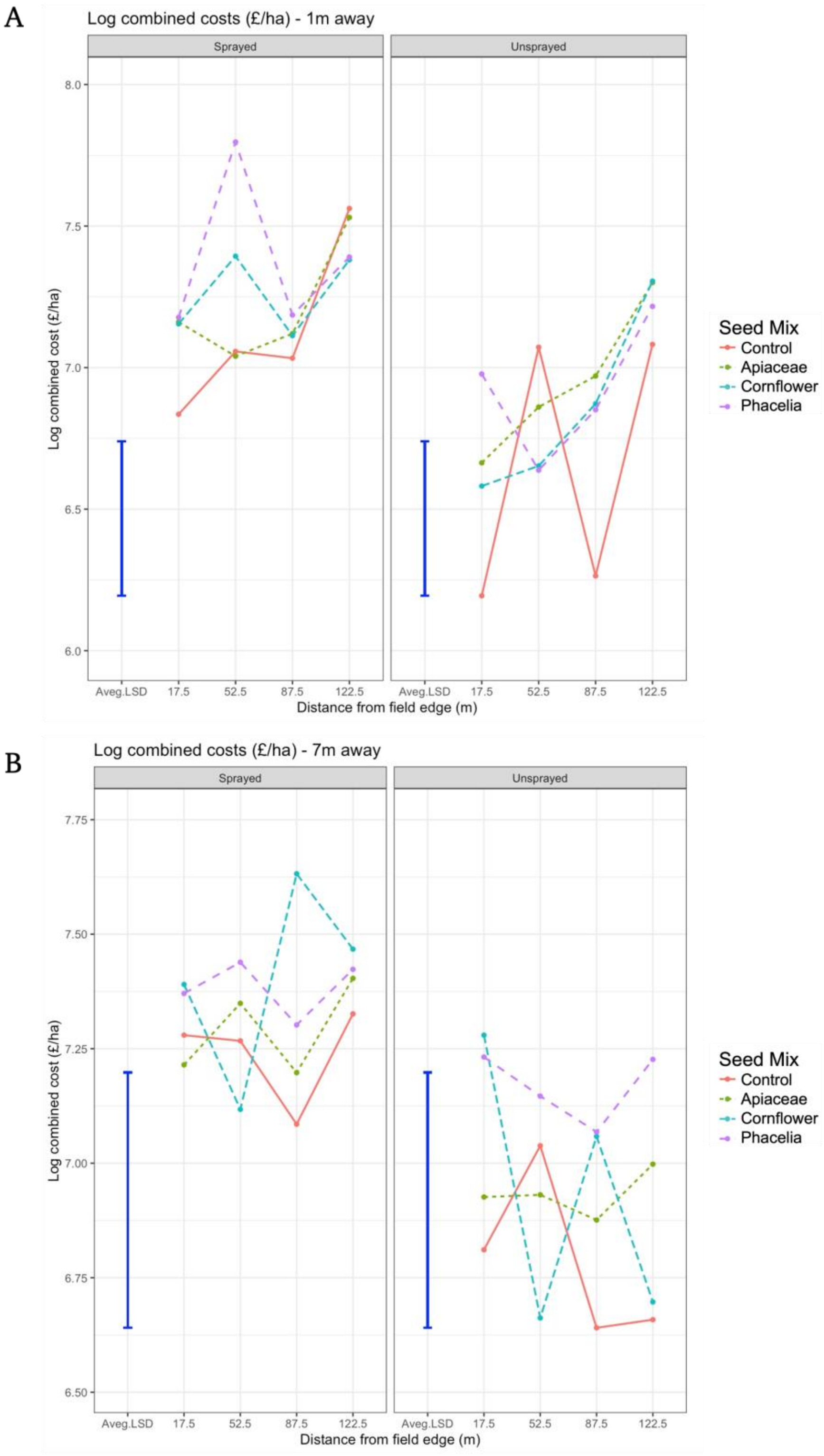

Figure 4.14. Predicted means for the log transformed combined costs from income lost and transportation of insect damaged carrots, insecticide applications, flowering mix seed costs and income forgone from the area taken out for samples 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the combined costs sampled at 1m. Blue reference bar denotes the Average LSD= 1.04. **B** – Predicted means for the combined costs sampled 7m. Blue reference bar denotes the Average LSD= 1.01. Seed mix: Control= —●—, Apiaceae= -●- , Cornflower= -●-, and Phacelia= —●

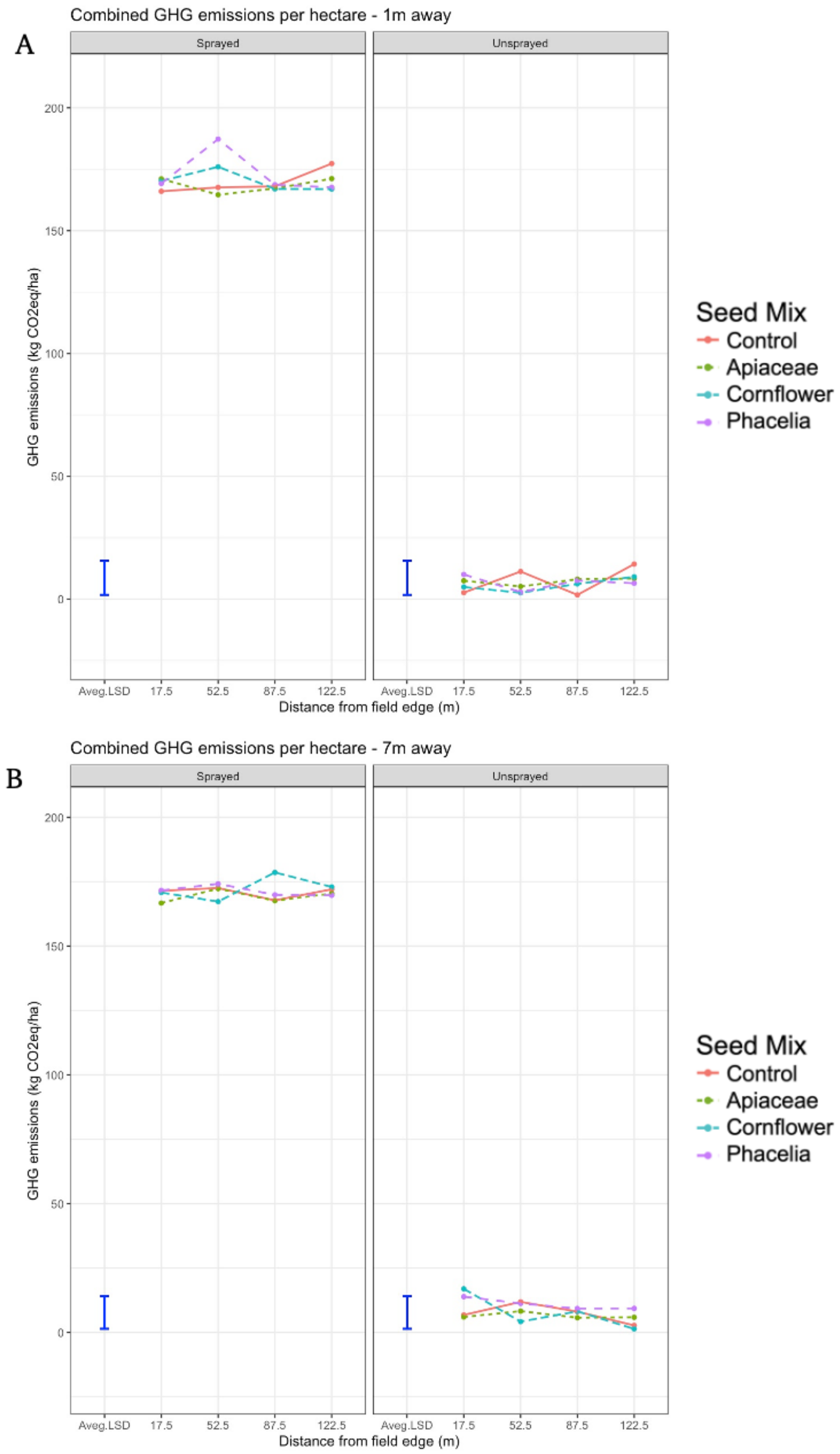

Figure 4.15. Predicted means for the combined greenhouse gas emissions from insecticide applications and transportation of insect damaged carrots sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the combined GHG sampled at 1m. Blue reference bar denotes the Average LSD= 13.4. **B** – Relative spraying benefit for combined costs sampled at 1m. Blue reference bar denotes the Average LSD= 12.6. Seed mix: Control= ●, Apiaceae= ●, Cornflower= ●, and Phacelia= ●.

4.3.5.4 Combined greenhouse gas emissions from insecticide applications and transportation of insect damaged carrots

At 1m away from the flowering strip, there was a significant effect of spray regime upon the combined GHG emissions per hectare ($F_{1,94}$= 8622, *p*<0.001) (Figure 4.15). Here, the predicted mean GHG emissions for the sprayed plots were 170 kgCO_2eq/ha which was significantly higher than 6.81 kgCO_2eq/ha (Figure 4..A). The influence of spray regime is seen again in samples taken at 7m away from the flowering strip. The predicted mean GHG emissions for the sprayed plots were 171 kgCO_2eq/ha which was significantly higher than 8.08 kgCO_2eq/ha ($F_{1,73.9}$= 17907, *p*<0.001) (Figure 4.15.B).

4.3.5.5 Impact of flower strips upon total productivity per hectare

The inclusion of flower strips into the carrot fields studied here can result in a net gain in productivity compared with 'business-as-usual' without strips. However, this is not uniformly seen throughout the field or across spray regimes, as there are instances where the productivity of a hectare would be higher without including flower strips (Table 4.25). However, when sprayed, at 1m there is a strong indication that flower mixes at the edge of the field the inclusion of the flower strips always resulted in a net gain of productivity (Table 4.25). The unsprayed carrots did not see such a large benefit as the sprayed plots from flower strips, suggesting they may be some additive role of pesticides and flower strips. At 7m away from the flower strip, there were less pronounced yield differences associated with flowering strips with increasing distance into the field.

Table 4.25. Predicted control net yields and flowering net yields, along with the yield lost assuming a 200m^2 area is removed for a flower strip, with increasing distance into the field from the edge and for two distances from the flowering strip for sprayed and unsprayed areas. The final two columns show in green when the net yield of carrots adjacent to flowering strips exceeds the control 'business-as-usual' net yield including the yield lost from flower strips indicating a net gain in productivity. When in highlighted blue, the net yield of the control is higher than flower strips and there would be a net loss of productivity. 1m Average LSD= 15.9. 7m Average LSD= 17.9.

		Control Net Yield (t/ha)		Flowering Net Yield (t/ha)			Flowering - Control Net Yield Difference (Inc. yield lost)	
Distance from flower strip	Distance from field edge (m)	Sprayed	Unsprayed	Sprayed	Unsprayed	Yield lost from 200m^2 flower strips (t/ha)	Sprayed	Unsprayed
1m away	17.5	38.0	42.5	60.9	51.1	0.76	22.1	7.78
	52.5	52.5	52.3	57.8	44.7	1.05	4.20	-8.61
	87.5	51.6	59.1	56.3	48.9	1.03	3.66	-11.2
	122.5	79.8	48.0	50.9	57.0	1.60	-30.5	7.40
7m away	17.5	57.9	43.1	61.6	47.6	1.16	2.58	3.38
	52.5	49.5	56.8	56.7	52.2	0.99	6.24	-5.56
	87.5	54.2	50.0	61.1	57.7	1.08	5.84	6.62
	122.5	68.5	49.9	61.3	64.6	1.37	-8.56	13.4

4.5 Discussion

There are indications that flower mixes can be tailored to support biological control and insect conservation, with the Cornflower and, to a lesser extent, the Phacelia mix performing well as assessed by their aerial insect communities. However, there was limited direct evidence for the delivery of pest control services as measured by aphid abundances, sentinel cards, virus damaged carrots, and insect-damaged carrots. A lack of direct evidence prevented the analysis of how seed mix, spray regime, distance from the field edge and spill-over from the flower strips influences direct ESD. However, the design of this trial has elucidated indirect evidence for the positive ecosystem service delivery that flower strips may provide. The net yield of carrots adjacent to all seed mixes was significantly higher than a 'business-as-usual' approach due to a prominent edge effect for control plots. Critically, this benefit was limited to the field edge. Moreover, positive impacts were most apparent at 1m next to the flowering strips, suggesting the sustainable intensification of carrot production relies upon the strength of any 'edge effect'. Together, with the increasing costs associated with flower strip inclusion further into the field, this suggests the potential for the spatial optimisation of flower strip placement to maximise ESD, depending on spray regime and edge effect. However, if located at the field edge, when sprayed, flower strips can improve carrot productivity, particularly for neighbouring carrots.

From a functional biological control perspective, the aerial insect community sampled in the cornflower mix had the significantly highest NE, total wasp and Ichneumonid wasp abundance. The strong association between Ichneumonid wasps and the Cornflower mix was also highlighted in the community composition analysis. Whilst not all Ichneumonid wasps are biological control agents, many are (Quicke, 2014). Although, it is vital to recognise that these ESPs are not static populations associated with the seed mixes. The strong performance of NE in Cornflower samples may be influenced by the high herbivore richness observed (Wilby and Orwin, 2013).

Suppose diversity is used as a proxy for insect conservation as higher insect diversity is thought improve community stability (Cadotte et al., 2012), NE diversity may lead to lower pest outbreaks (Wilby and Thomas, 2002), and community diversity can also improve resistance and response to insecticide applications (Greenop et al., 2020). In that case, the Cornflower mix appeared to perform strongly. The Cornflower mix had the highest Shannon Index and Simpson's diversity, higher bee abundance, as well as Diptera abundance and richness. The Apiaceae mix's support here was disappointing, although this may change with the inclusion of the second time point of samples. Clearly, the robustness of these conclusions would be strengthened by the second period of sampling and additional taxonomic resolution.

This work demonstrates that the inclusion of flower strips in carrot crops does not result in a measurable improvement in the delivery of pest control services into carrots seen via reduced aphid numbers in carrots adjacent to seed mixes in either year. Additionally, as the 144 foliage samples taken in August 2020 did not contain any aphids, it is challenging to conclude the efficacy of flower strips at controlling insect pests if there are no insect pests present. Whilst more sampling periods were planned, these were reduced in line with Covid-19 restrictions. It may be that more extensive trials over multiple years and regions would be required to detect the influence of seed mixes upon spatially and temporally variable pest outbreaks. Unfortunately, this was not possible despite planning a sentinel card regime with five time points for sampling due to challenges resulting from the Covid-19 pandemic. This is disappointing as arguably the most critical step in the standardised assessment of pest control delivery (McHugh et al., 2020, Greenop et al., 2019).

The seeming inability for seed mixes to improve the direct delivery of pest control delivery is not an unprecedented finding and may result from the influence of the landscape complexity and configuration around trial sites. Given that pest abundance as well as crop predators, subsequent pest control and crop damage show inconsistent responses to the landscape surrounding fields, it may be that four fields in one summer may not be sufficient to detect consistent trends (Karp et al., 2018). Additionally, it is also possible that the sown seed mixes did not provide sufficient support to natural enemies (Tscharntke et al., 2016), or were of a poor quality (Ashby, 2017).

The lack of significant difference between virus and insect-damaged carrots between sprayed and unsprayed could reflect a lack of aphids present across sites. As only 33 aphids were found in the direct aphid abundance counts, this perhaps strengthens this argument. However, it is challenging to draw robust conclusions about this given that this is only a snapshot of sampling with considerable associated limitations (Iuliano and Gratton, 2020). Alternatively, this lack of measurable impact from spraying could reflect the presence of insecticide resistance within *Cavariella aegopodii* populations (Supplementary 4.6.3). This aphid resistance to lambda-cyhalothrin has amplified Huntapac's and other growers' interest in the efficacy of flower strips at aphid control.

Notably, there was a common edge effect seen for control plots, whereby with increasing distance into the field, there were significant increases in mean carrot mass, gross yield, percentage of sellable carrots, and net yield. This edge effect was less apparent in unsprayed control plots, indicating that the benefit of spraying is greatest at the crop interior. This edge effect within sprayed control plots fits with growers' expectations and is in line with previous research (Pywell et al., 2015, Sparkes et al., 1998); therefore, this lends increased confidence that this sampling protocol is effective at detecting yield differences and trends.

However, the strength of this edge effect may dictate the bespoke and effective placement of these flower strips. Both the combined costs and total productivity impacts of flower strip inclusion are, in part, influenced by the yield and subsequent income lost from taking land out of production. Consequently, as this trial only ran for one year, the stability of this edge effect from year to year cannot be assessed. The lack of temporal replication may be a fatal flaw. Pywell et al.'s (2015) found field, crop and annual variation in this edge effect, Consequently, it is also likely spatio-temporal variability in carrot crops is present too. The scale of this edge effect will also alter the amount of land that can be taken out of production without compromising food production farm profits. More work is required to help growers optimise the placement of these strips.

As there were no significant changes in pest damage with increasing distance into the field, explaining why flower strips can increase yield at the field edge is challenging. One likely scenario is that there may have been poor sprayer application at the field edge. However, as each seed mix had an equal opportunity to be located at the field edge, this should reduce the effects of this bias. However, there may be indirect causes, such as the asymptomatic presence of viruses suppressing carrot yield (Collier et al., 2016). Of particular interest is investigating the presence of a suite of carrot viruses vectored by aphids, which hopefully will be answered following the processing and analysis of carrot foliage samples taken during this trial. It could also be that given the height of the flower strips, they may act as windbreaks or alter the microclimate, thereby influencing pest and pathogen distribution (Nguyen and Nansen, 2018) or additional soil compaction, weed pressure or shading factors (Pywell et al., 2015).

Work on in-field strips typically focuses on the spill-over effects away from the strip into the crop (Boetzl et al., 2020, Collins et al., 2002, Hatt et al., 2017, Juventia et al., 2021). This trial design contained a novel element by including distance from the edge of the field as a fixed effect. This, undoubtedly, added considerable complexity to the interpretation and communication of results. However, it would appear that seed mixes are not effective without this design element, as demonstrated by the lack of significant two-way interactions between spray regime and seed mix.

Whilst in-field harvest metrics demonstrated a potentially positive effect from the inclusion of flower strips at the field edge when the combined economic costs of these strips are evaluated, strips further into the field became more expensive. Indeed, the economic costs considered here may not fully capture the full financial picture incurred by implementing these flower strips, such as the time taken to establish and manage strips. Perhaps without overwhelming financial evidence demonstrating that flower strips outperform a business-as-usual approach, the strips will not be seen as an attractive option. While there is debate about the role and importance of finance within farmer decision making (Hayden et al., 2021, Naranjo et al., 2015), within farming, there is the often-quoted adage, ‘you can’t be green without being in the black’. Maximising economic profits might not be the only financial consideration growers make, as income stability is a known factor influencing the sustainability of farm businesses (Harkness et al., 2021). Carrot growers are also reliant upon being able to fulfil supermarket contracts for their produce. The findings from this trial show that despite the significantly lower costs incurred for unsprayed control plots than both unsprayed flower strips and the sprayed control plots and no measurable financial benefit of sprays reducing insect damage, growers continue to use frequent insecticide applications. Arguably, there will not be an impetus for this system to change without changes to supermarket and consumer demands, changing insecticide use regulation, or increasing uptake of funding for supporting ecosystem services. Consequently, CBC researchers must consider not just yield but also these economic costs, as this may highlight where barriers to uptake of sustainable agricultural practices exist (Johnson et al., 2021).

When evaluating these limited economic modelling efforts, it would also be relatively easy to state the ubiquitous phrase, ‘all models are wrong, but some are useful’. Thereby followed by detailed improvements to simplified or omitted variables, such as increasing sophistication through fluctuating carrot prices, or accounting for the tolerance thresholds for certain types of pathogen damage. Naturally, these endeavours would make these economic estimations closer to Huntapac's reality. However, sometimes how a model is wrong makes it useful (Wimsatt and Wimsatt, 2007). By using standardised figures for factors like insecticide applications and carrot prices, conceivably, the underlying narrative of CBC can be more easily understood and communicated. Moreover, due to the scarcity of examples documenting the economic impacts of CBC in all crops, the influence of these novel findings may reach beyond carrots. An overwhelmingly complex economic model in a minor horticultural is unlikely to be a persuasive argument to shift farming practices.

Unsurprisingly, there was a significant reduction in GHG emissions associated with unsprayed plots. This kind of decrease in GHG emissions following the uptake of wildlife friendly practices has also been seen on Hope Farm throughout the whole crop production system, but at the cost of food energy (Field et al., 2016). In this work, as there was no loss in insect damage in unsprayed plots compared with sprayed plots, this could be interpretated as a reduction in environmental impact without food losses. However, given the apparent additive benefit of spraying and flower strips at the crop edge, there is a quantifiable trade-off between pesticide applications, yield, and GHG emissions. As societal concern around emissions is only set to grow, and growers like Huntapac seek to reach 'net-zero', this is a fruitful avenue for further work.

Acknowledgements

I am grateful to Alex Dye for the swift processing of insects in the yellow water trap samples and to Stephen Foster for carrying out aphid insecticide resistance testing. I would also like to thank Todd Jenkins, Richard Hull, Jamie Hall, Debz Wright and Mike Storkey for their assistance harvesting carrots.

4.6 Supplementary Materials

4.6.1 Aerial Insect Taxonomic Groups

Table S.4.26 The total abundance of invertebrates, their functional categories and the literature used to assign these categories.

Taxonomic Unit	Total abundance	Reference	Categories
Acalyptrate	13		Other & Diptera
Agromyzidae	135	(Staton et al., 2021)	Herbivore & Diptera
Alticini	1		Herbivore
Anthocoris spp.	32		Other/various
Anthomyiidae	27	(Staton et al., 2021)	Herbivore
Aphidius spp.	4		NE & Wasp
Aphids	9	(Staton et al., 2021)	Herbivore
Apis melifera	30		Bee
Apocrita	17		Bee
Araneae	1	(Staton et al., 2021)	NE
Asteiidae	3	(Staton et al., 2021)	NE & Diptera
Athalia rosae	47	(Staton et al., 2021)	Herbivore
Bibionidae	1	(Staton et al., 2021)	Herbivore & Diptera
Bombus lapidarius	4		Bee
Bombus terrestris	1		Bee
Brachycera	2		Other & Diptera
Braconidae	20	(Staton et al., 2021)	NE & Wasp
Brassicogethese aenus	146	(Staton et al., 2021)	Herbivore
Calliphoridae	3	(Staton et al., 2021)	Other & Diptera
Camillidae	16	(Staton et al., 2021)	Other & Diptera
Carnidae	10	(Staton et al., 2021)	Other & Diptera
Cavariella spp.	2		Herbivore PEST
Cecidomyiidae	4	(Staton et al., 2021)	Herbivore & Diptera
Ceraphronidae	7		NE & Wasp
Ceutorhynchus spp.	1		Other/various
Chalcididae	6		NE & Wasp
Chalcidoidea	1	(Staton et al., 2021)	NE & Wasp
Chamaemyiidae	1		Other & Diptera
Chironomidae	1	(Staton et al., 2021)	Other & Diptera
Chloropidae	23	(Staton et al., 2021)	Herbivore & Diptera
Chrysidoidea	1	(Staton et al., 2021)	Herbivoreivore & Wasp
Chrysomelidae	1	(Staton et al., 2021)	Herbivore

Coccinella septempunctata	1	(Staton et al., 2021)	NE
Coleoptera	2		Other/various
Cryptophagidae	1	(Staton et al., 2021)	Other/various
Cynipoidea	5	(Staton et al., 2021)	Herbivoreivore & Wasp
Diapriidae	6		Wasp
Diastatidae	2	(Staton et al., 2021)	Other & Diptera
Dolichopodidae	7	(Staton et al., 2021)	NE & Diptera
Drosophilidae	1	(Staton et al., 2021)	Other & Diptera
Empidoidea	35	(Staton et al., 2021)	NE & Diptera
Ephydridae	23	(Staton et al., 2021)	Herbivoreivore & Diptera
Eucoilidae	1		Other & Diptera
Eupeodes spp.	1	(Staton et al., 2021)	NE & Diptera
Fanniidae	71	(Staton et al., 2021)	Other & Diptera
Heleomyzidae	1	(Staton et al., 2021)	Other & Diptera
Hemiptera	1		Other/various
Ichneumonidae	68	(Staton et al., 2021)	NE (& Wasp)
Lepidoptera	1	(Staton et al., 2021)	Herbivore
Liopteridae	3		Wasps
Lonchaeidae	1		Other/various
Lonchopteridae	6	(Staton et al., 2021)	Other & Diptera
Lucilia spp.	1		Other & Diptera
Lygus pratensis	18		Herbivore
Lygus spp.	2		Herbivore
Megaspilidae	11		NE & Wasp
Miridae	3		Herbivore
Muscidae	268		Other & Diptera
Mymaridae	2		Herbivore
Nematocera	137		Other & Diptera
Nephrotoma spp.	1		Other & Diptera
Neuroptera	1	(Staton et al., 2021)	NE
Odiniidae	2	(Staton et al., 2021)	Other & Diptera
Pallopteridae	2		Other & Diptera
Parasitica	1	(Staton et al., 2021)	NE & Wasp
Phoridae	30	(Staton et al., 2021)	Other & Diptera
Phylini	6		Other/various
Phyllotreta spp	1		Other/various
Platycheirus spp.	1	(Staton et al., 2021)	NE & Diptera
Platygastridae	3		NE & Wasp
Pollenia rudis	1		Other/various
Proctotrupidae	3		Other & Diptera
Psocoptera	5	(Staton et al., 2021)	Other/various

Psyllidae	1	(Staton et al., 2021)	Herbivore
Pteromalidae	4		NE & Wasp
Pterostichus spp.	1	(Staton et al., 2021)	NE
Rhagio tringarius	2		Other & Diptera
Sarcophaga sp.	7		Other & Diptera
Sarcophagidae	3		Other & Diptera
Scathophaga stercoraria	2	(Staton et al., 2021)	Other & Diptera
Scelionidae	1		NE & Wasp
Sciaridae	18	(Staton et al., 2021)	Herbivore & Diptera
Sepsidae	8	(Staton et al., 2021)	Other & Diptera
Sitobion avenae	1	(Staton et al., 2021)	Herbivore
Sitonia spp.	2		Other/various
Sphecodes spp.	1		Bee
Staphylinidae	5	(Staton et al., 2021)	Other/various
Tenthredo spp.	1		Other/various
Therevidae	1		Other & Diptera
Thysanoptera	113	(Staton et al., 2021)	Herbivore
Trigonalidae	1		Wasp
Triozidae	1	(Staton et al., 2021)	Herbivore

4.6.2 Control Net Yield Predicted Mean values

Table S.4.27. Table showing the predicted mean net yield and income lost from that area used in the calculation of the income forgone from an area taken out of production. Predicted mean net yield values are from linear mixed effects models for sprayed control carrot plots.

Distance from flower strip	Distance from field edge (m)	Predicted mean net yield (t/ha)	Income forgone (£/200m^2 plot)
1m	17.5	35.2	281.60
	52.5	53.2	425.60
	87.5	46.8	374.40
	122.5	82.2	657.60
7m	17.5	59.9	479.20
	52.5	47.5	380.00
	87.5	56.3	450.40
	122.5	66.6	532.80

4.6.2 Flowering vs Non-flowering Predicted Net Yield

At 1m, there was a significant effect of spray regime ($F_{1,92.2}$= 4.4631, *p*<0.05), whereby sprayed plots had a predicted mean net yield of 56.2t/ha compared with unsprayed samples of 49.7t/ha (Average LSD= 15.9) (Figure S.4.1). There was a significant interaction between seed mix, spray regime and distance into the field ($F_{3,90.6}$= 8.1280, p<0.001). There was a notable edge effect for control plots, whereas flowering plots saw net yield decrease in samples taken further into the field, although this is just within the average LSD bounds. Importantly, the seed mix net yield is higher than control plots at the edge of the field, suggesting close to the edge of the field seed mixes are more effective. At 7m, although there is no significant effect of spray regime ($F_{1,97.9}$= 3.65, *p*=0.0591), sprayed samples marginally had higher net yields (58.8t/ha) compared with unsprayed samples (52.8t/ha). All other main effects and interactions were not significant.

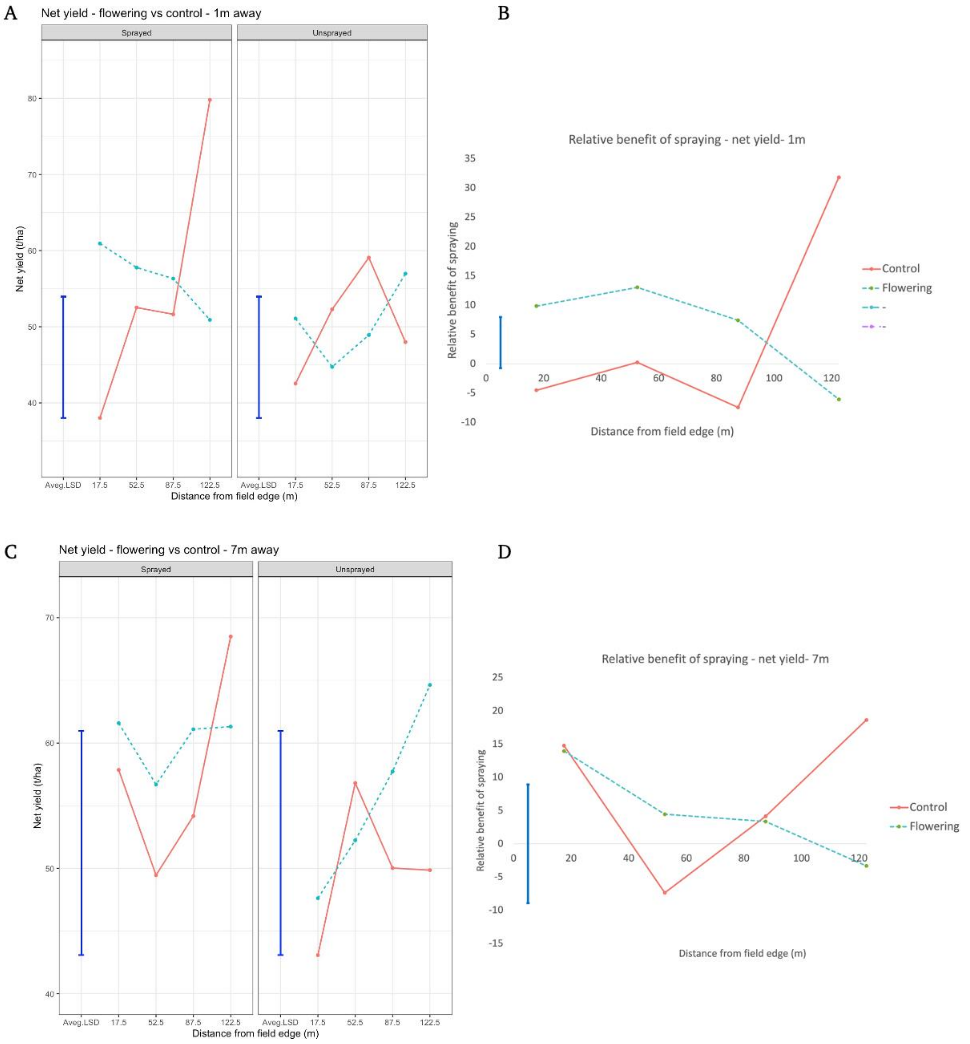

Figure S.4.1. Predicted means and relative spraying benefit for the net yield of flowering and control plots sampled 1m and 7m away from the flowering strip, for both sprayed and unsprayed plots, fitted using an LMM. **A** - Predicted means for the net yield sampled at 1m. Blue reference bar denotes the Average LSD=4.22. **B** – Relative spraying benefit for net yield sampled at 1m. Blue reference bar denotes the Average LSD=4.22. **C** - Predicted means for the net yield sampled 7m. Blue reference bar denotes the Average LSD=17.9. **D** - Relative spraying benefit for net yield sampled at 1m. Blue reference bar denotes the Average LSD=17.9. Seed mix: Control= -●-, Flowering= -●-

Table S.4.28. Cost of seed mixes planted in barley in 2018 & 2019 and in carrot fields in 2019 & 2020. Early and late flowering mixes were only sown in trials in cereals at Rothamsted in 2019, and not in carrot fields. Prices were collected in 2021. Table continues onto the next page.

		All	2018		2019		2020	
Mix Name	Species names	Seed price £/kg	30m^2 plot cost /£	Total cost per mix/£	30m^2 plot cost /£	Total cost per mix/£	35m^2 plot cost /£	Total cost per mix/£
Apiaceae	Dill – Anethum graveolens	40	0.8		0.72		0.98	
	Coriander – Coriandrum sativum	40	0.8		0.88		1.19	
	Wild carrot – *Daucus carota*	105	0.79	2.4	1.58	3.17	2.13	4.3
Phacelia	Coriander – Coriandrum sativum	40	0.8		0.88		1.61	
	Phacelia tanacetifolia	9.3	0.29		0.27		0.02	
	Mustard – Sinapsis alba	2.45	0.16	1.25	0.15	1.3	-	1.63
Cornflower	Field poppy – *Papaver rhoeas*	130	0.2		0.59		0.68	
	Cornflower - Centaurea cyanus	100	0.39		0.39		6.76	
	Crimson clover – Trifolium incarnatum	5.45	0.27	0.86	0.27	1.25	0.32	7.76

Mix Name	Species names	All Seed price £/kg	2018 30m^2 plot cost /£	Total cost per mix/£	2019 30m^2 plot cost /£	Total cost per mix/£
Buckwheat	Buckwheat – Fagopyrum esculentum	2.7	0.41		0.36	
	Corn chamomile – *Anthemis arvensis*	85	0.13		0.13	
	Corn cockle – *Agrostemma*	40	0.16	0.69	0.48	0.97
Grass	Westerwold ryegrass – Lolium multiflorum west.	2.85	0.35		0.26	
	Italian ryegrass – *Lolium multiforum*	3	0.34		0.25	
	White millet – Panicum miliaceu	2.85	0.23	0.91	0.29	0.8
‘Early’ Flowering	Corn marigold – Chrysanthemum segetum	120	-		9	
	Persian clover – Trifolium respinatum	7.3	-		0.25	
	Mustard – Sinapsis alba	2.45	-		0.15	9.4
‘Late’ flowering	Linseed – Linum usitatissimum	4.1	-		0.62	
	Forage rape – *Brassica napus*	3.75	-		0.13	
	Birdsfoot trefoil – *Lotus corniculatus*	13.5	-		0.34	1.08

4.6.3 Insecticide resistance testing of *Cavariella aegopodii* aphids from carrot fields

Adult Cavariella aegopodii aphids were collected from a commercial carrot crop on the 1/7/2021 (Shropshire, UK, 52.626179, -2.327338). This site was on the same farm as one of the trial sites from 2020 and is an area with a known high pest pressure. Multiple attempts were made in 2020 to collect aphids from trial sites but due to challenges with the Covid-19 pandemic and high parasitism of aphids, resistance testing was not possible for these samples.

On the 2/7/2021, resistance testing was conducted to identify the tolerance of Cavariella aegopodii to lambda-cyhalothrin pyrethroid. This was conducted via a standard insecticide-coated glass vial assay, where 7 different concentrations of insecticide were used (0.1 ng λ-cyhalothrin /cm2, 0.6 ng λ-cyhalothrin /cm2, 3 ng λ-cyhalothrin /cm2, 15 ng λ-cyhalothrin/cm2, 75 ng λ-cyhalothrin/cm2, 150 ng λ-cyhalothrin/cm2 (the field rate) and 480 ng λ-cyhalothrin/cm2, equivalent to 0.6%, 0.4%, 2%, 10%, 50%, 100% and 320% of the recommended UK field rate, respectively). Glass vials treated only with acetone were used as a control.

Vials were prepared by adding λ-cyhalothrin in 500 ul of acetone to each glass vial and then rotating vials on a roller for 2 hours before storing them upright in a fridge at 4oC for a further 24 hours and then sealing vials with plastic screw caps. Aphids were then added into vials with a fine paintbrush and scored for their response after 5 h, with the vials standing vertically. Aphids were scored as affected or dead as per the method used by Walsh et. al (2020). The environmental conditions whilst the tests were conducted were 20oC with 50% humidity and constant light.

The resulting percentage mortality for each lambda-cyhalothrin concentration, calculated via the summed dead and affected aphids, were analysed via a probit analysis creating a dose-response curve seen below in Figure S.4.2. Previous C. aegopodii samples from 2018 and 2019 taken from around the UK are shown in red and samples from this field are shown in blue. Although there is variation in the samples taken in 2021, there is still a clear indication of resistance to lambda-cyhalothrin. At the field rate, of 150 ng λ-cyhalothrin/cm2, only 30% of aphids were dead or affected after 5 hours exposure. This fits with the evidence for insecticide resistance from previous C. aegopodii samples from 2018 and 2019. Together the conclusion drawn from this is that lambda-cyhalothrin resistance is widespread in C. aegopodii across the UK.

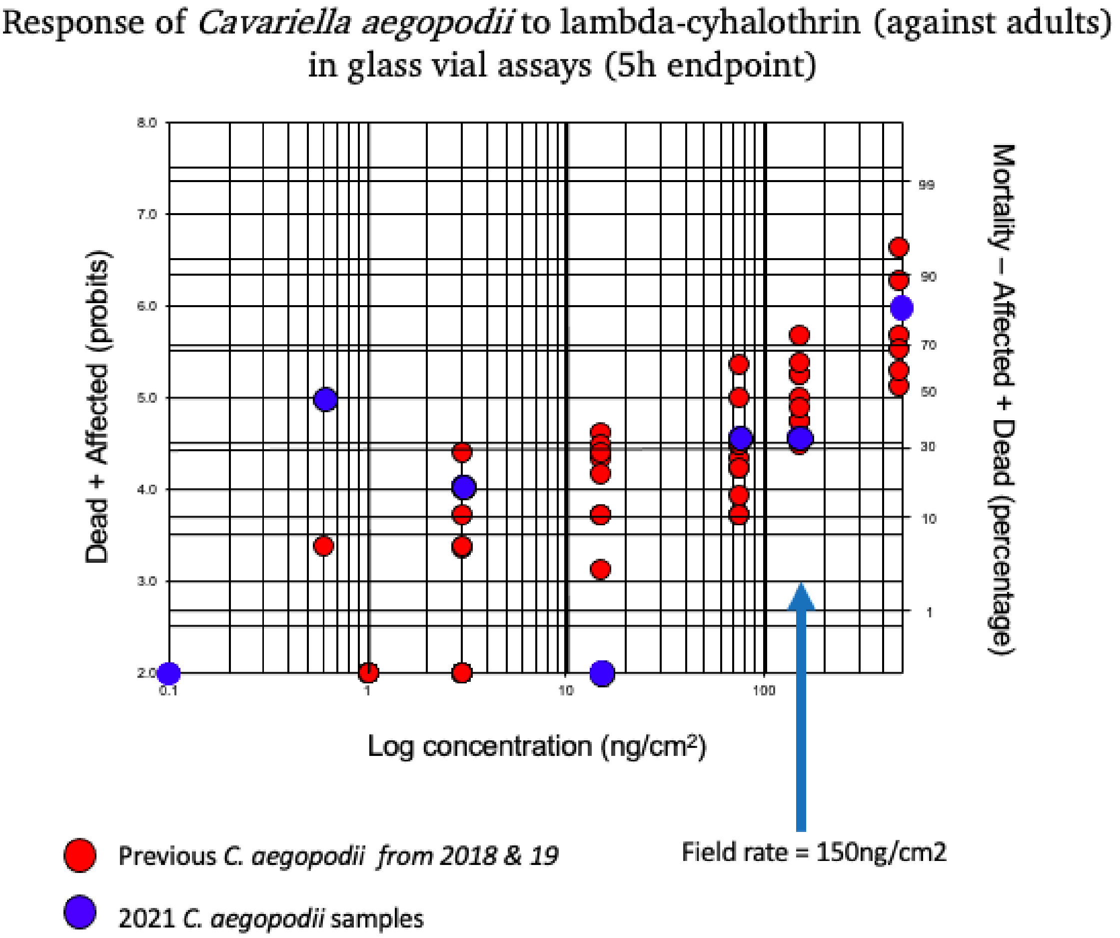

Figure S.4.2. Response of adult Cavariella aegopodii to lambda-cyhalothrin in glass vial assays after 5 hours exposure.

4.7 References

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Chapter 5 – Assessing flower visitors to seed mixes.

5.1 Abstract

Given the scarcity of floral resources across farmed landscapes, many growers wish to help provide increased support for pollinating insects and other flower visitors such as butterflies. Whilst flower strips can be included for pest control delivery, they also provide simultaneous support for flower visitors. Sown flower strips are commonly included in agri-environment schemes to support these insects. Plant-pollinator network analysis allowed the flower visitors of different mixes to be compared, allowing growers to make evidence-based decisions for insect conservation. Using the data collected during sampling across all three trials in this thesis, flower-insect visitor networks have been created. Results show that the Phacelia (*Phacelia tanacetifolia*, *Sinapis alba* and *Coriandum sativum*), and to a lesser extent, the Cornflower mix (*Centaurea cyanus*, *Trifolium incarnatum* and *Papaver rhoeas*) had a strong performance across a suite of relevant network properties across all three years. However, a few key visitors dominated the Phacelia mix networks (*Apis mellifera* and *Bombus terrestris*), whereas there was a higher species richness of visitors to the Cornflower mix. This work reveals that flower-insect visitor networks can be useful to aid growers, and researchers, to select species to include in multi-functional flower strips.

5.2 Introduction

There are widely acknowledged changes in insect populations across many taxa (Bell et al., 2020, Brooks et al., 2012, Clausnitzer et al., 2009, IPBES, 2016, Potts et al., 2010, Sánchez-Bayo and Wyckhuys, 2019). Drivers of change within pollinator populations have received focus due to our reliance upon pollination for over 75% of leading food crop types, the pollination of wild plant fauna underpinning biodiversity, and broader cultural value that we derive from insects (IPBES, 2016, Potts et al., 2016). Potts et al.'s review of this area reveals how land-use change, including habitat loss, fragmentation, and degradation alongside alien species, including pests and pathogens, and climate change are critical drivers of population changes that can also act synergistically (2010). The impact of land use is multifaceted. Land management can positively impact pollinator populations through direct provision of forage, nesting, reproductive and shelter resources. But also have an indirect negative effect, resulting from pesticide use or land-use change contributing to climate change (Senapathi et al., 2017). Across all global regions, Dicks et al. (2021) have concluded that there is well established and robust evidence regarding the role land management, land cover, and land configuration have as the most important drivers of pollinator declines. This is followed then by pesticide use and climate change in importance (Dicks, 2021). Pests and pathogens, pollinator management, invasive species, and genetically modified crops also have varying roles across different global regions (Dicks, 2021). Although, notable knowledge gaps exist.

Given our reliance upon pollination alongside these concerns and changes within pollinator populations, a common management technique is to supplement native pollinators with managed pollinator populations such as *Apis mellifera* and some *Bombus* spp., *Osmia* spp. and *Megachile* spp. (Eeraerts et al., 2017). However, the use of managed populations can come at a cost for wild pollinators under some conditions (Mallinger et al., 2017). For example, there are numerous examples where RNA viruses can spread from managed to wild pollinators through shared flower visitation (Alger et al., 2019, Dalmon et al., 2021, Furst et al., 2014), as well as evidence for the spread of parasites such as the *Varroa* mite and the microsporidian *Nosema ceranae* from managed to wild bees (Dalmon et al., 2021, Graystock et al., 2016), and debate about the impact of floral resource competition from honeybees upon wild bees (Wojcik et al., 2018).

Given our reliance upon pollination, changes in land management and farming methods are required, and fortunately, there is strong evidence that positive changes can be taken to aid wild pollinators (IPBES, 2016). As there has been a reduction in both plant taxonomic and functional diversity in intensified agricultural landscapes (Carmona et al., 2020, King, 2011), many efforts are directed at reversing this trend. Therefore, providing abundant, diverse floral resources and niches can improve pollinator diversity and abundance (Potts et al., 2003; Bartomeus et al., 2013; Vaudo et al., 2015). In turn, with increased pollinator diversity, there can be an improved delivery of pollination ecosystem services (Woodcock et al., 2019). More specifically, diverse habitat features such as semi-natural grassland and woodland areas can increase wild bee abundance and species richness (Mallinger et al., 2016), and field boundary features can support more stable and larger colony size in ground-nesting bumblebees (Gardner et al., 2021). Pollinators are broadly thought to respond positively and consistently to increased floral provision, especially at the local scale (Winfree et al., 2009). Notably, wild pollinators requirements for floral resources are not static within a year, and there are documented nectar availability shortages in March and late summer where common UK bumblebees may not be able to find sufficient resources (Timberlake et al., 2019).

In response to these collective findings, flower strips have been suggested as an approach that can increase the provision of floral nectar and pollen resources to pollinators throughout the flight season (Haaland et al., 2011). It is imperative to assess whether flower strips can successfully support pollinators as farming landscapes are highly productive land and the urgent need to support pollinators.

5.2.1 Plant-pollinator network metrics

One useful method to assess the ability of a floral community to support pollinators is the analysis of the architectural structure of plant-pollinator community networks. A network can be considered an often visual representation of the non-random interactions between two or more actors in a community. With networks, it is possible to quantify the frequency of interactions, which in the context of plants and pollinators typically represent a pollinator visiting a flower. Arguably, two central tenets one must be mindful of during the analysis of plant-pollinator networks. First, an insect visit to a flower does not automatically equal pollination, and second, not all flower visits are equal in their effectiveness as pollination vectors (Alarcon, 2010, Ballantyne et al., 2015, Willcox et al., 2017). In the context of this thesis, these concerns are relatively easy to address as it is sufficient to just consider flower visitation here, rather than measuring the effectiveness of an insect's visit resulting in pollen transfer and successful pollination. The mixes sown here are annual mixes which are not reliant upon effective pollination for their inclusion in margins in future years. Whereas, for perennial mixes that will persist for multiple years, it may be necessary to assess the effectiveness of flower visitors at pollination and subsequent seed set and reproductive success (Albrecht et al., 2007). Whilst carrot seed production relies upon pollination (Davidson et al., 2010), carrot roots are harvested a few months after sowing in the first year of the *Daucus carota* lifecycle before flowering in the second year. Therefore, from the perspective of a carrot grower, pollinators are not required to increase the yield of their crop, unlike many other crops (Garratt et al., 2014, Garratt et al., 2018).

The plant-pollinator networks arising from the insect visitors to sown flower mixes can subsequently be analysed to calculate metrics that provide insights into inherent properties or allow speculation about underlying complex ecological interactions and their drivers. These metrics can then allow more focused management of habitats for pollinators (Walton et al., 2021). There has been a focus on network complexity which is purported to ensure community resilience to disturbance. This focus on complexity and network properties has enabled the elucidation invasive plant species impact upon pollinators (Russo et al., 2019, Vila et al., 2009), highlighted the consequences of plant extinctions (Goldstein and Zych, 2016), allowed the investigation of the stability of pollinator communities to habitat fragmentation (Grass et al., 2018), and the increasing generality of pollinators with increasing agricultural land cover (Redhead et al., 2018). Common to this work is the use of the metrics; connectance, links per species, nestedness, weighted NODF (nestedness metric based on overlap and decreasing fill), and complementary specialisation (H2').

The **connectance** of a network reveals the "proportion of realized interactions out of total number of possible interactions" (Russo and Shea, 2017). With a high connectance and subsequently high number of potential interactions seen, typically thought to indicate a high generalisation (Bluthgen, 2010, Bosch et al., 2009). Often, connectivity indicates community complexity and subsequent robustness and stability of a community to change (Russo and Shea, 2017, Thebault and Fontaine, 2010). However, one must be mindful of the susceptibility of connectance to limited sampling effort, whereby connectance may be overestimated if rarer species interactions are not observed (Bluthgen, 2010). The distribution of this connectivity is elucidated by the **links per species** (Bosch et al., 2009), although this can also be liable to sampling bias (Bluthgen, 2010).

The concept of **nestedness** can be assessed via multiple metrics. Although a complex and much-debated concept, in its simplest form, nestedness can be considered as when specialist species mainly interact with generalist species, with high values indicating (Bluthgen, 2010). It has also been suggested that nestedness is another feature of a network that may confer some beneficial property to mutualistic networks (Bastolla et al., 2009). When real-life mutualistic networks have been studied, a positive relationship between increasing nestedness and increasing biodiversity has been observed (Bastolla et al., 2009). Additionally, many have proposed that nestedness demonstrates a network's ability to buffer any extinction cascade following the extinction of a species (Memmott et al., 2004, Thebault and Fontaine, 2010). However, this finding is not universally accepted, nor can it be equivocally applied to all scenarios (Burgos et al., 2007). As true 'specialist'-'specialist' interactions are extremely rare, there are also concerns that any nestedness present may simply be an artefact of insufficient sampling intensity or an analysis bias (Bluthgen, 2010). Given the notable criticisms of this consideration of nestedness (Almeida-Neto et al., 2008), **weighted NODF** (nestedness metric based on overlap and decreasing fill) has been proposed as an alternative.

Complementary specialisation (H2') is a network-level assessment that describes the deviation from a neutral network with no specialisation, thereby helping to remove bias from observation frequency (Bluthgen, 2010). When H2' is high (maximum of 1), there is high specialisation, and therefore niche differentiation, a posited optimal feature for plant-pollinator networks (Bluthgen and Klein, 2011). Although, one must be careful not to underestimate the stabilising role that a 'functional redundant' plant or pollinator species may play (Bluthgen and Klein, 2011). Despite this evident controversy within the literature, network analysis can be a useful tool to at least visually present insect-flower visitation and can allow communities to be compared if the interpretation is made carefully with these discussed limitations in mind.

5.2.2 Multifunctional, stackable benefits from flower strips

Beyond CBC and pollination, these flower strips can also provide support small mammals and their subsequent owl predators (Shields et al., 2019), other farmland birds (Thomas et al., 2001), soil erosion (Ali and Reineking, 2016), water run-off, wider rural aesthetic enhancement (Wratten et al., 2012) and improved consumer perception. This latter factor is an important consideration for growers. For instance, the deep-seated and divisive debate about neonicotinoid insecticide use was recently reignited following an application for a short-term exemption for sugar beet crops (Busby, 2021). As previously alluded to, whilst many crops are dependent on pollination for yield, carrot growers are not directly reliant upon pollination to enhance the yield of their crop. Nonetheless, carrot fields will be surrounded in the landscape by pollinator dependent crops which may benefit from a general enhancement of pollinator communities. Additionally, Huntapac are keen to explore the possibility of using wildflowers in their carrot crops to support pollinators as part of their corporate social responsibility efforts.

Consequently, given the urgent need to re-diversify agricultural landscapes, alongside the demands placed upon land for food production, it is necessary to consider how the benefits from flower strips can be optimised. Whilst the potential of these sown mixes to enhance pest control have been considered in previous chapters, it is also possible to quantify the support these mixes also provide to pollinators and other elements of wider biodiversity.

5.2.3 Research Questions

The aim of this chapter is to identify if there is an optimal mix for pollinators based upon flower-insect timed counts and the subsequent analysis of the resultant plant-insect network metrics.

The optimal mix can be selected based upon the suite of metrics used as it is hypothesised that these network metrics will significantly differ for each flower mix.

Do different seed mixes provide different support to pollinators as assessed by a suite of network metrics?

5.3 Materials and Methods

5.3.1 Study Sites

Each three study sites have been outlined in Chapter 2 (Rothamsted Farm 2019), Chapter 3 (single field carrot trial 2019) and Chapter 4 (multi-field carrot trial 2020).

5.3.2 Sampling

The sampling method utilised was loosely based upon that of the UK Pollinator Monitoring Scheme's so called 'Flower-Insect Timed' Counts (FIT counts) (UKPoMS, 2020). Sampling only occurred between 10:00 and 16:00, with the minimum weather conditions for temperature, sunshine and windspeed required for sampling as specified by the UK Butterfly Monitoring Scheme (UKBMS, 2020).

In 2019, a 0.6m^2 area of a plot was surveyed for a 15-minute period. During this time, any insect that visited a flower within the focal area was recorded. If the same insect visited multiple flowers of the same species, only one interaction was recorded. If an insect visited a different plant species, a new interaction was recorded. Although every effort was made to track insects that left and returned to the focal area, in plots with particularly high visitation this may not have been possible. The species of the insect visitor was observed and identified to the highest possible resolution. If there was any doubt over the identification of the insect, a sample would be collected and in addition, photographs were taken. Any samples were then identified to the maximum taxonomic resolution that could be confidently taken. Due to the nature of 'on-the-wing' observations, some taxonomic resolution was occasionally lost. For instance, when it was possible to make an identification to species level (e.g. *Episyrphus balteatus*) this was recorded. However, in instances where the taxonomic detail required to take the insect to species was not possible the highest resolution was recorded (e.g. Syrphidae). There were instances when this was not possible, and the order of many flower visitors was recorded (e.g. Diptera). These broader categories therefore may contain multiple species. Therefore, these categories may contain flower visitors which elsewhere have been identified to species level. In this context, richness therefore does not refer to a strict ecological species richness but rather the number of categories that flower visitors that were recorded. This will therefore be an underestimation of the true species richness of insect visitors.

In 2020, sampling time was shortened to 10-minute periods, in line with the UK pollinator monitoring scheme (UKPoMS, 2020). The focal area sampled changed each time but focused on the centre of plots or strips to reduce any 'spill over' effects from neighbouring mixes.

In 2019 on Rothamsted farm, with the first sampling occurring on 16/06/2019 and concluded on 23/08/2019. The sampling in a carrot field in 2019 began on 20/06/2019 and concluded on 11/08/2019. Sampling in 2020's multiple carrot fields began on 12/06/2020 and concluded on 12/08/2020.

5.3.4 Statistical Analyses

The following data has been analysed for each field trial separately. To identify any differences between the insect community visiting focal areas, the plant-pollinator networks for each plot have been studied. Analyses were conducted in R version 1.1.456 and quantitative network analysis was conducted using ‘Bipartite’ version 2.16 (Dormann et al., 2021).

Network properties were calculated at the network level. Specifically, connectance, links per species, web asymmetry, Shannon diversity index, weighted NODF, H2’ (complementarity specialisation), nestedness, interaction strength asymmetry (ISA), and specialisation asymmetry (SA). These metrics were calculated for each seed mix plot or strip in each replicate, therefore providing network metrics for each plot that can be used as replicates. For sampling in carrots in 2020, to overcome challenges throughout the Covid-19 pandemic, sampling was reduced. Therefore, networks were created for each field rather than each replicate.

To assess for an effect of seed mix upon these network properties LMMs were used, each weighted with the number of sampling occasions. For Rothamsted 2019, the fixed effect was seed mix treatment, and random effects were aspect nested within field. In Carrots 2019, the fixed effect used was seed mix treatment and the random effects were replicate nested within strip. In carrots in 2020, fixed effect used was seed mix treatment and the random effect was field. In each case, residual plots were all examined to ensure data met the assumptions of tests used. To present networks, the summed abundance of visits across the whole season have been collated for each seed mix. Whereby the size of each box at either plant or insect level represents visitor abundance and the width of links indicates interaction frequency.

5.3 Results

5.3.1 Rothamsted Farm 2019 Plant-Insect Network Analysis

Across the summer and the 118 flower-insect timed counts on Rothamsted Farm, 7332 insect visitors were recorded across all plots (Table 5.1). The Early-flowering mix had the highest abundance of insect visitors, but this was predominantly pollen beetle (*Meligethes* spp.). This mix also had the highest species richness with 19 different morpho-species recorded visiting (Table 5.1). The Cornflower and Phacelia mixes were the most visited by bees, although these were mainly *Apis mellifera* and *Bombus terrestris* (Table 5.1). The Late-flowering mix had the lowest recorded total insect visitor abundance and species richness (Table 5.1).

For four community level metrics calculated from the plant-pollinator networks, there were significant differences between seed mixes (Table 5.2). The only two metrics that did not yield significant differences were links per species and nestedness. The difference in connectance between seed mixes was driven by the high connectance and therefore generalisation of the Late-flowering mix ($F_{5,25.8} = 4.1$, $p<0.01$) (Table 5.3). Both Late and Early-flowering mixes had lower observed diversity as calculated by Shannon's index compared with the other flowering mixes ($F_{5,24.6} = 5.6$, $p<0.01$) (Table 5.3). A similar pattern was found in both the weighted NODF and H2', where the Apiaceae, Cornflower and Late-flowering mixes all had significantly higher NODF and lower H2'. Thus, suggesting that the Phacelia, Buckwheat and Early-flowering mixes had lower nestedness and greater niche differentiation.

Some of these properties are also apparent in Figure 5.1, Figure 5.2, and Figure 5.3 which represent the whole network for each seed summed across the summer. Within the Apiaceae mix, *Coriandrum sativum* supports the majority of the morphospecies visiting the mix and provides most of the support for Syrphidae (Figure 5.1.A). An element of functional redundancy appeared within the Buckwheat mix, with insect visitor like Syrphidae, Parasiticae and solitary bees visiting both *Anthemis arvensis* and *Fagopyrum esculentum* (Figure 5.1.B). The Cornflower mix was dominated by insect visitors to *Centaurea cyanus*, suggesting the flower may be a 'super-generalist' (Figure 5.2.A). Although, a high abundance of pollen beetle were present on *C. cyanus*. Similarly, the pollen beetle were prominent visitors to *Sinapis alba* in the Early-flowering mix, which could be providing an environmental disservice depending on the surrounding landscape (Figure 5.2.B). For supporting an abundance rather than range of species, *Phacelia tanacetifolia* appeared to be effective. This worked alongside *Coriandrum sativum* which had a more diverse range of visitors (Figure 5.3.A). Conversely to the Buckwheat mix, there did not appear to be niche overlap between insect visitors to the Late-flowering mix (Figure 5.3.A).

Table 5.1. The five most abundant insect visitors, total abundance, and total species richness at flower mixes across all sampling periods. Data from Rothamsted Farm 2019.

Apiaceae		Buckwheat		Cornflower		Phacelia		Early-flowering		Late-flowering	
Diptera	211	Syrphidae	151	*Meligethes* spp.	575	*Meligethes* spp.	882	*Meligethes* spp.	2029	*Parasiticae*	68
Syrphidae	109	Diptera	110	*A. mellifera*	242	B. terrestris	429	Syrphidae	153	Syrphidae	67
Cantharidae	55	Parasitica	85	B. terrestris	115	Syrphidae	190	Diptera	134	Diptera	55
E. balteatus	55	*Meligethes* spp.	59	*E. balteatus*	77	*A. mellifera*	120	*Parasiticae*	76	*Meligethes* spp.	15
Parasiticae	50	Solitary Apinae	41	*Syphidae*	69	E. balteatus	52	*A. mellifera*	69	T. sylvestris	8
Abundance	578	Abundance	590	Abundance	1333	Abundance	1892	Abundance	2696	Abundance	243
Richness	18	Richness	21	Richness	25	Richness	23	Richness	29	Richness	16

Table 5.2. Output from LMM analysis of plant-pollinator networks sampled from sown seed mixes planted on Rothamsted Farm in 2019. Significant results highlighted in bold, with significance levels indicated by: *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$.

Network metric	NumDF	DenDF	F value	Pr(>F)	Sig	Average LSD
Connectance	**5**	**25.8**	**4.10**	**0.007**	******	**0.13**
Links per species	5	24.0	1.44	0.248	NS	0.17
Shannon's Index	**5**	**25.4**	**2.78**	**0.039**	*****	**0.37**
Weighted NODF	**5**	**22.1**	**12.4**	**0.000**	*******	**9.50**
H2’	**5**	**22.9**	**22.2**	**0.000**	*******	**0.16**
Nestedness	5	21.3	1.25	0.319	NS	14.4

Table 5.3. Predicted means for various network metrics and their standard errors (SE) for each seed mix sown on Rothamsted Farm in 2019 and the group assigned to them based on their predicted means and average LSD.

Network metric	Flower mix	Mean	SE	Group
Connectance	Late-flowering	0.75	0.058	A
	Apiaceae	0.56	0.050	B
	Buckwheat	0.49	0.044	B
	Early-flowering	0.48	0.042	B
	Phacelia	0.48	0.044	B
	Cornflower	0.48	0.041	B
Shannon's index	Apiaceae	2.15	0.177	A
	Cornflower	2.00	0.154	AB
	Phacelia	1.69	0.162	BC
	Buckwheat	1.69	0.163	BC
	Early-flowering	1.64	0.158	C
	Late-flowering	1.57	0.196	C

Table 5.3 continued – predicted means from LMMs on plant-pollinator networks from Rothamsted Farm in 2019.

Network metric	Flower mix	Mean	SE	Group
Weighted NODF	Apiaceae	41.2	4.74	A
	Cornflower	36.3	4.10	A
	Late-flowering	31.6	5.61	A
	Phacelia	16.7	4.28	B
	Early-flowering	16.6	4.17	B
	Buckwheat	16.4	4.28	B
H2'	Early-flowering	0.87	0.05	A
	Buckwheat	0.86	0.06	A
	Phacelia	0.86	0.06	A
	Late-flowering	0.48	0.08	B
	Apiaceae	0.39	0.06	B
	Cornflower	0.37	0.05	B

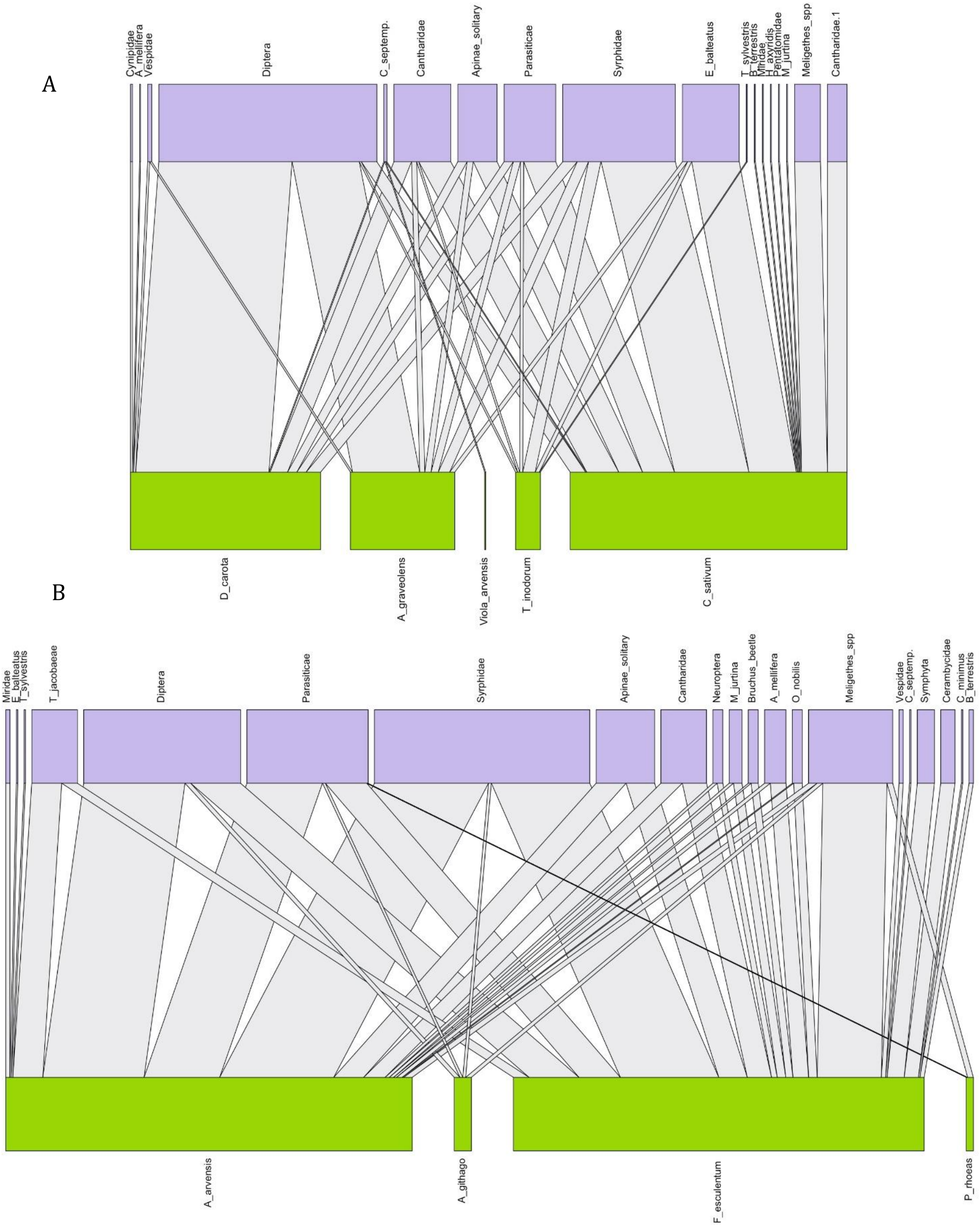

Figure 5.1. Plant-insect visitation network from plots on Rothamsted Farm throughout summer 2019 for **A** - all Apiaceae plots across 16 sampling points. **B** - all Buckwheat plots across 21 sampling points. Insects included to the maximum possible reliable resolution on the top in purple, with the sown species and any weeds present in green below. The size of each box at either plant or insect level represents visitor abundance and the width of links. indicates interaction frequency

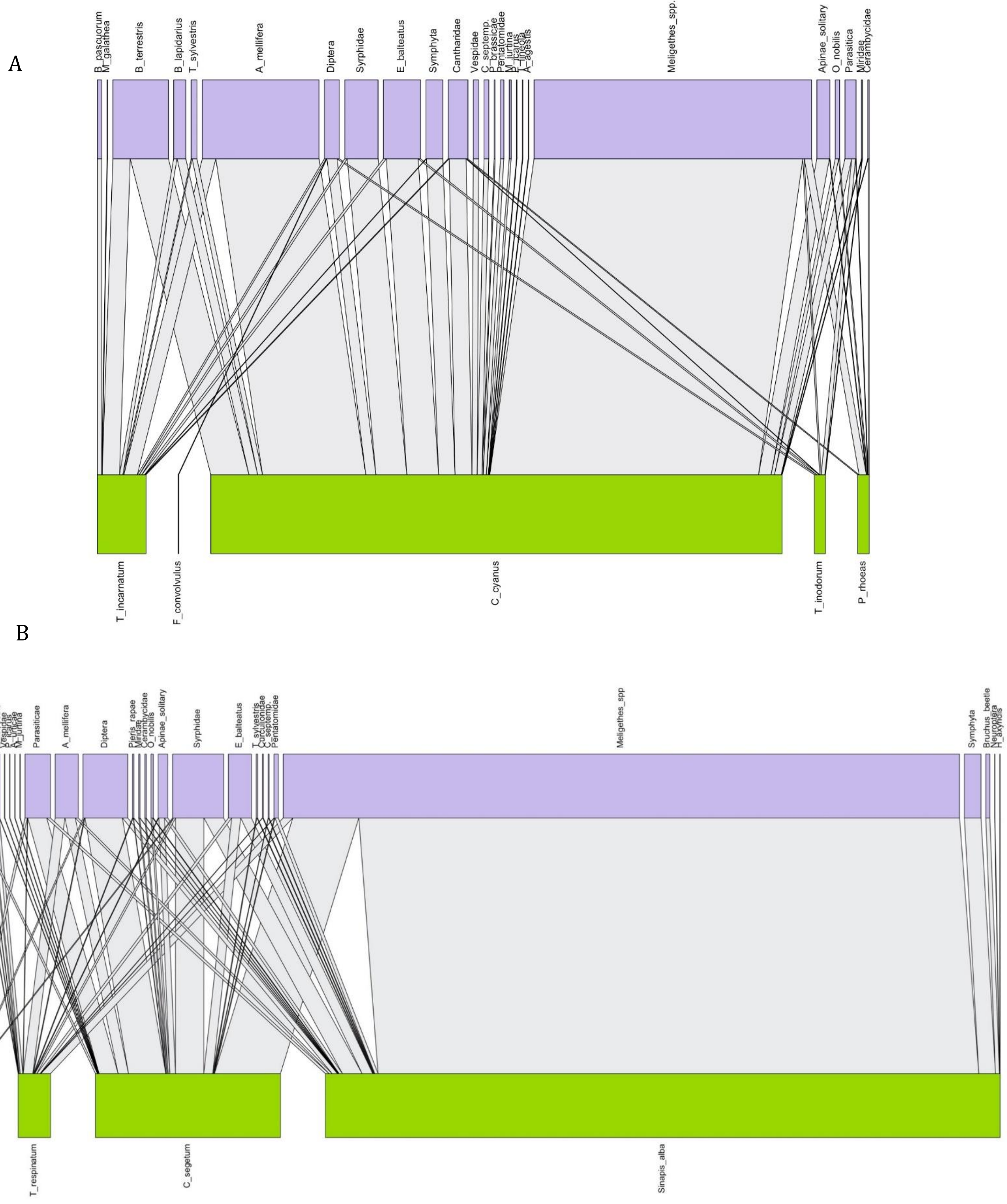

Figure 5.2. Plant-insect visitation network from plots on Rothamsted Farm throughout summer 2019 for **A** - all Cornflower plots across 25 sampling points. **B** - all Early-flowering plots across 23 sampling points. Insects included to the maximum possible reliable resolution on the top in purple, with the sown species and any weeds present in green below. The size of each box at either plant or insect level represents visitor abundance and the width of links indicates interaction frequency.

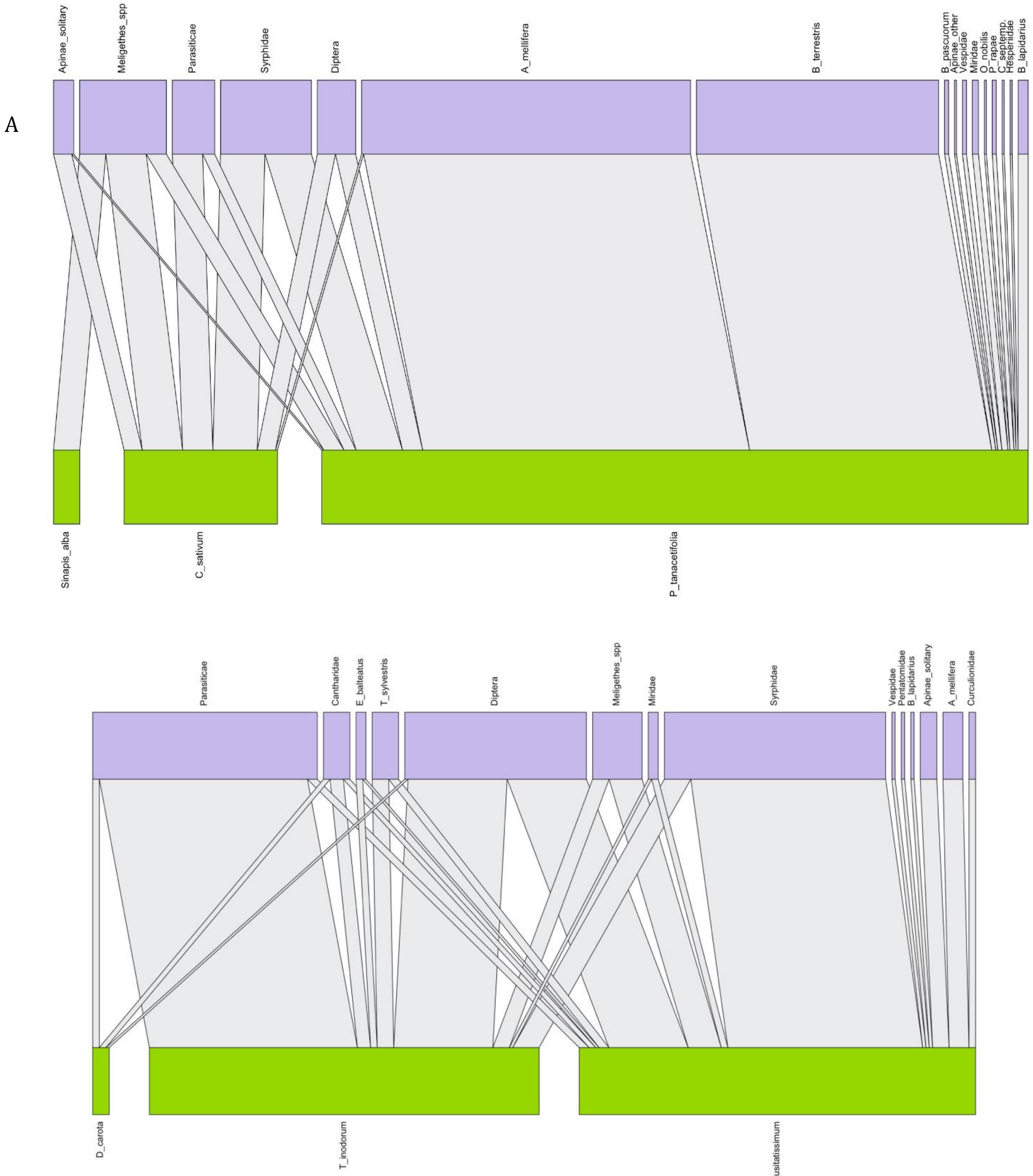

Figure 5.3. Plant-insect visitation network from plots on Rothamsted Farm throughout summer 2019 for **A** - all Phacelia plots across 12 sampling points. **B** - all Late-flowering plots across 21 sampling points. Insects included to the maximum possible reliable resolution on the top in purple, with the sown species and any weeds present in green below. The size of each box at either plant or insect level represents visitor abundance and the width of links indicates interaction frequency.

5.3.2 Carrot Field in Shropshire in 2019 Plant-Insect Network Analysis

During this summer, four time periods were sampled with 36 flower-insect timed counts undertaken across all flowering treatments. The Phacelia mix attracted the highest total visitor abundance, but this was dominated by pollen beetles (Table 5.4). Common to all mixes, Diptera were frequent visitors. The Cornflower mix had the highest species richness, with 23 morphospecies recorded.

There are far fewer network metrics that revealed significant differences between seed mixes from carrot fields in 2019, with only links per species differing significantly (Table 5.5), with Phacelia and Apiaceae mixes with significantly more links per species (Table 5.6). These networks are visualised in Figure 5.4, Figure 5.5 and Figure 5.6.

Table 5.4. The five most abundant insect visitors, total abundance, and total species richness at flower mixes across all sampling periods. Data from a carrot field in 2019.

Apiaceae		Buckwheat		Cornflower		Phacelia	
Diptera	213	Diptera	60	*A. mellifera*	183	*Meligethes* spp.	1000
Meligethes spp.	94	Syrphidae	43	Diptera	61	Syphidae	70
Syrphidae	55	Eristalis	33	Syrphidae	52	*A. mellifera*	29
Parasitica	38	*Meligethes* spp.	30	*B. lapidarius*	32	Diptera	28
E. balteatus	20	Solitary Apinae	16	*B. terrestris*	23	Solitary Apinae	18
Abundance	488	Abundance	235	Abundance	450	Abundance	1211
Richness	20	Richness	18	Richness	23	Richness	22

Table 5.5. Output from LMM analysis of plant-pollinator networks sampled from sown seed mixes planted in a carrot field in Shropshire in 2019. Significant results highlighted in bold, with significance levels indicated by: "*" = *p*<0.05.

Network metric	NumDF	DenDF	F value	Pr(>F)	Sig.	Aveg. LSD
Connectance	3	9.1	1.98	0.187	NS	0.15
Links per species	**3**	**9.0**	**4.98**	**0.026**	*****	**0.20**
Shannon's Index	3	8.9	0.73	0.558	NS	1.01
Weighted NODF	3	9.4	0.33	0.804	NS	19.5
H2'	3	9.3	0.16	0.922	NS	0.36
Nestedness	3	8.4	1.10	0.402	NS	14.8

Table 5.6. Predicted means for various network metrics and their standard errors (SE) for each seed mix sown in a carrot field in Shropshire in 2019 and the group assigned to them based on their predicted means and average LSD.

Network metric	Flower mix	Mean	SE	Group
Links per species	Phacelia	1.27	0.1210	A
	Apiaceae	1.26	0.1210	A
	Cornflower	1.02	0.1210	B
	Buckwheat	0.98	0.1218	B

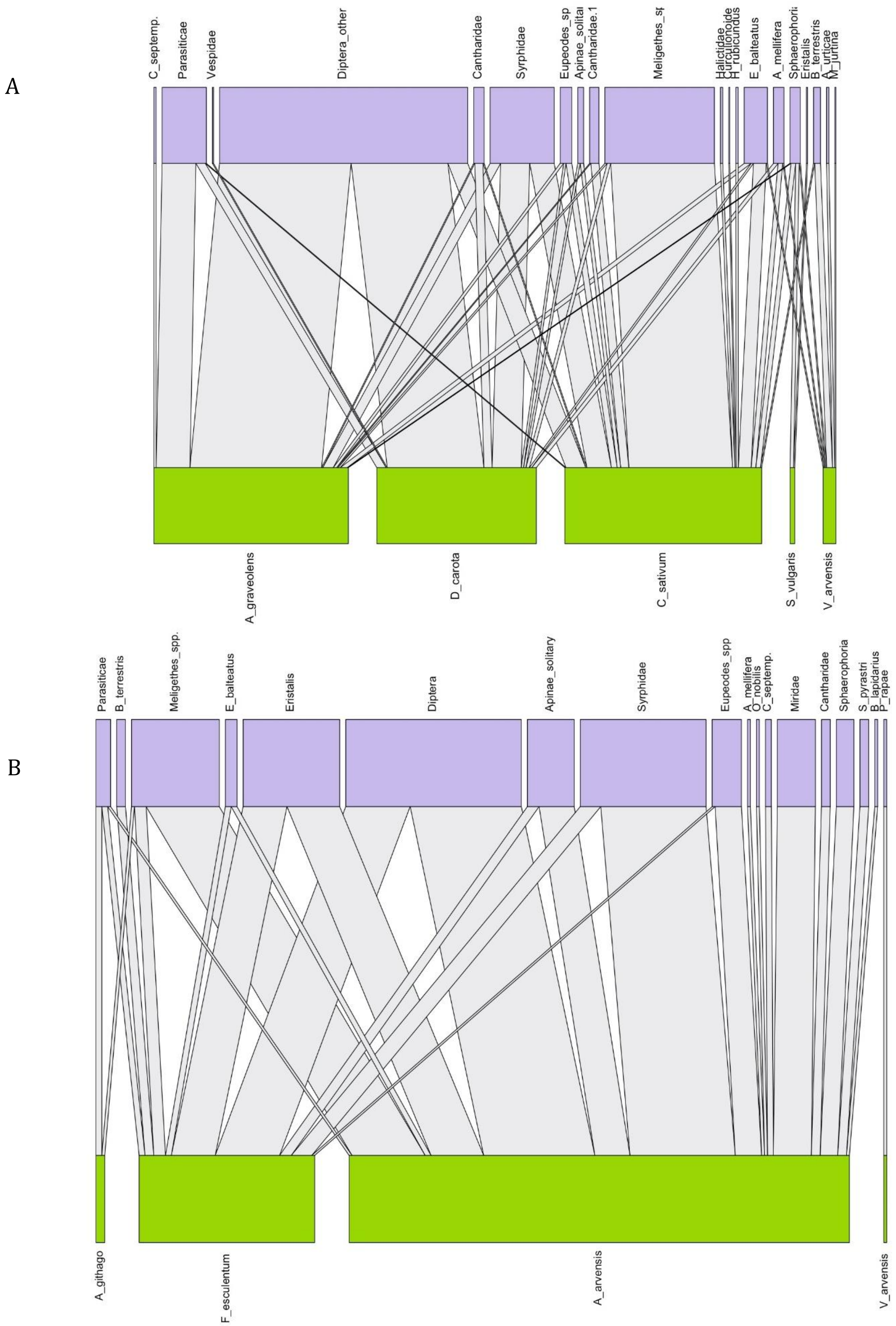

Figure 5.4 Figure legend overleaf.

Figure 5.4. Plant-insect visitation network from flower strips in a carrot field in Shropshire throughout summer 2019. **A -** all Apiaceae plots across 9 sampling points. **B -** all Buckwheat plots across 21 sampling points. Insects included to the maximum possible reliable resolution on the top in purple, with the sown species and any weeds present in green below. The size of each box at either plant or insect level represents visitor abundance and the width of links indicates interaction frequency.

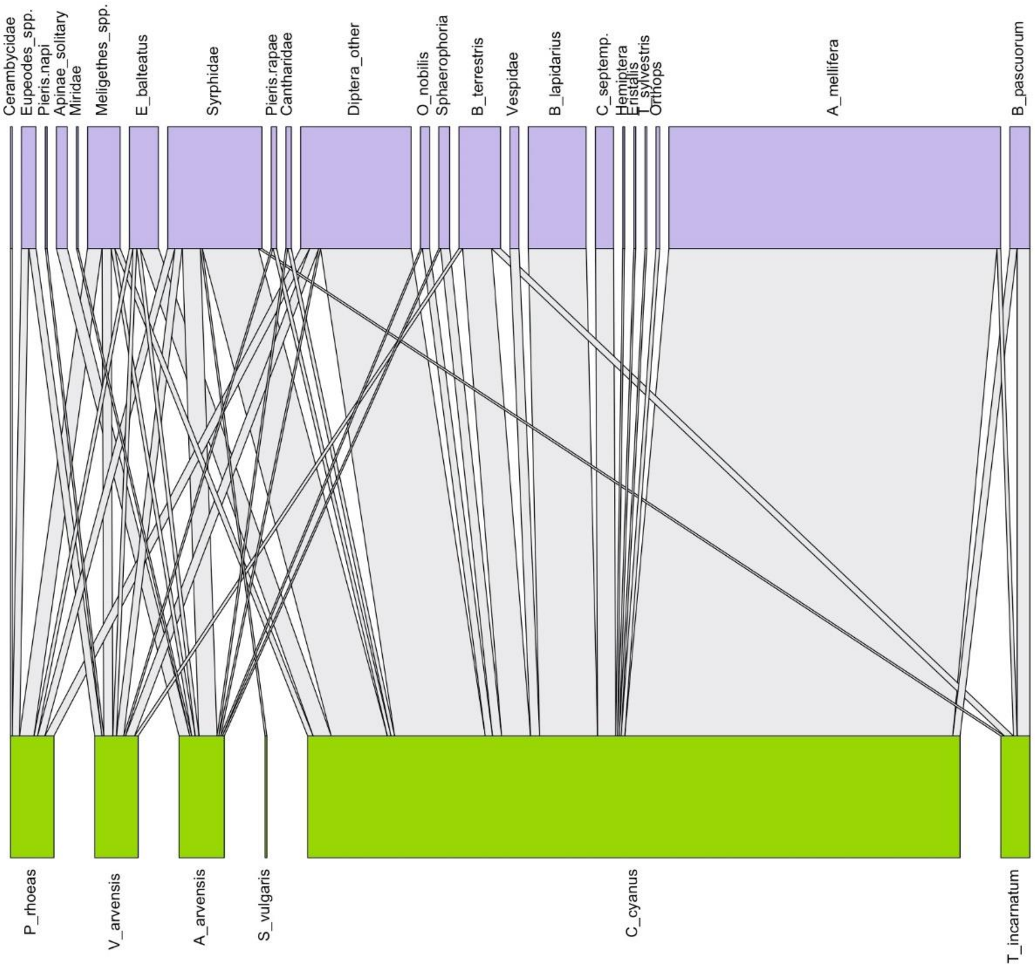

Figure 5.5. Plant-insect visitation network from created from sampling of all the Cornflower plots on in a carrot field in Shropshire throughout summer 2019, across 9 sampling points. Insect included to the maximum possible reliable resolution on the top in purple, with the sown species and any weeds present in green below. The size of each box at either plant or insect level represents visitor abundance and the width of links indicates interaction frequency.

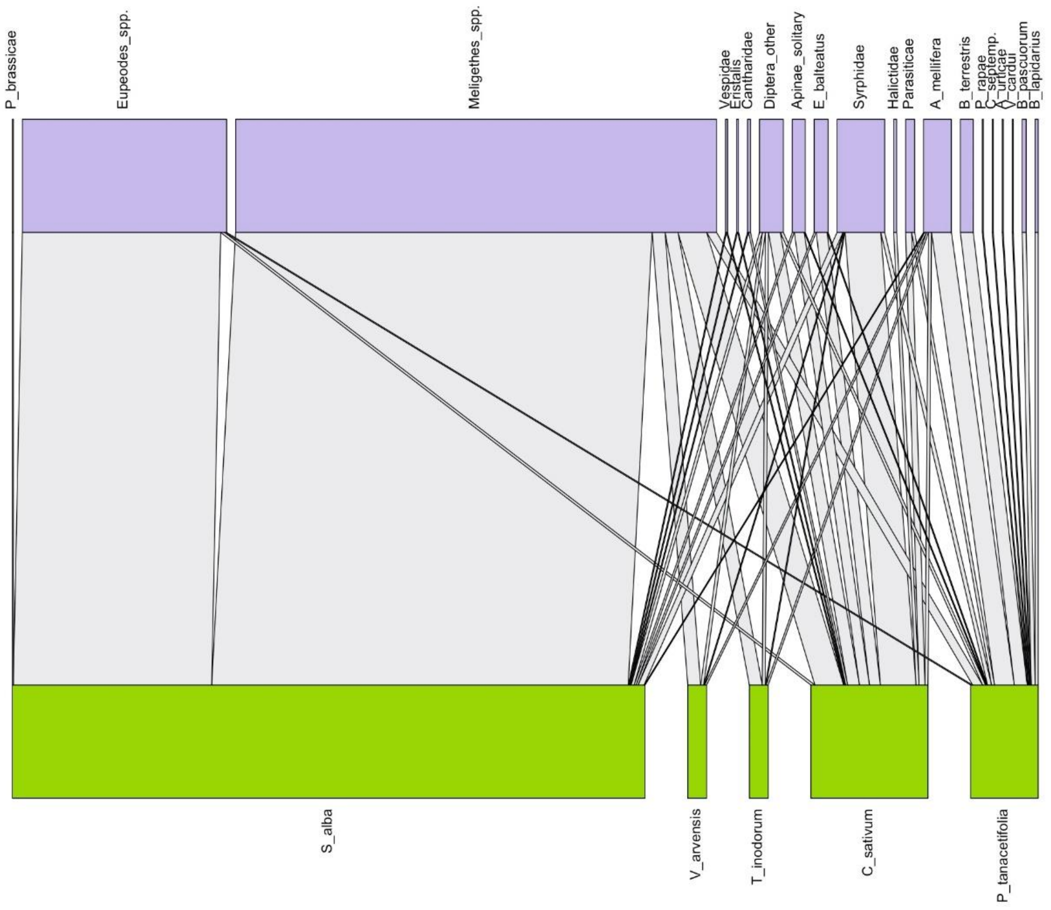

Figure 5.6. Plant-insect visitation network from created from sampling of all the Phacelia plots on in a carrot field in Shropshire throughout summer 2019, across 9 sampling points. Insect included to the maximum possible reliable resolution on the top in purple, with the sown species and any weeds present in green below. The size of each box at either plant or insect level represents visitor abundance and the width of links indicates interaction frequency.

5.3.3 Carrot Fields in Shropshire in 2020 Plant-Insect Network Analysis

During this summer, three time periods were sampling with 30 flower-insect timed counts undertaken across all flowering treatments. The Phacelia mix attracted the highest total visitor abundance, with this dominated by *A. mellifera* and *B. terrestris* (Table 5.7). The Apiaceae mix supported a high abundance of solitary bees (Table 5.8). Common to all mixes, pollen beetle were frequent visitors. The Phacelia mix had the highest species richness, with 16 morphospecies recorded.

There were no significantly different network property metrics from this round of sampling, although the weighted NODF and nestedness were marginally differed significantly (Table 5.8 & Table 5.9). The networks resulting from this 2020 sampling of Apiaceae (Figure 5.7), Cornflower (Figure 5.8), and Phacelia (Figure 5.9) mixes are much simpler with fewer interactions compared with networks from 2019.

Table 5.7. The five most abundant insect visitors, total abundance, and total species richness at flower mixes across all sampling periods. Data from carrot fields in 2020.

Apiaceae		Cornflower		Phacelia	
Solitary Apinae	67	*A. mellifera*	116	*A. mellifera*	163
Meligethes spp.	66	*B. terrestris*	32	*B. terrestris*	120
Syrphidae	60	*C. sepempuncta*	16	Syrphidae	45
A. mellifera	19	*B. lapidarius*	15	*Meligethes* spp.	43
Parasticae	12	*Meligethes* spp.	10	Parasticae	21
Abundance	264	Abundance	217	Abundance	439
Richness	12	Richness	15	Richness	16

Table 5.8. Output of LMMs assessing effect of seed mix upon various network metrics created from Flower Insect Timed Counts. Significant results highlighted in bold, with significance levels indicated by: “*” = $p<0.05$, “.” = $p<0.1$.

Network metric	NumDF	DenDF	F value	Pr(>F)	Sig.	Aveg. LSD
Connectance	2	6.0	0.26	0.781	NS	0.19
Links per species	2	9.0	0.15	0.861	NS	0.21
Shannon's Index	2	9.0	0.35	0.716	NS	0.61
NODF	2	5.8	4.08	0.078	.	9.5
H2	2	9.0	0.91	0.435	NS	0.42
Nestedness	2	8.0	2.99	0.108	NS	14.8

Table 5.9. Predicted means for various network metrics and their standard errors (SE) for each seed mix sown in four carrot fields in Shropshire in 2020 and the group assigned to them based on their predicted means and average LSD.

Network metric	Flower mix	Mean	SE	Group
Weighted NODF	Cornflower	33.73	5.88	A
	Apiaceae	27.11	7.39	AB
	Phacelia	9.062	7.39	B
Nestedness	Phacelia	29.47	5.72	A
	Cornflower	12.22	4.33	B
	Apiaceae	9.971	6.61	B

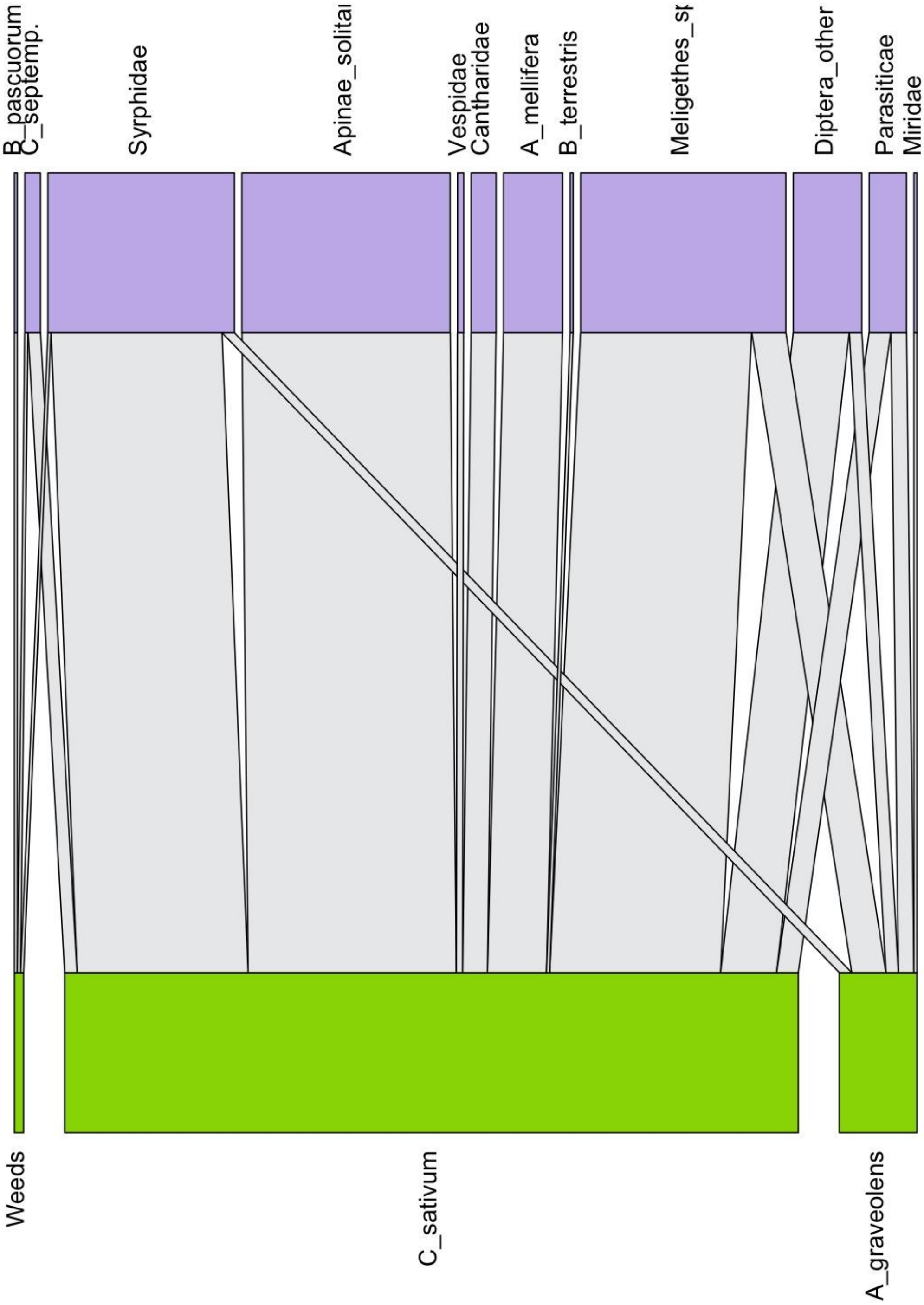

Figure 5.7. Plant-insect visitation network from created from sampling of all the Apiaceae plots in carrot fields 2020, across 8 sampling points. Insect included to the maximum possible reliable resolution on the top in purple, with the sown species and any weeds present in green below. The size of each box at either plant or insect level represents visitor abundance and the width of links indicates interaction frequency.

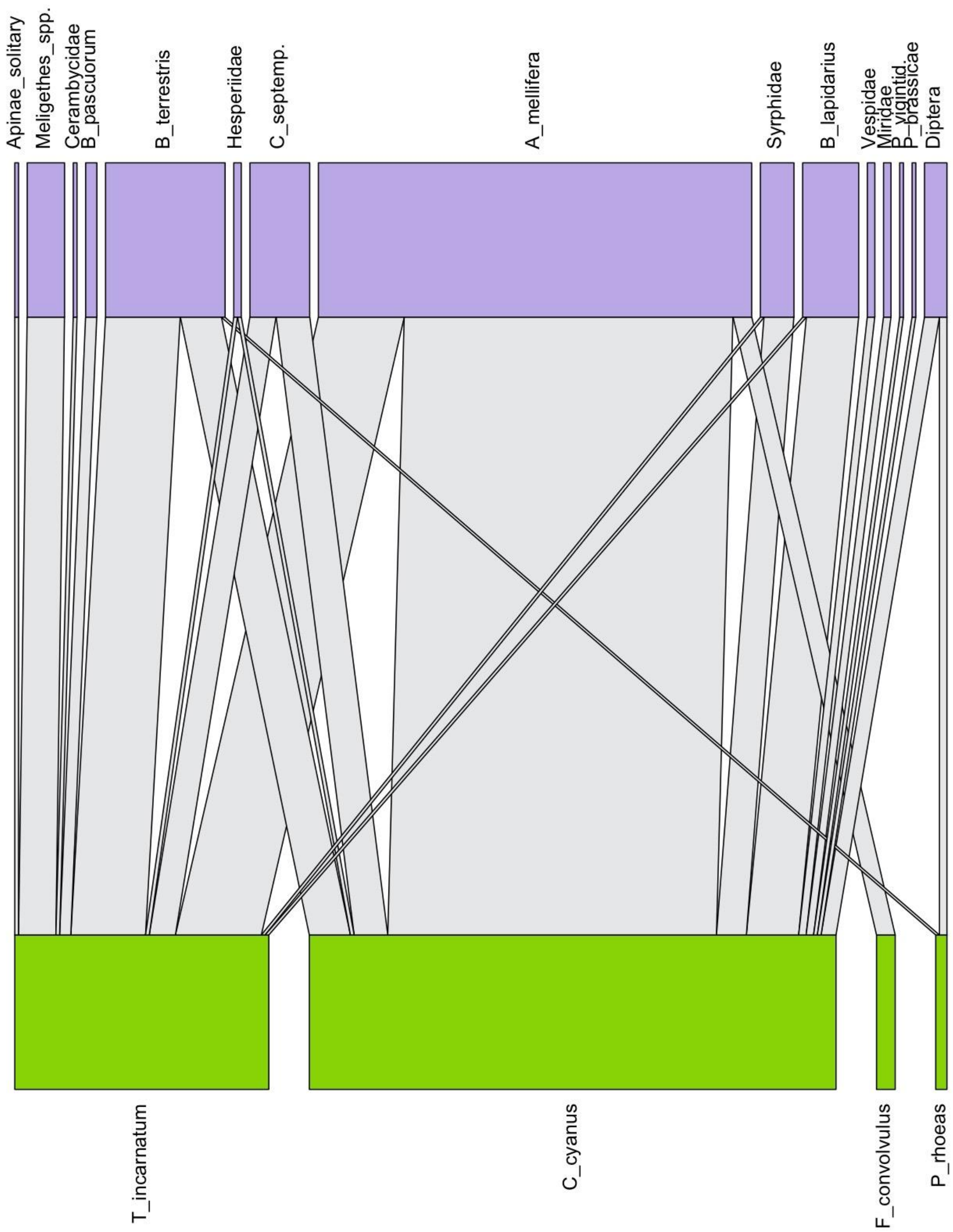

Figure 5.8. Plant-insect visitation network from created from sampling of all the Cornflower plots in carrot fields 2020, across 8 sampling points. Insect included to the maximum possible reliable resolution on the top in purple, with the sown species and any weeds present in green below. The size of each box at either plant or insect level represents visitor abundance and the width of links indicates interaction frequency.

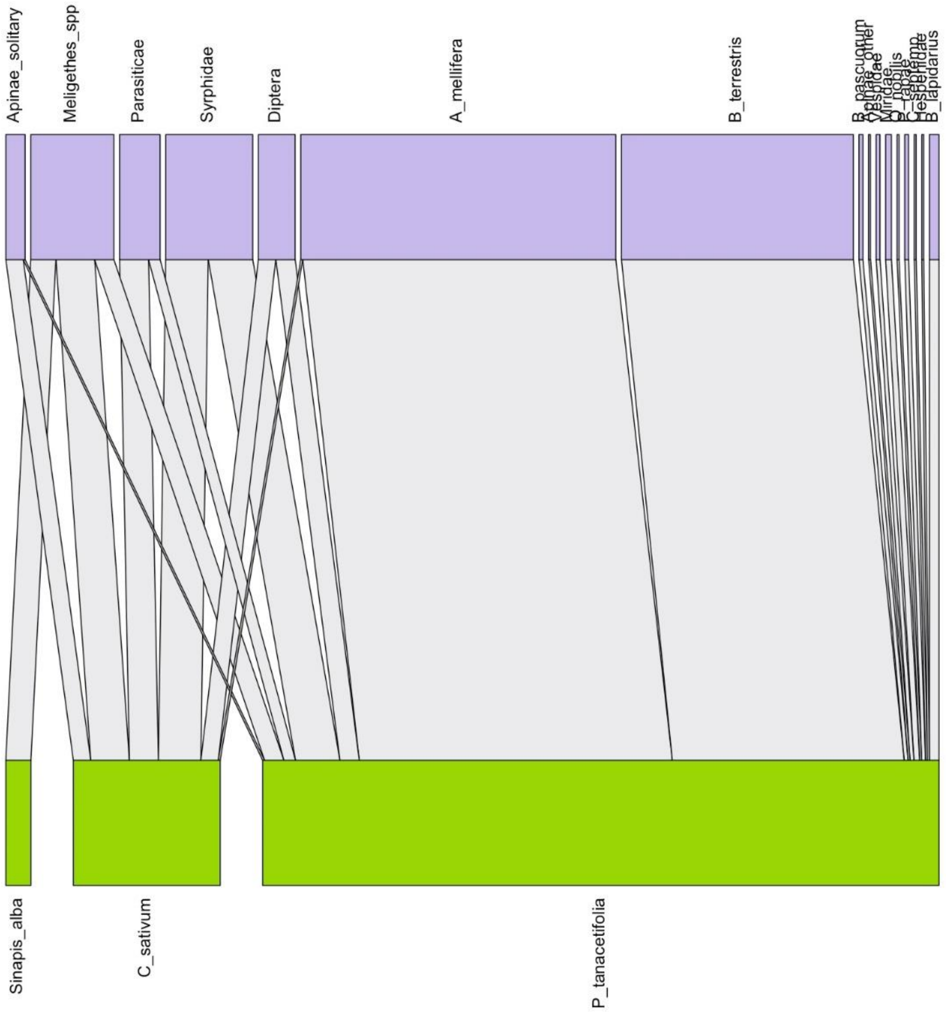

Figure 5.9. Plant-insect visitation network from created from sampling of all the Phacelia plots in carrot fields 2020, across 14 sampling points. Insect included to the maximum possible reliable resolution on the top in purple, with the sown species and any weeds present in green below. The size of each box at either plant or insect level represents visitor abundance and the width of links indicates interaction frequency.

5.3.4 Summary of Plant-Insect Network Analysis Across All Years

Across all three summers of sampling, the performance of each mix is summarised in Table 5.10. Demonstrating that across all three summers, the Phacelia mix performed highest in the range of network metrics used.

Table 5.1029. Qualitative combination of the number of instances a flower mix had a significantly enhanced performance in a network metric across three summers of flower-insect timed counts. The Early and Late-flowering mixes only occurred in Rothamsted 2019 sampling, and the Buckwheat mix did not occur in sampling done in four carrot fields in 2020.

	Network metrics across 3 years					*Rothamsted 19*		*Carrots 19*		*Carrots 20*		
Flower mix	Connectance	Shannon's Index	NODF	H2'	Links per species	Total abundance	Species richness	Total abundance	Species richness	Total abundance	Species richness	Times highest metric
Phacelia				+	+			+		+	+	5
Cornflower		+	+				+		+			4
Apiaceae		+	+		+							3
Late-flowering	+		+									2
Buckwheat				+	+							2
Early-flowering				+		+						2

5.4 Discussion

The Phacelia mix performed best across a suite of plant-insect network metrics resulting from the analysis of three years of flower-insect timed counts on Rothamsted Farm and in carrot fields in 2019 and 2020. However, this qualitative performance was skewed by its performance in carrot fields in 2019 and 2020 which be liable to a limited sampling bias. The Cornflower mix was the next best performing mix, which performed strongly in on Rothamsted Farm in 2019 which did not suffer from the same degree of bias due to low sampling effort. Thereby suggesting the Phacelia and Cornflower mixes may both be effective at supporting pollinators. Comparatively, the Buckwheat, Late and Early-flowering mixes performed poorly. This finding indicates that it is possible to tailor a range of flower mixes that differentially support pollinator communities. Thereby supporting the initial hypothesis that flower mixes would support significantly different plant-insect networks.

Given that carrot growers do not rely upon pollination for yield, there is no emphasis on attracting an effective key pollinator species. This therefore opens the possibility for growers to select plant species that are effective at increasing insect conservation, particularly providing floral resources for aerial insects. However, as sampling in 2019 and 2020 was limited, to draw conclusions, Rothamsted 2019 network properties will be considered.

Phacelia tanacetifolia flowers across two years revealed the highest abundance of insect visitors and was dominated by *Apis mellifera* and *Bombus terrestris* visitors, a feature of the mixes that was readily apparent to the Huntapac team in the field in 2020. This may superficially support the use of this plant. However, when *A. mellifera* visits to *P. tanacetifolia* flowers have been studied, it has been demonstrated that *A. mellifera* do not take pollen resources from Phacelia flowers (Sprague et al., 2016). This could lead to overestimations in the efficacy of floral support for pollinators or inefficient use of land taken out of production for pollinating insects (Sprague et al., 2016). Nevertheless, this lack of pollen utlisation is not common to all insects, as *Episyrphus balteatus* fitness is enhanced by feeding on Phacelia (Laubertie et al., 2012). However, the Phacelia *mix* had a significantly higher H2' than the Cornflower mix. This may suggest the Phacelia mix had higher complementary specialisation and niche differentiation. Thereby indicating the Phacelia mix have a high complementarity in visiting insects, a favourable feature of an insect community (Bluthgen and Klein, 2011). The significantly higher connectance of the Late-flowering plots, indicates that there was a high generalism in insect visitors, perhaps not a surprise given the mixes' poor establishment.

Limitations of this network analysis do remain as, like many forms of statistics, summarising complex ecological webs into a few network topology metrics is not a precise art. The quantitative network analysis used here is a often measure of interaction strength. Had we used a visit-based approach, where the number of flowers visited by an insect was also recorded, this could have provided different findings, especially regarding the specialisation of the networks (Novella-Fernandez et al., 2019). Although on sampling occasions with highly active insect communities, this would have been challenging.

The interpretation of these networks should also be conducted with caution as erroneous conclusions can be drawn depending on the network size, presence of rare species, and the sampling intensity (Dormann et al., 2009, Hemprich-Bennett et al., 2021). For instance, if sampling intensity is low, then a single insect visiting a flower may appear to be a 'specialist interaction', whereas in the true wider ecological network this event may common. Within these network metrics, this may lead to the over-estimation of network specialism. In this work, to help avoid this, sampling counts have been pooled into fields rather than separate time points or replicates in 2020. Additionally, as a timed observation approach was used rather than transect sampling, perhaps more unique visits have been recorded here, inflating specialisation (Gibson et al., 2011). However, whilst timed observations clearly need ample resources (Gibson et al., 2011), it was important to standardise sampling across mixes and minimise spill over. Moreover, as these focal observations have all taken place on artificially sown flower mixes mainly composed of three flower species, arguably this further amplifies the specialism of mixes.

The methodology used may also present results that have a bias towards larger, more visible insect visitors, due to the difficulty of seeing, catching and then identifying smaller insects on the wing. Moreover, inability to identify groups like Diptera to higher resolutions has the potential to bias the network structure, and subsequent metrics like connectance can be overestimated (Rodrigues and Boscolo, 2020, Renaud et al., 2020). However, as this study was not designed to elucidate highly specialised networks and given the consistent theme within thesis' focusing on functional insects, this method of analysis is still valid (Rodrigues and Boscolo, 2020, Renaud et al., 2020). Moreover, these are not the only type of insect sampling conducted on these flower mixes. Therefore, it is possible to draw conclusions both from these data and from those additional insects sampled in pitfall and water traps which allow insect identification to higher taxonomic resolutions.

Despite these concerns, networks are still a valuable method to standardise the assessments of the obvious visual differences present in the field. As the observation method used here was based around the UK Pollinator Monitoring Scheme, this has encouraged engagement from the growers. The method used is easy to understand and allows the growers to conduct this kind of focal observations themselves. Although results might be to a slightly reduced taxonomic resolution, they are still useful in developing farmer awareness of pollinators (Rodrigues and Boscolo, 2020).

Beyond assessing these bipartite networks from a pollination perspective, it is possible to consider their function from a CBC standpoint. For instance, across all years, pollen beetles are a dominant interaction, and given the potential disservice from pollen beetle to neighbouring crops, this may be a concern. Nonetheless, this work is a relatively simple foray into network analysis, given the desire to make trade-offs between CBC, pollination and a range of other ES, future work extending this approach such as Windsor et al.'s (2021) would be valuable.

Evidently, from a grower's perspective, these findings suggest that the inclusion of a variety of plants, beyond three deliberately sown here in each mix, is vital. Moreover, clearly a flower strip sown for a limited period of time will not provide the same degree of support as perennial, well managed semi-natural habitats. Including the surrounding habitat features (e.g. hedgerows) as a 'wild-type' control would have increased the power of this work to compare mixes. However, for growers farming on annually rented land, these flower strips can provide opportunities to delivery much needed floral resources, although this needs rigorous assessment to maximise multi-functionality.

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Chapter 6 – Discussion

Throughout this thesis, the answer to the question, ‘what is the best flower mix to increase pest control and insect conservation in carrots’ has been sought through three plot level and commercial field trials. The data collected to answer this question has been extensive, covering surveys of insect communities, predation estimates via sentinel prey cards, aphid counts, barley and carrot yield, along with derived carrot crop economic variables and the commercial implications of flower strips. The broad, diverse data analysed allows the efficacy of seed mixes to be considered from various perspectives. Although this breadth adds complexity when assessing the mixes to identify the ‘best’.

To visually summarise the performance of the seed mixes across the full suite of metrics used to assess both Ecosystem Service Providers (ESP) and Ecosystem Service Delivery (ESD), a qualitative assessment of mixes has been presented in Tables 6.1-6.4. In this thesis, the enhanced performance of a seed mix might reflect greater abundances of natural enemies, greater species richness and higher yields compared with control plots. Notably though there are instances where a reduced metric is preferable, such as reduced pest abundance. No seed mix performs well across the full suite of metrics used to assess both ESP and ESD (Table 6.1). Whilst, across all three trials, the cornflower performed highly on the most occasions, it also performed variably. In Barley in 2019 (Chapter 2), the Cornflower mix had a low abundance of NE whereas in Carrots in 2020 (Chapter 4), the Cornflower mix had a high abundance of NE. From an insect conservation perspective, the Cornflower mix did perform well, especially in the commercial carrot fields (Table 6.2). However, despite significant differences in the ESP present in the mixes, at no point across three years was there a significantly measurable difference in direct ESD (Table 6.3). Although, when indirect evidence of effects of the flower strips are considered (Table 6.4), all flowering mixes perform better than the ‘business-as-usual’ carrot controls (Chapter 4 results). However, it should be noted that improved flower mix performance is not equal distributed across the field, with flower strips only outperforming the controls at the field edge. Importantly, the indirect yield benefits from flower strips can offset the loss of production from the land taken out of production.

Table 6.130. Summary of seed mix efficacy across a range of metrics on Ecosystem Service Providers and biological control performance. A mix that performs well or has a significantly enhanced performance is highlighted in green and indicated with “+”; mixes with strongly significant performances are highlight in darker green and with “++”. A mix that does not perform well or has a significantly worse performance is highlighted in light orange and indicated with “–”; mixes with strongly significant poor performances are highlighted in darker orange and with “– –”. Mixes with an intermediate performance, or those not significantly different, are marked with “~”. For metrics not measured for a seed mix, cells are left blank. YWT = yellow water trap

Ecosystem Service Providers

Biological control

	YWT NE Abundance		YWT Wasp abundance		Pitfall NE abundance	Pitfall Herbivores	YWT Herbivore Abundance		YWT Pests	
Seed Mix	*Barley 2019*	*Carrots 2020*	*Barley 2019*	*Carrots 2020*	*Barley 2019*	*Barley 2019*	*Barley 2019*	*Carrots 2020*	*Barley 2019*	*Carrots 2020*
Apiaceae	~	~	~	~	~	~	~	-	-	~
Buckwheat	~		+		~	~	~		-	
Control- barley	~		++		~	--	~		~	
Control- carrots		~		~				~		~
Cornflower	-	+	~	+	~	~	~	+	-	~
Early-flowering	-		~		~	~	~		++	
Grass	+		~		~	~	~		~	
Late-flowering	~		-		~	~	~		-	
Phacelia	-	~	+	~	~	~	~	~	~	~

Table 6.2. Summary of seed mix efficacy across a range of metrics on Ecosystem Service Providers and insect conservation performance. A mix that performs well or has a significantly enhanced performance is highlighted in green and indicated with “+”, mixes with strongly significant good performances are highlight in darker green and with “++”. A mix that does not perform well or has a significantly worse performance is highlighted in light orange and indicated with “–”, mixes with strongly significant poor performances are highlighted in darker orange and with “– –”. For mixes with an intermediate performance or those not significantly different, they are marked with “~”. For metrics not measured for a seed mix, cells are left blank.

Ecosystem Service Providers

Insect conservation

	All species richness		NE richness		Bee abundance		Bee richness	Pollinator networks
Seed Mix	*Barley 2019*	*Carrots 2020*	*Barley 2019*	*Carrots 2020*	*Barley 2019*	*Carrots 2020*	*Barley 2019*	*Across 3 trials*
Apiaceae	~		~	~	+	~	~	-
Buckwheat	~		~		-		~	+
Control- barley	~		~		-		~	
Control- carrots				-		~		
Cornflower	~		~	++	~	++	~	++
Early-flowering	~		~		+		~	-
Grass	~		~		~		~	~
Late-flowering	~		~		~		~	-
Phacelia	~		~	+	~	+	~	+

Table 6.3. Summary of seed mix efficacy across a range of metrics on Ecosystem Service Delivery (ESD) and direct evidence for pest control. A mix that performs well or has a significantly enhanced performance is highlighted in green and indicated with "+", mixes with especially good performances are highlight in darker green and with "++". A mix that does not perform well or has a significantly worse performance is highlighted in light orange and indicated with "–", mixes with particularly poor performances are highlighted in darker orange and with "– –". For mixes with an intermediate performance or those not significantly different, they are marked with "~". For metrics not measured for a seed mix, cells are left blank.

Ecosystem Service Delivery

Direct ESD

	Crop Aphid Abundance		Sentinel prey cards			Gross yield		Insect damage	
	Carrots	*Carrots*	*Barley*	*Carrots*	*Carrots*	*Barley*	*Carrots*	*Carrots*	*Carrots*
Seed mix	*2019*	*2020*	*2019*	*2019*	*2020*	*2019*	*2019*	*2019*	*2020*
Apiaceae	~	~	~	~	~	~	~	~	~
Buckwheat	~		~	~		~	~	~	
Control- barley			~			~			
Control- carrots	~	~		~	~		~	~	~
Cornflower	~	~	~	~	~	~	~	~	~
Early-flowering			~			~			
Grass	~		~	~		~	~	~	
Late-flowering			~			~			
Phacelia	~	~	~	~	~	~	~	~	~

Table 6.4. Summary of seed mix efficacy across a range of metrics on Ecosystem Service Delivery (ESD) and indirect evidence for pest control and its impact upon derived economic variables. A mix that performs well or has a significantly enhanced performance is highlighted in green and indicated with "+", mixes with strongly significant and enhanced performance are highlight in darker green and with "++". A mix that does not perform well or has a significantly worse performance is highlighted in light orange and indicated with "–", mixes with strongly significantly poor performances are highlighted in darker orange and with "– –". For mixes with an intermediate performance or those not significantly different, they are marked with "~". For metrics not measured for a seed mix, cells are left blank.

	Ecosystem Service Delivery					
	Indirect ESD		Derived economic variables			
	Net yield		Turnover		Combined Costs	
Seed mix	*Carrots 2019*	*Carrots 2020*	*Carrots 2019*	*Carrots 2020*	*Carrots 2020 - sprayed*	*Carrots 2020 - unsprayed*
Apiaceae	~	+	~	+	~	~
Buckwheat	~		~			
Control- carrots	~	-	~	-	~	++
Cornflower	~	++	~	++	~	+
Grass	~		~			
Phacelia	~	+	~	+	~	~

While assessing these mixes across a broad range of factors is relatively novel (Johnson et al., 2021), this leaves the challenge of picking the 'best' performing mix across a range of metrics. Initially, in this thesis, this decision relied upon identifying the main NE guilds of *Cavariella aegopodii*. However, as these efforts ultimately failed, the consideration around optimal mix selection becomes more theoretical. For instance, a mix with a low herbivore or pest abundance may be favourable for a grower, but alternative prey is a core requirement for supporting NE populations (Gurr et al., 2017). As decisions become increasingly complex, with the proliferation of multiple metrics generated throughout the whole supply chain, a worthwhile route for further work would be to establish methods for effectively comparing results like this (Storkey et al., 2015). Furthermore, decision making becomes increasingly complex as this work has not included an exhaustive assessment of the mixes as it lacks social implications. For instance, the aesthetic, cultural value flower strips provide has not been assessed, nor has the time required to establish and manage strips. Studying these factors would enhance the holistic picture of flower strips to enable better decision making.

Selecting the optimal mix is also compounded by the variability in seed mix performance across multiple fields, crops, and years. Perhaps, this changeability reflects a seed mix interaction with field and landscape (Karp et al., 2018). If Huntapac chose to set aside 5% of their farmed area, this would require the bespoke tailoring of 50 hectares of wildflower areas each year, on top of their existing workload. Pragmatically, this will be, at best, challenging. However, without understanding and responding to this landscape context, manipulating ecosystem service delivery will be variable and maybe even unreliable.

This debate highlights that throughout this project there have been many instances of tension between traditional academic perspectives and commercial pragmatism. This was felt at the project's inception when arguably, a more substantial or more considered ecological focus could have improved the design of mixes. Such focus would have allowed more sophisticated hypotheses about plant traits or the functional diversity of the mixes and associated insect communities to be investigated. This conflict continued whilst studying the delivery of indirect pest control services onto carrot quality; the continuous methods used to measure carrot mass, diameter, and length offer the opportunity for extensive statistical investigation. However, the limited time resources were insufficient to harvest sufficient carrots to answer this project's overarching research question, both a commercial question whilst also satiating academic curiosity. In many ways, annual mixes are also a sub-optimal source of support for insect communities; for example, they do not provide support for overwintering, a critical resource for some, but not all, insects and key NE (Boinot et al., 2019, Gontijo, 2019). However, annual flower strips are available to growers working on annually rented land, allowing them to expand their control over land management for environmental outcomes over one growing season. At the field level, given the evident increases in species richness and abundance of insects in flower mixes seen in Chapter 4, this alone might be a sufficient justification for pursuing local scale CBC (Begg et al., 2017). Nonetheless, whilst annual flower strips may be better than ‘business-as-usual’, it remains to be seen whether annual strips are the best possible use of land in a farming landscape with multiple, often competing, demands. However, this tension is arguably the source of this project’s greatest strength: it's commercial applicability. With vast challenges facing humanity, evidence-based solutions that fit into large-scale agriculture are needed.

Sustainable Intensification may be possible for carrot growers (Chapter 4 results). Flower strips can be associated with indirect benefits of CBC with increasing carrot net yields at levels that can offset the land taken out of production. Although this is only true at the field edge, for sprayed carrots, adjacent to flower strips. Nonetheless, this supports previous work which found that semi-natural habitat may reduce pest outbreaks and improving pest control (Gontijo, 2019, Haaland et al., 2011, Holland et al., 2017). However, direct evidence of pest control delivery into crops at measurable levels has not been found here. There was no evidence across three years of trials suggesting that a variety of seed mixes could improve predation upon sentinel aphid cards (e.g. Chapters 2, 3 & 4 results). This lack of direct evidence is not an unexpected or unexplainable finding which could reflect concerns over the method to assess pest control (Boetzl et al., 2020), or a wider failure in pest control delivery (Tscharntke et al., 2016). However, the insufficient temporal replication of sentinel cards significantly hinders the ability to confirm that predation did not differ between mixes (Iuliano and Gratton, 2020). Likewise, direct aphid counts were conducted with limited success (Chapters 3 & 4 results), as they also did not reveal the trophic cascade of NE predation onto aphid numbers. However, the strength of this work is that by assessing crop yield and related economic factors, there are potentially indirect benefits at a grower-relevant level from flower strips (Chapter 4 results).

This thesis helps to add to the limited pool of research around the commercial implications of CBC (Griffiths et al., 2008, Johnson et al., 2021, Naranjo et al., 2015). Moreover, this thesis highlights that the most persuasive evidence for the potential of flower strips is apparent through the economic and food security considerations. A similar finding has been drawn from the Allerton Project's no-till trials, where the inclusion of a wide breadth of economic costs tipped the balance from economic losses from conservation agriculture to no-till becoming a viable alternative (Jarvis and Woolford, 2017). During this thesis, the skills required to consider the economic valuation of these pest control services were far more attainable than the vast entomological knowledge required for reliable insect identification. No doubt, this was partly due to the willingness of Huntapac to share their methods and financial details. The ease of access to commercially relevant factors may continue to grow with the proliferation of services allowing growers to track their activities. Although, in the scope of a resource-limited thesis, the time taken to explore these commercially relevant metrics arguably has come at a cost of more fundamental ecological analysis of data collected. Nonetheless, using these grower-provided resources is an avenue that would be valuable for CBC researchers to exploit, given the widespread lack of focus on these factors within the literature.

The inclusion of an admittedly complex experimental design in Chapter 4 elucidated the potential for the field-level spatial optimisation of pest control delivery. This finding is highly dependent on the magnitude of any 'edge effect' present. This suggests the inclusion of flowering strips that run the length of fields may be an ineffective and expensive endeavour for growers. Any reliance upon an 'edge effect' potentially complicates the long-term integration of flower strips as there will be annual yield and NE fluctuations, and there may be years where flower strips are not an effective option. This work may not directly translate into other carrot growing regions or crops. If the design used in Chapter 4 had been repeated over multiple years in more fields, these findings would have been strengthened. As is familiar in fieldwork, further work assessing whether these findings are consistent via more spatial and temporal replication and more extensive trials would be valuable and necessary for more definitive conclusions to be drawn. It is also critical for more work to establish the optimum land area within a field that can be 'set-aside' for these efforts. At a landscape scale, increasing SNH can lead to lower pest outbreaks, later pest migration and increased biological control (Gagic et al., 2021, Gardiner et al., 2009, Karp et al., 2018, McHugh et al., 2020, Paredes et al., 2021), and at a landscape scale, this is a feature that could be optimised for total productivity, cost and additional co-benefits. Similarly, this optimisation of the area required and the management of flower strips and pest control service delivery at the field level would be a valuable direction for research.

A common theme in this project is that the efficacy of a seed mix at supporting ESP can be assessed through two lenses, biological control and insect conservation (Begg et al., 2017). Within biological control, growers are keen to increase the abundance of key ESP that can measurably reduce pest populations. However, this may be a risky approach. If a singular ESP is targeted, such as a hoverfly species, it relies upon sufficient initial levels of this insect to be present within the regional species pool. If the targeted insect is absent, this jeopardies CBC success. Moreover, this thesis has found high temporal variability, whereby the abundance of key ESPs and insect community assemblage changes in pitfall and yellow water traps across years (Chapter 2 & 4 results). Such changes suggest that the temporal matching of floral resource phenology, ESPs lifecycles and pest outbreaks could be possible and should be explored further, although this has not been addressed in this work. It is hard to refute Iuliano and Gratton's conclusion that it is insufficient to study single snapshots of insects, as changes in NE resource provision across time and space must be considered (2020). However, with the resources available in this project, addressing this challenge thoroughly proved challenging, especially given the widely acknowledged need to consider ESD. Within this thesis, compromises between the assessment of ESP and ESD had to be made, especially whilst addressing a commercial problem. Additionally, despite attempts across two years, a disappointing knowledge gap remains about the fundamental ecology of the efficacy of epigeal and foliar NE guilds and their respective control of carrot specific aphid pests. This makes the identification of an effective ESP that controls *Cavariella aegopodii* challenging. Thereby this adds support to the recommendation that growers should seek to support a diversity of NE and diversity of NE traits (Greenop et al., 2018).

Caution must be used when interpreting ESP results as there often are many feasible explanations for a finding. For example, one must ask when considering direct aphid counts on carrots, are prey populations controlled by NEs? Or do prey populations influence the NE population? It is attractive to apply a unidirectional lens to interpretation (Root, 1973), where the finding of no pests next to a particular mix demonstrates the effectiveness of that seed mix at initiating a trophic cascade of NE-mediated pest control. This was seen in Chapter 2, where high Chalcididae wasp numbers were observed in Control barley plots which also had relatively high aphid abundance. Similarly, high herbivore richness was also seen in the Cornflower plots which also had high total wasp and Ichneumonidae abundance (Chapter 4 results). There are clearly both direct and indirect complex interactions between different NEs, and differing NE impacts upon prey populations operating over different time scales operating (Chailleux et al., 2014).

From an insect conservation perspective, the support for flower visitors has been explicitly considered through community-wide metrics, the abundance and richness of all bees, and specific flower visitor networks. Whilst pollinators extend beyond charismatic bee species; these were a focus for growers. It became evident that a mixture of plant species across the flowering mixes would provide various niches and flower types to support flower visitors across the summer. Building on this work, Huntapac have sown wildflower mixes in headlands and strips across their land containing buckwheat, crimson clover, linseed, Phacelia and millet; all species tested in this project. Their longer-term aim is to achieve 5% of their farmed land as set aside for these endeavours, and so far, Huntapac has planted 35 hectares. It would be interesting to visit these sites in the future to assess if there are changes in pollinator abundance or insect visitation networks compared with this thesis' work to identify if pollinator populations are rebounding as a result of Huntapac's efforts.

Like all projects with restricted budget and time resources, in this thesis, limitations are inescapable. The lack of focus on the landscape composition, quality, and configuration surrounding the seed mixes hinders this work's extension beyond carrots in Shropshire. This limitation arose from the decision to focus on identifying the 'best' seed mix, which subsequently reduced the capacity to study the influence of landscape upon ecosystem service delivery. However, the performance of a given mixture is relative to the landscape context it is found in, and the 'best' mix may vary across the landscape. With hindsight, it is impossible to assess mix efficacy without this information. Although in the assessment of insect community composition in Chapter 2 and Chapter 4, it was possible to consider the field's role in explaining community composition. The finding that aerial invertebrate communities differ by field, but epigeal communities do not, should be investigated further.

This thesis does not investigate farmer psychology around risk management; however, including more social science elements would have considerably strengthened this work. A consistent feature throughout conversations with carrot growers was the risk flower strips posed compared with insecticide use. It would be valuable to understand the underlying perception of spraying an insecticide product to control a known pest, compared with sowing flowering strips that differentially support NEs, versus taking no action. Throughout conversations during this work, the term pest 'control' had a dual meaning. Obvious was the reduction or elimination of insect pests, but the term also revealed the sense of control a grower might have over their livelihoods. Perhaps sowing a flower strip and relying upon relatively unknown insects is an uncomfortable proposition compared with the more active choice of spraying an insecticide. These are devastating consequences of getting pest control wrong which can decimate a farm's profits and affect the renewal of retailer contracts or tarnish a business's reputation.

It could be argued that these factors regarding farmer perception of risk are equally as important as the evidence basis around the efficacy of a sustainable action (Yorke, 2019). For instance, in South West France, farmers' perceive that landscape features threaten their crops due to the risk of harbouring pests (Salliou and Barnaud, 2017). Whilst there is debate around the consistency of crop pest responses to landscape composition (Karp et al., 2018), there are numerous instances where increasing semi-natural habitat in the surrounding landscape assists pest control suppression (Gagic et al., 2021, Gardiner et al., 2009, Paredes et al., 2021, McHugh et al., 2020). In the case of this work, the Apiaceae mix was included intentionally investigate whether it would harbour pest aphids or virus thereby worsen the situation for growers. Whilst virus levels from crop samples are outstanding, there were not significantly higher pest damage, or aphid numbers associated with the Apiaceae mix compared with control plots. By setting out to address grower concerns, perhaps this will lead to increase grower *trust* in the efficacy of flower strips for pest control. If we are to achieve truly sustainable farming, it is necessary to realise barriers to adoption and recognise that providing scientific evidence may not be sufficient.

This thesis opened with the powerful quotation, “To point out that the world is a better place than it once was does not mean that, for many, existence is not still cruel and brutish” (Dimbleby, 2021). As we strive to simultaneously tackle the vast challenges of biodiversity loss and climate change that pose an existential threat to humanity’s existence, we must not forget that farming’s function is to feed people. Consequently, the impact of flowering strips on food production, carrot growers’ businesses, and the natural environment has been considered throughout this work. Annual flower strips are not a panacea to all carrot growers’ problems, nor are such strips the optimal solution for all sustainable farming endeavours. However, this thesis has found that these flower strips do appear to be associated with increased net carrot yields at levels that can compensate for the land taken out of production, in limited circumstances. Moreover, these strips do not come at a higher cost than a ‘business as usual’ approach for growers. Simultaneously, they provide significantly more floral resources for wild pollinators. In a world that can seem increasingly conflicted, with fractious tensions between food production, profit, and environmental outcomes, it appears that a harmonious resolution is possible.

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