The use of habitat interventions to enhance natural pest regulation services in Spanish orange orchards

Alice Mockford

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Abstract

To meet the rising demand for food from a growing human population, modern agriculture has focused on intensification by mechanising agricultural processes, converting land to agriculture, removing ecological infrastructure to maximise field sizes, and increasing agrochemical inputs (synthetic fertilisers and plant protection products). This has resulted in habitat loss, environmental pollution, and increased greenhouse gas emissions which has contributed to the accelerated loss of biodiversity and global climate change. In turn, ecological processes have been disrupted, including economically important ecosystem services that underpin food production, such as pest regulation. Interventions which protect and support ecosystem services must be applied to agricultural systems to enable the increasing demand for food to be met sustainably. Wildflower habitats provide resources for natural enemies of crop pests, including carbohydrates (nectar, honeydew, and guttation), protein (pollen and alternative host/prey), and shelter, and can therefore support populations of natural enemies, which spill-over onto adjacent crops, and ultimately enhance pest regulation services. Orange is an economically important fruit crop in Spain, worth €1.24 billion annually. Spanish orange orchards are characterised by a high diversity of native and naturalised natural enemies which successfully regulate many potential citrus pests. However, some pests still avoid successful control. For the delivery of robust Integrated Pest Management programmes, habitat management strategies must therefore be developed for Spanish orange orchards to support natural enemies and their pest regulation services.

Replicated blocks established within larger commercial citrus orchards (>300 ha) in Huelva, Andalucía, were used to investigate the effect of a novel wildflower habitat sown in the orchard alleyways under different management strategies on natural enemy abundance and the delivery of pest regulation services in Spanish Navel orange orchards. Two management treatments were applied to the wildflower habitats: i) a standard management treatment, whereby the wildflower habitats were managed by cutting once annually in February, and ii) an active management treatment, for which the wildflower habitat received two additional cuts each year, once in May and again in July, to coincide with predicted incidence of a key pest of citrus, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae). These management treatments were compared against control alleyways whereby alleyways were managed conventionally by cutting the naturally occurring vegetation four to five times annually.

For the first time, it was demonstrated that species-rich wildflower strips can be established successfully in Spanish orange orchards, doubling plant richness in the alleyways, and supporting distinct plant communities compared to conventionally managed alleyways. In turn, this provided resources to support natural enemies. Carbohydrate feeding in parasitoids, a prerequisite for optimised host foraging, was increased and natural enemy abundance on orange trees was boosted in association with the standard wildflower treatment, without increasing the relative abundance of hyperparasitoids. This enhanced pest regulations services during a period of the year critical for the control of several economically important pest species (June-July). However, actively managing the wildflower alleyways by cutting resulted in a reduced abundance of the sown species during the three-year study. Rather than encouraging spill-over, the increased disturbance was associated with reduced abundance of natural enemies on the crop, decreased incidence of parasitoids recently fed on carbohydrate, and diminished pest regulation services.

This study has shown that wildflower habitats under standard management can enhance the delivery of pest regulations services in commercial orange orchards. To optimise this service, wildflower habitats should therefore be managed by cutting just once annually, allowing alleyways to grow throughout the rest of the year. The adoption of this strategy could allow growers to reduce the use of plant protection products and provide a more environmentally and economically sustainable approach to pest regulation in Spanish orange orchards.

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Chapter 1

Introduction

1.1. Sustainability in Agriculture

1.1.1. Global change, biodiversity loss and modern agriculture

Functioning ecosystems and their processes provide key services which have enabled humankind to thrive (Compton, 2011). Ecosystem services provide food, raw materials and freshwater, and regulate carbon sequestration, pollination, and pest regulation (Millennium Ecosystem Assessment, 2005). These services maintain ecosystem functions that are essential to human health and wellbeing (Millennium Ecosystem Assessment, 2005).

An estimated 60% of ecosystem services, however, are diminished (Millennium Ecosystem Assessment, 2005). Global systems are experiencing a period of unprecedented environmental change (Song *et al.*, 2018), with the greatest subsistence observed over the last 50 years (Millennium Ecosystem Assessment, 2005). Biodiversity can help safeguard ecosystem services against environmental change, serving as an insurance system for ecosystem function and the delivery of their services (Yachi and Loreau, 1999). As greater resilience to environmental change is observed in systems with high functional diversity (Elmqvist *et al.*, 2003), the loss of species and genetic diversity is associated with the loss of ecosystem services across the globe (Dirzo *et al.*, 2014), which could ultimately lead to ecosystem collapse (Downing *et al.*, 2012). Current extinction rates are estimated at 1,000 times above natural background rates which would be expected in the absence of human driven change (Pimm *et al.*, 2014). Flying insect biomass, for example, has declined by more than 75% over the last 27 years in protected areas in Germany (Hallmann *et al.*, 2017), with similar observations made elsewhere in the world (Lister and Garcia, 2018; Janzen and Hallwachs, 2019). Such drastic declines, driven by land-use change, chemical pesticides, invasive species, and climate change, represent a very real concern (Wagner, 2020). These declines represent not only loss of vital pollination, nutrient cycling and pest regulation services, but their cascading effects across trophic levels can restructure food webs and disrupt functioning ecosystems (Goulson, 2013; Tscharntke *et al.*, 2016; Lister and Garcia, 2018).

The human ability to engineer their surroundings has been fundamental to our success, from the domestication of the first livestock and crops to the mining of raw materials (Turner, Nakamura and Dinetti, 2004; Compton, 2011). By the end of the 20th century, between 30% and 50% of the Earth's surface had been transformed by human activity (Vitousek *et al.*, 1997), 62% of which is now devoted to agricultural production (cropland, managed grassland, commercial forestry and biofuels) (Poore and Nemecek, 2018). This land-use change has supported growing global economies (Bolt et al., 2014; FAO, 2017) which has resulted in income dependent changes in diet (Kearney, 2010; Tilman and Clark, 2014). Diets are shifting from low calorie and carbohydrate rich staples towards diets rich in dairy, meat, and sugar products, which furthers land clearance for high-intensity livestock industries (Kearney, 2010; Tilman and Clark, 2014). Such agricultural expansion leads to a reduction in habitat quality for biodiversity, loss of ecosystem services, and diminished benefits for human wellbeing (Polasky *et al.*, 2011).

Late 20th century land use has typically separated areas for agricultural production from natural and semi-natural areas designated for the conservation of biodiversity (Scherr and McNeely, 2008). This has resulted in the creation of large homogenous landscapes of high intensity farming practices which lack sufficient quality habitat to support beneficial insects (Scherr and McNeely, 2008). If the wider landscape fails to support the minimum population sizes of beneficial insects, they will fail to spill-over into the agri-environment (Letourneau, 1998). This land use system has therefore driven the degradation and loss of highly valuable ecosystem services that otherwise underpin many agricultural systems, such as pest regulation (Östman, Ekbom and Bengtsson, 2001; Tscharntke and Brandl, 2004; Tscharntke *et al.*, 2007; Tscharntke, Tylianakis, *et al.*, 2012).

To help meet yield demands, a dependency on chemical pesticides has developed, with global annual average use estimated at 2.68 kg ha^{-1} of active ingredient (FAO, 2019). In western Europe, usage is significantly higher at 3.97 kg ha^{-1} (FAO, 2019). Indirect effects of pesticides for the control of weeds, arthropods, and microorganisms are considered one of the most significant drivers in global biodiversity loss (Boatman *et al.*, 2004; Berger *et al.*, 2018; Sánchez-Bayo and Wyckhuys, 2019). The effects of chemical pesticides range from direct acute and chronic toxicity to sub-lethal behavioural and functional effects, which impact individuals, communities, and populations (Edwards, 2002; Boatman *et al.*, 2004). Insecticides have been linked to declines in arthropod populations, including soil and aquatic arthropods (Sánchez-Bayo *et al.*, 2007; Beketov *et al.*, 2013), species of conservation concern (Krishnan *et al.*, 2020), crop pollinators (Brittain *et al.*, 2010; Hahn *et al.*, 2015) and natural enemies of crop pests (Geiger *et al.*, 2010). Declining densities of these arthropod populations can disrupt the functioning of ecosystem processes and the provision of services (Benayas *et al.*, 2009; Brittain *et al.*, 2010; Easton and Goulson, 2013; Hahn *et al.*, 2015).

The global food supply chain is considered one of the leading contributors to climate change, producing 25-30% of anthropogenic greenhouse gas (GHG) emissions (Poore and Nemecek, 2018; Mbow *et al.*, 2019). Critically, agriculture itself is becoming increasingly vulnerable to this changing climate (Mbow *et al.*, 2019). Since 1880, the average global temperature has increased by 0.85°C (IPCC, 2015) which has impacted crop yield both directly (Asseng *et al.*, 2013; Challinor *et al.*, 2014), as well as indirectly by shifting spatial and temporal ranges of interacting species (Mooney *et al.*, 2009; Campbell *et al.*, 2016). Plant pollinator interactions, for instance, are being disrupted by temporal shifts in plant and pollinator phenology (Burkle, Marlin and Knight, 2013). Under increasing regional temperatures, some species are responding by shifting their ranges to higher latitudes (Musolin, 2007) or higher altitudes (Konvicka *et al.*, 2003). If the responses to climate change between insects and their obligate host plants differ, the interacting species can become spatially isolated (Schweiger *et al.*, 2008). Range shifts driving immigration of exotic species due to climate change is expected to exceed incidental introduction rates, which is currently considered one of the leading drivers of biodiversity loss and disruption to ecosystem services (Sala *et al.*, 2000; La Sorte and Jetz, 2010). In systems degraded due to biodiversity loss, resilience to invasion by exotic species is reduced (Lyons and Schwartz, 2001). In turn, this can disrupt pest regulation services through host-enemy dynamics, herbivory, host-plant associations, competition, and mutualisms (see Thomson, Macfadyen and Hoffmann, 2010).

The disruption of vital ecosystem services in agricultural has highlighted the vulnerability to ineffective management, pollution, and resource depletion (Baldos and Hertel, 2014; Godfray, 2015). Over the next 30 years, global human population is projected to grow to 9.73 billion (FAO, 2018). To meet future demands, food production will have to increase by an estimated 50% which will require increased intensification and an additional 6-21% of land cleared for agriculture (FAO, 2018). Greenhouse gas emissions are simultaneously expected to rise by 80% by 2050, further constraining agricultural production (Tilman and Clark, 2014). The challenge now is to increase agricultural production to meet this growing demand yet mitigate the negative impacts agriculture exerts on the environment (Matson *et al.*, 1997; Tilman, 1999b; Tilman *et al.*, 2002, 2011; Foley *et al.*, 2011; Bommarco, Kleijn and Potts, 2013; Godfray, 2015). This paradox of 'sustainable intensification' has become one of the 21st century's greatest challenges (Matson *et al.*, 1997; Tilman, 1999a; Baulcombe *et al.*, 2009).

1.1.2. The need for an ecological approach to sustainable intensification

The objective of sustainable intensification is to optimise resource utilisation and management to increase agricultural yields, without adverse environmental impact nor the conversion of yet more land to agriculture (Godfray, 2015). However, there is no specified optimal way in which sustainable intensification can be achieved. As such, all possible approaches should be considered and rigorous research conducted across all agricultural systems in order to meet global sustainable intensification targets (Garnett *et al.*, 2013). The term encompasses all aspects of the supply chain from food production, access, consumption, and waste (Foresight, 2011). Sustainable intensification highlights the need to introduce new agricultural approaches which simultaneously consider environmental sustainability, while safeguarding the needs of the world's most vulnerable, through increased food supply (Baulcombe *et al.*, 2009; Foresight, 2011). Achieving this requires collaboration between growers, policy makers and ecologists alike, particularly as different interpretations of the term by these sectors has hindered more widespread adoption (Godfray, 2015). A three-part interpretation has been suggested to consider; i) Ecological intensification: concerned with conserving and harnessing ecosystem services, ii) Genetic intensification: concerned with plant and animal breeding, and iii) Market intensification: concerned with investment in growers and market access (Conway, 2012). In this thesis, the focus will be on ecological intensification.

Ecological intensification aims to improve performance and efficiency of agricultural systems and safeguard farmers' livelihoods, through optimal management of ecosystem processes (FAO, 2013). Environmentally damaging practices must be replaced with mechanisms to support biodiversity and harness the regulating and supporting ecosystems services they provide (Bommarco, Kleijn and Potts, 2013). As ecological communities are governed by landscape and local scale processes (Gaston, 2000)(Gaston, 2000), ecological intensification must utilise practices which harness landscape-wide processes, as well as adopt farm-scale practices to support ecosystem services (Bommarco, Kleijn and Potts, 2013). A key aspect for the success of ecological intensification is for growers to have suitable decision-making tools to safeguard these ecosystem processes on individual farms, across the agricultural landscape, and into the wider interacting ecosystem (Geertsema *et al.*, 2016).

1.1.3. Integrated Pest Management

Integrated Pest Management (IPM) is a multidisciplinary approach to pest regulation in agricultural crops which relies on a sound understanding of pest and natural enemy population dynamics and their interaction with their external environment (FAO, 1974). Integrated Pest Management considers all the possible techniques available and prioritises those of least impact to bringing pest populations under economic injury levels and minimising adverse environmental effects (FAO, 1974). As such, IPM serves as a decision-making support tool in which pest control strategies can be coordinated into management programmes (Kogan, 1998). Growers have a suite of methods which can be used individually or in combination in order to bring about control by; i) mechanical, physical and cultural means, ii) host resistance through classic plant breeding or genetic engineering, iii) biological control, iv) mating disruption including sterile insect technique, v) biopesticides, and vi) conventional pesticides (Figure 1.1) (Eilenberg, Hajek and Lomer, 2001).

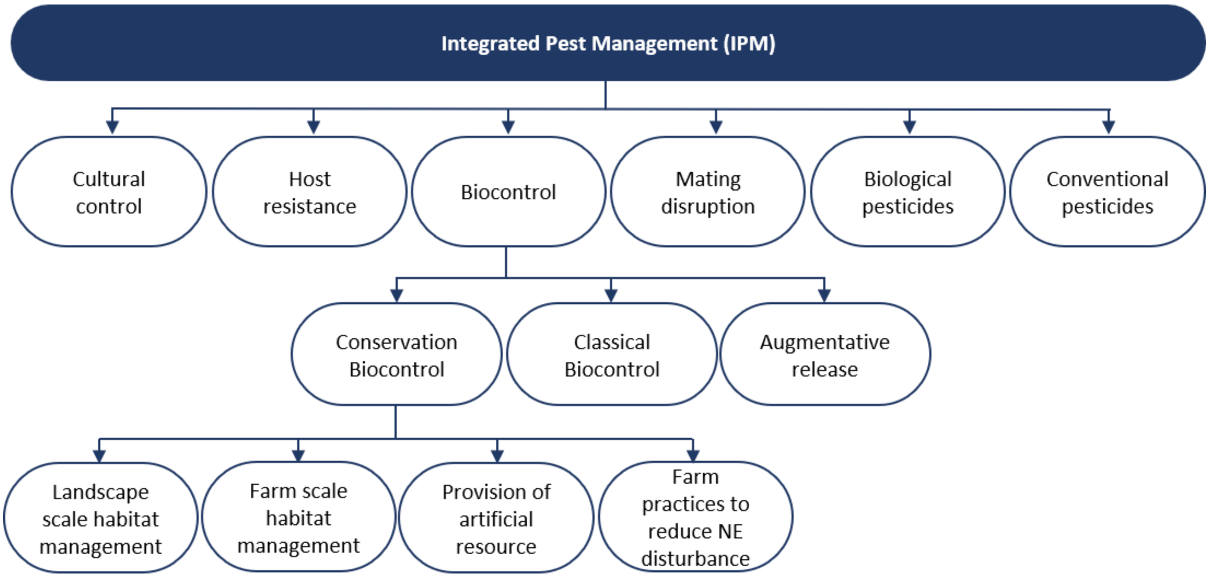

Figure 1.1 The relationship between Conservation Biological Control (CBC) strategies and other biological control strategies within the broader decision-making framework provided by Integrated Pest Management. Modified from Eilenberg, Hajek and Lomer, (2001) with CBC principles from Begg et al. (2016).

Pest control strategies under IPM are deployed using an eight-tier hierarchical framework. The framework aims to reduce the use of environmentally damaging plant protection products (PPPs) and encourage the adoption and deployment of the least ecologically damaging methods as the first line of defence (Barzman *et al.*, 2015):

1. Cropping system should be designed to be robust, implementing cultural control measures to prevent the establishment of pest organisms.
2. Early warning and pest forecasting systems should be used to anticipate pest establishment and once established they should be monitored.
3. Economic injury levels serve as a reference for actual pest load on the crop and guild decisions of which pest control strategies to deploy either independently or in combination.
4. Sustainable biological, physical and other non-chemical methods should be preferentially selected before the use of chemical control measures.

5. When chemical measures are necessary, specific/narrow-spectrum pesticides should be used and timed to limit effects on non-target organism.
6. Chemical pesticides should be applied using precision application techniques to reduce pesticide leaching and drift.
7. Anti-resistance strategies should be employed to mitigate the risk of pesticide resistance developing in pest species.
8. The protection measures used, and the programme developed should be evaluated to assess its efficacy and guild future decision making (European Commission, 2009; Barzman et al., 2015).

Since 2014, it has been mandatory for all commercial growers within EU member states to follow IPM principles for the use of sustainable biological, physical, and non-chemical methods in preference to chemical control (Lefebvre, Langrell and Gomez-y-Paloma, 2014). Calculating IPM adoption rates, however, has proven difficult (European Commission, 2017). The EU overview report on sustainable use of pesticides (European Commission, 2017) highlighted disparity between the adoption of IPM in member states and its potential across all eight levels. For instance, in Spain it has not been possible to quantify IPM adoption rates due to data gaps in the use of pest monitoring strategies (European Commission, 2018). As such, there is much to be achieved before the full potential of IPM is harnessed across European member states (European Commission, 2017).

In 2009, a framework was established (Directive 2009/128/EC) to achieve sustainable pesticide usage within the European Union (European Commission, 2009). Biological control is one of the strategies for sustainable pest regulation which takes advantage of the services provided by natural enemies, typically predators, parasitoids and pathogens, to control target pest species (Eilenberg, Hajek and Lomer, 2001). There are three common approaches to biological control (Figure 1.1); i) classical biocontrol is the importation and release of non-native species with the aim to establish them within the new habitat, ii) augmentative release is the periodic release of mass-reared naturalised or native natural enemies, and iii) Conservation Biological Control (CBC) is the conservation of native and naturalised natural enemies within the agricultural environment (DeBach and Rosen, 1991; Letourneau, 1998; Eilenberg, Hajek and Lomer, 2001).

1.2. Habitat management for pest regulation services

1.2.1. Conservation Biological Control

Conservation Biological Control (CBC) utilises practices originally developed for the conservation of rare and endangered species and applies them to support natural enemy populations in the landscape to provide pest regulation services (Letourneau, 1998). Agricultural landscapes typically exhibit low landscape-scale diversity with patches of higher diversity scattered scarcely across them. It is in these intermediate landscapes, where the greatest benefits from habitat creation and conservation can be harnessed (Haenke *et al.*, 2009; Chaplin-Kramer and Kremen, 2012). Though schemes designed for specific ecosystem services rarely increase biodiversity, restoring biodiversity at the farm scale often provides valuable ecosystem services (Díaz *et al.*, 2006); a high diversity of natural enemies in the wider landscape can increase pest regulation services (Letourneau *et al.*, 2009).

Management practices aimed to enhance pest regulation services should increase crop diversification across the landscape, increase the quantity and quality of semi-natural habitats, and minimise pesticide pressure on natural enemies, both at the within-farm and landscape scales (Bommarco, Kleijn and Potts, 2013; Rusch *et al.*, 2016). Conservation biological control strategies at both spatial scales may be classified as those which protect and those which enhance natural enemies (Eilenberg, Hajek and Lomer, 2001). Strategies to minimise impact on natural enemies may include the use of specific (narrow-spectrum) insecticides applied using precision techniques (Cassman, 1999; Jacas and Urbaneja, 2010), reduced tillage and soil disturbance (Choudhary *et al.*, 2018), the provision of artificial refugia, such as earwig shelters (Lordan *et al.*, 2014), establishing within field 'beetle banks' (MacLeod *et al.*, 2004), and conserving seminatural habitats within the wider landscape, such as fallow fields, hedgerows and woodlands (Östman, Ekbom and Bengtsson, 2001). Strategies to enhance natural enemies may include directly providing sugar (carbohydrates) or protein (amino acids) resources to the crop (Wäckers *et al.*, 2017), establishing wildflower field boundaries (Rodríguez-Gasol *et al.*, 2019), conserving wildflower meadows, or putting previously cropped fields into fallow (Vialatte *et al.*, 2017). For growers, the establishment and/or preservation of such non-cropped habitats to support and enhance natural enemies can be an appealing option as a cost-effective strategy to pest regulation (Griffiths *et al.*, 2008).

1.2.2. Habitat management

Habitat management is a subset of CBC concerned with establishing, preserving, or enhancing existing habitats within the agricultural landscape for the delivery of pest regulation services (Landis, Wratten and Gurr, 2000). By improving the availability and quality of resources for natural enemies, habitat management aims to maximise natural enemy performance and enhance pest regulation services (Landis, Wratten and Gurr, 2000). These non-cropped habitats, which can be specifically created or preserved semi-natural areas, must serve as a source of natural enemies when pest populations increase in the crop and as a sink when crop pests are scarce (Pulliam, 1988; Ortiz-Martínez, Ramírez and Lavandero, 2013). As such, effective management relies on provisioning essential resource across suitable spatiotemporal scales, enabling pest regulations services to be supported throughout the year (Landis, Wratten and Gurr, 2000). Before adopting habitat management strategies, it is important to understand the resource requirements of natural enemies so that longevity and fecundity can be maximised (Landis, Wratten and Gurr, 2000; Jervis, 2005). However, resource requirements are dynamic, they are dependent on both the species of natural enemy and the biology of their hosts/prey (thermal limits, feeding ecology, dispersal patterns, development, oogenesis, gravidity-age trade-off), as well as a complex of environmental conditions across space and time (Fellowes, van Alphen and Jervis, 2005).

1.2.3. Natural enemy resource requirements

1.2.3.1. Dispersal and residency in response to resource availability

Natural enemies rely on cues in the environment to identify suitable patches of resources (Fellowes, van Alphen and Jervis, 2005). Many natural enemies are attracted to patches based on olfactory cues (Raguso *et al.*, 2015) as well as visual cues which are associated with their host/prey or host plants (Lorenzetti *et al.*, 1997; Williams and Cook, 2010). Natural enemy response then depends on availability of carbohydrates (e.g., nectar and/or honeydew), protein (e.g., hosts, prey and/or pollen), and appropriate shelter and refuge (Gurr *et al.*, 2017; Snyder, 2019). The individual response of natural enemies to these resources depends on internal stimuli and individual experience; external stimuli are traded-off against nutritional state, egg-load, age, and experience (Charnov, 1976; Wäckers, 1994). Natural enemies will not disperse into patches where the resource they require is absent (Tscharntke *et al.*, 2016), or will disperse away from patches with poor quality resource (Charnov, 1976; Schellhorn, Bianchi and Hsu, 2014; Tscharntke *et al.*, 2016; Heimpel, 2019). In agricultural landscapes, resource can be limited to brief and discrete periods, such as the crop flowering period or during pest infestation (Landis, Wratten and Gurr, 2000; Wackers, van Rijn and Bruin, 2005). Therefore, establishing, preserving and managing resources within cropped areas can help to attract natural enemies into the crop, increase their residency time, and ultimately boost pest regulation services (Landis, Wratten and Gurr, 2000; Schellhorn, Bianchi and Hsu, 2014; Gurr *et al.*, 2017). For habitat management to be effective, the time natural enemies spend in the cropped environment must be maximised (Heimpel, 2019). This may be achieved by establishing or preserving plant communities within or around the cropped habitat to provide natural enemies with the necessary resources required: carbohydrates, proteins, and shelter and refuge (Landis, Wratten and Gurr, 2000; Gurr *et al.*, 2017).

1.2.3.2. Carbohydrates as a resource for natural enemies

Despite being defined by their entomophagous life history stage, many natural enemies benefit from consuming carbohydrate (e.g., nectar and honeydew), increasing both longevity and fecundity (Wackers, van Rijn and Bruin, 2005). Carbohydrates are available to natural enemies via floral nectar, extrafloral nectar and hemipteran honeydew (Tena *et al.*, 2016; Benelli *et al.*, 2017). However, seasonal variation can influence the abundance of these resources, leading to periods of limited availability (Vollhardt *et al.*, 2010). In the field, nectar and honeydew availability increases sugar feeding in parasitoid populations and communities (Pekas *et al.*, 2010; Kishinevsky, Cohen, *et al.*, 2017), which translates to increased longevity and fecundity and can maximise pest regulation services (Tena *et al.*, 2015).

Jervis *et al.* (1993) described flower visitation and nectar feeding behaviour in over 1,000 parasitoid species. Floral and extrafloral nectar is composed of glucose, sucrose and fructose in varying compositions across different plant taxa (Wäckers, 2001). Higher nectar water content and humidity facilitates its exploitation by natural enemies (Winkler *et al.*, 2009). Nectar also contains several secondary metabolites which influence its attractiveness and suitability for natural enemies (Palmer-Young *et al.*, 2019). The architecture of the flower itself affects accessibility of the nectar to natural enemies (van Rijn and Wäckers, 2016). Nectaries in some plant taxa are concealed and only accessible to specialised pollinators, while the nectaries of some flowers are exposed and nectar is accessible for non-specialist (generalist) arthropods, including natural enemies (van Rijn and Wäckers, 2016).

Nectar is considered a superior food source for natural enemies compared to other carbohydrate sources (Wäckers, 2001). For example, nectar from *Anchusa azurea* Miller (Boraginales: Boraginaceae), was found to significantly increase parasitoid longevity compared to a diet of *Aphis gossypii* honeydew (Pinheiro *et al.*, 2013a). However, a more ubiquitous carbohydrate source, not limited to brief flowering periods, is hemipteran honeydew (Wäckers, van Rijn and Heimpel, 2008), the sugary substance secreted by phloem feeding Hemiptera (Wilkinson *et al.*, 1997). Despite its lower nutritional value for natural enemies than nectar, honeydew can still significantly increase natural enemy survival when no other sugar source is available (Tena, Llácer and Urbaneja, 2013; Pinheiro *et al.*, 2015). Varying fitness effects on natural enemies have been observed across different honeydew sources, largely due to honeydew composition (Hogervorst, Wäckers and Romeis, 2007; Tena, Llácer and Urbaneja, 2013). In the field, when honeydew producers are at high densities, carbohydrate is no longer limiting, natural enemy fitness is increased and pest regulation services optimised (Vollhardt *et al.*, 2010). However, when pest populations reach lower densities and honeydew is scarce, floral nectar becomes the most important source of carbohydrate in the field (Vollhardt *et al.*, 2010).

The provision of carbohydrates can also relax competition for limited shared resources between natural enemies and ants (Calabuig, Garcia-Marí and Pekas, 2015; Wäckers *et al.*, 2017). Ants, such as *Lasius grandis* Forel and *Tapinoma erraticum* (Latreille) (Hymenoptera: Formicidae), are often considered indirect pests through their tending behaviour of honeydew producing Hemiptera from which they have a supply of carbohydrates (Hölldobler and Wilson, 1990; Calabuig, Garcia-Marí and Pekas, 2014). This protective behaviour reduces predation and parasitism rates in the ant-tended hemipteran citrus pests and inhibits pest regulation services (Calabuig, Garcia-Marí and Pekas, 2014; Wäckers *et al.*, 2017). Additionally, attack rates of non-honeydew producing pests are reduced as the ants' foraging activity disrupts natural enemies foraging for their prey/hosts (Pekas *et al.*, 2011) and prevents access to otherwise readily available honeydew resources, reducing natural enemy fitness (Calabuig *et al.*, 2015). However, when higher-quality alternative carbohydrates sources are provided, foraging ants in the canopy are attracted away from honeydew producers and their activity in the crop canopy is reduced (Wäckers *et al.*, 2017). This indirectly increases pest mortality rate by increasing predation/parasitism rates by natural enemies through reduced resource competition and increased natural enemy fitness by carbohydrate-feeding (Wäckers *et al.*, 2017).

1.2.3.3. Protein as a resource for natural enemies

Insect predators and parasitoids require protein throughout developmental life history stages to complete their lifecycles (House, 1961; Chen, 1966). Protein can be obtained both from arthropod and plant-derived sources, depending on life history strategy (Jervis, 2005). Some natural enemies, e.g., Coccinellids, display predatory behaviour in both the adult and juvenile stages (Hodek, Honek and Van Emden, 2012). However, other insect predators, such as the Chrysopid *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), only display predatory behaviour in their juvenile stage, obtaining protein during their adult stage by pollen feeding (New, 1975). Parasitoids display entomophagous behaviour during their larval stages when they obtain the protein required by feeding on their hosts (Godfray, 1994). Additionally, some parasitoid species, such as female *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), require protein during their adult stage for oogenesis, which they obtain by host feeding (Heimpel, Rosenheim and Kattari, 1997; Mondy *et al.*, 2006). Similarly, adult female *C. carnea* display a greater rate of protein consumption than their male counterparts (Villenave *et al.*, 2005).

In the absence of crop pests, agricultural landscapes can be limited in arthropod derived amino acids (Tscharntke *et al.*, 2007). The establishment or preservation of non-cropped habitat increases the niche breadth for non-pest phytophages and can help attract natural enemies into the agri-environment and sustain them during periods of pest scarcity (Berryman and Hawkins, 2006). Grasses are considered good candidates to support non-pest phytophages which serve as alternative hosts/prey (Gómez-Marco, Urbaneja and Tena, 2016).

Pollen can serve as a complimentary protein source for some predatory arthropods when other sources are scarce (Villenave *et al.*, 2005; Vandekerkhove and De Clercq, 2010; Wong and Frank, 2013; Vacacela Ajila *et al.*, 2019). The consumption of pollen under arthropod-protein scarcity can help increase survival and fecundity and shorten developmental time in many predacious arthropods, such as Chrysopidae (Venzon *et al.*, 2006), Heteroptera (Vandekerkhove and De Clercq, 2010; Wong and Frank, 2013), Coccinellidae (De Clercq *et al.*, 2005; Berkvens *et al.*, 2008), adult Syrphidae (Pinheiro *et al.*, 2015) and Phytoseiidae (van Rijn and Tanigoshi, 1999). Patch residency time of the phytoseiid *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) was also increased through the provision of pollen, even after successful control of the pest, *Tetranychus urticae* Koch (Acari: Tetranychidae) (Vacacela Ajila *et al.*, 2019). This suggests that supplementary protein may help retain natural enemies in the environment even when prey is scarce.

Under resource competition, the provision of pollen can alter the structure of predator communities which could disrupt biological control services (Shakya, Weintraub and Coll, 2009; Aguilar-Fenollosa *et al.*, 2011b; Pina *et al.*, 2012; Calabuig, Pekas and Wäckers, 2018). Not all pollen is of the same nutritional quality however, and different taxa respond in varying ways in terms of feeding preference, developmental time, longevity, and fecundity, according to the nutritional quality of the pollen (van Rijn and Tanigoshi, 1999; Goleva and Zebitz, 2013). Under prey scarcity, the provision of high-quality pollen can significantly decrease the intraguild predation between generalist predators (Calabuig, Pekas and Wäckers, 2018). However, the availability of high-quality pollen promotes establishment of generalist Phytoseiids which are more specialised to pollen-feeding (McMurtry, Moraes and Sourassou, 2013) and may outcompete Phytoseiids which are more specialised to a predatory life history (Pina *et al.*, 2012; McMurtry, Moraes and Sourassou, 2013). Competition for this shared resource can lead to a greater abundance of less effective predatory species e.g., *Euseius stipulatus* (Acari: Phytoseiidae) (Pina *et al.*, 2012). In contrast, the provision of lower nutritional-quality grass pollen reduces competition and enhances the abundance of more effective predatory species e.g., Type I and II Phytoseiids (Aguilar-Fenollosa *et al.*, 2011a; Pina *et al.*, 2012).

1.2.3.4. Shelter as a resource for natural enemies

The cropped environment provides an unstable habitat for natural enemies due to high levels of regular disturbance (Thorbek and Bilde, 2004). In the absence of suitable shelter, disruptive agricultural practices, such as harvesting, tilling, and pesticide applications, expose natural enemies to unfavourable temperatures, pesticides, and intraguild predation (Landis, Wratten and Gurr, 2000). Non-cropped habitats significantly reduce soil surface temperatures (Orr and Landis, 1997; Diehl, Wolters and Birkhofer, 2012) and disrupt the thermal boundary layer, creating a microclimate which extends above the soil surface (Luff, 1965).

Most insects are governed by strict laws of thermoregulation and as such, microclimates serve as vital resources to shelter from upper and lower temperature limits (Jackson, 1986). In temperate latitudes, overwintering sites for natural enemies are essential for their persistence (Geiger, Wäckers and Bianchi, 2009). Alternative habitat in cropped areas during the summer months can also provide vital shelter for natural enemies which otherwise would not survive in the cropped environment (Dennis and Fry, 1992). Insect activity is reduced, and mortality rates increased, when upper temperature and lower humidity thresholds are reached (Rahim, Hashmi and Khan, 1991; Hailemichael, Smith Jr. and Wiedenmann, 1994). In turn, this directly impacts natural enemies, and their pest regulation services (Rahim, Hashmi and Khan, 1991; Hailemichael, Smith Jr. and Wiedenmann, 1994; Landis, Wratten and Gurr, 2000).

Shelter exists on various scales, from specific plant-structures (e.g., trichomes) to the whole plant and plant communities (Walter, 1996; Agrawal and Karban, 1997; Cortesero, Stapel and Lewis, 2000). On the whole plant scale, trichomes and cavities create refugia for predatory mites, anthocorids and thrips and can help increase pest regulation services (Walter, 1996; Agrawal and Karban, 1997). At the plant community scale, grasses forming dense tussocks, such as *Dactylis glomerata* L. (Poales: Poaceae), support favourable microclimates both during cold and wet winters as well as hot and dry summers (Luff, 1965). The microclimates created by tussock-forming grasses can help increase predator abundance within the non-cropped habitat (Woodcock *et al.*, 2005), as well as dispersal into the adjacent crop (Thomas, Wratten and Sotherton, 1991).

When insecticide treatments are applied to target key pest species, the deleterious effect on natural enemies can facilitate secondary pest species in exceeding economic injury levels (Li and Yang, 2015). The provision of refugia can help shelter natural enemies from insecticide treatments, reducing their mortality, and supporting pest regulation services (Lester, Thistlewood and Harmsen, 1998; Lee, Menalled and Landis, 2001; Heimoana *et al.*, 2017). For example, within-tree artificial refugia can shelter Phytoseiid predators from insecticide applications, allowing them to persist in apple orchards and subsequently control secondary pest species (Lester, Thistlewood and Harmsen, 1998). At the field scale, the deployment of habitats composed of grasses and wildflowers can provide shelter and significantly increase recolonisation and predator activity in the adjacent crop after insecticide applications (Lee, Menalled and Landis, 2001; Heimoana *et al.*, 2017).

Alternative habitat may also provide refuge for predators under intraguild predation (Finke and Denno, 2002; Janssen *et al.*, 2007). Increased habitat structural complexity can decrease encounter rates between predators, create predator-free space and relax predation risk between intraguild predators (Janssen *et al.*, 2007). Refugia from intraguild predation within complex structural habitats can therefore enhance predation of the shared pest and enhance pest regulation services (Finke and Denno, 2002). Additionally, the intensity of intraguild predation might be relaxed in more structurally complex habitats which harbour greater diversity and abundance of prey items (Tixier *et al.*, 2013).

1.2.4. The influence of scale on pest regulation services

Different taxa are susceptible to processes at different scales (Schmidt *et al.*, 2008), and the strength of these effects on pest regulation services is dependent on pest and natural enemy dispersal capacity (Chaplin-Kramer *et al.*, 2011). Specialist natural enemies, such as parasitoids, respond most strongly to habitat complexity at the local scale (Rand and Tscharntke, 2007; Chaplin-Kramer *et al.*, 2011; Bianchi *et al.*, 2015), whilst generalist natural enemies, respond more strongly at spatial scales of 1.5 to 2 km (Chaplin-Kramer *et al.*, 2011). Consequently, both scales need to be considered in conjunction with relevant IPM strategies. In simple landscapes the arrival of natural enemies may be delayed, even when complexity exists at the local scale, suggesting that pest regulation services may be dramatically affected in simple, homogenous landscapes (Chaplin-Kramer and Kremen, 2012).

1.2.5. Habitat composition and structure influence pest regulation services

To maximise ecosystem services in the field, resources required by natural enemies (outlined in section 1.2.3) must be available throughout the season (Landis, Wratten and Gurr, 2000). Ramsden *et al.* (2015) investigated the relative importance of floral diversity, pest abundance, and overwintering habitat on aphid parasitoids and predators. The presence of wildflower habitats increased predator and parasitoid abundance and enhanced regulation in the adjacent crop, both before the arrival of the aphid prey/host and during the aphid critical period (Ramsden *et al.*, 2015). This suggests that diverse nectar carbohydrates are a vital resource in augmenting and sustaining natural enemies in the agri-environment, even when honeydew is not limited (Ramsden *et al.*, 2015).

The diversity of flower architecture within a wildflower habitat can have significant effects on arthropod community structure (Campbell *et al.*, 2012, 2017; van Rijn and Wäckers, 2016).

The benefits of exposed nectaries on natural enemies are greatest during summer months, following the aphid critical period when honeydew-carbohydrate is likely to be limited (Campbell *et al.*, 2017). There is therefore a strong rationale to increase plant species diversity to provide flowers in succession and extend the combined flowering period throughout the year (Dale *et al.*, 2020).

The quantity of floral resource provided by annual and biennial plant species can vary significantly between years. Perennial species, on the other hand, provide a more consistent year-on-year resource (Carvell *et al.*, 2006), with the habitat becoming more attractive to natural enemies as it matures (Fiedler and Landis, 2007). Furthermore, habitats composed of perennial species often begin flowering earlier in the season and exhibit a longer flowering period over the length of the year (Fiedler and Landis, 2007). Though some annual species support higher abundances of natural enemies (Buchanan, Grieshop and Szendrei, 2018), non-cropped habitats comprised of perennial species are able to provide suitable resource to enhance pest regulation in orchard systems (Tschumi *et al.*, 2016; Cahenzli *et al.*, 2019). As such, perennial species might provide a more economically viable solution, as they do not have to be re-sown yearly (Miettinen *et al.*, 2014), and the delivery of pest regulation services increases with time (Fiedler and Landis, 2007).

The composition of the non-cropped habitat can further play a key role in the availability of alternative prey/hosts (Berryman and Hawkins, 2006). Plant species richness drives the abundance of phytophagous arthropods which serve as alternative prey/hosts (Woodcock and Pywell, 2010) and can support greater abundance of natural enemies (Marshall and Moonen, 2002). Non-native herbaceous plants, however, tend to harbour greater proportions of potential pest species than native vegetation and can facilitate early colonisation of pest species on the crop (Parry *et al.*, 2015). In contrast, native vegetation, sustains a greater predator-to-pest-ratio than on the crop, and can serve as a source of natural enemies to spill-over into the cropped environment (Bianchi, Schellhorn and Cunningham, 2013; Parry *et al.*, 2015). Furthermore, the complexity of the non-cropped habitat, taken as the vertical distribution of plant functional groups, is considered a key driver in both natural enemy and phytophage diversity (Woodcock *et al.*, 2007, 2009).

Structural complexity within the non-cropped habitat can be enhanced by increasing not only plant species, but plant trait and functional group diversity (Woodcock *et al.*, 2007). Therefore, use of a diverse range of native plant species from distinct functional groups, such as a mix of both grasses and forbs, can increase plant species diversity and help to augment natural enemy diversity and abundance (Woodcock *et al.*, 2007, 2009; Woodcock and Pywell, 2010; Bianchi, Schellhorn and Cunningham, 2013; Parry *et al.*, 2015; Ramsden *et al.*, 2015).

1.2.6. Manipulation of the non-cropped habitat

Management of non-cropped habitat, e.g., cutting and scarification, can have significant effects on plant community composition and structure (Westbury *et al.*, 2008). The strategic management of non-cropped habitat can therefore be used to differentially influence assemblages of higher tropic levels (Westbury *et al.*, 2011), and might be used to enhance pest regulation services.

A key strategy applied to manipulate habitat composition and structure is cutting (Bugg and Waddington, 1994). Cutting is recommend once annually to 15cm, to support nectar feeding species (Natural England, 2013). The timing of cutting, however, can affect plant species composition (Marriott, Bolton and Fisher, 2003; Bretzel *et al.*, 2012) as well as abundance of higher tropic levels (Ausden and Treweek, 1995). By cutting late in the season (autumn), plant species diversity and evenness can be increased when compared with spring and summer cuts, as annuals are allowed to set seed (Bretzel *et al.*, 2012). However, spring cutting preferentially selects for perennial species by promoting vegetative shoot growth and preventing annuals from setting seed (Marriott, Bolton and Fisher, 2003; Westbury *et al.*, 2008).

Cutting can also be used as a strategy to manipulate the availability of required resources, e.g., shelter, carbohydrate, and protein throughout the year (Bugg and Waddington, 1994). Cutting prior to bud burst, for example, can extend the flowering period by delaying flowering (Nowakowski and Pywell, 2016). Cutting a portion of the wildflower habitat could be used to increase structural heterogeneity which can boost natural enemy abundance within the sward (Woodcock *et al.*, 2007). Furthermore, wildflower habitats augment natural enemy populations which are then able to spill-over onto the adjacent crop canopies (Mateos-Fierro *et al.*, 2021), with many important citrus natural enemies found within the alleyway habitats themselves (Monzó, Molla, *et al.*, 2011; Vercher *et al.*, 2012). There is, therefore, strong rational that cutting the wildflower habitats might force spill-over movement of natural enemies onto the crop (Bugg and Waddington, 1994). Timing the cut to coincide with peak pest incidence could be a strategy to boost natural enemies and their pest regulations services on the crop at key points in the year. The extent to which a forced spill-over of natural enemies can be achieved has not been fully investigated (Landis, Wratten and Gurr, 2000).

To enhance habitat heterogeneity at the local scale, habitat management and manipulation strategies need not be applied homogeneously throughout a farm (Bugg and Waddington, 1994). Different management strategies may be applied in alternating alleyways in orchards to maximise benefits. This can include different plant composition between alleyways and different manipulation techniques, or any combination of composition and manipulation strategies (Bugg and Waddington, 1994). This results in swards of different composition, providing a more diverse range of resources, which could potentially reduce competition between beneficial arthropods (Bennett, Peterson and Gordon, 2009; Campbell *et al.*, 2012).

1.3. Citrus cultivation and pest management

1.3.1. The Citrus *genus and its cultivation in Spain*

The *Citrus* genus (Geraniales: Rutaceae) has a long history of cultivation and trade (Ramón-Laca, 2003). Citrus has a high tendency to hybridise, leading to the development of numerous varieties which was also facilitated through ancient trade routes, resulting in the modern Citrus lineages (Ramón-Laca, 2003; Wu *et al.*, 2014, 2018). Citrus is commonly grouped into four categories based on fruit characterises characteristics; lemons and limes, tangerines, oranges, and grapefruits (Ramón-Laca, 2003; FAO, 2017).

Modern citrus is an important global cash crop traded all over the world, with annual total production of 124 million tonnes (FAO, 2017). Spain is considered the leading global exporter of citrus (FAO, 2017), producing 7.5 million tonnes in 2018, 41.3% of which were exported (MAPA, 2019). In the Mediterranean basin, Spain comprises the largest land use area for citriculture (Berk, 2016), totalling 269,677 ha in 2018 (Berk, 2016; MAPA, 2019). Of this, orange production represents the largest area (47%), compared to 37% for mandarin, 14% for lemon and 2% for other citrus (e.g., grapefruit). In total, 3.9 million tonnes of sweet oranges were produced in 2018 (MAPA, 2019). Most of the sweet orange grown in Spain is destined for the high value fresh fruit market (FAO, 2017) for which high quality and high aesthetic standards are expected (Urbaneja, Grout, *et al.*, 2020). Fruit failing to meet these standards due to the presence of pests or their damage, is downgraded or discarded (Urbaneja *et al.*, 2015).

In 2013, over 2.99 million kg pesticide active ingredient was applied in Spanish citriculture with over two thirds (67.4%) of which were to control arthropod pests (MAPAMA, 2013). However, since the 2014 EC directive was enforced (see section 1.1.3), insecticide application in Spain has decreased by an average of 2.5% per year (Eurostat, 2020). Consumer demand for products low in pesticides residuals and organically produced has increased (Shafie and Rennie, 2012). Due to the economic significance of Navel oranges in Spain coupled with the high cosmetic standards of the fruit and a drive for sustainable approaches to agriculture, this study focuses on the ecological intensification of Navel orange orchards in Andalucía. Andalucía is one of the most important citrus growing regions in Spain, where production has increased over the last decade and now produces 1,722.6 tonnes of sweet oranges annually (almost half the Spanish total of 3,488.5 tonnes) (MAPA, 2021).

1.3.2. Navel oranges growth and development

The ancestor of Navel oranges (*Citrus sinensis* L.) arose from the hybridisation of two pummelo-mandarin mixes, with the modern varieties radiating by stomachic mutation from this single ancestor (Wu *et al.*, 2018). The Washington or Bahia navel (*C. sinensis* L.) arose from a mutation in the Selecta variety in Brazil in the early 19th Century (Reuther, Batchelor and Webber, 1967). Due to a combination of desirable characteristics, fruit quality, large fruit size and vigour of the tree itself, the Washington variety became popular and was introduced to Australia in 1824 (Reuther, Batchelor and Webber, 1967). It was in Australia where further mutations gave rise to the Lane Late, Powell, Chislett, and Rohde varieties of Navel orange (Reuther, Batchelor and Webber, 1967; United States Patent, 1991; IVIA, 2016). Despite numerous varieties, modern Navel oranges show very little genomic variation(Wu *et al.*, 2018).

Navel orange trees display three distinct growth phases per year, spring, summer and autumn flushes (Guardiola, 1997). The summer and autumn phases are characterised by vegetative growth, while the spring growth phase is characterised by the onset of flower bud development (Guardiola, 1997). The timing and vigour of these phases are affected by climate (temperature, photoperiod, light intensity), water availability, and fruit load (Ramana *et al.*, 1981; Krajewski and Rabe, 1995; Guardiola, 1997; Valiente and Albrigo, 2004). For example, Navel orange yields are closely associated with autumn temperatures, and yields are reduced when maximum daily temperatures exceed 30°C from September to November (Ramana *et al.*, 1981). In Mediterranean regions such as Spain, vegetative shoots typically occur during spring, summer and autumn (Guardiola, 1997; Valiente and Albrigo, 2004). Flowering and fruiting patterns depend on the variety; citrus breeding has selected for a range of fruit maturation dates from October (such as the Navelina variety M-7) to June (such as the Valencia late variety Lavalle) (IVIA, 2016). Late varieties are harvested between late January and early May (IVIA, 2016). The fruit develops over approximately 68 weeks, resulting in fruit and flowers being present simultaneously on the same tree (Ramana *et al.*, 1981). During the seven to eight month period after flowering, the fruit passes through a rapid growth phase, where the juice content increases, reaching a maximum at the end of this phase in autumn (Ramana *et al.*, 1981). Low winter temperatures then trigger fruit ripening and initiates flower development (Guardiola, 1997; Valiente and Albrigo, 2004).

1.3.3. Key Navel orange pests and their management

Despite extensive efforts to identify and implement biological control strategies in Spanish citrus, several species still evade satisfactory control and regularly require the use of additional control methods (Urbaneja *et al.*, 2015). In sweet orange, when present, California Red Scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae); Medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae); *Eutetranychus* mites, *E. banksi* (McGregor) and *E.*

orientalis (Klien) (Acari: Tetranychidae); and Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae), all require the use of chemical insecticides to maintain populations beneath economic injury levels (Llorens Climet and Martín Gil, 2014).

Increased trade and movement of plant material and fresh produce across the globe, has accelerated the introduction of invasive species (Urbaneja, Grout, *et al.*, 2020). Two such recent introductions to Spain have resulted in the establishment of new and significant pest species: the mealybug *Delottococcus aberiae* (De Lotto) (Hemiptera: Pseudococcidae) and the thrips *Chaetanophothrips orchidii* (Moulton) (Thysanoptera: Thripidae) (Campos Rivela et al., 2017; Urbaneja, Grout, et al., 2020). Both *D. aberiae* and *C. orchidii* are capable of inflicting significant economic damage to the Spanish citrus industry (Campos Rivela et al., 2017; Urbaneja, Grout, et al., 2020). The urgency to control these species, in the absence of a biological and ecological control strategies, leads to dependency on insecticides which in turn compromises conservation biological control (Pérez-Rodríguez *et al.*, 2017; Urbaneja, Grout, *et al.*, 2020). Huanglongbing (HLB) for example, is an insect vectored citrus disease which currently represents a real threat to the Spanish citrus industry (Cocuzza *et al.*, 2017). In 2014, one of HLB's insect vectors, *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae), was detected in Galicia, north-western Spain, and has since dispersed southwards into Portugal (Pérez-Otero, Mansilla and del Estal, 2015). If not successfully managed, *T. erytreae* could reach neighbouring citrus cultivation regions in Andalucía, south-western Spain (Pérez-Otero, Mansilla and del Estal, 2015). HLB is considered the most threatening citrus disease worldwide, having devastated citrus cultivation regions in North and South America, Africa and Asia (Urbaneja, Grout, *et al.*, 2020). The significant economic impact of this disease has resulted in insecticide dependency and increased application rates to control HLB's insect vectors, *T. erytreae* and *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Urbaneja, Grout, *et al.*, 2020). The severe economic threat of HLB has led to the abandonment of biological and ecological management strategies in regions where the disease is now present (Monzó and Stansly, 2020).

1.3.3.1. Aonidiella aurantii *(California Red Scale)*

Despite being polyphagous, *A. aurantii* is only considered a significant pest in citrus (Ebeling, 1959). *Aonidiella aurantii* feeds on the cell sap of the parenchyma cells by extending its mouthparts into the host plant, puncturing non-target plant cells along the way (Washington and Walker, 1990). Feeding activity can therefore cause chlorosis of the leaves and twig drop (Ebeling, 1959). However, the most typical damage caused by *A. aurantii* is cosmetic damage of the mature fruit by the presence of scales and fruit tissue scarring caused by their feeding action (Grafton-Cardwell *et al.*, 2003; Urbaneja *et al.*, 2015). Fruit with *A. aurantii* scales present are downgraded, resulting in significant economic losses for growers (Urbaneja *et al.*, 2015). The economic injury level is exceeded when just 2% of the fruit at harvest is colonised by 10 or more scales (Llorens Climet and Martín Gil, 2014).

Aonidiella aurantii attacks all above ground parts of the tree (Beardsley, González and Gonzalez, 1975) and is therefore able to exploit the citrus tree year-round (Carroll and Luck, 1978). During the winter months, *A. aurantii* resides in the woody parts of the tree and disperses outwards through the season to colonise younger branches and twigs, new leaf flushes, and ultimately descend onto the ripening fruit (Carroll and Luck, 1978; Rodrigo *et al.*, 2004). This migration is facilitated by the first instar ‘crawler’ stage, which apart from alate adult males, are the only mobile life history stage (Forster, Luck and Grafton-Cardwell, 1995). The crawlers disperse from the mother scale over six to twelve hours (Forster, Luck and Grafton-Cardwell, 1995), and migrate onto more favourable and newly available plant organs and citrus tree growing season continues towards fruit maturation (Carroll and Luck, 1984).

Temperature impacts the developmental time of *A. aurantii*, with minimum and maximum threshold temperatures of 11.7°C and 37.8°C respectively and a generation time on sweet orange of 576.8 degree days (Grout *et al.*, 1989). In Andalucía, three to four generations of *A. aurantii* can be expected in the crop per year (Campos-Rivela, Martinez-Ferrer and Fibla-Queralt, 2012). The first generation appears in mid-May, the second towards the end of July, the third at the end of September and depending on climatic conditions (temperature) a fourth generation occurs at the end of October (Campos-Rivela, Martinez-Ferrer and Fibla-Queralt, 2012). Fruit is most susceptible to colonisation by *A. aurantii* during the second generation when the fruit enters the cell enlargement and water accumulation stage (Bain, 1957; Rodrigo *et al.*, 2004). In autumn, when the fruit begins to ripen, the majority of the *A. aurantii* population can be found colonising the fruit (Bain, 1957; Carroll and Luck, 1984).

Cultural recommendations for the management of *A. aurantii* typically include regular pruning of the citrus tree canopy and the removal of infested debris, such as fruit and branches, from the orchard floor (Krajewski and Krajewski, 2011; Llorens Climet and Martín Gil, 2014). Pruning to reduce canopy density aims to increase airflow into the trunk, preventing microclimate refuge for the scale insects and increasing early instar mortality (Llorens Climet and Martín Gil, 2014; Germán Casado *et al.*, 2016).

Classical biological control programmes for *A. aurantii* have seen the introduction of the coccinellid predator, *R. lophanthae*, and five hymenopteran parasitoid species, *Encarsia perniciosi* (Tower) (Hymenoptera: Aphelinidae), *Aphytis lingnanensis* Compere and *A. melinus* (Hymenoptera: Aphelinidae), and *Comperiella bifasciata* Howard (Hymenoptera: Encyrtidae) into Spain (Urbaneja, Jaques and Garcia-Marí, 2009). The introduced parasitoid *A. melinus* and its native congeneric *A. chrysomphalus* (Mercet) are considered the most successful and widespread *A. aurantii* parasitoids in Spain (DeBach and Rosen, 1991; Forster and Luck, 1996), with up to 60% of natural mortality attributed to these species (Vanaclocha *et al.*, 2011). However, *A. melinus* and *A. chrysomphalus* (Mercet) are resource competitors, exploiting the scale host during the second and third instar (Pekas *et al.*, 2016). Under competition, the introduced species, *A. melinus*, will outcompete the native *A. chrysomphalus* (Cebolla *et al.*, 2017, 2018). Smaller scales may serve as a refuge for *A. chrysomphalus* in the field (Pekas *et al.*, 2016), however in southwestern Spain, *A. chrysomphalus* has now been displaced by *A. melinus* (Vela *et al.*, 2007; Boyero *et al.*, 2014). Although *Encarsia perniciosi* and *C. bifasciata* are also outcompeted by *Aphytis* species in second and third instar hosts, they are able to attack a broader range of host developmental stages (Forster, Luck and Grafton-Cardwell, 1995). *Encarsia perniciosi* and *C. bifasciata* can therefore escape competition by exploiting first instars or mated- and gravid-females (respectively) which serve as refuge in the field (Yu, Luck and Murdoch, 1990; Forster, Luck and Grafton-Cardwell, 1995; Boyero *et al.*, 2014). Additionally, *A. melinus* shows preference towards *A. aurantii* on the orange fruit and leaves, enabling *E. perniciosi* and *C. bifasciata* to exploit those on the twigs, branches, and inner canopy (Forster, Luck and Grafton-Cardwell, 1995; Boyero *et al.*, 2014). Hence, where *Aphytis* species are present with either *E. perniciosi* or *C. bifasciata*, greater control of *A. aurantii* can be achieved (Sorribas, Rodríguez and Garcia-Mari, 2010). In south-western Spain, *E. perniciosi* has been reported present with *A. melinus*, however, the literature is scarce with regards to the establishment success and range of *C. bifasciata* (Pina, 2008; Sorribas, Van Baaren and Garcia-Marí, 2012; Boyero *et al.*, 2014).

Despite the significance of parasitoids in the mortality of *A. aurantii* in the field, several studies have highlighted the importance of predators, whose value may have been previously underestimated (Vanaclocha *et al.*, 2011; Bouvet *et al.*, 2019). Specialist Coccinellid predators of *A. aurantii* have been well documented in the literature, including *Chilocorus bipustulatus* (L.), *Rhyzobius lophanthae* and *Coccidophilus citricola* Brèthes (Sorribas and Garcia-Marí, 2010; Vanaclocha *et al.*, 2011). Generalist predators, such as Chrysopidae (Neuroptera), Coniopterygidae (Neuroptera), Cecidomyiids (Diptera) and Eupalopsellidae (Acari), have also been associated with *A. aurantii* (Sorribas *et al.*, 2008; Vanaclocha *et al.*, 2011). However, the recent use of molecular techniques has revealed a much more diverse range of natural enemies of *A. aurantii* than previously considered, and field mortality rates of up to 97% have been attributed to predatory enemies (Bouvet *et al.*, 2019). Nonetheless, regulation by natural enemies fails to maintain *A. aurantii* under economic injury levels and further control measures are typically required (Llorens Climet and Martín Gil, 2014).

Mating disruption techniques can be employed to help control *A. aurantii* populations in the field (Llorens Climet and Martín Gil, 2014; Germán Casado *et al.*, 2016). The use of pheromone dispensers has been shown to reduce the number of downgraded fruits by 70% (Vacas *et al.*, 2009, 2010). However, it remains unclear as to whether mating disruption enhances (Vacas *et al.*, 2012) or disrupts (Pekas *et al.*, 2015) pest regulation services by natural enemies. It is therefore recommended that the first instars of the first generation are treated with insecticides as these life history stages are most susceptible to insecticides (Urbaneja *et al.*, 2015).

1.3.3.2. Ceratitis capitata *(Mediterranean fruit fly / Medfly)*

Ceratitis capitata is highly polyphagous and can develop in more than 250 species of fruit worldwide (Christenson and Foote, 1960; Liquido, Shinoda and Cunningham, 1991; Liquido, McQuate and Suiter, 2015). Females lay their eggs under the skin of ripening or ripened fruits and the developing larvae feed on the fruit tissue causing significant economic damage (Christenson and Foote, 1960). As such, globally, *C. capitata* is considered one of the most significant pests of fruit crops (Szyniszewska and Tatem, 2014). Countries with established *C. capitata* populations are constrained by strict trade limitations, with an exportation threshold of zero individuals per fruit (Szyniszewska and Tatem, 2014).

In Spain, adult *C. capitata* are present in citrus orchards year-round (Martínez-Ferrer *et al.*, 2010). The population growth is related to spring and the previous winter climate, with cooler temperatures delaying the onset of the population growth phase (Martínez-Ferrer *et al.*, 2010). Typically, populations begin to increase in late spring (April-May) displaying a peak in mid-summer (July). Gravid females are found throughout the year, with the highest proportion found in summer, during the peak of the population (Martínez-Ferrer *et al.*, 2010). Due to its high polyphagy, the potential for reservoir populations of *C. capitata* to be harboured year-round in ornamental fruit trees and abandoned orchards within the wider landscape is high, and so is its persistence in the landscape (Sciarretta and Trematerra, 2011).

Cultural control methods for *C. capitata* include the monitoring, removal and destruction of infested fruit from the canopy and fallen fruit from the orchard floor (Llorens Climet and Martín Gil, 2014). Reservoir populations in the local landscape should also be managed through regular monitoring of alternative host plants and the removal and destruction of infested fruit (Llorens Climet and Martín Gil, 2014). A diverse guild of natural enemies attack *C. capitata* in Spain, including three introduced (non-native) parasitoids: *Diachasmimorpha tryoni* (Cameron), *D. longicaudata* (Ashmead), and *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae); two native parasitoids: *Pachycrepoideus vindemmiae* (Rondani) and *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae); and two native predators: *Pardosa cribata* Simon (Araneae: Lycosidae) (Monzó *et al.*, 2009) and *Pseudophonus rufipes* (De Geer) (Coleoptera: Carabidae) (Monzó, Molla, *et al.*, 2011; Monzó, Sabater-Muñoz, *et al.*, 2011).

However, despite this range of natural enemies, collectively they are not able to maintain *C. capitata* below the tight economic injury levels (Urbaneja, Jaques and Garcia-Marí, 2009). As a consequence, in Spain, a combined approach of mass trapping (Martinez-Ferrer, Campos and Fibla, 2012), chemosterilisation (Navarro-Llopis *et al.*, 2007), Sterile Insect Technique (SIT) and chemical control is used to control populations of *C. capitata* (Urbaneja *et al.*, 2015).

1.3.3.3. Eutetranychus *mites*

Like other Tetranychid mites, *Eutetranychus orientalis* and *E. banksi* cause necrosis to the plant tissue and discolouration of the plant organs where it feeds (Ferragut, Navia and Ochoa, 2013). *Eutetranychus* mites feed along the midvein of the upper leaf surface and as density increases, the mites spread along the secondary veins (Ferragut, Navia and Ochoa, 2013). Under heavy infestations, leaf drop, and twig die back have been reported (Ferragut, Navia and Ochoa, 2013). However, Hall and Simms (2003) found that when sweet orange trees were properly irrigated, no negative relationship existed between *Eutetranychus* feeding damage and leaf drop. Hence, there remains some questioning over whether *Eutetranychus* mites represents significant economic threat to the citrus industry in Spain and wider Mediterranean citrus growing regions (Vacante, 2010; EFSA Panel on Plant Health, 2013; Ferragut, Garzón-Luque and Pekas, 2013). Invasion where present has been rapid and exponential growth under favourable conditions leads to high population densities in the tree (Ledesma *et al.*, 2011; Vela *et al.*, 2017). Furthermore, *E. orientalis*, in particular, shows high potential to invade other citrus growing areas within the Mediterranean (EFSA Panel on Plant Health, 2013). However, the economic impact from downgrading fruit or loss of yield due to *Eutetranychus* feeding damage has been classed as minor concern by the European Food Safety Authority (EFSA) Panel on Plant Health (2013). Nonetheless, in the regions where Eutetranychus are present in Spain, they are typically controlled by the use of chemical pesticides (EFSA Panel on Plant Health, 2013; Llorens Climet and Martín Gil, 2014).

The two species of *Eutetranychus* invaded the Iberian Peninsula within short succession of one-another. In 1999, *E. banksi* was detected in Portugal and in 2001, *E. orientalis* was reported in Malaga (Andalucía) (Ferragut, Navia and Ochoa, 2013). In Spain, *E. banksi* is constrained to Huelva in south west Andalucía while *E. orientalis* has spread throughout all the citrus growing regions of Andalucía (Márquez *et al.*, 2003). However, due to the difficulty in separately identifying these two species in the field, some debate exists over the true ranges of these congeneric species in Andalucía (Ferragut, Navia and Ochoa, 2013). *Eutetranychus orientalis* populations increase in the field when mean temperatures decrease to 23-25°C, and mean relative humidity increases to 60% (Vela *et al.*, 2017). In Spain, *E. orientalis* populations therefore display a single peak in autumn, when climatic conditions are favourable, then swiftly decline as temperatures decrease in winter months (Ledesma *et al.*, 2011; Vela *et al.*, 2017). Field population dynamic data of *E. banksi* in Spain are limited, however, optimal developmental time when reared on sweet orange in the laboratory has been reported at temperatures in the range of 28-31°C (Badii *et al.*, 2003). Differing optimal developmental temperatures of the two congenerics may explain their distinct geographic ranges in Andalucía (Ferragut, Navia and Ochoa, 2013).

Populations of the polyphagous predatory mite, *Euseius stipulatus* (Athias-Henriot) (Acari: Phytoseiidae), coincide with *Eutetranychus* species in the field, and represent as much as 89% of the Phytoseiid fauna present on sweet orange in Andalucía (Ledesma *et al.*, 2011). Nonetheless, *E. stipulatus* is unable to bring the pests under effective control (Ledesma et al., 2011). This is most likely due to poor suitability of *Eutetranychus* as a food source *for E. stipulates*; under laboratory conditions *E. stipulates* displays low fecundity and high mortality rates when reared on *E. orientalis* (Garzón, 2010). However, surveys in north eastern Spain have described a diverse range of insect natural enemies for control of other Tetranychid mites (Acari: Tetranychidae) in citrus, the most abundant being the coccinellid *Stethorus punctillum* (Weise) (Coleoptera: Coccinellidae), the neuropterans *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Conwenzia psociformis* (Curtis) (Neuroptera: Coniopterygidae), and the thrips *Scolothrips longicornis* Priesner (Thysanoptera: Thripidae) (León and García Marí, 2005; Abad-Moyano *et al.*, 2009). With a diverse guild of Tetranychid natural enemies in citrus, there is potential for natural pest regulation if pest regulation services are not interrupted by deleterious orchard management practices (EFSA Panel on Plant Health, 2013). However, as a newly emerged pest in Spain, no cultural or biological control measures specifically exist for *Eutetranychus* species in citrus, and as such, chemical control measures are recommended once populations reach the economic injury level of 20% (August to October) or 80% (the rest of the year) of leaves infested (Monzó *et al.*, 2016).

1.3.4. Biological Control in Spanish citrus

1.3.4.1. Classical Biological Control in Spanish citrus

The long history of citrus cultivation in Spain has been coupled with the invasion of non-native arthropod species (Urbaneja, Jaques and Garcia-Marí, 2009). In the late 1800s, the mealybug, *Planococcus citri* Risso (Hemiptera: Pseudococcidae), along with the brown soft scale, *Coccus hesperidum* L. (Hemiptera: Coccidae) were considered some of the most economically important citrus pests in Spain (Garcia-Marí, 2012). The Diaspidid scales were then identified in Spain in 1903. Next, the cottony cushion scale, *Icerya purchasi* (Maskell) (Hemiptera: Monophlebidae), which devastated the Californian citrus industry (Doutt, 1958; Garcia-Marí, 2012), was recorded in Spain in 1922 (Doutt, 1958). These new pest species prompted the release of non-native natural enemies to establish and to harness their pest management services. First was the release of the exotic ladybird, *Rhyzobius lophanthae* (Blaisdell) (Coleoptera: Coccinellidae) for the control of Diaspidid scales (Limón *et al.*, 1976). In 1922, the Australian specialist predator *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) was introduced leading to the effective control of *I. purchasi* (Jacas, Urbaneja and Viñuela, 2006). Shortly after, in 1927, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) was introduced for the control of *Planococcus citri* Risso (Hemiptera: Pseudococcidae) (Jacas, Urbaneja and Viñuela, 2006). *Cryptolaemus montrouzieri* has established, but population recovery after the cool winter period is slow, and so it is unable to bring *P. citri* under complete control (Jacas, Urbaneja and Viñuela, 2006). Instead, annual augmentative release strategies are deployed (Jacas, Urbaneja and Viñuela, 2006).

DIRECCIÓN GENERAL DE AGRICULTURA
Sección de Fitopatología y Plagas del Campo

DEFENSA SANITARIA DE LOS CULTIVOS

Las «serpetas» del naranjo y limonero
(Mytilococcus gloverii y M. Beckii)

Son dos cóccidos, «cochinillas», llamadas vulgarmente «serpeta fina», la primera, y «serpeta gruesa» la segunda, que atacan a los agrios infestando hojas, ramas y frutos. Ambas están provistas de un escudo en forma alargada y de coloración pardo rojizo, más claro en la «fina» y más obscuro en la «gruesa». Debajo del caparazón se encuentran los huevos, como se ve en el dibujo. La «fina» es más estrecha y alargada, mientras que la «gruesa» tiene ostensible forma de coma. La fruta infestada queda depreciada, y en los ataques intensos se puede producir la defoliación y hasta la muerte de las ramas. En algunos naranjales se encuentran simultáneamente las dos especies, pero en general domina la «serpeta» fina, que constituye la plaga más importante de estos árboles.

Tratamiento. – Se emplea indistintamente la fumigación cianhídrica y las pulverizaciones con emulsiones de aceites minerales de las que prepara el comercio, al 1 ½ y 2 %. El gas cianhídrico puede obtenerse: 1.°, por reacción del cianuro sódico y el ácido sulfúrico hidratado (método del generador o del «pote»); 2.°, introduciendo debajo de las tiendas el líquido ácido cianhídrico; y 3.° utilizando el cianuro de calcio. Cada procedimiento tiene su técnica operatoria, sus productos y tablas, y exige determinadas condiciones atmosféricas.

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Figure 1.2. Infographic card produced c. 1950 for the identification of *Lepidosaphes gloverii* and *L. beckii* and their chemical control in the field.

The subsequent decades, from 1922 to 1973, saw an increased dependency on chemical insecticides in Spain and no further introductions of classical biological control agents (Urbaneja, Jaques and Garcia-Marí, 2009) (Figure 1.2). In 1973 however, *Cales noacki* Howard (Hymenoptera: Aphelinidae) was introduced to Spain for the control of *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) (Soto, Ohlenschlager and Garcia-Marí, 2001), which marked the start of a new era for classical biological control in Spanish citrus (Urbaneja, Jaques and Garcia-Marí, 2009). To date, at least 28 exotic natural enemies have been introduced into Spain for the control of citrus pests (Jacas, Urbaneja and Viñuela, 2006). Many of these natural enemies have successfully established in Spain and have continued roles in pest regulation, reducing reliance on chemical control measures (Table 1.1.) (Urbaneja, Jaques and Garcia-Marí, 2009; Urbaneja, Grout, *et al.*, 2020).

Table 1.1 Status of citrus pests in Spain and the principal control strategies used for their control. Adapted from Urbaneja, Jaques and Garcia-Marí (2009) using pest status and incidence data from Tena and García-Marí (2011) and control measures from Urbaneja *et al.* (2015) '**' = Excellent control; '*' = Satisfactory control; '-' = Insufficient control. CBC: Conservation Biocontrol; AR: Augmentative release; MD: Mating disruption; MT: Mass trapping; SIT: Sterile insect technique; and CC: Chemical control

Pest species	Efficiency of natural control	Status of the key natural enemy(s) involved in pest regulation	Principal control strategies used
Icerya purchasi (Hemiptera: Margarodidae)	**	Introduced	CBC
Lepidosaphes gloverii (Hemiptera: Diaspididae)	**	Introduced	CBC
Chrysomphalus dyctiospermi (Hemiptera: Diaspididae)	*	Native	CBC
Aonidiella aurantii (Hemiptera: Diaspididae)	-	Native and introduced	MD, AR, CC
Ceroplastes sinensis (Hemiptera: Coccidae)	*	Native	CBC
Coccus hesperidium (Hemiptera: Coccidae)	*	Native	CBC
Saissetia oleae (Hemiptera: Coccidae)	*	Native	CBC
Planococcus citri (Hemiptera: Pseudococcidae)	*	Introduced	AR, CC
Aphis gossypii (Hemiptera: Aphididae)	-	Native and introduced	CBC
Aphis spiraecola (Hemiptera: Aphididae)	-	Native and introduced	CBC, AR, CC
Toxoptera aurantii (Hemiptera: Aphididae)	-	Native and introduced	CBC
Aleurothrixus floccosus (Hemiptera: Aleyrodidae)	*	Introduced	CBC
Phyllocnistis citrella (Lepidoptera: Gracillariidae)	*	Introduced	CBC
Ceratitis capitata (Diptera: Tephritidae)	-	Native and introduced	CBC, SIT, MD, MT, CC
Tetranychus urticae (Acari: Tetranychidae)	-	Native	CBC
Eutetranychus orientalis and *E. banksi* (Acari: Tetranychidae)	-	Unknown	CC
Panonychus citri (Acari: Tetranychidae)	*	Native	CBC, CC
Delotococcus aberiae (Hemiptera: Pseudococcidae)	-	Unknown	CC
Chaetanaphothrips orchidii (Thysanoptera: Thripidae)	-	Native	CC
Pezothrips kellyanus (Thysanoptera: Thripidae)	-	Native	CBC, CC

1.3.4.2. Augmentative release programmes in Spanish citrus

Despite the success of many classical biological control programmes, some concerns have been raised over the ecological implications of importing non-native natural enemies (Jacas and Urbaneja, 2010). European Union directives require substantial risk assessment and environmental fait analysis to be conducted prior to the authorisation of candidate natural enemies for release (Ward, 2016). As such, focus has turned to alternative methods, such as augmentative release programmes and conservation of native and naturalised natural enemies within the agricultural landscape as means to supplement classical biocontrol in Spanish citrus (Jacas and Urbaneja, 2010; Ward, 2016). Those introduced natural enemies, such as *A. melinus* and *C. montrouzieri*, and native natural enemies, such as *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and *Gaeolaelaps aculeifer* Canestrini (Acari: Laelapidae), which fail to satisfactorily regulate their pests under natural population levels, can be mass-released to increase their abundance at critical periods (Jacas, Urbaneja and Viñuela, 2006; Abad-Moyano *et al.*, 2010; Zappalà *et al.*, 2012; Navarro-Campos *et al.*, 2020). If properly managed, the release of these natural enemies can increase populations sufficiently to manage pest populations and prevent pest load reaching economic injury levels (Jacas, Urbaneja and Viñuela, 2006; Jacas and Urbaneja, 2010). In the case of *P. citri*, for example, two commercialised natural enemies are available for augmentative release, *C. montrouzieri* and *A. pseudococci* (Jacas and Urbaneja, 2010; Urbaneja, Pérez Hedo, *et al.*, 2020). *Cryptolaemus montrouzieri*, can be released into orchard systems at a rate of 20-50 individuals per infested tree, from August onwards, and *A. pseudococci* is at a rate of 500-2000 individuals per hectare, depending on season and infestation rate (Urbaneja, Pérez Hedo, *et al.*, 2020). As the production costs of such natural enemies are relatively low, this can be both an economically viable and environmentally sustainable approach to pest management (van der Blom, 2002).

1.3.4.3. Conservation Biological Control in Spanish citrus

1.3.4.3.1. Narrow spectrum pesticides

To help reduce reliance on mass produced natural enemies purchased for release, the use of conservation strategies to sustain natural enemies within Spanish citrus orchard systems have gained momentum (Monzó *et al.*, 2020). A key element to this approach is the use of specific and narrow spectrum pesticides to help conserve non-target organisms, including natural enemies (Urbaneja *et al.*, 2015). Indeed, many of the most common pesticides used to control key citrus pests in Spain, such as spirotetramat, spinosad, spirodiclofen and mineral oils, are recommended for their low acute and sublethal toxicity against non-target organisms, including natural enemies (Urbaneja *et al.*, 2008, 2009; Planes *et al.*, 2013; Argolo, Jacas and Urbaneja, 2014; Urbaneja, Pérez Hedo, *et al.*, 2020). However, to prevent the development of pest resistance, it is recommended that the active ingredient used in pesticide treatments are rotated, which can result in the occasional use of pesticides which are more highly toxic to non-target species (Urbaneja *et al.*, 2015). Since this study was conducted, the authorisation of spirodiclofen for use within the European Union has expired (EFSA *et al.*, 2021).

1.3.4.3.2. Host plants to support alternative prey and hosts

Over the past two decades, significant research has resulted in the development of habitat management strategies in Spanish citrus to help promote natural pest regulation services (Urbaneja *et al.*, 2015; Monzó *et al.*, 2020; Urbaneja, Pérez Hedo, *et al.*, 2020). Historically, two plant species have been used in Spanish citrus as suitable habitat for alternative prey and hosts (Jacas and Urbaneja, 2010). Hedges of *Nerium oleander* L. (Gentianales: Apocynaceae), which is host to non-pest aphid species, can help maintain populations of aphid parasitoids of the genus *Lysiphlebus* and *Aphidius* L. (Hymenoptera: Braconidae) as well as predators of aphid citrus pests (Tena *et al.*, 2011; Laamari *et al.*, 2012; Bañol *et al.*, 2017). Vercher-Aznar *et al.* (2009) observed greater relative abundance of alternative prey and hosts on hedgerows composed of mixed Mediterranean species than on the adjacent citrus canopy. Additionally, the relative abundances of natural enemies, particularly Neuroptera and Hymenoptera, were greater on hedgerows than on naturally occurring orchard vegetation (Vercher-Aznar *et al.*, 2009). *Oxalis pes-caprae* L. (Oxalidales: Oxalidaceae), which is a host to non-pest Tetranychid mites and can act as a reservoir for predators of acarine pests of citrus (del Rivero and García Mari, 1982). However, since the invasion of *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae) into Spain, the use of *O. pes-caprae* has decreased due to its potential to harbour this key citrus pest and increase its persistence in citrus orchards (Vassiliou, 2010a; Navarro-Campos *et al.*, 2013).

1.3.4.3.3. Maintaining naturally occurring orchard vegetation

Two main intervention strategies are now typically used in Spanish citrus systems for the maintenance of naturally occurring vegetation on the orchard floor and the sowing of grass alleyway strips (Monzó *et al.*, 2020). The maintenance of resident vegetation benefits, epigeal predatory spider communities, significantly increasing richness as well as the abundance of *Pardosa cribata* Simon (Araneae: Lycosidae), a predator of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Monzó *et al.*, 2010; Monzó, Molla, *et al.*, 2011). Furthermore, the abundance of important natural enemies, such as hymenopteran parasitoids, Chrysopidae and Araneae, in the adjacent citrus tree canopy can be increased when the resident vegetation is allowed to grow (Silva *et al.*, 2010). However, little evidence exists that the presence of resident vegetation in the orchard alleyways can successfully suppress citrus pest species below economic injury levels (Silva *et al.*, 2010; Aguilar-Fenollosa *et al.*, 2011b; Monzó, Molla, *et al.*, 2011; Monzó *et al.*, 2020). Furthermore, some common plant species naturally found in Spanish citrus orchards are associated with economically important citrus pests (Aucejo *et al.*, 2003; Vassiliou, 2010b; Jacas and Aguilar-Fenollosa, 2013; Pereira *et al.*, 2014; Gómez-Marco, Urbaneja and Tena, 2016). As such, attention is beginning to focus on sowing specific plant species with an aim to control specific pest species (Monzó *et al.*, 2020).

1.3.4.3.4. Grass strips

The other main intervention strategy deployed in Spanish citrus are grass strips composed of *Schedonorus (Festuca) arundinaceus* Schreber (Poales: Poaceae) which target acarine pests, although they can also help regulate other pest species (thrips and aphids) (Jacas and Aguilar-Fenollosa, 2013; Monzó *et al.*, 2020). Of 45 plant species which commonly grow in Spanish citrus orchards, the tussock-forming grass species *S. arundinaceus* was found to have the highest ratio of Phytoseiid predatory mites to the pest species *T. urticae* (Aucejo *et al.*, 2003; Jacas and Urbaneja, 2010). It was therefore selected for habitat creation to encourage spill-over of Phytoseiids onto the crop and regulate spider mite on the citrus canopy (Aguilar-Fenollosa *et al.*, 2011a).

Orchard alleyways sown with *S. arundinaceus* support races of *T. urticae* specialized to feed on *S. arundinaceus* and non-pest thrips species*,* which serve as alternative prey for natural enemies of acarine pest species (Aguilar-Fenollosa *et al.*, 2011a, 2011b). Additionally, *S. arundinaceus* strips support non-pest aphid species, which serve as alternative hosts and prey for an altogether different feeding guild, with potential to spill-over onto the crop (Gómez-Marco, Urbaneja and Tena, 2016). In the citrus tree canopy adject to the sown *S. arundinaceus* habitat*,* greater abundances of specialist phytoseiids predators and aphid natural enemies were supported (Aguilar-Fenollosa *et al.*, 2011a, 2011b; Gómez-Marco, Urbaneja and Tena, 2016). As a result, a significant reduction of *Panonychus citri* (McGregor) (Acari: Tetranychidae) and *T. urticae* was achieved in the citrus tree canopy (Aguilar-Fenollosa *et al.*, 2011b). The establishment of two pest thrips species, *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), were delayed in clementine orchards established with *S. arundinaceus* (Jacas and Aguilar-Fenollosa, 2013).

Despite the success of the *S. arundinaceus* grass strips for the control of mites and thrips, other citrus pests still evade successful management (Urbaneja *et al.*, 2015). One potential hypothesis is that due to the absence of suitable carbohydrate sources and the limited period of pollen availability when the grass flowers, the pest regulation services of other important natural enemies are limited (Landis, Wratten and Gurr, 2000; Tena *et al.*, 2015). Tena *et al.* (2013) tested field captured *Aphytis melinus,* a parasitoid of *A. aurantii*, for carbohydrate consumption and found almost 60% to have a carbohydrate deficit i.e., starving. The addition of sugar and protein sources (e.g., Biogluc® and Nutrimite^{TM}) directly onto the citrus trees themselves enhances the pest regulation services of both parasitoids (*Aphytis melinus*) and predators (Phytoseiids) (Tena *et al.*, 2015; Beltrà *et al.*, 2017), supporting the rational that pest regulation services in citrus could be boosted if these resources were present. Satisfactory regulation of *Aphis gossypii* Glover and *A. spiraecola* Patch (Hemiptera: Aphididae) can be achieved using *S. arundinaceus* grass strips, but only when the strips are mature (>5 years old) and naturally occurring forbs species are established within the swards (Gómez-Marco, Urbaneja and Tena, 2016). This study highlights the importance of wildflowers for provisioning resource in Spanish citrus systems and their potential to deliver pest regulation services to the crop (Gómez-Marco, Urbaneja and Tena, 2016). This approach might be highly site specific due to differences in flora between sites and is further reliant on forbs species which provision accessible and high-quality resource being present and able to establish in the strips.

1.3.4.3.5. Wildflower habitats for natural pest regulation in citrus

As in other crops, sowing perennial forbs to diversify plant traits, increasing resource availability, may be a sustainable approach in citrus (Landis, Wratten and Gurr, 2000; Tena *et al.*, 2016; Gurr *et al.*, 2017). For example, floral resources adjacent to Mediterranean pomegranate orchards can provide a suitable and accessible source of carbohydrates for parasitoids (Kishinevsky, Cohen, *et al.*, 2017). Additionally, non-cropped habitat might provide greater niche breadth and reduce ant and natural enemy competition for limited carbohydrate and protein resource in the canopy (Snyder, 2019). Currently, however, there is scarce empirical evidence that the provision of natural carbohydrate (e.g., nectar) and protein (e.g., pollen) resources, such as flowers, can increase natural enemy diversity and abundance to enhance pest regulation in citrus (Monzó *et al.*, 2020). Silva *et al.*, (2010) observed increased abundance of hymenopteran parasitoids, Coccinellids, Chrysopids and spiders in lemon tree canopies adjacent to sown strips of non-tussock-forming grasses and leguminous forbs (*Trifolium* and *Medicago* species) when compared with bare soil but not when compared to plots consisting of the naturally occurring vegetation (Silva *et al.*, 2010). However, this failure to augment natural enemy abundance could be due to plant species selection (van Rijn and Wäckers, 2016). Though *Trifolium* and *Medicago* species can support alternative hosts and prey and provision pollen (Venzon *et al.*, 2006; Bertolaccini, Núñez-Pérez and Tizado, 2011), the nectar is inaccessible to many natural enemies (Wäckers, 2004; Vattala *et al.*, 2006) and provision of both pollen and carbohydrates can have a synergistic effect on natural enemy fitness (Venzon *et al.*, 2006). To support natural enemies of citrus pests, plants to be included in wildflower habitats must be carefully considered to maximise the provision of different resources along the length of the year. As such, there is a need to investigate whether different sown forbs species may enhance natural enemy diversity, abundance, and pest regulation services in citrus (Monzó *et al.*, 2020).

1.4. Aims

To support the ecological intensification of commercial orange orchards, the aim of this study was to investigate the use of a sown wildflower habitat to deliver pest regulation services to the adjacent crop. A key aim was to determine whether the provision of a sown diverse habitat and subsequent management techniques can deliver a sustainable conservation biological control approach for growers as part of Integrated Pest Management (IPM).

1.5. Thesis Outline

A full outline of the experimental design and its rationale is given in Chapter two. Chapter three explores the effect of habitat provision and management on natural enemy diversity and abundance and investigates the importance of habitat structure and composition on the natural enemy community within citrus orchards. Chapter four explores whether the habitat intervention management techniques can enhance the delivery of pest regulation services to the crop. Chapter five investigates whether the provision of the sown wildflower habitat increases carbohydrate feeding in adult parasitoids and details an investigation into the relative importance of different carbohydrate resources provided by the wildflower habitat and their accessibility to different parasitoid species with distinct life history traits. A discussion of the findings, with recommendations for best practice, is given in Chapter six. Ideas for further research are also provided.

1.6. References

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Chapter 2
Experimental design

Abstract

The study was conducted over a three-year period (2016 to 2019) in the sweet orange cultivars: Powell, Lane Late, Chislett and Rhode; using 0.5-hectare treatment plots within a replicated randomised block design in Huelva, Andalucía, Spain. Treatment plots were situated within larger commercial citrus orchards (>300 ha). Plots were at least 150m from one another to help ensure independence between treatments. To reduce edge effect, the outermost alleyways were excluded from sampling and 20m buffer zones at either end of the alleyways were established. Four orchards across Huelva, south west Spain, were selected for the study and the plots were established with the wildflower habitat in November 2016. The wildflower seed mix consisted of twelve forb species and two tussock-forming grass species, selected to provide carbohydrate, protein (pollen, hosts/prey) and shelter and refuge, for natural enemies throughout the season. In year one (2017), all plots sown with the wildflower habitat were cut twice in spring to aid establishment of the sown species and minimise competition with annual species. Two treatments were investigated that first year, i) a control treatment in which the alleyways were managed conventionally by cutting to ≤5cm four to five times throughout the season to keep the vegetation low and ii) the establishing wildflower habitats. In years two and three (2018 and 2019), two distinct management strategies (cutting regimes) were applied to the wildflower habitats so that there were three treatments; i) the control treatment as before, ii) a sown wildflower habitat managed with cutting once a year in February to a height of approximately 10cm (standard management wildflower treatment), and iii) the same wildflower habitat described for the standard management treatment but managed by cutting two additional times each year, once in May and once in June (≈10cm) (active management wildflower treatment).

2.1. Study sites

The study was conducted in four navel orange (*Citrus sinensis*) orchards within three farms (sites), Madre del Agua, La Calvilla and Montepinos, at two different localities in the province of Huelva, south-west Andalucía, Spain (Figure 2.1). Huelva is an important citrus growing region of Spain; characterised by a sub-tropical Mediterranean climate, with an average annual temperature of 17°C, and annual precipitation of 525mm (AEMET, 2020). The farm of

Montepinos was situated in the north of Huelva (37°47’43.21N 6°56’21.11W) at 351 m elevation and characterised by clay soil. The farms Madre del Agua and La Calvilla, were situated in the south of Huelva (37°26’28.42N 7°09’55.50W) at 128 m elevation and characterised by sandy soil. The two localities were 42.59 km apart.

Figure 2.1 Location of the three orchards in relation to the Iberian Peninsula a) and the province of Huelva, south west Spain b).

An additional farm, Manzorrales, was also established with two replicate blocks at the start of the study. However, due to poor drainage, which led to prolonged periods of standing water after sowing, establishment of the sown wildflower habitat was unsuccessful, and these two replicate blocks were dropped.

2.1.1. Site selection

Orchards were selected to meet the following selection criteria:

1. **Orchards must be managed by Martinavarro and Vincente Giner.**
   The study aims to support ecological intensification within commercial citrus orchards. It was therefore considered important to conduct the study in commercial citrus orchards. The citrus producers, Martinavarro and Vicente Giner, who supply oranges to the project's co-funders (Waitrose & Partners and Primafruit Ltd.), kindly offered the use of their orchards for the study.

2. **Orchards must be planted with Navel oranges, consisting of varieties with similar phenology and management strategies.**
   Plant phenology significantly affects the pest status of phytophagous arthropods (Williams and Dixon, 2007). Citrus varieties with different phenology may experience pest outbreaks at different times of the year (Franco *et al.*, 2004). As such, all treatment plots were established in late cropping Navel varieties, Lane Late, Rohde and Powel, which all have similar phenology and management requirements (IVIA, 2016).

3. **Orchards must be at least eight years old and no older than 50 years.**
   Citrus trees take eight years to reach full productivity (Ferguson and Grafton-Cardwell, 2014) and peak productivity typically extends no further than 50 years of age (Ferguson and Grafton-Cardwell, 2014; Wang *et al.*, 2015). The maturity of the citrus trees also influences their susceptibility to attack from different pest species (Llorens Climet and Martín Gil, 2014).

4. **Orchards must be on flat land with the rows of orange trees forming straight lines. Terraced orchards planted along contour lines must be excluded.**
   Linear objects in the landscape act as corridors, along which natural enemy dispersal is facilitated (Burel, 1996), but their movement across them is inhibited (Mauremooto *et al.*, 1995). Terraces were therefore expected to restrict natural enemy movement within plots by acting as a barrier between alleyways, increasing dispersal out of alleyways as the area will be perceived by natural enemies as smaller, isolated plots (Englund and Cooper, 2003).

5. **Orchards must not contain any other sown habitat or have natural habitat within 150m of the treatment plots.**
   The presence of alternative non-cropped habitat within foraging distance of the treatment plots may increase spill-over of natural enemy into the experimental plots or dispersal out of the experimental plots (Lindgren, Lindborg and Cousins, 2018). Even small fragments of non-cropped habitat can sustain natural enemies and act as islands, which facilitate dispersal between experimental plots and would reduce independence within the study design (Knapp and Řezáč, 2015).

6. **Orchards must be of sufficient area to accommodate the randomised block design.**
   To reduce interference between plots, the three experimental treatment plots, each 100m x 50m (0.5ha) and spaced at least 150m from one another, must be situated within the same orchard. Each plot must be no closer than 7.5m from the orchard boundary or any other anomalies in the topography such as trenches and gullies.

Martinavarro and Vicente Giner manage orchards in both Andalucía and Valencia. However, due to the demands of the selection criteria, only those in Huelva were suitable. The long history of citrus cultivation in Valencia has led to reduced orchard size through generations of inheritance and the subsequent division of the land. Orchards available in Valencia did not meet the size criteria (6) and hence the study took place in Huelva.

2.1.2. Management of orchard sites

The study was conducted within commercial citrus orchards managed under IPM guidelines. All treatment plots within a single block were managed the same, which included applications of chemical pesticides when deemed necessary by the growers (Table 2.1). Cyclic pesticide applications for the control of California red scale, *Aonidiella aurantii* (Hemiptera: Diaspididae), were used across all farms. In the orchard managed by Vicente Giner (Montepinos), 15% spirotetramat, a systemic acetyl-CoA carboxylase inhibitor, was applied once annually to coincide with the first generation of *A. aurantii*. In orchard managed by Martinavarro (Madre del Agua and La Calvilla), two annual pesticide treatments to control *A. aurantii* were applied to coincide with the first two generations. In these orchards (Madre del Agua and La Calvilla), spirotetramat, organophosphates (chlorpyrifos) and juvenile hormone mimics (pyriproxyfen) were applied in rotation. In Vicente Giner orchards, annual applications of flonicamid, a chordotonal organ inhibitor, were applied in spring to control Aphids, organophosphates in mid-summer for the control of Coccids (Coccidoidea), and abamectin, a chlorine channel activator, in late summer to control citrus leaf miner (*Phyllocnistis citrella*). Across all orchard sites, another acetyl-CoA carboxylase inhibitor, spirodiclofen, was applied at the end of summer, at least once annually for the control of *Eutetranychus* mites. The orchard Madre del Agua (Martinavarro) was treated at least once annually with spinosyns, a nicotinic acetylcholine receptor allosteric modulator, when populations of Medfly, *Ceratitis capitata* (Diptera: Tephritidae), reached the action threshold. Likewise, population of *C. capitata* at La Calvilla (Martinavarro) were treated when they approached the action threshold, typically with the pyrethroid pesticide, lambda cyhalothrin. The approval of spirodiclofen for use in the European Union (EU) expired on 31 July 2020, as such, since this study was conducted, spirodiclofen is no longer authorised in the EU (EFSA *et al.,* 2021).

Table 2.1 Chemical intervention for the control of citrus pests in the study orchards, Madre del Agua[1], La Calvilla[2], and Montepinos[3]: the target organisms, products used, typical timing of application, and the type of monitoring used to determine intervention.

Target organism	Product	Month	Criteria for intervention (life cycle dependent / field captures / crop sampling)
Aonidiella aurantii	Spirotetramat[1,2], chlorpyrifos[1,2], or pyriproxyfen[1,2]	May/June and July/August	Life cycle dependent
Aphidoidea	Flonicamid[3]	May	Crop sampling
Tetranychidae	Spirodiclofen[1,2,3]	August to October	Crop sampling
Phyllocnistis citrella	Abamectin[3]	August	Crop sampling
Coccidae	Chlorpyrifos[3]	August	Crop sampling
Ceratitis capitata	Spinosyns[1] or lambda cyhalothrin[2]	October	Field captures

All orchards were irrigated via underground systems, and fertilisers applied directly into these irrigation systems (fertigation). Conventional management practices of the orchard alleyways included regular cutting of the naturally occurring vegetation to less than 5cm in height, four to five times annually. During the winter months, the naturally occurring vegetation of the alleyways was allowed to grow until the first cut of the year. Debris pruned from the citrus trees, typically in late spring, was discarded in the alleyways and a tractor mounted disc mulcher used to shred it. Shredded plant material was then left *in situ.*

2.2. Study design and treatments

2.2.1. Experimental orchard treatments

Across the three farms, four replicate experimental blocks were established in November 2016 to investigate three different alleyway management strategies (Figure **2.2**). Within each block, a control treatment was established in which the alleyways were managed conventionally by cutting to ≤5 cm throughout the season to keep the vegetation low. This was compared with plots sown with the wildflower habitat in the alleyways between the rows of fruit trees receiving either 'standard management' or 'active management'. For the standard management wildflower treatment, alleyway vegetation was cut once annually in February, whilst for the active management wildflower treatment, the habitat was cut three times annually to approximately 10cm to encourage spill-over of natural enemies onto the crop at times of predicted pest outbreaks (Figure 2.2). With all three treatments, cut vegetation was left *in situ.*

The three treatments investigated were therefore:

1. **Control**

   A control treatment in which the naturally occurring vegetation of the alleyways was managed under standard farm management practices by cutting to ≤ 5 cm, four to five times per year to keep the vegetation low (Figure 2.2a).

2. **Standard management of the wildflower habitat**

   A sown habitat comprised of 12 forb and two tussock-forming grass species, established in alternate alleyways between the rows of orange trees, cut once annually in spring in years two and three but otherwise allowed to grow tall and complex throughout the year (hereon in standard management wildflower treatment) (Figure 2.2b). Alleyways not established with wildflowers were managed as with the control.

3. **Active management of the wildflower treatment**

   The same sown habitat of 12 forb and two tussock-forming grass species the standard management wildflower treatment, established in alternate alleyways between the rows of orange trees, cut three times annually in years two and three to approximately 10cm to encourage spill-over of natural enemies onto the crop at times of predicted pest outbreaks (hereon in active management wildflower treatment) (Figure 2.2c). Alleyways not established with wildflowers were managed as with the control.

Figure 2.2 Treatment plots; a) Control treatment, managed conventionally by cutting throughout the season to keep the vegetation low; b) Standard management wildflower treatment, cut once annually, allowed to grow tall and complex; c) Active management wildflower treatment, cut three times annually to approximately 10cm to encourage spill-over of natural enemies onto the crop.

2.2.2. Study design

A randomised block design was used to reduce spatiotemporal variation between treatments (Underwood, 1996); each of the four blocks contained all three treatments (Figure **2.3**) (Underwood, 1996). The three treatments were randomly assigned to one of three orchard plots. To reduce interference between treatments and ensure independence, plots were located at least 150 metres apart (Figure **2.3**). Spill-over of both predators and parasitoids from non-cropped habitat into orchard systems is significantly reduced at distances greater than 60-120 m (Ries *et al.*, 2004; Miliczky and Horton, 2005; Zappalà *et al.*, 2012; Thomson and Hoffmann, 2013). However, as distance increases between plots, spatial heterogeneity is expected to increase, and hence unexplained variation between plots may be increased (Fox, Negrete-Yankelevich and Sosa, 2015). A complete compliment of the three experimental treatment plots was therefore contained within a single orchard. All plots were separated from field boundaries or other anomalies in the local topography by at least 7.5 m (Figure **2.3**).

To account for edge effects (Vacas *et al.*, 2010) whilst ensuring a sufficient sampling area in the centre of each plot, a plot size of 0.5 ha was used. Perimeter to area ratio affects the plot residency of natural enemies, with smaller plots increasing the likelihood that arthropods will cross habitat edges, and hence decrease independence (Englund and Cooper, 2003). Each treatment plot was 100 m in length by 50 m wide and consisted of eight rows of orange trees and seven non-cropped alleyways in-between rows of trees (hereon in referred to as 'alleyways'). The three treatments were randomly allocated to individual plots and treatments were applied to alternate rows within plots (Figure 2.3). Vacas *et al.* (2010) proposed non-sampling buffers of at least 15 m to reduce edge effect in citrus orchards. The two outer alleyways of each plot (14 m) were therefore excluded from sampling as well as a 20 m buffer area at either end of the alleyways (Figure 2.3). Consequently, sampling was conducted from the central two alleyways. In each plot, eight trees were randomly selected and paired with the tree adjacent across the treatment alleyway. These same 16 trees were then used throughout the three-year study (Wan *et al.*, 2014; Calabuig *et al.*, 2015). Sampling from the tree canopy was conducted from the side facing the treatment alleyway.

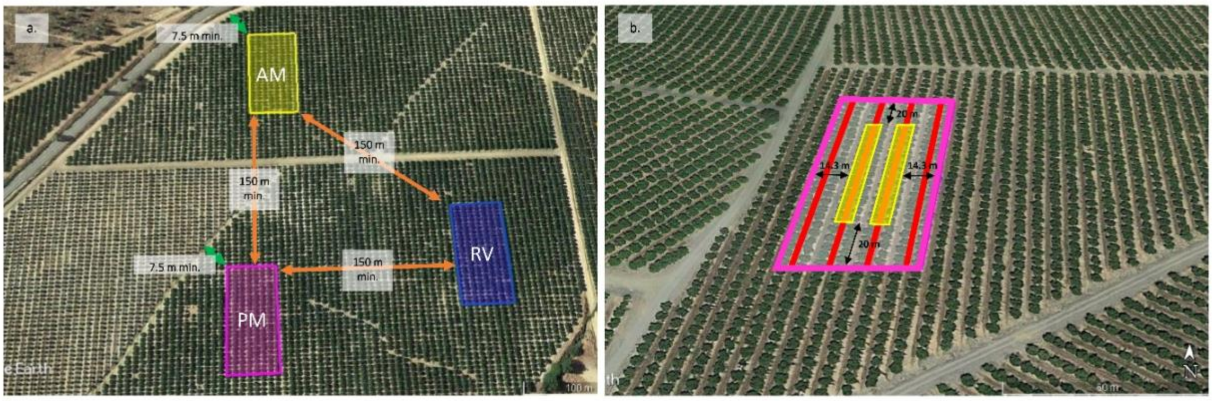

Figure 2.3 The three treatments were randomly allocated within each experimental block, two treatments sown with the wildflower habitat (pink and yellow) and control treatment unsown and managed under standard farm practices (blue) a). The wildflower habitat was deployed in alternate alleyways within plots as demonstrated by the red lines, the two outermost alleyways and 20 m of each alleyway formed a buffer zone (white shading), leaving two 60 m long central sampling areas (yellow shading) b).

2.3. Designing a sown habitat for pest regulation

2.3.1. Plant species selection

To provide key resources for natural enemies throughout the year, which performed consistently throughout the three-year study, the selection of species was based on the following criteria:

1. All species were perennial.

   The quantity of floral resource provided by annual and biennial plant species can vary significantly between years, whereas perennial species provide more consistent year-on-year resource (Carvell *et al.*, 2006), and may become more attractive to natural enemies as the wildflower habitat matures (Fiedler and Landis, 2007). Additionally, perennial species often exhibit a longer flowering period over the year (Fiedler and Landis, 2007; Hicks *et al.*, 2016; Pellissier and Jabbour, 2018). As perennials do not have to be re-sown yearly, they are expected to provide a more economically sustainable option for growers (Miettinen *et al.*, 2014).

2. All species were native to the region of Andalucía, Spain.

   Native species are expected to have greater potential for native arthropod species (Fiedler and Landis, 2007).

3. Species were selected to provide a range of traits

   Diversity of plant species traits within a habitat can increase richness of arthropod natural enemies (Woodcock *et al.*, 2007; Campbell *et al.*, 2012), plant species exhibiting a range of different traits (flowering period, flower morphology, taxonomy) were selected for the study (Figure 2.4).

4. Species were selected to include different functional groups (grasses and forbs).

   Functional group diversity broadens the range of provisioned resource, including shelter, nectar, alternative prey/hosts and protein, and can increase structural complexity and augment natural enemy diversity (Woodcock *et al.*, 2007, 2009; Woodcock and Pywell, 2010; Parry *et al.*, 2015).

5. **Tussock-forming grasses were selected to provide microclimate shelter.** Microclimates are formed within the dense tussock structure (Luff, 1965), which enhance predator abundance within the non-cropped habitat (Woodcock *et al.*, 2005), and facilitate spill-over into the adjacent crop (Thomas, Wratten and Sotherton, 1991).

6. **Species were selected to extend the flowering period across the season.** Flowers provide both carbohydrates and protein for natural enemies (Landis, Wratten and Gurr, 2000; Gurr *et al.*, 2017). Using multiple forb species, the flowering period of the non-cropped habitat can be extended (Fiedler and Landis, 2007) which can help fulfil natural enemy resource requirements even when alternative resources are scarce (Fiedler and Landis, 2007).

7. **Species known to be associated with citrus pests were excluded from the seed mix** (see Kalaitzaki, Amara and Tsagkarakis, 2013; Gómez-Marco, Urbaneja and Tena, 2016).

In total, twelve perennial flowering plant species and two tussock-forming grasses were selected for use in the mix (Table 2.2).

Figure 2.4 Examples of the variety of floral traits, the capitulum of *Achillea millefolium* are clustered into umbels a); long stamen of *Plantago lanceolata* project anthers with pollen 3-5mm out of the flower heads b).

Table 2.2 Flowering period of species included in the seed mix. Combined flowering period was designed to extend throughout the length of the year. These species are more commonly known as [1]Yarrow, [2]Bugloss, [3]Chicory, [4]Common shrubby everlasting, [5]St John's wort, [6]White hore-hound, [7]Apple mint, [8]Yellow restharrow, [9]Ribwort plantain, [10]Arabian pea, [12]Wild clary, [12]Tansy, [13]Orchard grass, and [14]Tall fescue.

Species	Family	Flowering period											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Achillea millefolium[1]	Asteraceae					🟨	🟨	🟨					
Anchusa azurea[2]	Boraginaceae			🟨	🟨	🟨	🟨						
Cichorium intybus[3]	Asteraceae					🟨	🟨	🟨					
Helichrysum stoechas[4]	Asteraceae				🟨	🟨	🟨	🟨					
Hypericum perforatum[5]	Hypericaceae					🟨	🟨	🟨	🟨				
Marrubium vulgare[6]	Lamiaceae		🟨	🟨	🟨	🟨	🟨	🟨	🟨				
Mentha suaveolens[7]	Lamiaceae						🟨	🟨	🟨				
Ononis natrix[8]	Fabaceae				🟨	🟨	🟨	🟨					
Plantago lanceolata[9]	Plantaginaceae			🟨	🟨	🟨	🟨	🟨	🟨	🟨			
Psoralea bituminosa[10]	Fabaceae				🟨	🟨	🟨	🟨					
Salvia verbenaca[11]	Lamiaceae	🟨	🟨	🟨	🟨	🟨							
Tanacetum vulgare[12]	Asteraceae							🟨	🟨	🟨			
Dactylis glomerata[13]	Poaceae					🟨							
Festuca arundinacea[14]	Poaceae					🟨	🟨						

2.4. Establishing the wildflower habitat

The wildflower habitat was established in alternate alleyways to allow growers access to the citrus trees via the conventionally managed unsown alleyways for activities such as pruning, harvesting, and observations under IPM management (Bugg and Waddington, 1994). Not only is varied strip management useful for the growers, but it also helps protect the sown habitat from disturbance or being destroyed. In early spring during harvest, heavy machinery is used to position crates in the alleyways adjacent to the trees, and up to five workers all harvest the fruit from a single tree. In early summer, trees are pruned to remove unproductive branches and to increase airflow and minimise microclimate effects in the tree interior which can harbour pest species (Llorens Climet and Martín Gil, 2014). Pruned branches are discarded in the alleyways where they are mechanically chipped, and the chippings are left *in situ.* The non-sown alleyways therefore provide suitable space so that these essential practices can be conducted without disrupting the provision of resource in the wildflower habitat (Bugg and Waddington, 1994).

The wildflower habitat was established in November 2016 in 2m wide strips between rows of trees. The soil needs to be sufficiently moist and warm when sowing (Nowakowski and Pywell, 2016), therefore mid to late autumn is optimal for habitat establishment in Spain (Ramírez and Lasheras, 2015). To increase establishment success, competition from existing vegetation was eliminated by spraying the naturally occurring vegetation in alleyways with the broad-spectrum herbicide glyphosate using a backpack-mounted sprayer (Natural England, 2013). To ensure the glyphosate had broken down and would not inhibit germination of the sown species, the alleyways were left for one week before being cultivated to create a seedbed (Westbury *et al.*, 2017). A tractor-mounted power harrow was used to create approximately 60% soil disturbance on the top 2.5 cm of the soil surface (

a)(Westbury *et al.*, 2017). The seed mix (Table **2.3**) was then sown at a rate of 5.66 gm^{-2} in orchard alleyways for both wildflower treatments (standard and active management) between 15th and 19th of November 2016. Following sowing, the alleyways were immediately rolled to ensure the seed was bound within the substrate (Figure **2.5**). During the subsequent spring, the wildflower habitat was cut twice, during the weeks of 13th March and the 17th April 2017, to a length of 5-10cm and the cuttings were left *in situ*. Cutting during the first year is essential for good establishment of the sown species, reducing completion from fast growing annual species and stimulating root growth of the sown perennials (Woodcock *et al.*, 2008). In the second and third years, all sown plots were cut in February to reduce competition with annual species and the active management treatment was introduced, which involved cutting the sown alleyways two additional times per year, once in May and once at the beginning of August, leaving all cuttings *in situ*.

Figure 2.5 A suitable seed bed was achieved by spraying-off naturally occurring alleyway vegetation with glyphosate and cultivating a). After sowing, a tractor-mounted roller was used to ensure the newly sown seeds were firmed to the substrate to improve establishment success.

Table 2.3 Species and sowing rates (expressed as the percentage weight of seeds sown and actual number of seeds sown per species per metre^2) used to create the wildflower habitat in the experimental orange orchards. The seeds were sown at a total rate of 5.66 gm^{-2}.

Scientific name	Common name	Sowing rate (%)	Actual sowing rate (seeds m^2)
Anchusa azurea	Bugloss	1.12	19
Salvia verbenaca	Wild clary	1.53	26
Psoralea bituminosa	Arabian pea	2.47	42
Hypericum perforatum	St John's wort	6.11	104
Mentha suaveolens	Apple mint	7.35	125
Ononis natrix	Yellow restharrow	8.23	140
Plantago lanceolata	Ribwort plantain	8.23	140
Dactylis glomerata	Orchard grass	8.29	141
Schedonorus (Festuca) arundinaceus	Tall fescue	8.29	141
Helichrysum stoechas	Common shrubby everlasting	8.29	141
Marrubium vulgare	White hore-hound	8.29	141
Achillea millefolium	Yarrow	8.58	146
Tanacetum (Chrysanthemum) vulgare	Tansy	8.58	146
Cichorium intybus	Chicory	14.64	249
	Total	100.00	1699

2.4.1. Forecasting cutting of the Active Management treatment plots

A phenology model (Grout *et al.*, 1989) was used to forecast the arrival of *A. aurantii* in the study orchards by using climate data and male flight data obtained from La Calvilla (one of the study sites). The climate data was obtained from the Gibraleón-Manzorrales meteorological station (37°18'31N 07°00'55W) that is part of a network maintained by the Andalucían Council of Agriculture, Fisheries and Rural Development. It was selected based on its proximity to the study sites and a minimum of 10-years historical climate data. The thermal constant, the time required to complete development, taken from peak male flight to peak of male flight, was 576.8 Degree Days (DD) (°C), with a minimum threshold temperature of 11.7°C and maximum of 37.8°C (Grout *et al.*, 1989). Generation time was then calculated using peak crawler activity reported by Campos-Rivela, Martinez-Ferrer and Fibla-Queralt (2012) as 184.2 DD (°C) after peak male flight activity. This model was then fitted to the mean accumulative Degree Days (°C) calculated from climate data taken from 1st

January 2008 to 31st December 2017 (mean daily temperature - minimum developmental threshold = number of DD).

The first generation was modelled to appear in the crop on 22nd May (380.8 DD), the 2nd generation on 11th July (957.6 DD) and the 3rd generation 21st August (1524.4 DD). Peaks in male flight activity were expected on 26th April (196.6 DD), 28th June (773.4 DD), 8th August (1350.2 DD), and finally 23rd September (1927 DD) (Figure **2.6**).

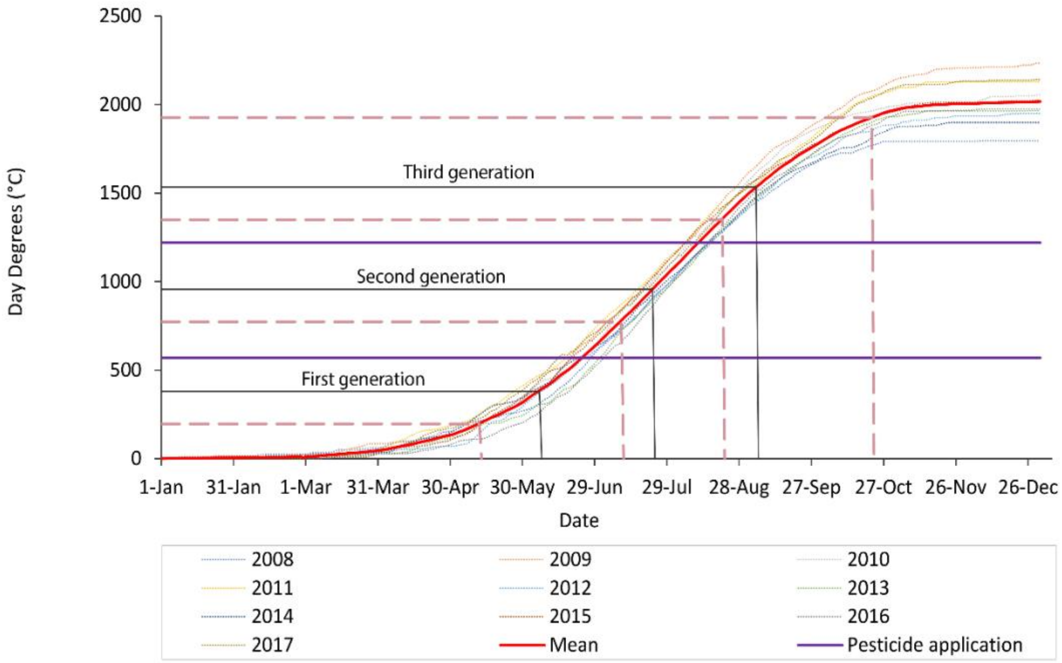

Figure 2.6. Expected *Aonidiella aurantii* generations were modelled using the phenology model proposed by Grout *et al.* (1989), showing the mean accumulative degree days (red solid line), male flights (red dashed lines), and expect generations (grey solid lines). The solid purple lines show when pesticide treatments are typically applied at 570 DD and 1220 DD.

The active management wildflower habitat was cut no more than five days before the first generation of *A. aurantii* was modelled to arrive in the crop, enabling the natural enemies to spill-over into the orange trees. In 2018, the first and second cuts were conducted on 17th May (259 DD) and 11th July (779 DD), respectively. As the actual (observed) accumulative degree days (°C) in 2018 were behind those of the predictive model, the timings of the cuts for the active management treatment were adjusted in 2019 to reflect those of 2018. In

2019, the active management treatment was therefore cut on 3rd May (267.1 DD) and again on 28th June (773 DD).

2.5. References

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Chapter 3

The establishment and success of wildflower habitats in orange orchards and their impact on plant communities

Abstract

To enhance natural enemies and their associated pest regulation services in commercial citrus orchards, there is a need to support populations with suitable habitat. Wildflower seed mixes have been successfully used in a range of fruit crops, including apple, cherry, and blueberries, to support pest regulation services. However, the value of native perennial seed mixes has never been investigated in Spanish citrus. A wildflower seed mix was designed to include twelve forb species and two tussock-forming grass species and was sown in alternate alleyways between rows of orange trees. To maximise the benefits of this approach, two different management strategies of the wildflower habitat were investigated for two years after the establishment year. One approach was standard management of the wildflower habitat, in which alleyways were cut annually in February. This was compared with wildflower habitat that was actively managed by cutting to coincide with the predicted maximum incidence of vulnerable instars of *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), a key crop pest of citrus, with the aim of forcing spill-over of natural enemies onto the crop. Over a three-year period, the establishment and longer-term success of the sown species was compared between the two wildflower treatments. The influence of sowing and alleyway management on the number of plant genera (richness) and community structure were compared with a control treatment in which alleyways were unsown, consisting of naturally occurring vegetation which was managed conventionally with regular cutting. The influence of these alleyway treatments on the provision of resource for natural enemies, determined by the cover class, was also investigated. For the first-time, this study has shown that native perennial wildflower strips can be successfully established in Spanish citrus orchards. Irrespective of management, the wildflower treatments increased richness of plant genera and were characterised by less bare soil and alleyway leaf litter than the control. Furthermore, the standard management wildflower treatment increased the relative abundance of forbs species which were in the vegetative and budding stages, suggesting the extended provision of floral resource. Of the 14 species sown in the experimental seed mix, ten successfully established and persisted in the orchard alleyways throughout the three-year study and could be recommended for inclusion in seed mixes in Spanish citrus orchards. However, the active management regime resulted in the decline of *Salvia verbenaca*, *Psoralea bituminosa* and *Cichorium intybus* from the wildflower strips.

Further investigation is required to establish longer term effects of management strategy and to determine whether specific strategies might encourage re-colonisation of species which declined over the three-year study.

3.1. Introduction

The loss of semi-natural habitats is being driven by agricultural intensification (Robinson and Sutherland, 2002). In turn, biodiversity is being lost at an alarming rate along with the services it provides for functioning ecosystems, including pest regulation (Millennium Ecosystem Assessment, 2005; Dirzo *et al.*, 2014). As such, there is a need to develop sustainable approaches to food production that meet the rising global demand for food whilst simultaneously reducing the impact on biodiversity and the associated loss of ecosystem services (Oliver *et al.*, 2015). Such approaches are referred to as the 'Ecological Intensification' of agriculture (Oliver *et al.*, 2015). One such approach to support ecosystem services is to increase heterogeneity at the farm scale through the inclusion of ecological infrastructure, such as wildflower strips, field margins, hedgerows, or other semi-natural habitats (Bommarco, Kleijn and Potts, 2013). The inclusion of habitat patches and corridors within agricultural landscapes has been found to significantly increase biodiversity and the delivery of ecosystem services (Holland *et al.*, 2012, 2016; Grass *et al.*, 2016). However, variable responses to habitat management are observed across geographic regions and cropping systems (Albrecht *et al.*, 2020). Hence, successful habitat management interventions require careful consideration in terms of plant species composition and management if targeted ecosystem services are to be delivered (Duru *et al.*, 2015). To support natural enemies and the delivery of pest regulation services, new habitats must provide carbohydrate (nectar and honeydew), protein (pollen and alternative hosts/prey), and shelter and refuge. To date, the creation of sown wildflower habitats within Spanish citrus orchards to reduce the reliance of growers on Plant Protection Products (PPPs) and support sustainable food production has not been investigated.

In Spanish citrus, it is typical for alleyways to be maintained as bare soil by treating naturally occurring vegetation with herbicide or by scarification (Monzó *et al.*, 2020). However, it is becoming increasingly common for growers to leave naturally occurring vegetation in alleyways between rows of fruit trees, which is then managed with regular cutting (Fibla-Queralt *et al.*, 2002; Jacas and Urbaneja, 2010). More recently, the value of sowing alleyways with a single grass species (*Schedonorus arundinaceus* (Schreb.)) for the management of spider mite and thrips has been investigated (Aguilar-Fenollosa *et al.*, 2011b; Jacas and Aguilar-Fenollosa, 2013). Unsown forbs, which enrich these otherwise species-poor habitats, can further enhance pest regulation (Gómez-Marco, Urbaneja and Tena, 2016). The benefit of a legume-based seed mix, containing non-tussock-forming grass species, has been demonstrated in Portuguese lemon orchards, however natural enemy abundance was only enhanced in comparison with bare soil but not with naturally occurring vegetation (Silva *et al.*, 2010). The use of native perennial wildflower habitats and the role of subsequent management has not previously been investigated in Spanish citrus.

To investigate whether the creation and management of a perennial wildflower habitat can support natural enemies and deliver enhanced pest regulation services in Spanish citrus, three alleyway management approaches were investigated; i) a control treatment whereby alleyways were managed conventionally by cutting the naturally occurring alleyway vegetation to a height of ≤5 cm, four to five times throughout the season, ii) a wildflower habitat sown in alternative alleyways between rows of orange trees and managed by cutting once annually (hereafter standard management wildflower treatment), and iii) the same sown wildflower habitat as in the standard treatment, managed actively by cutting three times per year (≈10cm), the second two cuts were timed to coincide with the predicted maximum incidences of susceptible instars of the key pest, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) (hereafter active management wildflower treatment). The aims of this study were to; i) determine performance and success of the sown wildflower habitats in Spanish orange orchards, ii) determine the influence of alleyway management on the composition of the wildflower habitat, and iii) investigate differences in resources, determined by cover class, for natural enemies between the different management strategies. The cover classes were defined as bare soil, leaf litter, vegetative forbs, vegetative grasses, budding forbs, budding grasses, flowering forbs, flowering grasses, seeding forbs, seeding grasses.

3.2. Materials and Methods

3.2.1. Study System

The study was conducted in commercial Spanish citrus orchards in Huelva, south-west Spain. (Figure 2.1). All orchards used in this study contained naturally occurring vegetation in alleyways between rows of trees and were managed with regular cutting to a height of ≤5cm four to five times annually. A novel seed mix (Table 2.3) targeted to deliver pest regulation was deployed in alternative alleyways of wildflower treatment plots and managed under two distinct cutting regimes, standard management or active management. All orchards were treated with herbicides directly under the orange trees and treated with insecticides and acaricides as described in section 2.1.2 (Chapter two).

3.2.2. Site Description

The study was conducted across four navel orange (*Citrus sinensis*) orchards within three orchard sites: Madre del Agua, La Calvilla, and Montepinos, in two different localities in the province of Huelva, south-west Andalucía, Spain (Figure 2.1).

3.2.3. Study Design

The randomised block design used to investigate the effect of alleyway treatment and wildflower habitat management was established in November 2016 (See Chapter two). The seed mix for the experimental habitat consisted of twelve forb species and two tussock-forming grass species and were all native perennials (Table 2.2). During the establishment year (2017) following sowing, all wildflower habitat plots were cut once in March and once in April to ≈10cm to promote establishment of the sown species (Woodcock *et al.*, 2008) Further details, including species and sowing rates, can be found in Chapter two.

3.2.4. Botanical Surveys

Botanical surveys were conducted annually in May of each year to determine plant richness and community composition according to alleyway treatment. During the establishment year (2017), as all eight wildflower plots were managed the same, only four replicate plots were sampled to provide baseline data on establishment success, equivalent to the same number of control plots. From year two, all eight wildflower plots were surveyed. The management strategies were randomly allocated to the plots sampled in year one, so that half received the standard management treatment and half received the active management wildflower treatment. Only the two alleyways located towards the centre of each 0.5ha plot were surveyed, with 20m buffer at either end to reduce possible edge effects (Englund and Cooper, 2003; Vacas *et al.*, 2010). Early spring (May) was selected for surveys to ensure spring ephemeral species (unsown) were also recorded prior to the application of the cutting regimes. Twelve replicate 0.5m x 0.5m quadrats were randomly placed and assessed within each 0.5ha plot, six times in each of the two 60m x 2m sampling areas of the central two alleyways. Quadrats were always placed towards the centre of alleyways to reduce edge effects.

All plant species present within quadrats were identified to species where possible, except for unsown Poaceae which were identified to family. Each species from within the quadrat was assigned a percentage cover score according to an eight-point scale (1 = <1%, 2 = 1-5%, 3 = 6-10%, 4 = 11-20%, 5 = 21-40%, 6 = 41-60%, 7 = 61-80%, and 8 = 81-100%). Bare soil and alleyway leaf litter (unattached) were also recorded using absolute percentage cover values. The reproductive status of each species was also recorded to indicate whether individuals of a species were; i) vegetative only, or had ii) flower shoots present or budding, iii) flowers open in bloom, and iv) seeds in formation, ripe of dehiscent. A four-point scale was used to assign the proportion of individuals at each stage (1 = 1%-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%) (Westbury *et al.*, 2017).

3.2.5. Statistical Analysis

All statistical analysis were performed using R studio Version 1.3.1056 (RStudio Team, 2015) for R version 4.0.2 (R Core Team, 2019). Data manipulation was carried out using the tidyr and dplyr packages (Wickham, 2020; Wickham *et al.*, 2020).

3.2.5.1. The effect of wildflower habitat management on the performance of sown species

To assess the inherent differences in establishment success between wildflower management strategies, cover values of the 14 sown species were converted from the eight-point scale to a cover mid-point score (1 = 1%, 2 = 3%, 3 = 8%, 4 = 15%, 5 = 30%, 6 = 50%, 7 = 70%, and 8 = 90%) and regressed against wildflower management treatment using the manyglm function of the mvabund package (Wang *et al.*, 2012). The response was a 14-vector matrix and the independent variable a signal two-level factor vector, where each level corresponded to one of the two wildflower management treatments (standard and active management), year (one, two and three) as well as their interaction. A negative binomial model was fitted, and the goodness of fit was verified by plotting the residuals (y) against the estimated responses (x) to check for equal distribution about y = 0, and by plotting a quantile-quantile (QQ) plot to check for dispersion. Test statistics were generated via Likelihood-Ratio-Test and the *P*-value estimated via Monte Carlo bootstrapped resamples, run 999 times. To account for the replicated block design, restricted permutations were implemented: an integer matrix of bootstrap labels was defined in permute (Simpson, 2019) for each resample run to sample within orchard blocks and to thus test for treatment effect within orchard blocks. Univariate GLMs fitted to each of the vector of the multivariate response variable matrix were then explored to identify which species had the strongest responses to the treatment across the three-year study (Wang *et al.*, 2012).

3.2.5.2. The effect of alleyway management treatment on plant richness

The number of plant genera (richness) was calculated for each quadrat. Unsown Poaceae and some other individuals were only identified to family (totalling eight unidentified groups). It was assumed that each one of these unidentified groups contained at least one genus other than those already identified and were therefore counted as a single genus. A negative binomial generalised linear mixed affects model (GLMM) was used from the lme4 package (Bates *et al.*, 2015) to infer differences in the number of genera (plant richness) between treatments and years. A negative binomial error structure was applied to account for zero inflated count data (i.e., lack of consistent presence of a particular genus). To account for the randomised block design, random intercepts were fitted for each orchard block (site). A single vector consisting of the number of genera for each quadrat was specified as the response variable and was regressed against treatment (control, standard management wildflower, and active management wildflower) and year. To check for normal distribution of residuals, a QQ-plot was generated, and the dispersion checked. The modes of the random effects were extracted, and the variance plotted to ensure their inclusion within the model was justified (Bates *et al.*, 2015).

Stepwise reduction of the model was then conducted, and the Akaike Information Criterion (AIC) values compared to determine the most parsimonious model (Burnham and Anderson, 2002). The ANOVA function of the package CAR (Fox and Weisberg, 2019) for the negative binomial GLMER-type object was then used to assess significance of the terms within the selected model.

3.2.5.3. The effect of alleyway management treatment on plant abundance and plant community composition

To assess differences in plant community composition between treatments, multivariate analysis of the alleyway communities was conducted in mvabund (Wang *et al.*, 2012) and the results visualised using ordination in BORAL (Hui, 2016).

The response variable was a multivariate-vector matrix consisting of the cover scores (converted to the mid-point as described above) for each plant genera identified across all the plots during the three-year study (the total number of recorded genera was 71). To account for the zero-inflated count data, a negative binomial model was fitted. The plant abundance matrix was regressed against treatment using the manyany function of the mvabund package and composition set to true (Wang *et al.*, 2012). Goodness of fit and dispersion were verified as previously described (section 1023.2.5.1). A null model was then fitted in which treatment affect was removed from the model. ANOVA was performed with 999 bootstrapped resamples via PIT-trap resampling to compare the nested models.

To visualise the result of the multivariate compositional analysis, an unconstrained latent variable model was constructed in BORAL and the posterior medians plotted in an ordination plot (Hui, 2016). This approach was selected as BORAL is a model-based approach for the analysis of multivariate abundance data without the need for data transformations or dissimilarity measures (Hui, 2016). The response was the same 71-vector matrix containing the abundance of plant genera described above. To account for randomised block design, a random row effect structure was specified and included in the model. Goodness of fit and dispersion were again verified as previously described (section 1023.2.5.1). The posterior medians were then extracted from the model output and plotted as an ordination plot using ggplot2 (Wickham, 2016).

To explore the multivariate differences of total abundance (as opposed to relative abundance as in compositional analysis), the same 71-vector matrix was regressed against treatment using the manyglm function in mvabund (Wang *et al.*, 2012). One negative binomial model was constructed for each of the study years and restricted permutations were implemented for each as previously described (section 1023.2.5.1). Goodness of fit and distribution were verified as described in section 1023.2.5.1. Univariate GLMs were fitted and examined as previously described (section 1023.2.5.1).

3.2.5.4. Difference in cover class between habitat management strategies

The differences in percentage cover of each resources class according to treatment were investigated using linear models in R. The ten cover classes were bare soil, leaf litter, vegetative forbs, vegetative grasses, budding forbs, budding grasses, flowering forbs, flowering grasses, forbs having set seed, and grasses having set seed. Bare soil and leaf litter are considered non-reproductive resource classes, while grasses and forbs in vegetative, budding, flowering, and seed-set stages are considered reproductive resource classes. Non-reproductive resource classes were recorded as absolute percentage cover values, and these were used in the analysis. The percentage cover of each reproductive resource classes, however, had to be calculated.

To calculate the percentage cover scores for the reproductive resource classes (grasses and forbs), a reproductive percentage cover score was first estimated for each species sampled form within the quadrat. The reproductive percentage cover score was estimated from; i) the reproductive scores per species and ii) percentage cover score per species (both described in section 3.2.4). Firstly, reproductive scores for each species were back-transformed to their mid-point values to give an estimated percentage of each of the four reproductive stages for each plant species (1 = 12%, 2 = 38%, 3 = 63%, and 4 = 88%). Secondly, the estimated percentage cover that each species occupied within the whole quadrat (0.5%, 2.5%, 8%, 15.5%, 30.5%, 50.5%, 70.5%, or 90.5%) was divided by the estimated reproductive scores to calculate the reproductive percentage cover scores for each species at each reproductive status. Once the reproductive percentage cover score for each species had been calculated, these scores were summed across all grasses or all forb species to give the percentage cover scores for the reproductive resource classes for each group (vegetative forbs, vegetative grasses, budding forbs, budding grasses, flowering forbs, flowering grasses, forbs having set seed, grasses having set seed).

The response was the percentage cover assigned to each one of the ten cover classes (reproductive and non-reproductive). As the response was a continuous variable, it was first logit transformed (Warton and Hui, 2011; Douma and Weedon, 2019). An alternative model was created in which the logit-transformed response was then regressed against treatment, resource class, and orchard block. Interaction terms were included between treatment and resource class, treatment and orchard block, and resource class and orchard block. The summary function was used to verify the inclusion of each of the terms in the model. Goodness of fit was verified as previously described (section 3.2.5.2). To infer differences in resource class across the treatments, a null model was then constructed with the same structure, but the treatment*cover scores interaction removed. The alternative model was then compared against the null model using ANOVA. LSmeans was used to make pairwise comparisons between treatments (Lenth, 2016).

3.3. Results

3.3.1. The effect of wildflower habitat management on the performance of sown species

The performance of the sown species based on percentage cover values varied significantly between alleyway treatments (manyglm: LRT = 21.72, df=1, *P* = 0.009) and between years (manyglm: LRT = 74.78, df=2, *P* = 0.001). Moreover, the treatment response changed with time (manyglm: LRT = 48.66, df= 2, *P* = 0.002) (Figure **3.2**).

During the establishment year of 2017, before any management strategies had been applied, the abundance of sown species varied significantly (manyglm: LRT = 31.38, df= 2, *P* = 0.001). *Mentha suaveolens* accounted for 30.27% of the variation in the model due to treatment and represented 0.88% cover in the plots which would receive the standard management treatment from year two but was not recorded from strips to receive the active management treatment. *Dactylis glomerata* accounted for a similar amount of variation in the model (28.68%) and represented 9.25% of the cover in plots which would receive the standard management, and 0.625% in those which would receive the active management treatment (Figure **3.1**).

In year two, before implementation of the two wildflower management treatments, differences in sown species composition were no longer apparent (manyglm: LRT = 10.69, df= 2, *P* = 0.488) (Figure **3.1** and Figure **3.2**).

In year three, following a full year of applying the treatments to the wildflower alleyways, the abundance of sown species between the two wildflower treatments differed significantly (manyglm: LRT = 21.48, df = 2, *P* = 0.023). Differences in abundance scores of *P. bituminosa* between the two management treatments accounted for 41.5% of the variation due to treatment within the model, representing 5.6% of the cover in the standard management, but was not recorded from the active management in 2019. *Salvia verbenaca* accounted for 19.7% of the variation in the model and was also more abundant in the standard management (1.5% cover) than the active management treatment (0.06% cover). *Hypericum perforatum* and *C. intybus* accounted for 13.2% and 12.2% of the variation in the model, both more abundant in the standard management treatment (0.9% cover and 3.1% cover respectively) than in the active management treatment (0.0% cover and 0.3% cover respectively) (Figure 3.1).

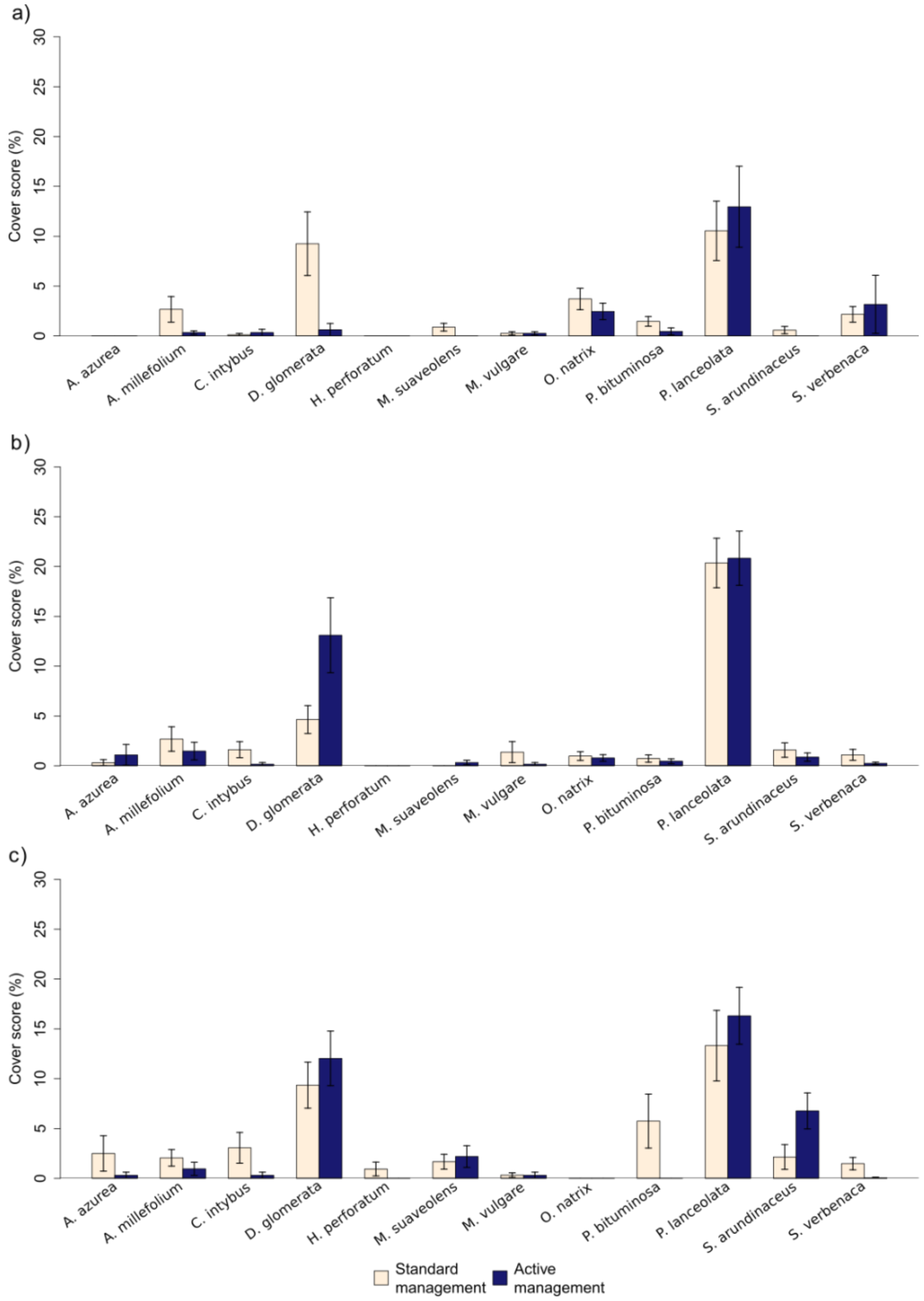

Figure 3.1. Response of sown species managed under different cutting regimes (standard and active management) across the three years of the study. The mean cover scores are presented for each of the sown species compared for years one (a), two (b), and three (c). Error bars represent ± 1 SEM.

Across the three-year study *O. natrix* decreased in abundance from 3.1% cover in 2017, to 0.9% cover in 2018, to 0.0% in 2019, and accounted for 54.6% of the year effect, irrespective of treatment. In contrast, *S. arundinaceus,* which accounted for the second most variance in the model due to year effect (10.3%) displayed an increase in cover throughout the study, irrespective of the management strategy applied to the wildflower strips. *Schedonorus arundinaceus* increased form 0.3% cover in 2017, to 1.3% cover in 2018, and to 4.5% cover in 2019 (Figure **3.1** and Figure **3.2**).

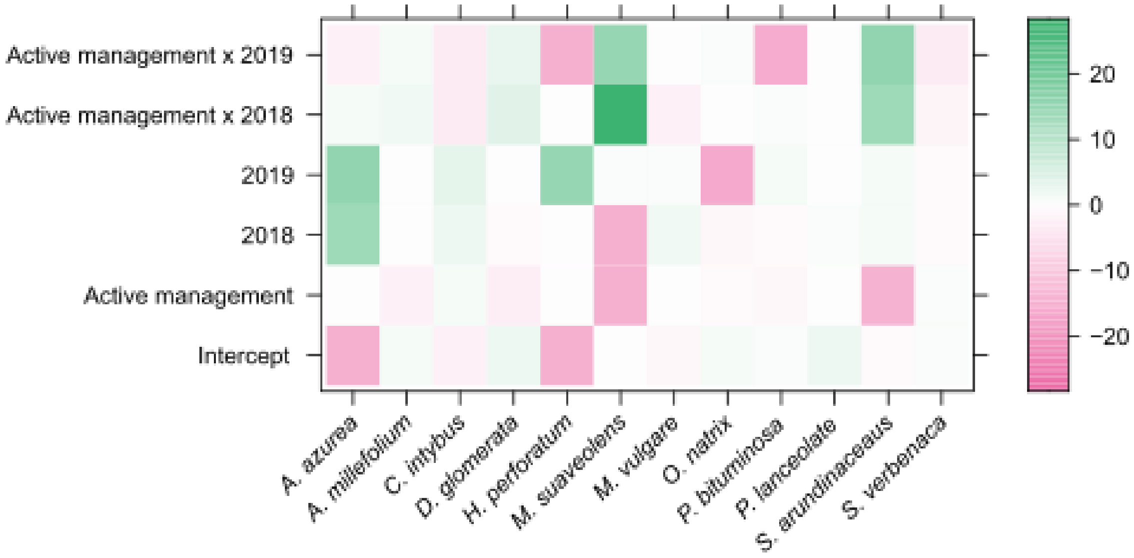

Figure 3.2. The response in abundance of sown species managed by standard or active management across the three years of the study. The coefficients from mvabund model are plotted to show direction and magnitude of the response; no difference from the mean is white, an increase is green, and a decrease is pink. The scale represents the mean change in the response from the predictor and therefore denotes the magnitude of the response, with the sign indicating direction.

Psoralea bituminosa accounted for 27.1% of the treatment*year affect in the model, increasing from 1.5% cover in the standard management treatment in 2017 to 5.8% in 2019 (Figure **3.1** and Figure **3.2**). However, in the active management treatment, the percentage cover decreased from 0.5% in 2017 and 2018 to being unrecorded in 2019. In contrast, *M. suaveolens*, which accounted for 21.4% of the management strategy*year affect, increased in abundance across the three-year study (Figure **3.2**). *Mentha suaveolens* showed a greater increase in abundance score in the active management wildflower treatment (not recorded in 2017 to 2.2% in 2019) than in the standard management treatment (0.9% in 2017 to 1.7% in 2019). In the standard management treatment, *D. glomerata* decreased in abundance between 2017 and 2018 from 9.3% cover to 4.7% but increased again to 9.4% cover in 2019. In the active management treatment, there was an increase between 2018 and 2018 from 0.6% cover to 13.1% and remained at a similar abundance in 2019 (12.0% cover) (Figure **3.1** and Figure **3.2**). *Dactylis glomerata* accounted for 20.4% of the overall treatment*year affect in the model (Figure **3.2**).

3.3.2. The effect of alleyway management treatment on plant richness

The number of plant genera within alleyways was affected by the treatment (GLMM: χ^2 = 94.90, df = 2, $P < 0.001$) (Figure **3.3** and Table **3.1**) and changed significantly across the three-year study (GLMM: χ^2 = 20.95 df = 2, $P < 0.001$) (Figure **3.4** and Table **3.1**). Furthermore, there was no change in treatment effect between years, as indicated by the non-significant interaction (GLMM: χ^2 = 6.78 df = 2, $P = 0.148$).

Table 3.1 Mean plant richness per quadrat (± SE) of all genera, sown genera, and unsown genera recorded from the three different treatments, control, standard management wildflower treatment, and active management wildflower treatment, across each of the three study years, 2017, 2018 and 2019.

Alleyway treatment	2017	2018	2019
Control	2.40 (± 0.15)	1.98 (± 0.17)	1.90 (± 0.17)
Standard management wildflower	5.25 (± 0.23)	4.23 (± 0.28)	3.21 (± 0.24)
Active management wildflower	3.96 (± 0.25)	4.02 (± 0.29)	3.19 (± 0.22)

Irrespective of sampling year, alleyways established with wildflower habitat under either management strategy contained double the number of plant genera (4.03 ± 0.16 genera in the standard management wildflower alleyways and 3.67 ± 0.15 genera in active management wildflower alleyways) compared to the control alleyways (2.09 ± 0.10 genera) containing regularly cut naturally occurring vegetation. There was no significant difference between the two wildflower treatments (Figure **3.3**). Richness irrespective of treatment decreased by almost a quarter throughout the three years, from a mean of 3.5 genera per quadrat in 2017 to 2.76 per quadrat in 2019 (Figure **3.4**). There was no difference in plant richness between 2017 and 2018 (3.40 genera per quadrat).

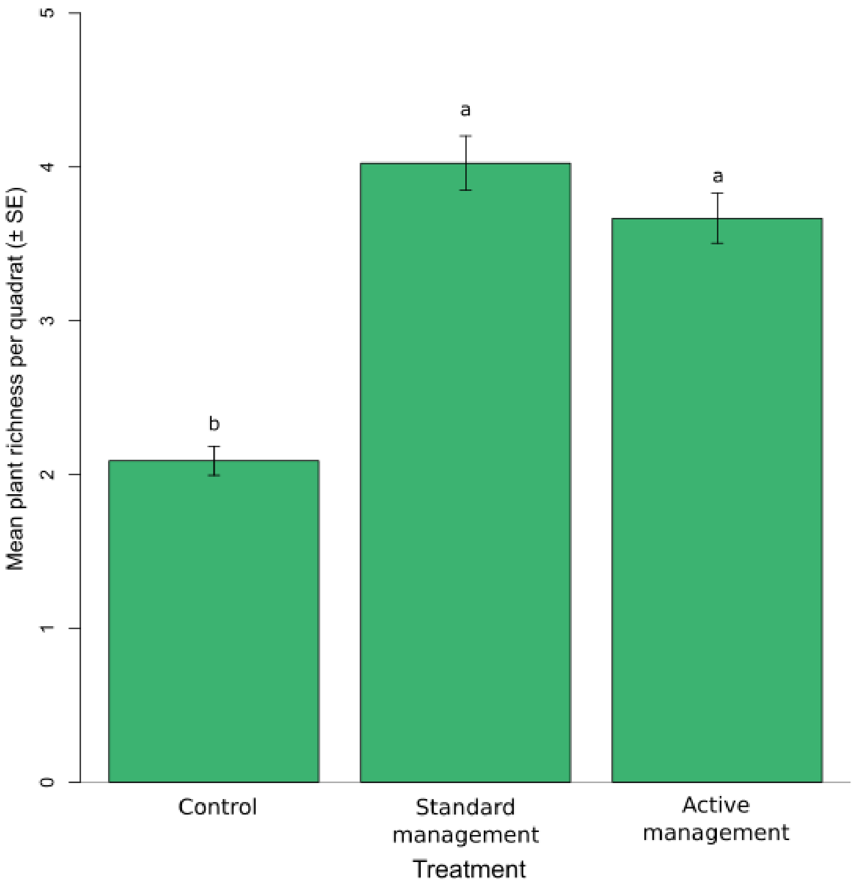

Figure 3.3 Mean richness of plant genera between the three treatments; control, standard management wildflower treatment and active management wildflower treatment, summed across the three study years. Error bars represent ± 1 SEM. Different superscripts represent significant differences between management strategies (Tukey's pairwise contrasts; *P* < 0.05).

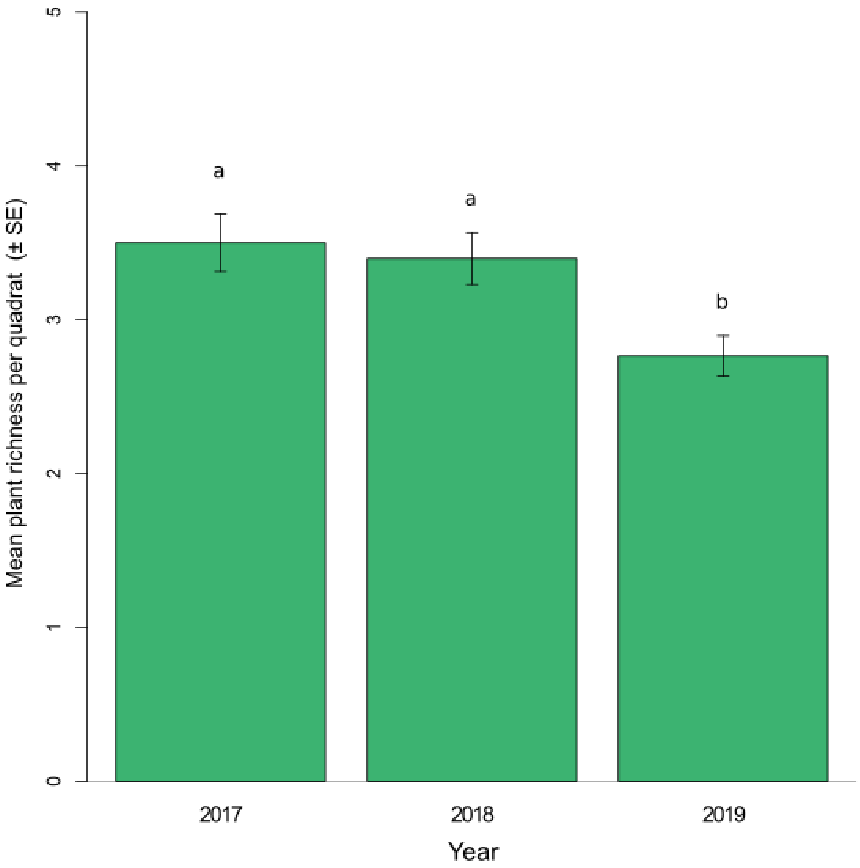

Figure 3.4 Mean richness of plant genera between three study years, one (2017), two (2018) and three (2019), summed across treatments. Error bars represent ± 1 SEM. Different superscripts represent significant differences between management strategies (Tukey's pairwise contrasts; *P* < 0.05).

3.3.3. The effect of alleyway management treatment on plant abundance and plant community composition

In all three study years, the species/genera abundances (based on percentage cover values) varied significantly between the three alleyway management treatments (manyglm: 2017: LRT = 354.8, df = 2, *P* = 0.001; 2018: LRT = 364.5, df = 2, *P* = 0.001; 2019: LRT = 270.6, df = 2, *P* = 0.001) (Appendix 1.1). These are not only differences in total abundance of plant species, but furthermore, the composition of plant communities varied significantly between treatments (manyany: LRT = 405.39, df = 1, *P* < 0.001). Visualisation of the communities across the three treatments using unconstrained ordination of latent variables show that the community composition of the control treatment is most different from the standard and active management treatments (Figure 3.5).

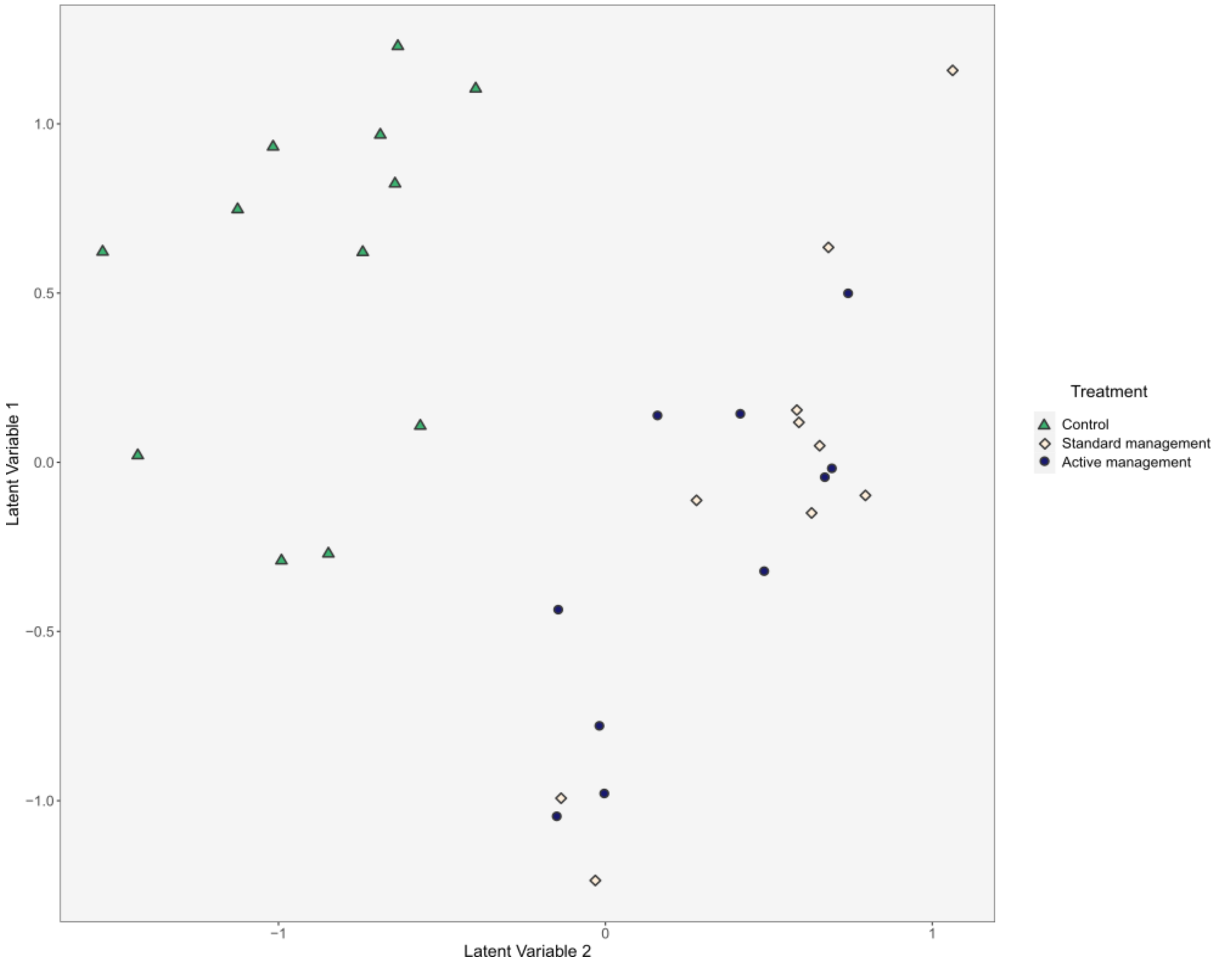

Figure 3.5 Biplot with 15 indicator species based on the negative binomial latent variable model (boral). The points correspond to site indices, where control sites are green coloured triangles, standard management are cream coloured rhombi, and active management are blue coloured circles. Two clusters appear, one of which is composed entirely of sites applied with the control treatment, while the other is composed of sites applied with the two wildflower treatments (standard and active management).

In 2017, ten genera accounted for 68.1% of the treatment effect (Table 3.2). Four sown forbs established consistently well across all sites: *P. lanceolata* with an average cover of 11.8%, *O. natrix* (3.1%), *A. millefolium* (1.5%), *P. bituminosa* (1.0%). The sown grass species *D.*

glomerata also established consistently with an average cover of 4.9%. The most abundant unsown genera present in wildflower alleyways was *Matricaria* accounting for 4.1% of the average cover per quadrat. Unsown control alleyways were dominated by *Sonchus* (4.8% cover) and *Crepis* (3.6% cover). *Portulaca* and *Sonchus* were not recorded in the wildflower habitats.

Table 3.2 Species/genera found to be significantly different in abundance between treatments during the year one (2017). The likelihood ratio for each univariate test is given as well as the percentage contribution of the total treatment affect. Mean percentage cover scores for each of the treatments, control and establishing wildflower plots are also specified.

Species	Sown / unsown	Test statistic (LR)	Percent of total treatment effect (%)	Mean % cover (± SE)	
				Control	Establishing wildflower plots
2017					
Plantago lanceolata	Sown	69.45	19.6	-	11.8 (±2.5)
Ononis natrix	Sown	35.79	10.1	-	3.1 (±0.7)
Dactylis glomerata	Sown	26.51	7.5	-	4.9 (±1.7)
Achillea millefolium	Sown	20.36	5.7	-	1.5 (±0.7)
Salvia verbenaca	Sown	18.52	5.2	-	2.7 (±1.5)
Psoralea bituminosa	Sown	17.28	4.9	-	1.0 (±0.3)
Coleostephus sp.	Unsown	15.65	4.4	-	1.8 (±0.6)
Crepis spp.	Unsown	13.84	3.9	3.6 (±1.4)	0.00
Portulaca sp.	Unsown	10.51	3.9	0.9 (±0.4)	0.00
Sonchus sp.	Unsown	15.65	3.0	4.8 (±2.0)	0.00

In 2018, three species accounted for more than 50% of the treatment effect, all of which were sown species (Table **3.3**). *Plantago lanceolata* was the most abundant species in the standard and the active management wildflower alleyways and was equally abundant in both, accounting for an average of 20.4% and 20.8% cover, respectively. *Dactylis glomerata* was the second most abundant sown species and was at least three times more abundant in the plots receiving the active management treatment (13.1% cover) than those managed under the standard management wildflower treatment (4.7% cover). *Achillea millefolium* was marginally more abundant in the passive management treatment (2.7% cover) than the active management treatment (1.5% cover).

In 2019, seven species were most responsive to the effect of alleyway treatment and accounted for more than 52% of this variance (Table **3.3**). Five of these species were sown forbs, one an unsown forb genus (*Crepis*), and one was the sown grass *D. glomerata*. *Plantago lanceolata* accounted for the greatest variation due to treatment affect and represented an average cover of 13.3% in the standard management, 16.3% in the active management treatment, and was not recorded from the control. *Dactylis glomerata* was also more abundant in the active management (12.0% cover) than the standard management treatment (9.4% cover) and was not recorded from the control. Conversely, *C. intybus* and *S. verbenaca* were more abundant in the standard management than the active management treatment. Similarly*, P. bituminosa,* accounted for 5.8% of the cover in the standard management treatments, however it was not recorded from the active management or control treatments. *Crepis* spp. were twice as abundant in the control treatment (3.8% cover) than the standard management treatment (1.8% cover) and not recorded from the active management treatment.

Table 3.3 Species/genera found to be significantly different in abundance between treatments during the years two (2018) and three (2019). The likelihood ratio for each univariate test is given as well as the percentage contribution of the total treatment affect. Mean percentage cover scores for each of the treatments, control and establishing wildflower plots are also specified.

Species	Sown / unsown	Test statistic (LR)	Percent of total treatment effect (%)	Mean % cover (± SE)		
				Control	Standard management wildflower	Active management wildflower
2018						
Plantago lanceolata	Sown	133.85	35.8	-	20.40 (±2.48)	20.80 (±2.73)
Dactylis glomerata	Sown	35.87	9.6	-	4.65 (±1.40)	13.10 (±3.77)
Achillea millefolium	Sown	12.69	3.4	-	2.69 (±1.23)	1.48 (±0.89)
2019						
Plantago lanceolata	Sown	50.31	18.5	-	13.30 (±3.54)	16.30 (±2.85)
Dactylis glomerata	Sown	35.89	13.2	-	9.35 (±2.31)	12.00 (±2.74)
Psoralea bituminosa	Sown	13.96	5.13	-	5.75 (±2.71)	-
Crepis spp	Unsown	10.93	4.0	3.75 (±1.30)	1.83 (±1.30)	-
Cichorium intybus	Sown	10.35	3.8	-	3.06 (±1.54)	0.31(±0.31)
Salvia verbenaca	Sown	10.16	3.7	-	1.48 (±0.62)	0.06 (±0.06)
Mentha suaveolens	Sown	9.63	3.5	-	1.67 (±0.75)	2.19 (±1.09)

3.3.4. Resource abundance according to alleyway treatment

Across all the study years, the proportion of the different resource classes, bare soil, leaf litter, vegetative, budding, flowering, and seed-set, for both forbs and grasses, were significantly affected by treatment (ANOVA, 2017: F = 9.53, df = 9, $P < 0.001$, 2018: F = 11.98, df = 18, $P < 0.001$ and 2019: F = 5.18, df = 18, $P < 0.001$) (Figure **3.6**Figure 3.7).

In 2017, control alleyways were characterised by significantly more leaf litter (21.8% mean cover) and forbs which had reached seed-set (8.1% mean cover) than plots sown with the wildflower seed mix (leaf litter: 13.8% mean cover, and forbs having set seed: 0.7% mean cover). Plots sown with the wildflower seed mix were characterised by almost nine times the cover of forbs in the vegetative stage (17.8% mean cover) than the control alleyways (2.0% mean cover). None of the other resource classes were significantly different (Figure **3.6** and Appendix 1.2).

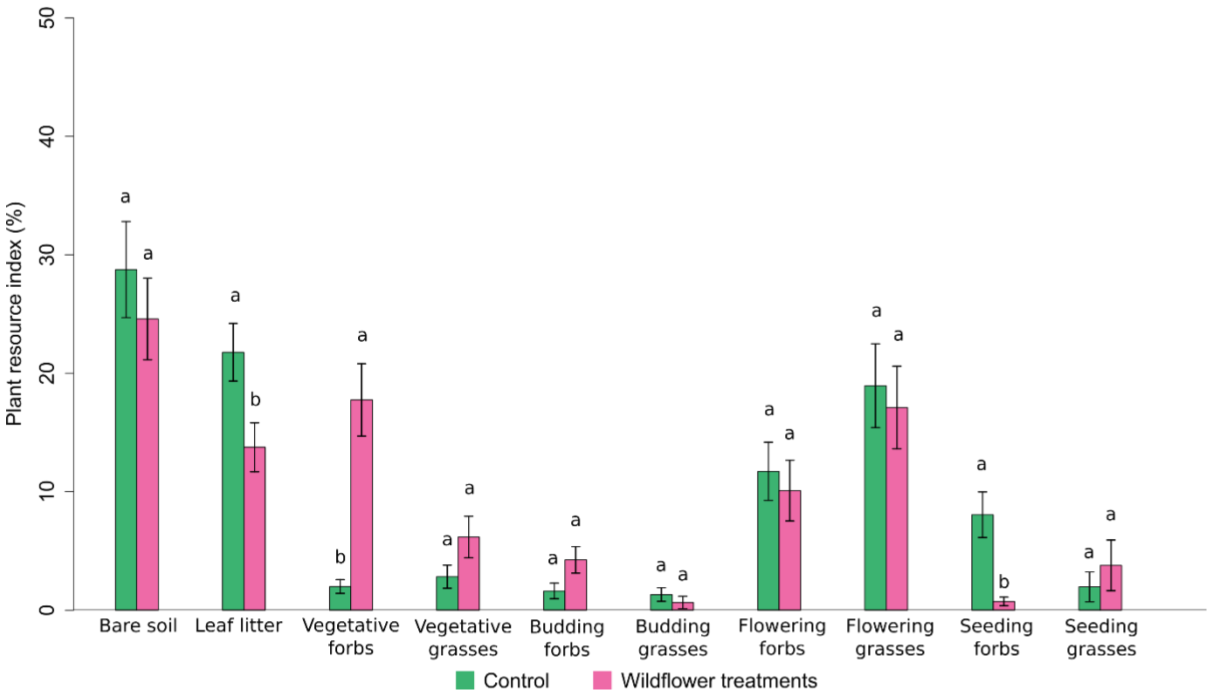

Figure 3.6. Differences in the plant resource index (%) by resource class, bare soil, leaf litter, vegetative forbs, vegetative grasses, budding forbs, budding grasses, flowering forbs, flowering grasses, seeding forbs, seeding grasses, irrespective of being sown or unsown between treatments during the establishment year, 2017. Different superscripts denote significant differences in the plant resource index between treatments, control or establishing wildflower plots (Tukey's pairwise contrasts; $P < 0.05$).

In 2018, control alleyways were characterised by more than double the cover of bare soil (37.8% mean cover) than both standard management plots (12.2% mean cover) and active management plots (15.5% mean cover). Control alleyways were covered in almost 40% more flowering grasses (23.2% mean cover) than the standard management treatment (16.7% mean cover) and almost three times the cover than the active management treatments (8.0% mean cover). Budding grasses represented six times more cover in the active management treatment plots (9.2% mean cover) than the control (1.5% mean cover). In the standard management plots (3.2% mean cover), the cover of budding grasses was neither significantly different from the control nor the active management treatment. Similar to the establishment year, plots under the standard and active management strategy were characterised by significantly greater cover of vegetative forbs (standard management: 14.8% mean cover and active management: 17.1% mean cover) than the control (4.0% mean cover). Similarly, budding forbs were about five times more abundant in the standard (11.1% mean cover) and the active (10.6% mean cover) management treatments than the control (2.2% mean cover). None of the other resource classes were significantly different (Figure **3.7** and Appendix 1.3).

In 2019, the standard (13.0% mean cover) and active (16.6% mean cover) management wildflower alleyways were covered by more than twice the cover of vegetative forbs than the control (5.0% mean cover). The control treatment was characterised by significantly more bare soil cover (38.9% mean cover) than the standard management wildflower alleyways (23.1% mean cover), however the cover of bare soil in the active management (28.8% mean cover) was not different from the control or the standard management wildflower treatment. Vegetative grasses and budding grasses however, represented almost twice the amount of cover in the active management treatment (vegetative grasses: 16.6% mean cover and budding grasses: 20.3% mean cover) than both the control (vegetative: 8.7% mean cover and budding: 3.1% mean cover) and the standard management treatments (vegetative: 8.5% mean cover and budding: 3.5% mean cover). Budding forbs represented almost three times the cover in the standard management treatment (14.4% mean cover) than the control treatment (5.5% mean cover) but was not significantly different from the active management treatment (7.6% mean cover). The cover of budding grasses in the active management treatment was also not different from the control. Similarly, grasses having set seed represent three times more cover in the standard management treatment (11.5% mean cover) than the control (1.3% mean cover) or the active management (3.9% mean cover) treatments. However, both control and active management plots were characterised with the same amount of cover of budding grasses. All other resource classes were not significantly different between treatments (Figure **3.7** and Appendix 1.3).

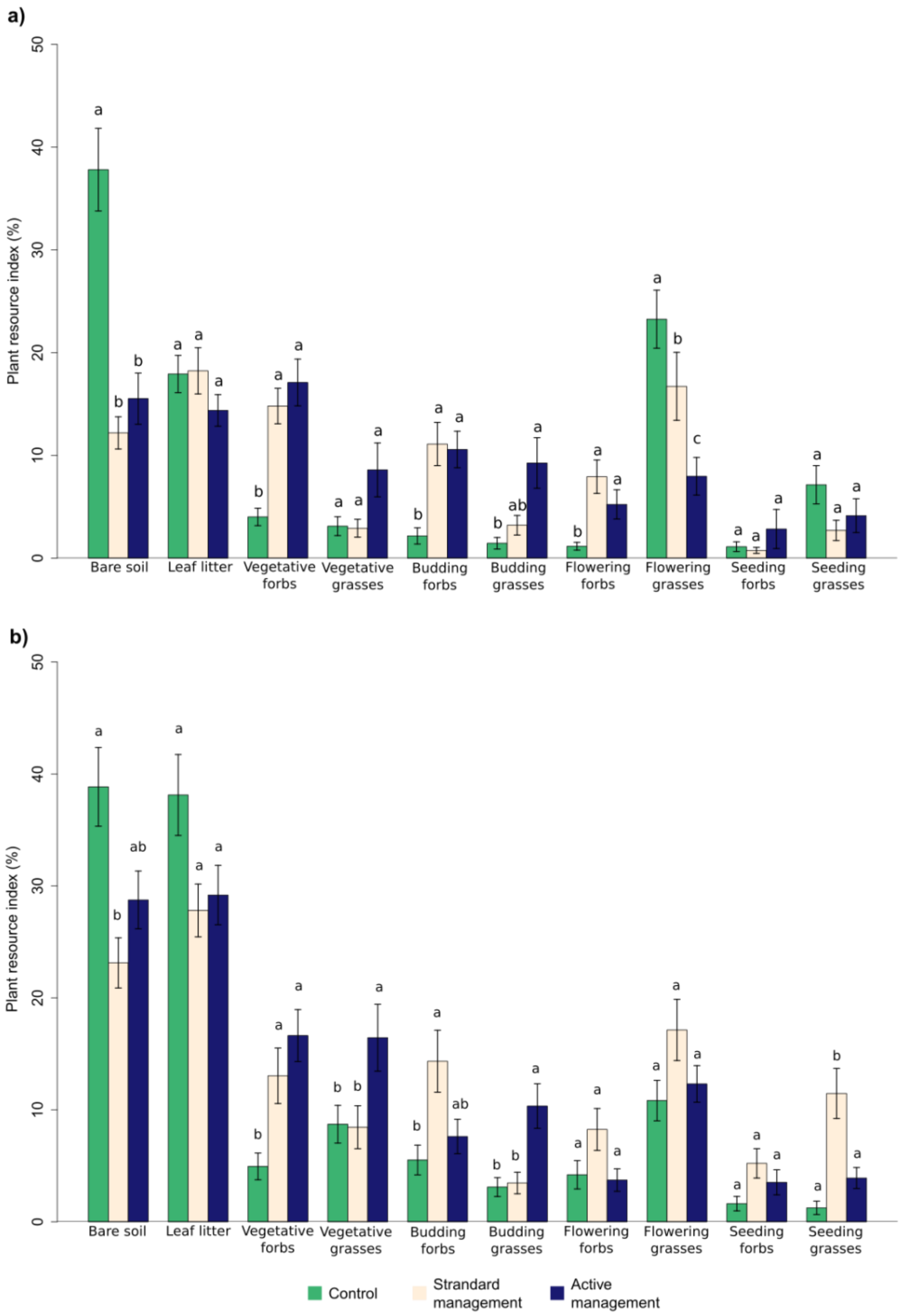

Figure 3.7 Differences in the plant resource index (%) by resource class, bare soil, leaf litter, vegetative forbs, vegetative grasses, budding forbs, budding grasses, flowering forbs, flowering grasses, seeding forbs, seeding grasses, irrespective of being sown or unsown, between treatments during two (2018a) and year three (2019b). Error bars represent ± 1 SEM. Different superscripts denote significant differences in the plant resource index between treatments, control, standard management wildflower or active management wildflower plots (Tukey's pairwise contrasts; $P < 0.05$).

3.4. Discussion

For the first time, this study has demonstrated that it is possible to successfully establish a native perennial wildflower mix in alleyways between rows of citrus in Spanish orchards. As a consequence, the richness of plant genera within alleyways was increased, relative to the unsown control alleyways. The standard and active management of the wildflower habitats were also shown to differentially influence plant composition and the resources provided, which has important implications for supporting natural enemies within citrus orchards (Parry *et al.*, 2015). Active management wildflower habitats, cut three times across the year to promote the movement of natural enemies onto the crop, were associated with an increased cover of species which can reproduce vegetatively. The two sown grass species *D. glomerata* and *S. arundinaceus* both responded positively to the active management wildflower treatment, and their cover increased throughout the study. *Mentha suaveolens*, another species capable of vegetative reproduction (Yahia *et al.*, 2019), also responded positively to the active management treatment. However, although *A. millefolium* can reproduce vegetatively via rhizomes (Kannangara and Field, 1985), it did not increase during the study. In situations where water availability can limit seed germination and successful establishment of perennial species, the ability to reproduce vegetatively can be advantageous (Clary, 2008). Species which can reproduce both sexually and asexually show preference for vegetative reproduction when under stressed conditions, such as water stress and as a short-term strategy, it is considered a key driver to dominance in perennial species (Yang and Kim, 2016). As a result, in climates characterised by low summer precipitation, spring annual species dominate (Clary, 2008). Species with deep roots, such as *Plantago lanceolata*, can also dominate in dry conditions. The competitive advantage of species spreading vegetatively in dry climates can also be facilitated by regular cutting which can induce more vegetative growth (Bricca *et al.*, 2020) and have a significant effect on the establishment and persistence of annual (ephemeral) species (Westbury *et al.*, 2008; Blake *et al.*, 2011). As a consequence, this can lead to a reduction in the abundance of species reproducing solely by seed. For example, despite the initial successful establishment of *P. bituminosa* and *S. verbenaca* in all orchard alleyways, values of cover in the active management wildflower alleyways were significantly lower, compared to the standard management wildflower alleyways, which were managed only once with annual cutting.

Competitive exclusion through the increased grass abundance and the associated lower values of bare ground in active management wildflower alleyways is an on-going issue for newly created wildflower habitats (Westbury *et al.*, 2008). In contrast, the unsown alleyways managed conventionally with regular cutting exhibited greater percentage cover of bare soil and leaf litter, than alleyways established with wildflower habitat. Cutting Mediterranean plant communities typically increases the abundance of annual grass and forbs species for several years before perennials typically re-establish (Merou, Tsiftsis and Papanastasis, 2013). The regular cutting associated with the conventional alleyway management would be expected to reduce the ability for many plant species to flower and set seed, including spring annuals, resulting in greater values of bare ground. In temperate regions, regular cutting may lead to dominance of perennial grass species (Westbury and Dunnett, 2008). In arid climates however, the proportion of bare soil and leaf litter in areas of high disturbance are highly variable across time as a response to changes in the environment, particularly such as water stress (Souther *et al.*, 2019).

Irrespective of alleyway treatment, four sown species did not perform well during the three-year study: *Hypericum perforatum*, *O. natrix, T. vulgare* and *H. stoechas*. Both *H. perforatum* and *T. vulgare* were only recorded in one plot, whilst *H. stoechas* was not recorded in any quadrats assessed, nor observed while conducting surveys. Oliveira *et al.* (2012) similarly, failed to establish *H. stoechas* when testing its suitability as part of a seed mix for erosion control in the Mediterranean. Despite the initial establishment success of *O. natrix*, it was absent from all plots by year three (2019). Atallah *et al.* (2008) recorded *O. natrix* only as scattered individuals in open vegetation. This suggests that *O. natrix* may be outcompeted in closed vegetation associated with low amounts of bare ground, as in this study. *Ononis natrix* is also more typical of nutrient poor soils (Alard *et al.*, 2005; Atallah *et al.*, 2008). Fertigation methods used to deliver nitrogen and phosphorus to the orange trees may have created less favourable conditions for this species. In addition to direct competition, shading may have altered the success of these species within a community context as light intensity affects plant response to stress and their interactions with other organisms (Valladares *et al.,* 2016). Species establishment might have also been influenced by the sowing rates used (Stevenson, Bullock and Ward, 1995). Higher sowing rates can increase success by reducing latent factors confounding establishment, ensuring at least a few individuals can persist

(Stevenson, Bullock and Ward, 1995; Jaksetic *et al.*, 2018). Further investigation is therefore required to determine if the successful establishment of these species might be enhanced by altering the initial sowing rate.

Several unsown species, *Malva sylvestris*, *M. parviflora*, *Medicago intertexta*, *M. truncatul*a, *Trifolium glomeratum and T. scabrum,* were abundant in the wildflower treatments and are known to support non-pest alternative prey and/or hosts as well as their predators and parasitoids (Bertolaccini, Núñez-Pérez and Tizado, 2011; Gómez-Marco, Urbaneja and Tena, 2016). The active management treatment was also associated with the unsown species *Solanum nigrum,* which is considered a problem species in citrus (Ferreira and Sousa, 2011; Celepci *et al.*, 2017), though also recorded from the standard management treatment later in year three (Chapter five). It is associated with both pest aphid, mealybug and mite species (Ferreira and Sousa, 2011; Celepci *et al.*, 2017). *Solanum nigrum* is an annual species and typically germinates in late spring to early summer and performs best in unshaded open locations (Roberts and Lockett, 1978; Keeley and Thullen, 1989). The reduction in sward height associated with the active management wildflower treatment is therefore likely to have opened up the sward, enhancing conditions for its establishment (Keeley and Thullen, 1989). In contrast, vegetation in the standard management wildflower treatment was associated with lower values of bare ground and therefore a denser sward; values of *S. nigrum* were no different with this treatment compared to the control. Overall, despite the concern for *S. nigrum*, the control treatment was associated with a greater range of plant species considered to be problematic, including *Portulaca oleracea*, *Polygonum aviculare* and *Amaranthus spp*, which are known to harbour pest species (Ferreira and Sousa, 2011; Celepci *et al.*, 2017). *Polygonum aviculare* L (Caryophyllales: Polygonaceae) also showed a positive response to the active management strategy. This species is also considered an economically important weed species in arable and horticultural crops and can cause significant impacts in the Mediterranean (Radicetti, Mancinelli and Campiglia, 2012) due to its competitive dominance. *Polygonum aviculare* also has an allelopathic effect and reduces germination of competitors (Alsaadawi and Rice, 1982). Its seeds are also hard-coated and can remain dormant in the seed bank until germination conditions are optimal (Costea and Tardif, 2005; Malavert, Batlla and Benech-Arnold, 2017; Fernández Farnocchia, Benech-Arnold and Batlla, 2019). As such, the cultivation of the alleyways prior to sowing the wildflower seed is likely to have increased its abundance in the active management treatment (Costea and Tardif, 2005). As with *S. nigrum*, *P. aviculare* was also facilitated by the cutting regime of the active management wildflower treatment as a low growing species (Costea and Tardif, 2005).

3.5. Conclusions

Based on the establishment success and performance of the 14 species sown, ten are recommended for sowing to establish wildflower habitat in alleyways in Spanish citrus orchards: the two tussock-forming grasses, *Dactylis glomerata* and *Schedonorus (Festuca) arundinaceus;* and the eight forbs, *Anchusa azurea, Salvia verbenaca, Psoralea bituminosa, Mentha suaveolens, Plantago lanceolata, Marrubium vulgare, Achillea millefolium, and Cichorium intybus.* However, depending on the provision of pest regulation services in each of the two sown treatments, some of these ten species might be omitted from this recommendation. This is because the active management of the sown wildflower habitat resulting the loss of *S. verbenaca*, *P. bituminosa* and *C. intybus*, the inclusion of these species would not be recommended for this management regime. Further investigation is required to establish longer term effects of management strategy applied to this habitat and to determine whether specific strategies may be applied to encourage re-colonisation of species which declined over the three-year study.

3.6. References

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Chapter 4

The influence of wildflower habitats and their management on natural enemy abundance and pest regulation services in the tree canopy

Abstract

Human driven degradation of the environment is driving the collapse of ecosystem processes. Many of these processes provide vital services to human health and wellbeing (ecosystem services). The contemporary approaches to agriculture must be reimaged to include ecological techniques which maximise the delivery of ecosystem services so that food can be produced sustainably whilst meeting the growing yield demands. Pest regulation services, harnessed through the conservation of natural enemies in the agri-environment, are an economically important service degraded by conventional citrus production practices. For the first time, a sown wildflower strip composed of native forbs and tussock-forming grasses has been investigated for its influence on natural enemy richness and abundance and their pest regulation services in Mediterranean citrus. A novel management strategy was applied during May and June, using the predicted generation times of *Aonidiella aurantia,* a key pest in citrus, to determine whether cutting the wildflower habitat at critical points in the year could displace natural enemies onto the adjacent crop, enhancing pest regulation services. Three treatments were compared in orange orchard alleyways: i) a control treatment, the standard orchard practice of regular cutting to 5 cm throughout the year, ii) a sown wildflower treatment managed with cutting once a year in February to a height of 10cm (standard management wildflower treatment), and iii) the same sown wildflower treatment but managed with two additional cuts each during May and June (active management wildflower treatment). The orange tree canopies were sampled for natural enemies and pest regulation services were quantified using cards baited with *Ephestia kuehniella* eggs. Natural enemy richness and abundance were greater in orange tree canopies with the standard management wildflower treatment in the alleyways compared to the control and active management treatments. Furthermore, this was associated with enhanced pest regulation services (depletion of sentinel prey from baited cards), especially during the early summer months, which coincided with a crucial period in which to control *A. aurantii*. In contrast, the active management treatment did not enhance natural enemy richness and abundance, with little evidence that natural enemy abundance in the canopy could be boosted subsequent to cutting. Pest regulation services were also lower in the active management treatment compared to the standard management wildflower treatment, and in some cases the control. The outcome of this study suggests that leaving the wildflower habitat uncut throughout the

season, as in the standard management wildflower treatment, helps to mitigate pest incidence through enhanced pest regulation services. However, further studies are required to determine how this would influence populations of target pests.

4.1. Introduction

Modern agricultural practices are challenged to feed the growing human population whilst simultaneously reducing environmental impact (Bommarco, Kleijn and Potts, 2013; Godfray, 2014; Oliver *et al.*, 2015). The current system of intensive agriculture has simplified once complex landscapes, now characterised by a few crop species distributed as monocultures within large fields, and coupled with high agrochemical inputs (Tilman, 1999a; Macfadyen *et al.*, 2012; Godfray and Garnett, 2014). Such intensive landscapes have left little space for non-cropped habitats (Tscharntke, Clough, *et al.*, 2012) which has come at a great cost to biodiversity (Tilman, 1999a; Dirzo *et al.*, 2014; Sánchez-Bayo and Wyckhuys, 2019; Wagner, 2020). In turn, the very processes which support and regulate ecosystems are being undermined (Downing *et al.*, 2012; Tscharntke, Clough, *et al.*, 2012; Oliver *et al.*, 2015) and ever-increasing inputs are required to mitigate the loss of these ecosystem services (Matson *et al.*, 1997). As such, it is increasingly apparent that the exploitation of land for human consumption must be reimagined to reap the benefits provided by biodiversity (Bommarco, Kleijn and Potts, 2013). To ameliorate environmental degradation and biodiversity loss, habitat interventions to enhance ecological infrastructure have become a cornerstone of sustainable 'ecological' intensification (Bommarco, Kleijn and Potts, 2013; Garnett *et al.*, 2013). Ecological intensification aims to optimise the provision of ecosystem services through the implementation of ecological practices without adverse environmental impact nor further conversion of land to agriculture (The Montpellier Panel, 2013; Godfray, 2014; Ghosh *et al.*, 2019).

One key economically important agricultural ecosystem service undermined by standard farm practices is the regulation of crop pests by their natural enemies (Naranjo, Ellsworth and Frisvold, 2015). Plant resources which are fundamental to natural enemies, such as carbohydrates, protein, and shelter and refuge, are scattered across large spatial scales (Kuemmerle *et al.*, 2016) and when they are present, are typically limited to brief periods such as the crop flowering period (Woodcock *et al.*, 2005). This spatial and temporal isolation from fundamental resources fails to support natural enemy populations within or adjacent to target crops, reduces natural enemy fitness, and can limit the potential for biological control (Martin *et al.*, 2016, 2019). Furthermore, in the absence of natural pest control, dependency on chemical plant protection products (PPPs) has increased (Pimentel, 2005; Popp, Pető and Nagy, 2013). The overuse of PPPs further constrains the establishment of natural enemies both on the crop and in the surrounding environment which disrupts pest regulation services (Desneux, Decourtye and Delpuech, 2007; McKerchar *et al.*, 2020). To support natural enemies and reduce the use of PPPs, habitat creation and management techniques have been developed to maximise natural enemy fitness and enhance pest regulation services (Landis, Wratten and Gurr, 2000).

Habitat management encompasses a variety of different strategies to diversify otherwise fragmented landscapes (Landis, Wratten and Gurr, 2000; Gurr *et al.*, 2017). Approaches can be largely divided into the preservation of existing semi-natural, non-cropped habitats and the creation of new habitats, which can be applied to a range of scales from landscapes to within-field patches (Tscharntke, Clough, *et al.*, 2012). While landscape scale interventions might include the preservation of woodlands and the extension of hedgerows as habitat corridors, within-field interventions might focus on resource requirements of specific beneficial insects (Bianchi, Booij and Tscharntke, 2006). Creating wildflower habitats at field boundaries or in alleyways between rows of crop plants has successfully increased natural enemy abundance in fruit crops such as cherry, apple, and blueberry and can help contribute to pest regulation services (Whitehouse, Sial and Schmidt, 2018; Albrecht *et al.*, 2020; Mateos-Fierro *et al.*, 2021). However, such strategies must be tailored for each agroecosystem (Gurr *et al.*, 2017). Moreover, the use of cutting as a tool to encourage the movement of natural enemies from the alleyways to the adjacent crop and boost pest control at key periods needs further investigation.

In Mediterranean biomes, habitat interventions have been found to increase natural enemy diversity and abundance in grape (Rosas-Ramos *et al.*, 2019), pear (de Pedro *et al.*, 2020), olive (Carpio, Castro and Tortosa, 2019; Nave *et al.*, 2021), lemon (Silva *et al.*, 2010) and pomegranate (Kishinevsky, Keasar, *et al.*, 2017) production systems. In these systems, increasing structural complexity at the farm scale has been found to support natural enemies (Rosas-Ramos *et al.*, 2018). In Spanish citrus, orchard alleyways are typically maintained as bare soil, with vegetation either mechanically or chemically removed (Monzó et al., 2020). However, in Andalusia agri-environmental schemes for Integrated Production promote the naturally occurring vegetation on the orchard floor to mitigate soil erosion (Anonymous, 2002). Also, over the past decade, there has been an increase in the use of sown *Schedonorus arundinaceus* (Schreb.) grass strips (Monzó et al., 2020), predominantly for the regulation of mites and thrips (Aguilar-Fenollosa et al., 2011; Aguilar-Fenollosa and Jacas, 2013). The value of naturally occurring unsown forbs species establishing within these grass strips for the control of aphids, principally by predatory arthropods, has also been demonstrated (Gómez-Marco, Urbaneja and Tena, 2016). However, other Mediterranean citrus pest species still escape satisfactorily control (Urbaneja *et al.*, 2015; Urbaneja, Grout, *et al.*, 2020) and responses might be very site-specific depending on which naturally occurring plant species colonise each site. The value of sowing a seed mix composed of legumes and non-tussock-forming grasses to support natural enemies in crop canopies has been investigated in Portuguese lemon orchards, but pest regulation services were not assessed (Silva *et al.*, 2010). The inclusion of tussock-forming grasses in wildflower strips can increase resources for natural enemies in comparison to non-tussock-forming species by providing favourable microclimates during weather extremes (Luff, 1965; Collins *et al.*, 2003). Furthermore, by increasing the number of forb species and their associated traits, the flowering period of wildflower strips can be extended to support a greater diversity of natural enemies (Fiedler and Landis, 2007; Wäckers and van Rijn, 2012). There is therefore a strong rationale for creating wildflower habitats in commercial citrus orchards that include tussock-forming grass species and a wide diversity of forbs species to support natural enemies and their pest regulation services.

The effect of wildflower habitat management by cutting on arthropod spill-over and pest regulation services has seldom been investigated. Goller, Nunnenmacher and Goldbach (1997) observed increased abundance of Coccinellids in the crop two weeks after cutting the leguminous strips which was attributed to forced spill-over. In citrus orchards, Vercher *et al.* (2012) described many economically important parasitoids, such as those of the genera *Aphytis* (Hymenoptera: Aphelinidae), *Metaphycus*, and *Microterys* (Hymenoptera:

Encyrtidae), and predators, such as *Propylea quatuordecimpunctata* and *Scymnus interruptus* (Coleoptera: Coccinellidae), inhabiting both the alleyway vegetation and the tree canopy, but reported no spill-over effect onto the citrus canopy due to cutting. However, the study was only conducted over 17-days of one year. As the diversity of plant species and traits as well as the age of the sward has a significant effect on the arthropod community (Fiedler and Landis, 2007; Campbell *et al.*, 2017), a species-rich perennial wildflower habitat designed to support natural enemies may readily facilitate spill-over onto the crop following cutting. In this study therefore, wildflower habitats were managed with two different cutting regimes (treatments), to determine whether the creation and management of a perennial wildflower habitat can support natural enemies on the crop and deliver enhanced pest regulation services in Spanish citrus. To test the principal of forcing spill-over of natural enemies onto the crop by cutting, alleyway management was timed to coincide with predicted generations of *Aonidiella aurantii* (Hemiptera: Diaspididae) a key pest in Spanish citrus (Urbaneja, Grout, *et al.*, 2020) (see Chapter two, section 2.4.1).

4.2. Material and methods

4.2.1. Site Description

A full site description including the selection criteria can be found in Chapter two (section 2.1). The study was conducted in three large (>300ha) commercial Spanish citrus orchards (sites); Madre del Agua, La Calvilla and Montepinos, in two different localities in the province of Huelva, south-west Andalucía. In these orchards, the standard practice was to manage the naturally occurring alleyway vegetation with cutting four to five times a year to a height of ≤5cm, and the cuttings left *in situ*. All orchards were treated with herbicides directly under the orange trees and the crop was treated with insecticides and acaricides as described in Chapter two (section 2.1.2).

4.2.2. Study Design

A full description of the study design can be found in Chapter two (section 2.2). Three alleyway management approaches were investigated, i) a control treatment where alleyways were managed by standard farm practice, ii) a wildflower habitat sown in alternate alleyways between rows of orange trees, managed by cutting once annually (hereafter standard management wildflower), and iii) the same sown wildflower habitat as in the standard management wildflower treatment, managed actively by cutting three times per year (≈10cm), the second two cuts were timed to coincide with the predicted maximum incidence of susceptible instars of the key pest, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), in May and July (hereafter active management wildflower treatment).

The different alleyway treatments were established in 0.5ha plots randomised within orchard blocks. Treatment plots were separated by at least 150m to help ensure independence between treatments (Ries et al., 2004; Miliczky and Horton, 2005; Zappalà et al., 2012; Thomson and Hoffmann, 2013). One replicate block was situated at Madre de Agua and one at La Calvilla, and two blocks within Montepinos. In year three (2019), the orange variety was changed in one of the treatment plots in a block at Montepinos. This involved the removal of the tree canopy, and so this whole replicate block was discounted in the final year of study, and as such, three replicate blocks were investigated in year three.

The wildflower seed mix consisted of twelve forb species and two tussock-forming grass species. All species were native perennials, selected to provide carbohydrate resource, shelter, pollen, and alternative hosts/prey for natural enemies throughout the year. Further details, including species and sowing rates, can be found in Chapter two (section 2.3). During the establishment year (2017) following sowing, all wildflower habitat plots were cut once in March and once in April, to ≈10cm to promote establishment of the sown species (Woodcock *et al*., 2008). No further alleyway management was applied to any of the wildflower plots in year one and they were all managed the same to promote successful establishment of the wildflower habitats. In years two and three, the different management strategies were then applied to the alleyways where the wildflower habitat was established. To reduce edge effect, the outermost alleyways of each plot were excluded from sampling and a 20m buffer zone at either end of the alleyways was established. Samples were collected from May to October in year one and April to October in years two and three at 4-week intervals, except for September of year two when sampling was delayed by one week. Each orchard block at a site was sampled in a single day and the order that the plots were sampled was randomised on each visit. All responses were compared to the control treatment.

The aims of this study were to determine whether the creation of wildflower habitats and their subsequent management could i) increase natural enemy richness and abundance in the crop canopy, and ii) enhance pest regulation services.

4.2.3. Timing the active management of the wildflower habitats

To investigate the principle that natural enemies could be forced to spill-over onto the crop and boost pest regulation services, the management (cutting) of the active management wildflower treatment was timed to coincide with predicted first and second generations of *A. aurantii*. These were forecast by applying the phenology model developed by Grout *et al.* (1989) to climate data collected from a meteorological station at the farm La Calvilla (Gibraleón-Manzorrales) and male flight data obtained from one of the farms (Manzorrales). The active management wildflower alleyways were then cut in May and July of years two and three according to these predictions. See Chapter two (section 2.4.1) for a full description.

4.2.4. Arthropod sampling

To investigate the response of natural enemies to the active management treatment applied to the wildflower habitats (May and July), alleyways were cut 3-5 days prior to sampling for natural enemies. Insects were sampled from the orange tree canopies facing the wildflower habitats using a vacuum sampler adapted from a commercial leaf-blower (Stihl BG 86 C-E) and modified for arthropod sampling on foliage. The modification consisted of an extension to the input vent tube which increased the aperture to a diameter of 21cm (Tena, Soto and Garcia-Marí, 2008; Planes *et al.*, 2015). A wire mesh prevented leaves and twigs entering the sample bag. Sixteen trees, paired across the alleyway, were randomly selected at the start of the study and sampled for the three-year duration. The tree canopies were vacuumed randomly to a height of 2m with four suctions, each for a duration of ten seconds. Each sample consisted of a set of paired trees, with eight 10-second sucks and eight samples taken per plot. Samples were placed on ice and returned to the laboratory for processing and identification. All samples collected with the vacuum device were stored frozen until identification after which they were stored in ethanol for reference.

Where possible, all Araneae, Coleoptera, Hemiptera, Neuroptera, Hymenoptera, and Diptera (Syrphidae) implicit in biological control (Bellows and Fisher, 1999) were identified to morpho-species. If morpho-species could not be determined, individuals were identified to the next lowest rank which enabled the functional group, based on trophic function, to be determined (genus or family). Many ant species are able to predate citrus crop pests but can also disrupt biological control by attacking natural enemies and so were classed in their own functional group (Pekas *et al.*, 2011) (Figure 4.3). As a result, five non-taxonomic functional groups were determined, parasitoids, predators, hyper-parasitoids, omnivores, and ants (Table 4.1) (Figure 4.1 Figure 4.3).

Figure 4.1. *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), a predator, feeding on *Aphis spiraecola* Patch. (Hemiptera: Aphididae) in the citrus canopy. Aphids parasitized by Aphidiinae (Hymenoptera: Braconidae), identified by the characteristic "aphid mummy" appearance, are present within the colony.

Table 4.1 Different orders and families which were considered in the different functional groups

Functional group	Order	Family
Predator	Araneae	**Salticidae**
Predator	Araneae	**Araneidae**
Predator	Araneae	**Clubionidae**
Predator	Araneae	**Cheiracanthiidae**
Predator	Araneae	**Sparassidae**
Predator	Araneae	**Lycosidae**
Predator	Araneae	**Oxyopidae**
Predator	Araneae	**Thomisidae**
Predator	Araneae	**Other Araneae**
Predator	Coleoptera	**Coccinellidae**
Predator	Coleoptera	**Carabidae**
Predator	Coleoptera	**Staphylinidae**
Predator	Hemiptera	**Nabidae**
Predator	Hemiptera	**Reduviidae**
Predator	Hemiptera	**Anthocoridae**

Functional group	Order	Family
Predator	Neuroptera	Chrysopidae
Predator	Neuroptera	Hemerobiidae
Predator	Neuroptera	Myrmeleontidae
Predator	Neuroptera	Coniopterygidae
Predator	Diptera	Syrphidae
Primary parasitoids	Hymenoptera	Aphelinidae
Primary parasitoids	Hymenoptera	Braconidae
Primary parasitoids	Hymenoptera	Encyrtidae
Primary parasitoids	Hymenoptera	Eulophidae
Primary parasitoids	Hymenoptera	Pteromalidae
Primary parasitoids	Hymenoptera	Scelionidae
Primary parasitoids	Hymenoptera	Chalcididae
Ants	Hymenoptera	Formicidae
Hyperparasitoids	Hymenoptera	Aphelinidae
Hyperparasitoids	Hymenoptera	Encyrtidae
Hyperparasitoids	Hymenoptera	Pteromalidae
Hyperparasitoids	Hymenoptera	Figitidae
Hyperparasitoids	Hymenoptera	Cynipidae
Hyperparasitoids	Hymenoptera	Megaspilidae
Other parasitoids	Hymenoptera	Aphelinidae
Other parasitoids	Hymenoptera	Braconidae
Other parasitoids	Hymenoptera	Encyrtidae
Other parasitoids	Hymenoptera	Trichogrammatidae
Other parasitoids	Hymenoptera	Eulophidae
Other parasitoids	Hymenoptera	Pteromalidae
Other parasitoids	Hymenoptera	Scelionidae
Other parasitoids	Hymenoptera	Figitidae
Other parasitoids	Hymenoptera	Ceraphronidae
Other parasitoids	Hymenoptera	Diapriidae
Other parasitoids	Hymenoptera	Eurytomidae
Other parasitoids	Hymenoptera	Ichneumonidae
Other parasitoids	Hymenoptera	Tetracampidae
Omnivores	Dermaptera	-

Figure 4.2 Coniopterygidae larvae (Neuroptera), a predator, feeding on phytophagous mites on the underside of a citrus leaf.

Figure 4.3 *Lasius grandis* Forel (Hymenoptera: Formicidae) attacking a Coniopterygid adult on an immature orange fruit.

4.2.5. Pest regulation services

Assessments of pest regulation services and natural enemies in the canopy were conducted concurrently so that observed predation rates directly related to natural enemy abundance. Due to low pest incidence levels recorded during year one (2017), cards baited with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs were used to quantify pest regulation services in subsequent years (Campbell et al., 2017; McKerchar et al., 2020; Mateos-Fierro et al. 2021). To determine whether cutting the alleyways in the active management wildflower treatment boosted pest regulation services in the canopy, baited cards were deployed within the trees 3-5 days after the alleyways were cut. The baited cards consisted of 60mm x 10mm strips of graph paper. The upper most 10mm x 10mm of the graph paper strip was covered with Henkel® Pritt roller tape and covered with *E. kuehniella* eggs. Excess eggs were removed with a paint brush so that the remainder covered the paper as a single layer. The number of eggs within half of the prepared 10mm x 10mm area (five of the ten columns of the graph paper) was counted for each baited card and then multiplied to estimate the total number of eggs on the card prior to predation. Each prepared card held approximately 450 *E. kuehniella* eggs. Cards were mounted in the tree canopy by wrapping the excess graph paper around a twig and stapling it to itself (Figure **4.4**). The baited cards were left *in situ* for seven days after which the number of eggs remaining was estimated by counting half the area, as previously described. This was repeated with fresh baited cards every 28 days for the months of May, June, and July 2018 (year two), and from April to October 2019 (year three). Six to eight cards were mounted per plot in 2018, whilst in 2019 the number was increased to 16 per plot.

Figure 4.4. A baited card, prepared by sticking approximately 450 *Ephestia kuehniella* eggs to a 10mm x 10mm area of graph paper, mounted in the citrus canopy using a staple and left *in situ* for seven days before being collected and the predation rate assessed.

4.2.6. Statistical analyses

All statistical analysis were performed using R studio (RStudio Team, 2015) Version 1.3.1056 for R version 4.0.2 (R Core Team, 2019). Data manipulation was carried out using the tidyr and dplyr packages (Wickham, 2020; Wickham *et al.*, 2020).

4.2.6.1. Natural enemy richness

The influence of alleyway management on natural enemy richness across the sampling season was investigated using a Poisson distribution generalised linear model (GLM). The number of natural enemy species was set as the response variable. Where it was not possible to identify an individual to species, it was assumed that the identified rank (genus or family) contained one species. The explanatory variables were alleyway treatment, orchard block, sample month, and study year. Richness (y) was plotted against sample month (x) and their relationship observed to be non-linear, as such, the polynomial term month^2 was also included in the model. Goodness of fit was verified by plotting the residuals (y) against the estimated responses (x), observing equal distribution about y = 0, and by plotting a quantile-quantile (QQ) plot to check for dispersion. The influence of each of the fixed terms was obtained via model comparisons using ANOVA Chi square. Factors and their interactions were sequentially removed from the global model and then compared using ANOVA.

4.2.6.2. Functional abundance and functional composition

To investigate the influence of alleyway treatment on the abundance of the different functional groups recorded (parasitoids, predators, hyper-parasitoids, omnivores, and ants) a negative binomial multivariate model was fitted for each sampling year, one (2017), two (2018), and three (2019), in the mvabund package (Wang *et al.*, 2012). The response variable in each was a 5-vector multivariate matrix. The vectors corresponded to the different function groups recorded and contained the total number of individuals counted from each sample. The explanatory variables were alleyway treatment and month. As sampling month was a non-linear function of family abundance, the polynomial term month^2 was also fitted to the model. Non-significant terms were removed from the model and then tested against the null model. Multivariate test statistics were obtained via Likelihood-Ratio-Test and the *P*-value estimated via 999 PIT-trap resamples (Warton, Thibaut and Wang, 2017). To account for the replicated block design, restricted permutations were implemented. An integer matrix of bootstrap labels was defined in permute (Simpson, 2019) for each resample run to sample within orchard blocks to test for treatment effects within orchard blocks. Goodness of fit was verified by plotting the residuals (y) against the estimated responses (x) to check for dispersion and equal distribution about y = 0, and by plotting a quantile-quantile (QQ) plot to check for dispersion. Univariate GLMs, fitted for each of the families (vectors) of the multivariate response matrix, were then explored to identify which families showed the strongest responses to treatment across the three-year study (Wang *et al.*, 2012). The univariate models applied to each of the vectors within the multivariate matrix were then investigated to identify which species infer most variance within the models (Wang *et al.*, 2012). A resampling-based implementation of Holm's step-down method was used within the manyglm function to make pairwise contrasts between significant treatments within each model.

To investigate the influence of alleyway treatment on the relative abundance of different functional groups (functional composition) a negative binomial multivariate compositional model was fitted for each sampling year, one, two, and three, via the manyany argument in the mvabund package (Wang *et al.*, 2019). The response variables were the same 5-vector multivariate matrices described before and the explanatory variable was alleyway treatment. As the anova.manyany argument only compares two manyany objects, a null model was fitted with the treatment variable removed. Model comparisons were conducted to determine whether there was evidence for an overall treatment affect. Multivariate test statistics were obtained via Likelihood-Ratio-Test and the *P*-value estimated via 999 PIT-trap resamples (Warton, Thibaut and Wang, 2017). Goodness of fit was verified in the same way as explained for the non-compositional (manyglm) model above.

4.2.6.3. Natural enemy family abundance

To investigate the influence of alleyway treatment on the abundance of different natural enemy families, a negative binomial multivariate model was fitted for each sampling year, one, two, and three, in the mvabund package (Wang *et al.*, 2012). As ants have a dual role in citrus as both antagonists and predators, the Formicidae were included in this analysis. The response variable in each model was therefore a 27-vector multivariate matrix with each vector corresponding to the different families recorded and contained the total number of individuals counted from each sample. The analysis was conducted and fit verified as explained for functional abundance (section 4.2.6.2).

4.2.6.4. Pest regulation services

To investigate the influence of alleyway treatment on pest regulation services, a quasibinomial GLM was fitted to each of the three sampling years. The response was a two-vector variable containing the number of eggs remaining on the card after seven days and the number predated from the cards during the same period. The explanatory variables were alleyway treatment, orchard block, and month. In year three the polynomial argument month^2 was also included in the model as the samples were taken across the whole sampling season (April to October), and displayed a non-linear response to sampling month. Goodness of fit for each model was determined as explained for natural enemy richness (4.2.6.1). ANOVA Chi-square tests were then conducted for each model.

4.3. Results

Over the three-year study, a total of 13,691 arthropods were identified from the tree canopy following vacuum sampling. Of these, 9,539 individuals (69.4%) were natural enemy species belonging to 26 different families. 2,306 (16.8%) of the natural enemies were identified as primary parasitoids, 7,233 were predators (52.8%), and 38 (0.3%) were omnivores. A further 416 of the total arthropods recorded were identified as hyperparasitoids (3%) belonging to eight genera and 1,999 ants belonging to four genera. An additional 1,699 parasitoids were collected and categorised as an ‘other parasitoids’ functional group category (12.4% of total individuals collected), as they were only identified to family which prevented identification of their function in relation to pest regulation.

4.3.1. Natural enemy richness in the canopy

In year one, irrespective of sampling month, the richness of natural enemy species recorded in the citrus canopy was not influenced by alleyway treatment (ANOVA: df = 1, χ^2 = 0.125, P = 0.724). There was also no difference in species richness across sampling months according to alleyway treatment (ANOVA: df = 7, χ^2 = 10.951, P = 0.141) (Figure **4.5**). In contrast, species richness in the canopy was influenced by the alleyway treatment in years two (ANOVA: df = 2, χ^2 =18.740, $P < 0.001$) and three (ANOVA: df = 2, χ^2 =36.349, $P < 0.001$), with greater values being recorded with the standard management wildflower treatment compared to control alleyways (Figure **4.5**).

In year two, natural enemy richness was similar between standard management and active management wildflower alleyways (standard management: 2.33 species ± 0.11 per pair of trees sampled as summed average across the year; active management: 2.14 ± 0.11), although both had a greater species richness than the control treatment (1.75 ± 0.10). However, by year three, new species were recorded in the canopies with the standard management wildflower alleyways as the richness more than doubled from year two (4.52 ± 0.20), whilst values for the active management wildflower treatment (3.61 ± 0.17) were similar to the control treatment (3.36 ± 0.17) (Figure **4.5**).

Additionally, the influence of sampling month on natural enemy richness varied between alleyway treatments in years two (ANOVA: df = 14, $\chi^2 = 53.35$, $P = 0.002$) and three (ANOVA: df = 10, $\chi^2 = 32.420$, $P < 0.001$) (Figure **4.5**). During year two, across all three alleyway treatments, natural enemy richness decreased throughout the season until autumn, however, the greatest decline was observed in the control plots (Figure **4.5**). In contrast, in year three, natural enemy richness increased until early July in all three treatments after which it plateaued. In all three treatments, richness then steadily decreased in September and October (Figure **4.5**).

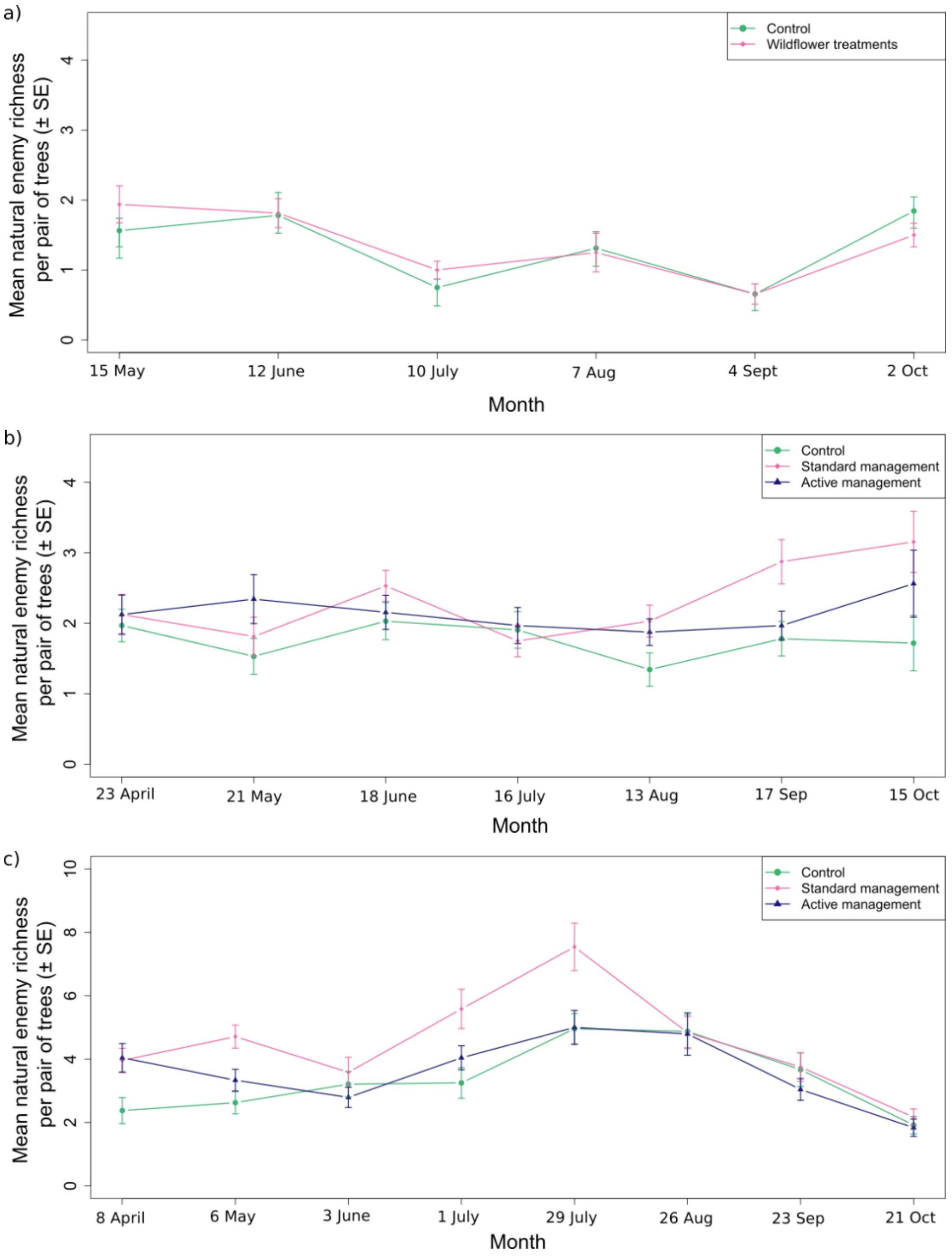

Figure 4.5 Response of natural enemy richness to alleyway treatment between sample months during year one (a), year two (b), and year three (c). The fitted lines are means predicted from the optimal generalized linear model and the points represent partial residuals. Error bars represent ± 1 SEM.

4.3.2. Functional abundance in the canopy

4.3.2.1. Influence of alleyway treatment

Alleyway treatment significantly influenced the number of individuals represented in each of the functional groups in years two (anova.manyglm: LR = 44.73, df = 2, *P* = 0.002) and three (anova.manyglm: LR = 92.19, df = 2, *P* = 0.001), although not in year one (anova.manyglm: LR = 6.62, df = 1, *P* = 0.254) (Figure **4.6**).

In year two, there were differences in the abundance of the other parasitoids functional group in the canopies of the different treatment alleyways (Figure **4.6**). Other parasitoids were responsible for almost half (49.1%) of the treatment effect within the model; they were more abundant within the standard management and active management alleyways than in the control alleyways (standard: 1.54 individuals per pair of trees ± 0.11; active: 1.42 ± 0.14; control: 0.87 ± 0.11). Ants contributed to a quarter (25.0%) of the treatment effect in the year two model (Figure **4.6**) and were more abundant with the control and standard management wildflower treatments than the active management treatment (control: 2.08 individuals per pair of trees ± 0.46; standard: 1.63 ± 0.24; active: 0.96 ± 0.15). Primary parasitoids were responsible for 12.4% of the treatment effect within the model (Figure **4.6**) and were 1.5 times more abundant with the standard management wildflower treatment (1.29 individuals per pair of trees ± 0.14) than with the control (0.86 ± 0.12 per pair of trees), and similarly abundant with the active management wildflower treatment (1.04 ± 0.14 per pair of trees) as with the control.

In year three, primary parasitoids accounted almost two thirds (64.6%) of the total treatment effect within the model (Figure **4.6**). Their abundance was more than double (2.57 times) with the standard management wildflower treatment (4.23 individuals per pair of trees ± 0.45) than with the control alleyways (1.65 ± 0.15 per pair of trees). Abundance of primary parasitoids was similar with the active management wildflower treatment (1.72 ± 0.16 per pair of trees) as that with the control treatment. Hyperparasitoids accounted for 10.8% of the treatment effect within the model (Figure **4.6**). While their abundances were similar in the canopy with the standard management wildflower treatment (0.45 individuals per pair of trees ± 0.07) and with the control treatment (0.42 ± 0.07 per pair of trees). Hyperparasitoid abundance in the canopy with the active management wildflower treatment (0.20 ± 0.04 individuals per pair of trees) was less than half (48.0%) that with the control (0.42 ± 0.07) and with the standard management wildflower treatment (45.0%; 0.45 ± 0.07 individuals per pair of trees). Predators accounted for 9.5% of the treatment effect within the year three model (Figure 4.6). Predators were most abundant with the standard management wildflower treatment (7.77 individuals per pair of trees ± 0.41), 1.28 times more abundant than in the canopy with the control treatment (6.06 ± 0.39 per pair of trees). Predators with the active management wildflower treatment (6.59 ± 0.35 per pair of trees) were similarly abundant as those with the control treatment.

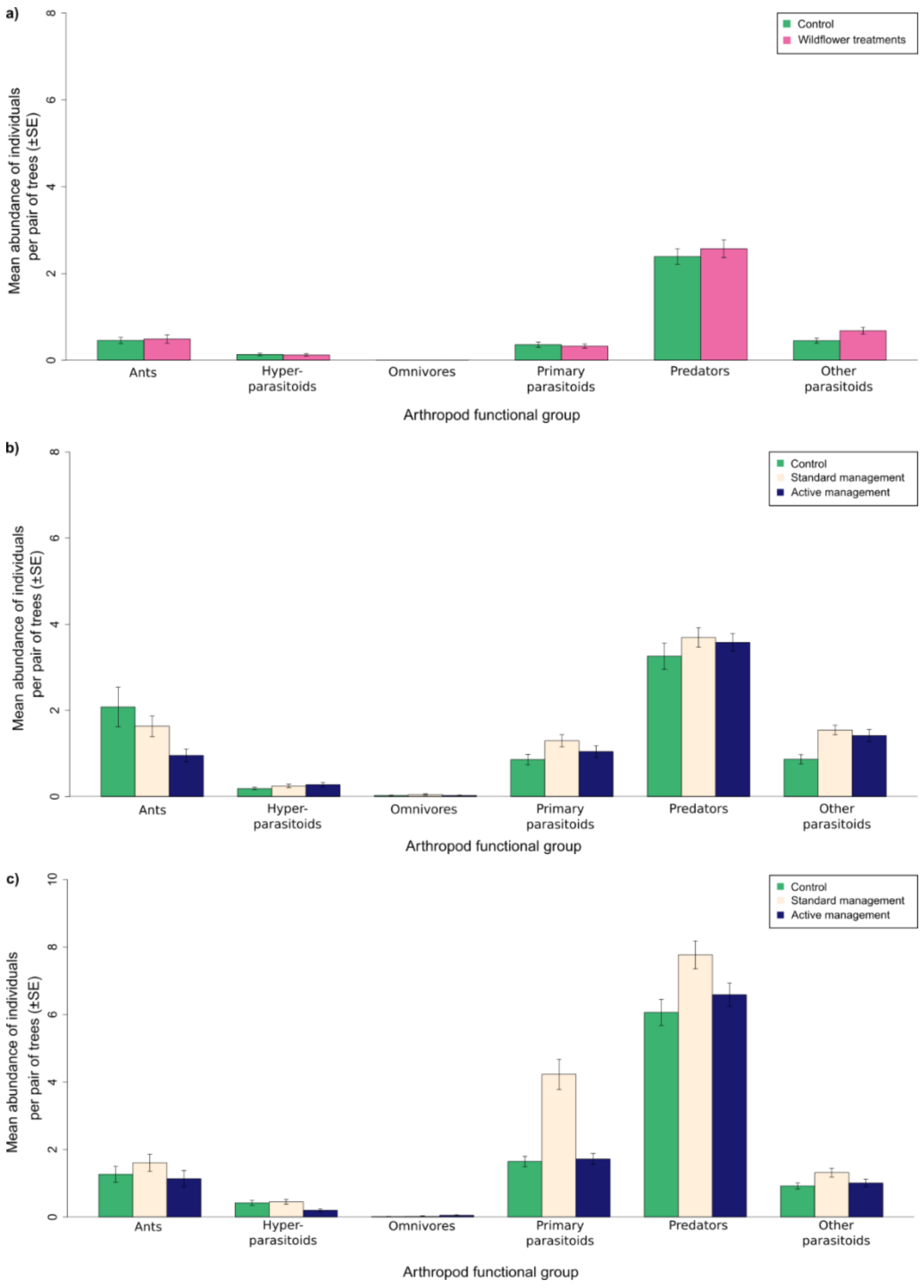

Figure 4.6 Mean abundance of arthropods sampled across year one (2017) (a), year two (2018) (b), and year three (2019) (c). Arthropods are grouped by functional group: ants, hyperparasitoids, predators, omnivores, primary parasitoids, and other parasitoids, sampled from the canopy with the two alleyways treatments in year one: control (green) and wildflower treatment (pink), and the three alleyway treatments in years two and three: control (green), standard management wildflowers (cream), and active management wildflowers (blue). Tails represent standard error about the mean.

4.3.2.2. Treatment response in relation to sampling month

The monthly abundance of the different functional groups recorded from the citrus canopies was significantly different between alleyway treatments in years one (anova.manyglm: LR = 15.73, df = 1, *P* = 0.018) and three (anova.manyglm: LR = 22.40, df = 2, *P* = 0.043) (Table **4.2**) but not in year two (anova.manyglm: LR = 18.10, df = 2, *P* = 0.167) (Table **4.2**).

In year one, 50.16% of the treatment*month effect within the model was due to differences in the monthly abundance of ants between the two treatments (control and the establishing wildflower habitats). In year one, the abundance of ants was initially similar in May, but was followed by a decline in canopies with both treatments, the control and the establishing wildflower habitats, until July in the control and August in the wildflower treatments (Table **4.2**). While with the control treatment the abundance of ants in the canopy remained constant until October, with establishing wildflower treatments, ant abundance in the canopy increased steeply in September before decreasing back to a level similar to that of the control in October (Figure **4.7**). Hyperparasitoids accounted for 19.8% of the treatment*month effect, predators 18.2%, other parasitoids 11.4%, primary parasitoids 0.4% and omnivores 0% (Table **4.2**).

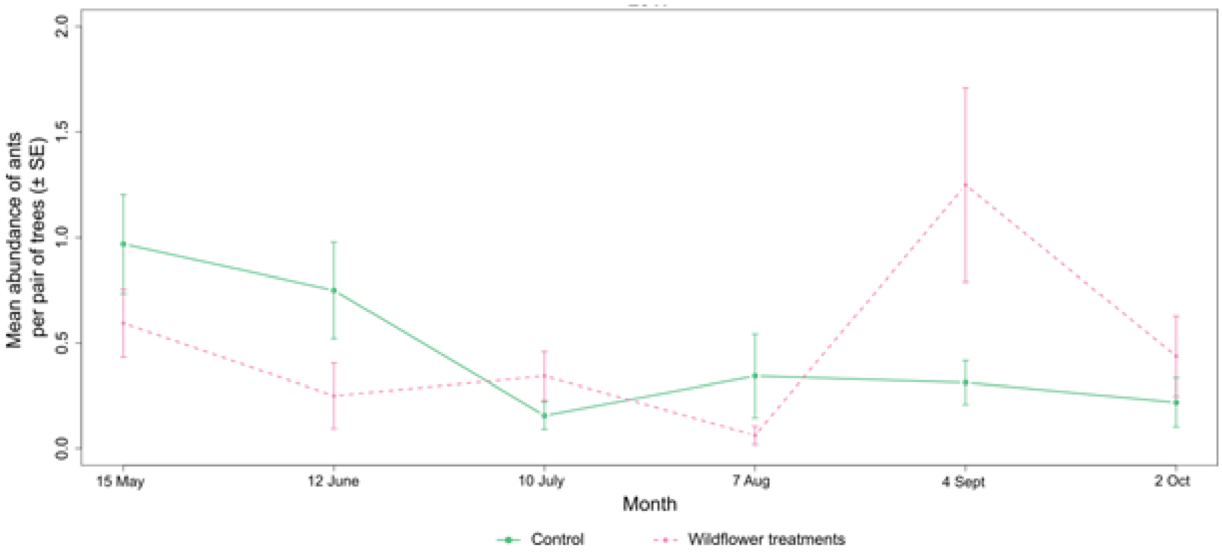

Figure 4.7 Mean abundance of ants sampled from the citrus tree canopy across year one (2017), between the two alleyway treatments: control (green) and the establishing wildflower treatments (pink). Error bars represent ± 1 SEM.

In year three, differences in the monthly abundance of primary parasitoids between alleyway treatments were responsible for more than one third (36.1%) of the total treatment*month effect observed in the model. In April of year three, primary parasitoids were most abundant with the standard and active management wildflower treatments and showing a declining trend towards June in all three treatments. The abundance of primary parasitoids in the canopies associated with the control and active management wildflower treatments were similar throughout the sampling season. With the standard management wildflower treatment, however, primary parasitoid abundance in the canopy increased significantly until late July (Figure **4.8**). Monthly trends in abundance of the other five functional groups, predators, ants, omnivores, hyperparasitoids, and other parasitoids, tended to be similar between treatments and accounted for 63.9% of the total month*treatment effect within the model combined. Of this, 23.4% was attributed to differences in other parasitoids between treatment and month, 16.2% was attributed to differences in ant abundance, 11.9% for omnivore abundance, 7.6% for predator abundance, and 4.8% attributed to hyperparasitoid abundance (Table **4.2**).

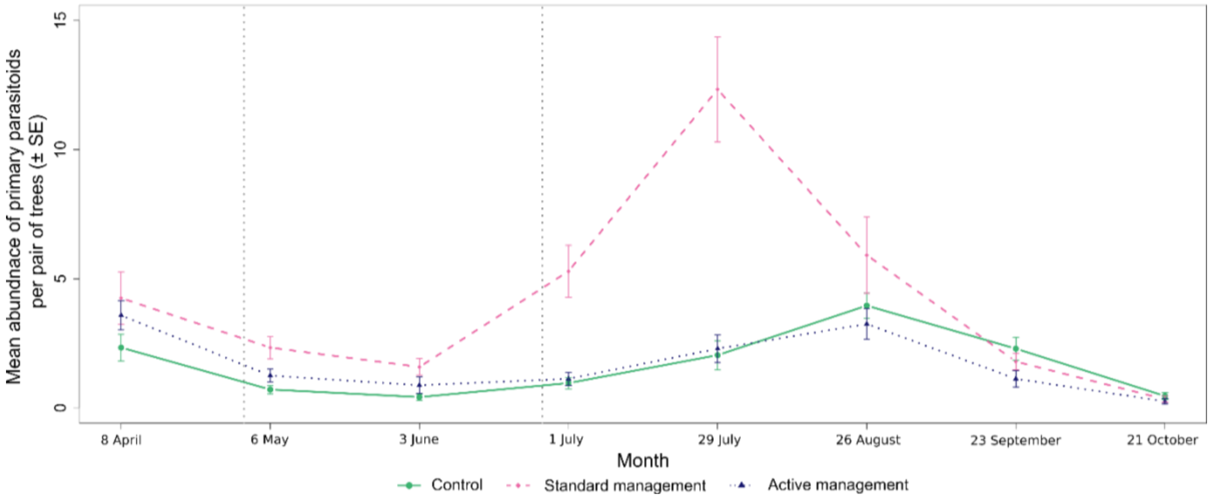

Figure 4.8 Mean abundance of primary parasitoids recorded from the citrus tree canopy across year three (2019), between the three alleyway treatments: control (green), standard management wildflowers (pink), and active management wildflowers (blue). Grey dashed vertical lines represent when the alleyways were cut in the active management treatment. Error bars represent ± 1 SEM.

Table 4.2 The estimated effect (log-likelihood ratio) of each independent variable extracted from the manyglm (mvabund) models to explore differences in functional abundance in the citrus canopies associated with the three different alleyway treatments: control, standard management wildflower treatment, and active management wildflower treatment, and from the monthly samples along the length of the sampling season, and polynomial expression month^2. Three models were fitted, one for each of the study years one (2017), two (2018) and three (2019). Asterisks (*) denote the level of significance within the global model.

Functional group	Test statistic (LR)			
	Treatment	Month	Treatment * Month	Month^2
2017				
Total	**6.62**	**51.12*** **	**15.73***	**120.12*** **
Primary parasitoids	0.18	0.31	0.07	4.93
Predators	0.38	10.25	2.87	51.26
Ants	0.07	1.55	7.89	3.18
Omnivores	-	-	-	-
Hyperparasitoids	0.06	9.62	3.12	6.27
Other parasitoids	5.93	29.39	1.79	54.50
2018				
Total	**44.7****	**136.5*** **	**18.1**	**326.5*** **
Primary parasitoids	5.55	4.80	4.25	188.30
Predators	1.69	0.20	2.93	47.47
Ants	11.64	0.12	2.39	71.29
Omnivores	1.39	8.25	3.23	7.57
Hyperparasitoids	2.82	14.19	0.81	7.52
Other parasitoids	21.65	108.92	4.51	4.36
2019				
Total	**92.19****	**118.54*** **	**22.40***	**199.64*** **
Primary parasitoids	59.56	1.75	8.08	22.28
Predators	8.77	2.94	1.71	70.39
Ants	2.30	2.05	3.63	24.57
Omnivores	4.23	18.00	2.66	0.46
Hyperparasitoids	9.99	53.36	1.08	50.84
Other parasitoids	7.32	40.48	5.25	31.08

4.3.2.3. Effect of sampling month

The sampling month (month^2) affected the abundance of the different functional groups across all three years; year one (manyglm: LR = 120.12, df = 1, *P* = 0.001), year two (manyglm: LR = 326.5, df = 1, *P* = 0.001), and year three (manyglm: LR = 199.64, df = 1, *P* = 0.001).

4.3.3. Functional composition in the canopy

Although total abundance of the different functional groups was affected by alleyway treatment, the composition of the different functional groups was not affected in either year one (anova.manyany: df = 1, LR = 1.590, *P* = 0.867), year two (anova.manyany: df = 1, LR = 8.068, *P* = 0.813) or year three (anova.manyany: df = 1, LR = 27.765, *P* = 0. 523).

4.3.4. Family abundance

4.3.4.1. Influence of alleyway treatment

In year one (2017), there were no differences in abundance of the different natural enemy families between the control and the establishing wildflower treatments (manyglm: LR = 28.49, df = 1, *P* =0.150; (Figure **4.9**a) However, in years two (2018) and three (2019), alleyway treatment had a significant influence on the abundance of different natural enemy families sampled from the canopy (year two: manyglm: LR = 77.3, df = 2, *P* = 0.008; year three: manyglm: LR = 92.5, df = 2, *P* = 0.002; (Figure **4.9**b and c and Appendix 2.4 and Appendix **2.5**).

In year two, family abundance in the canopy with the standard management wildflower treatment was similar to those in canopies with the control treatment, and both were greater than with the active management wildflower treatment. In year two, Encyrtidae accounted for 15.2% of this treatment effect within the model and were more than twice as abundant with the standard management wildflower treatment (0.29 ± 0.06 individuals per pair of trees) than the control (0.12 ± 0.03), and the active management wildflower treatment (0.10 ± 0.03) (Figure **4.9**b). Araneidae accounted for 11.8% and were similarly abundant in the control (0.05 ± 0.02) and standard management (0.06 ± 0.02) treatments but were at least six times less abundant in the active management treatment by comparison (0.01 ± 0.01; Figure **4.9**b). Scelionidae accounted for 10.7% of the treatment effect in year two and were most abundant with the standard management treatment (0.26 ± 0.04) when compared with the control (0.13 ± 0.03) and active management treatments (0.17 ± 0.04) (Figure **4.9**b). Coccinellidae accounted for 9.3% of the treatment effect within the model; the highest abundance was found with the active management wildflower treatment (0.38 ± 0.05), then the standard management wildflower treatment (0.31 ± 0.04), with lower abundance with the control (0.24 ± 0.03; Figure **4.9**b).

In year three, differences in the composition of natural enemy families between alleyway treatments were greater than in year two. The composition of natural enemy families within the standard management treatment was different to those found within the active management and control treatments (Figure **4.9**c). Coccinellidae accounted for 31.5% of the variation in the model due to the effect of treatment, and while they were similarly abundant in canopies with the control alleyways (0.65 ± 0.07) and active management wildflower alleyways (0.067 ± 0.08), they were almost twice as abundant with the standard management wildflower alleyways (1.32 ± 0.14; Figure **4.9**c). Encyrtidae accounted for 14.5% of the treatment effect within the model, again with similar abundances in the control (0.46 ± 0.08) and active management wildflower (0.43 ± 0.08) alleyways, but much more abundant in the standard management wildflower alleyways (0.86 ± 0.11, Figure **4.9**c).

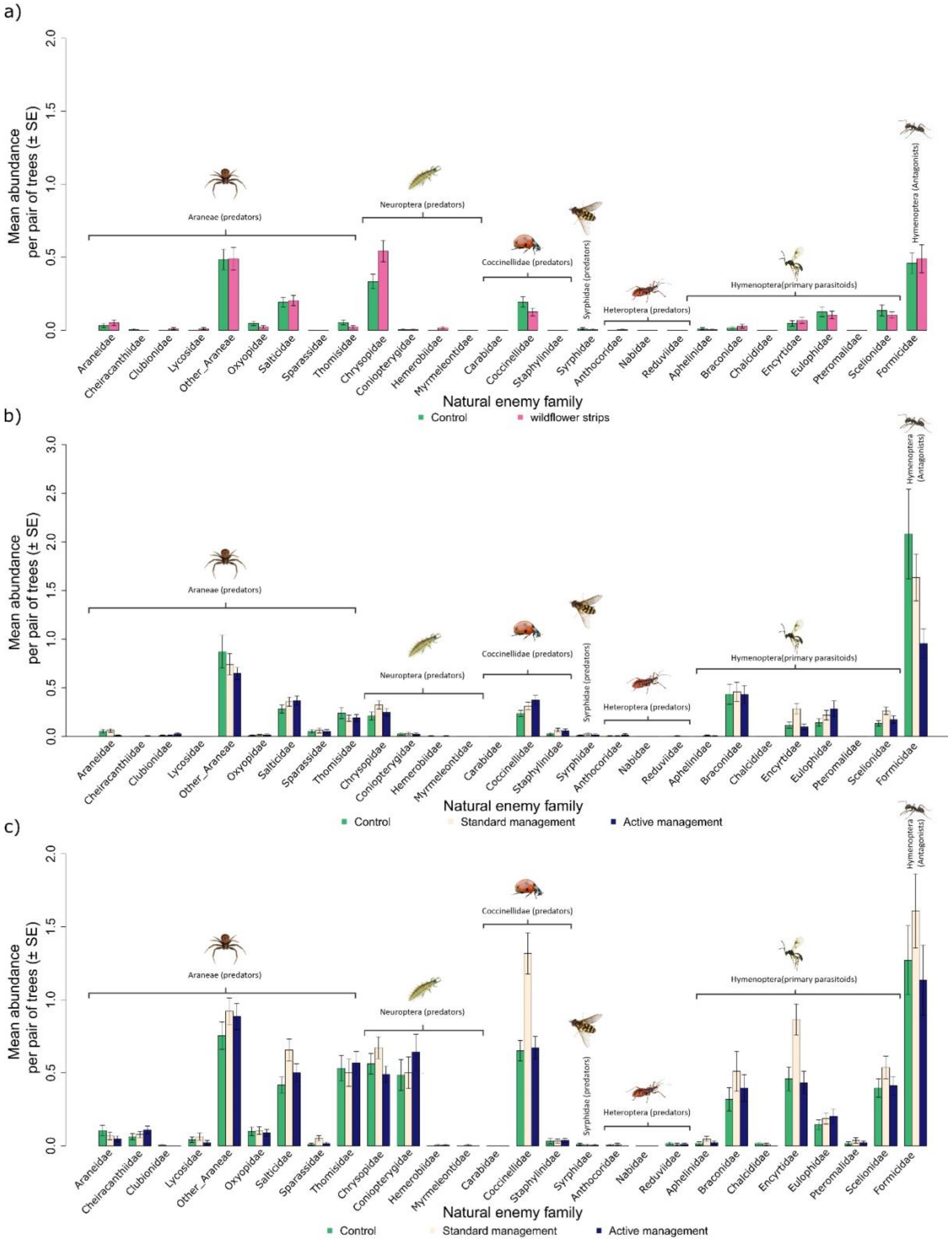

Figure 4.9 Mean natural enemy abundance according to family across the three years; year one (a), year two (b), and year three (c), between treatments; control (green) and wildflower treatments (pink) in year one, and treatments; control (green), standard management wildflowers (cream), and active management wildflowers (blue) in years two and three. Error bars represent ± 1 SEM.

4.3.4.2. Treatment response in relation to sampling month

Across all years, natural enemy family composition changed across the year and showed differences according to alleyway treatment (treatment*month interaction: year one manyglm: LR = 28.43, df = 1, $P = 0.012$, Appendix 2.1; year two - manyglm: LR = 77.6, df = 2, $P = 0.005$, Appendix 2.2; and year three - manyglm: LR = 100.4, df = 2, $P = 0.001$, Appendix 2.3). In year two, 29.2% of the variation within the model associated with differences in monthly trends in abundance of natural enemies between alleyway treatments was due to Clubionidae (Appendix 2.6), 21.3% due to Anthocoridae (Appendix 2.7), and 14.2% due to Thomisidae (Appendix 2.8). In year three, Scelionidae were responsible for the majority of the treatment*month effect within the model (21.9%) (Appendix 2.9) and though rarely recorded, the Anthocoridae contributed 9.6% (Appendix 2.10).

4.3.4.3. Effect of sampling month

The sampling month ($month^2$) had a significant effect on the abundance of the different natural enemies in all three years; year one (manyglm: LR = 201.72, df = 1, $P = 0.001$), year two (manyglm: LR = 329.9, df = 1, $P = 0.001$), and year three (manyglm: LR = 554.9, df = 1, $P = 0.001$). Braconidae, for example, were most abundant in the canopy in April and May and then were absent from the canopy for the rest of the year. Encyrtidae were most abundant later in the year, showing two peaks in abundance, one towards the end of spring (May) and the second during summer (July to September). Coccinellidae followed a similar trend as Encyrtidae. Coccinellidae were present across all sampling months of all three years, although they tended to be most ubiquitous during the summer months. In year three, when Coccinellidae abundance was greatest, there were two distinct peaks in abundance, one towards the end of spring (May) and the second during summer (July to late August). Coniopterygidae were most abundant towards the end of the year, from the end of summer through to autumn (late July to October) (see Appendix 2.1 to 2.3).

4.3.5. Pest regulation services

In year two during May, June and July, alleyway treatment had a significant effect on predation rates in the canopy (GLM: df = 2, χ^2 = 3799.1, $P < 0.001$), as the proportion of eggs predated was consistently greater in citrus canopies with the standard management wildflower treatment. Furthermore, irrespective of alleyway treatment, predation rates were not different between months (GLM: df = 2, χ^2 = 1303.6, $P = 0.058$). The effect of alleyway treatment did not change according to month (GLM: df = 4, χ^2 = 644.7, $P = 0.590$) (Figure **4.10**a).

In year three, predation rates in the canopies were also affected by alleyway treatment (GLM: df = 2, χ^2 = 2002.0, $P = 0.018$), with the proportion of eggs predated typically greater in citrus canopies with the standard management wildflower treatment (Figure **4.10**). Additionally, in year three, the sampling month impacted predation rates independently of treatment (GLM: df = 7, χ^2 = 16078.8, $P < 0.001$), with the highest predation rates in June and early July. Similarly, as in year two, the effect of alleyway treatment on the predation rates within the canopy did not change across the sampling months (GLM: df = 14, χ^2 = 5360.8, $P = 0.087$) (Figure **4.10**b).

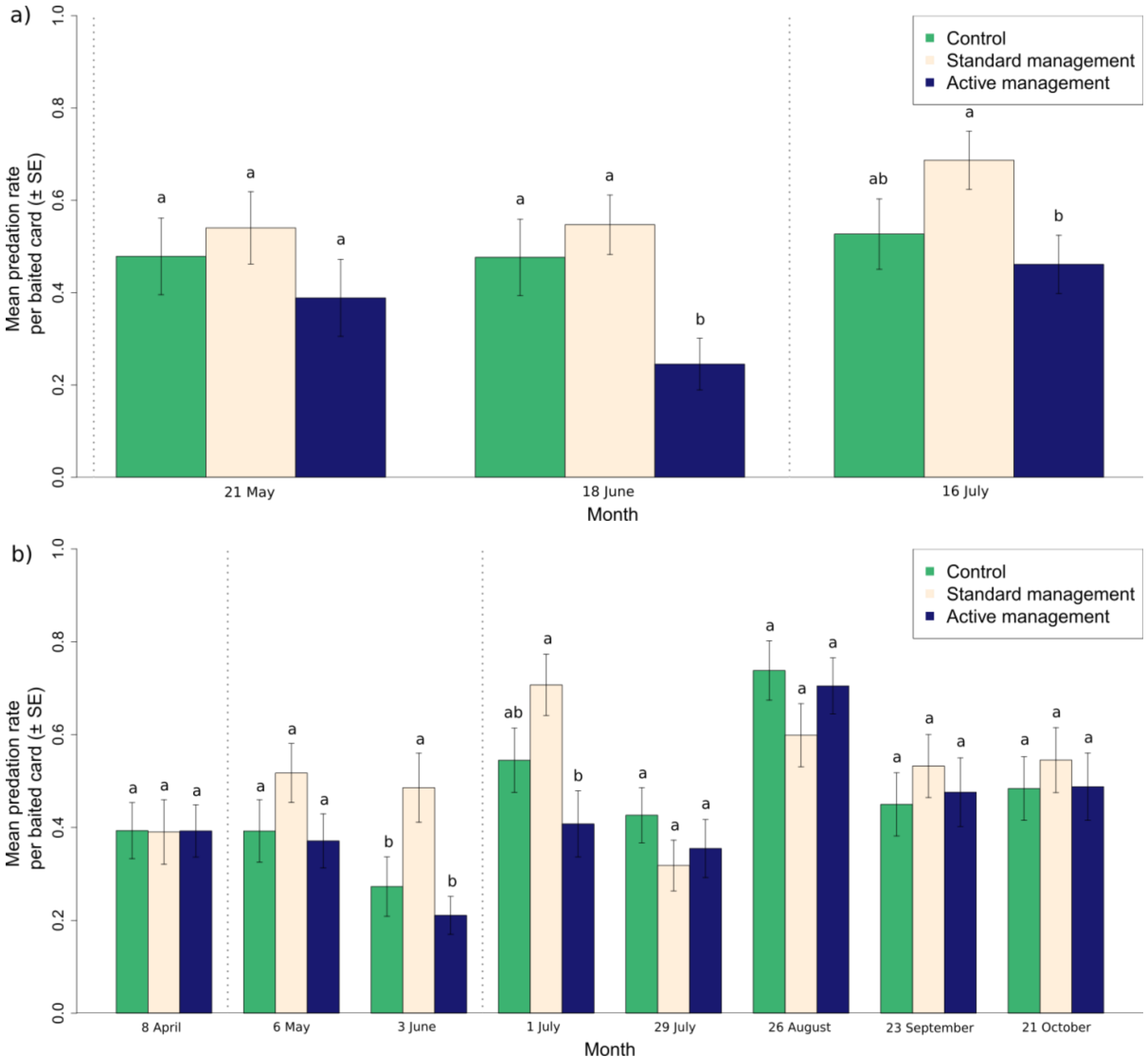

Figure 4.10 Predation rates per baited card, represented as the mean proportion of eggs depleted per card between the three alleyway treatments: control (green), standard management (cream), and active management (blue), in years two (a) and three (b). The grey dashed vertical lines represent when the alleyways were cut for the active management wildflower treatment. Error bars represent ± 1 SEM.

4.4. Discussion

This is the first study to demonstrate that wildflower interventions, composed of native perennial forb and grass species, can increase the abundance and richness of natural enemies in the adjacent orange tree canopies, leading to enhanced pest regulation services. However, this was not realised until the third year of study, and then only in association with the standard management wildflower treatment. During the establishment year (year one) and year two, when the distinct management strategies were applied to the sown wildflower alleyways, the wildflower treatments did not increase natural enemy abundance in the citrus canopies relative to the control (standard farm practice) treatment. This finding is similar to that observed by Silva *et al.* (2010) in lemon and is most likely a consequence of the wildflower habitats only just establishing (Woodcock *et al.*, 2005; Westbury *et al.*, 2017; Mateos-Fierro *et al.*, 2021), and a delayed response to the new treatments in year two (Denys and Tscharntke, 2002). In year three, however, the standard management wildflower treatment showed significantly greater natural enemy richness and abundance in the citrus canopy over other treatments. Furthermore, this was associated with enhanced pest regulation services.

Relative to the conventional alleyway management strategy (control treatment), the standard management wildflower treatment enhanced pest regulation services in the crop canopy during late spring and early summer (June and July) of both years. This is a crucial period for several key citrus pest species, such as *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), *Planococcus citri* Risso (Hemiptera: Pseudococcidae), *Panonychus citri* (McGregor) (Acari: Tetranychidae), and *Aleurothrixus floccosus* (Maskell) (Hemiptera, Aleyrodidae) (Urbaneja, Jaques and Garcia-Marí, 2009; Urbaneja *et al.*, 2015; Urbaneja, Grout, *et al.*, 2020). Pest regulation services provided by native and naturalised natural enemies during June and July are crucial to successfully regulate these pests and mitigate economic losses (Martínez-Ferrer, García-Marí and Ripollés Molés, 2003; Urbaneja, Jaques and Garcia-Marí, 2009; Vanaclocha *et al.*, 2011; Bouvet *et al.*, 2019). However, when the wildflower habitats were managed under the active management treatment, pest regulations services were no different from or sometime diminished relative to the control. Taller uncut vegetation, as in the standard management treatment, supports greater natural-enemy-to-pest ratio within the habitat (Dobbs and Potter, 2014; Meyer *et al.*, 2019; Zanettin *et al.*, 2021). As such, under increased resource competition, natural enemies spill-over from these habitats and bolster pest regulation services on the crop (Rand, Tylianakis and Tscharntke, 2006; Campbell *et al.*, 2017). Indeed, the standard management treatment supported greater abundances of predators in the canopy than the control and active management treatments, of which the Coccinellidae (Coleoptera), Salticidae (Araneae), and Chrysopidae (Neuroptera) tended to show the strongest positive responses. The significance of such polyphagous predators had been overlooked (Symondson, Sunderland and Greenstone, 2002) and their contribution to pest regulation called into question as direct and indirect interactions with other natural enemies, such as intra-guild predation, competition for shared resource and presence of optimal non-pest prey, could disrupt satisfactory pest management (Harwood and Obrycki, 2005; Michalko, Pekár and Entling, 2019). Recent studies however have emphasised the important role of polyphagous predators in modulating pest incidence in citrus (Calabuig, Garcia-Marí and Pekas 2016; Bouvet *et al.*, 2019; Mansour *et al.*, 2021). It is likely that the increased abundance of polyphagous predators in the canopy with the standard management wildflower enhanced the pest regulation services.

In contrast to the standard management wildflower treatment, the active management wildflower treatment was associated with reduced abundance of predators and diminished pest regulations services, suggesting that the strategy of cutting to increase spill-over was not successful. As wildflower habitats can support greater abundance and diversity of generalist predators (Franin *et al.*, 2021) and natural enemy dispersal is a strong predictor for pest regulation (Samaranayake and Costamagna, 2018), it was hypothesised that cutting the wildflower habitats would displace these natural enemies onto the adjacent crop, promoting pest regulation services. However, there was little evidence that natural enemies moved onto the crop in response to cutting; Anthocorids were the only family increased in the canopy in the active management wildflower treatment, but their low numbers imply there would be little functional advantage. In contrast to what was expected, the wildflower habitat under active management may not offer enough high-quality resources to augment natural enemy populations nor facilitate spill-over (Herz *et al.*, 2019). Additionally, the regular disturbance itself might lead natural enemies to migrate further away. Natural enemies optimise foraging to enhance energy reserves whilst minimising fitness costs incurred through searching, disturbance, and predation/parasitism (Charnov, 1976; Andersson, 1981). Cutting increases direct arthropod mortality from the machinery itself (up to 88% dependent on the machinery) with non-flying species most impacted (Humbert, Ghazoul and Walter, 2009; Mazalová *et al.*, 2015), and causes indirect mortality by the removal of refuge (Humbert *et al.*, 2012).

Parasitoid abundance in the canopy was also influenced by the alleyway treatment; the standard management wildflower treatment consistently supported more primary and other parasitoids in the citrus canopy than the control or active management wildflower treatments. This difference was again greatest in year three. These differences were mainly driven by the increased abundance of Encyrtidae in association with the standard management wildflower treatment. The most common encyrtid primary parasitoids recorded were the coccid parasitoids: *Metaphycus* and *Microterys;* the pseudococcid-parasitoids: *Anagyrus, Leptomastix, and Leptomastidea;* and the *Aonidiella aurantii* parasitoids: *Comperiella.* Vercher *et al.* (2012) recorded a significant decline in primary parasitoids abundance after the alleyway habitat was cut, which supports the observation made here. Additionally, in the canopy with active management wildflower treatment the abundance of hyperparasitoids in year three was half that of the control. Hyperparasitoids are known to disrupt the regulation of aphids in citrus by their primary parasitoids, especially later into the year (Gómez-Marco *et al.*, 2015), and hence a reduction in their abundance might translate to extended pest regulation services. Though a lower abundance of hyperparasitoids might be expected in the active management wildflower treatment as abundance of primary and other parasitoids were also lower, the control treatment was also characterised by low primary and other parasitoid abundance, suggesting the relative abundance of hyperparasitoid abundance in the control was much greater than that of the two wildflower treatments (active and standard management).

Despite values of natural enemy richness and abundance in the active management wildflower treatment being similar to the standard management wildflower treatment in year two, in year three, the active management wildflower treatment supported no greater abundance of richness than the conventional management practice (control treatment). This suggests alleyway resources in year three were limiting and unable to support natural enemies, and hence not able to facilitate spill-over of natural enemies into the crop canopy. Although active management promoted the sown tussock-forming grass species within the sown alleyways (Chapter three), which are typically associated with a more stable microclimate and can shelter insects from adverse climatic conditions (Luff, 1965; Collins *et al.*, 2003; MacLeod *et al.*, 2004), cutting to a height of 10 cm in both May and July is likely to have strongly affected the structure and function of the alleyway habitat, and removed shelter during the hottest and driest part of the year, preventing the habitat from recovering (Morris, 2000). Cutting also reduced the cover abundance of some of the sown forb species, such as *Psoralea bituminosa*, *Salvia verbenaca, Hypericum perforatum,* and *Cichorium intybus* (Chapter three), which without cutting are expected to help support natural enemies by increasing the abundance and diversity of open flowers throughout the year (Mateos-fierro *et al.*, 2021). *Salvia verbenaca*, for example, provides flowers in early spring and autumn (Blamey and Grey-Wilson, 2004). It is likely that the removal of above-ground biomass for two consecutive years impacted the ability for the plant community to recover (Morris, 2000) and led to the reduced abundance and richness of natural enemies observed in year three. In the standard management treatment however, sown forbs species were retained in the wildflower habitat and tussock-forming grasses were able to grower taller through the less intensive cutting frequency, which boosted plant richness and the provision of resource for natural enemies.

4.5. Conclusions

This study has demonstrated for the first time that natural enemy richness and diversity in the citrus canopy can be enhanced by sowing wildflower habitats in alleyways, but only when they are allowed to grow throughout the season, as in the standard management wildflower treatment. Furthermore, such wildflower habitats, designed to provide crucial resource in the alleyways, such as microclimate shelter from tussock-forming grasses, nectar and pollen from sown forbs species, and alternative hosts and prey through a diverse plant community, can bolster pest regulation services in the adjacent canopy. This study has highlighted the importance of wildflower strip management, especially under the Mediterranean climate. The repeated removal of the resource in the active management wildflower habitat, without sufficient time to recover, alters not just plant communities but also the natural enemy community structure, and ultimately diminishes pest regulation services.

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Chapter 5

The influence of wildflower habitats and their management on the availability of carbohydrate resources for foraging parasitoids

Abstract

Parasitoids are economically important natural enemies of crop pests. Their longevity and fecundity can be increased by the provision of nectar and honeydew, which lead to enhanced pest regulation services. However, the effects of wildflower habitats and their management on carbohydrate feeding in parasitoids has not previously been studied in the field. In this study, a randomized block design was used to investigate three experimental alleyway treatments in orange orchards, i) a control treatment where naturally occurring alleyway vegetation between rows of orange trees was managed under standard farm practice, ii) a standard management wildflower treatment in which sown wildflower habitats in the alleyways were managed by cutting once a year, and iii) an active management wildflower treatment, in which the same sown wildflower habitat in the alleyways were managed by cutting three times a year. The influence of these alleyway treatments on the abundance of accessible carbohydrate sources (nectar and/or honeydew) between alleyway management treatments was determined. Parasitoids and the citrus pest *Phyllocnistis citrella* were sampled from the three treatments and tested to determine if they had recently fed on carbohydrates. Orchard characteristics, such as structural heterogeneity of the alleyway habitats, and parasitoid phenotypic and functional traits, such as head width and functional group, were used to predict carbohydrate feeding in the field. The abundance of carbohydrate resources was consistently greater in the standard management treatment than the control or the active management treatments. Parasitoids were also more likely to have recently fed on carbohydrate in the standard management treatment, including in autumn when resources were limiting in the other two alleyway treatments. Two sown plant species, *Salvia verbenaca*, and *Mentha suaveolens*, and an unsown plant species *Heliotropium europaeum,* were found to be accessible to almost all parasitoids (>95%). *Salvia verbenaca* provided nectar during all three of the seasons sampled. Furthermore, greater carbohydrate feeding in parasitoids was associated with increased structural heterogeneity within the alleyways. From these results, *S. verbenaca*, *M. suaveolens*, and *H. europaeum* can be recommended for inclusion in wildflower habitat aimed at feeding parasitoids throughout the year in Mediterranean citrus systems. Furthermore, to enhance carbohydrate feeding, the management of wildflower habitats should be aimed at increasing the structural

heterogeneity of vegetation. This study has emphasised the importance of studying the management of wildflower habitat when targeting specific resource requirements.

5.1. Introduction

Natural enemies of crop pests provide a valuable pest regulation service to agriculture (Jervis, 2005). These include both parasitoids and predators which feed on and kill their hosts or prey during at least one of their life history stages (Jervis, 2005). Parasitoids are a key group of natural enemies as they are responsible for successfully regulating a diverse range of agricultural pest species (Jervis, 2005). Consequently, there is a strong rationale for conserving populations of parasitoids within the agri-environment and maximising their pest regulation potential by providing the resources they require (Lundgren, 2009; Gillespie, Gurr and Wratten, 2016; Tena et al., 2016; Gurr *et al.*, 2017). This can be achieved through the provision of suitable carbohydrate resources, such as nectar and honeydew, which increase both the longevity and fecundity of adult parasitoids (Lee, Heimpel and Leibee, 2004; Winkler *et al.*, 2006; Pinheiro *et al.*, 2013b; Tena *et al.*, 2015). However, in modern agricultural landscapes, the availability of such carbohydrate resources is often limited both spatially and temporally (Matson *et al.*, 1997). In intensively managed agricultural landscapes, carbohydrate resources are typically limited to brief flowering periods of nectar producing plants (predominantly crops), when honeydew-producing hemipteran pests are present, or from guttation (Lundgren, 2009a; Tena *et al.*, 2016; Urbaneja-Bernat *et al.*, 2020). As such, parasitoids are more likely to be carbohydrate deficient in cropped habitats compared to semi-natural areas typically associated with a higher diversity of flowering plants (Kishinevsky, Cohen, *et al.*, 2017). This imbalance led to the development of the ‘parasitoid nectar provision hypothesis’, which states that diversification of crop monocultures can decrease pest pressure through the provision of nectar (Heimpel and Jervis, 2005).

A potential ecosystem disservice associated with the provision of nectar is that it could also increase the fitness of phytophagous crop pests (Heimpel and Jervis, 2005; Wäckers, Romeis and van Rijn, 2007) as some economically important crop pests, particularly Lepidoptera, also require nectar for increased longevity and fecundity (Alder and Bronstein, 2004; Lavandero I *et al.*, 2006; Winkler *et al.*, 2009). Similarly, arthropods of the fourth trophic level, such as hyperparasitoids and parasitoids of predators (Figure **5.1**) might also benefit from the provision of nectar (Araj *et al.*, 2009; Tougeron and Tena, 2019). Under these circumstances, any benefits gained by enhancing fitness of the primary parasitoids of crop pests may be counteracted. Therefore, understanding how the management of wildflower habitat affects crop pests and the fourth trophic level is necessary when managing for pest regulation services.

The parasitoid nectar provision hypothesis is now a cornerstone of habitat management for pest regulation (Landis, Wratten and Gurr, 2000; Gurr *et al.*, 2017). Though studies have investigated whether the provision of nectar via wildflower habitat can increase the abundance and fitness of parasitoids in the field (Dively, Leslie and Hooks, 2020), the management of these habitats and the effects these interventions have on parasitoids has not been studied. Perennial wildflower habitats require annual management with cutting to maintain the plant communities. Different outcomes can be achieved by manipulating the management strategy, through altering the cutting frequency and/or height (Storkey and Westbury, 2007; Westbury *et al.*, 2008). For example, different management techniques can be used to promote establishment and retention of sown species, to create germination niches for annual species, or to facilitate easy access to the crop for farm workers (Westbury *et al.*, 2008). Management can affect the heterogeneity of the habitat and this has been found to be important for arthropods (Woodcock *et al.*, 2008; Blake *et al.*, 2013). Management might also be used as a tool to help move natural enemies onto the crop at critical periods of pest incidence (Goller, Nunnenmacher and Goldbach, 1997). Therefore, to develop an effective approach to boost parasitoids and their pest regulation services, there is a need to understand how wildflower management strategies can influence the availability of carbohydrate resources for parasitoids. In the study system, citrus orchards are typically maintained bare, with vegetation either mechanically or chemically removed so that sources of nectar for parasitoids are scarce (Monzó *et al.*, 2020).

The main objective of this study was to determine the impact of wildflower habitat and their management on the availability and use of carbohydrates for parasitoids. The overall aims of the study were to; i) determine the abundance of accessible carbohydrate resource according to alleyway treatment, ii) to determine the effect of alleyway treatment on carbohydrate feeding in parasitoids and a key citrus pest, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), iii) to investigate how alleyway treatment may alter the abundance of different parasitoid functional groups, and iv) to determine which orchard characteristics and parasitoid phenotypic and functional traits best predict carbohydrate feeding.

5.2. Methods

5.2.1. Site Description

A full site description including the selection criteria can be found in Chapter two (section 2.1). The study was conducted in three large (>300ha) commercial Spanish citrus farms (sites); Madre del Agua, La Calvilla and Montepinos, in two different localities in the province of Huelva, south-west Andalucía. In these farms, the standard practice was to manage the naturally occurring alleyway vegetation with cutting four to five times a year to a height of ≤5cm, and the cuttings left *in situ*. All orchards were treated with herbicides directly under the orange trees and the crop was treated with insecticides and acaricides as described in Chapter two (section 2.1.2).

5.2.2. Study design

The study was conducted in 2019 in three Navel orange (*Citrus sinensis*) orchards. All samples were collected and surveys conducted in May (spring), July (summer), and October (autumn). No surveys were conducted during the citrus tree flowering and aphid critical period (April), as carbohydrate from citrus flower nectar and aphid honeydew was not expected to be limited in any of the alleyway management treatments. The orchards were situated within three different farms (sites), Madre del Agua, La Calvilla and Montepinos, at two localities in the province of Huelva, south-west Andalucía, Spain.

A randomised block design was used to investigate the effect of alleyway management on parasitoid carbohydrate feeding. The three treatments investigated were, i) a control treatment in which the naturally occurring vegetation of the alleyways was managed conventionally by cutting to ≤5cm four or five times throughout the year, ii) wildflower habitat sown in alternate alleyways between rows of orange trees, managed by cutting once annually in February (hereafter standard management), and iii) wildflower habitat sown with the same mix as used for the standard management wildflower treatment, managed actively by cutting three times per year (≈10cm) in an attempt to increase the dispersal of natural enemies into the crop prior to the forecasted peak population of a key pest (California red scale) (hereafter active management).

To reduce edge effects, the two outermost rows of citrus trees from each plot were excluded from sampling and a 20m buffer zone at either end of each row was established. As such, all carbohydrate resource surveys and insect sampling were conducted from the inner most four rows of fruit trees along a row length of 60m for all treatments.

5.2.3. *Carbohydrate resource surveys within the orchard alleyway*

All surveys were conducted in the spring, summer, and autumn of 2019. From the alleyways, the carbohydrate resource available to parasitoids was recorded from ten randomly placed 0.5m x 0.5m quadrats, so that a total of 20 quadrat samples were collected from each plot. In each quadrat, all the floral units of each plant species and all the colonies of honeydew-producing species were identified and recorded. One floral unit was defined as a flower or group of flowers from which a 3mm long parasitoid could visit without having to fly between them (adapted from Baldock *et al.*, 2015). For example, the capitulum of a Composite was considered a single floral unit. Carbohydrate resource units were defined as the number of floral units and the number of honeydew-producing colonies per species from within each 0.5m x 0.5m quadrat.

5.2.4. *Carbohydrate resource surveys in citrus tree canopies*

Canopy carbohydrate resource surveys were conducted in the trees adjacent to the alleyways surveyed for alleyway carbohydrate resources. All canopy resource surveys were conducted on the same day as the adjacent alleyway surveys. From each plot and for all treatments, 16 paired trees were surveyed for colonies of honeydew producing Hemiptera and citrus flowers. In each tree, a $25cm^2$ plastic hoop was randomly placed on the canopy adjacent to the alleyway and the number of carbohydrate resource units (honeydew-producing colonies and/or floral units) from within the hoop was recorded (Tena et al., 2015).

5.2.5. Vegetation structural heterogeneity

Additionally, for each alleyway, the coefficient of variation of the habitat surface height measurements was calculated to provide a measure of structural heterogeneity. For this, a wooden disc of a standardised diameter (30cm) and weight (200g) was dropped down a 1m rule and the height it rested on the sward was recorded (Stewart, Bourn and Thomas, 2001). Twenty drop disc measurements were taken along each 60m alleyway sampling area.

5.2.6. Sampling for parasitoids and Phyllocnistis citrella *in the tree canopy*

Insect sampling and carbohydrate resource surveys were conducted concurrently so that carbohydrate state of the sampled insects could be directly related to resource availability. Insects were sampled from orange tree canopies using a vacuum sampler adapted from a commercial leaf-blower (Stihl BG 86 C-E), modified as described in Chapter four (section 4.2.4). Sixteen trees, paired across the alleyway, were randomly selected and sampled per plot. The same 16 trees were then sampled for each season (spring, summer, and autumn). In summer and autumn, an additional 16 paired trees from within the same rows were sampled, so that 32 trees were sampled per plot during those two seasons. This increase in sampling effort in summer and autumn was to account for an expected lower abundance of parasitoids during these seasons. The tree canopy adjacent to the alleyway was randomly vacuumed up to a height of 2m with four suctions, each for a duration of ten seconds. Each sample consisted of a set of paired trees, with eight 10 second sucks. Thus, there were eight samples per plot in spring and 16 in summer and autumn. In summer, the citrus leaf miner, *P. citrella* was also collected according to the above methodology. All samples collected with the vacuum device were immediately frozen in the field at -196 °C in liquid nitrogen to stop enzyme activity, halting digestion and preserving any carbohydrates in the gut. The samples were then stored on dry ice and transported to the lab where they were stored in a freezer at -80°C.

5.2.7. *Parasitoid identification, head width measurements, and testing for the presence of carbohydrates*

All identification and measuring of all insects were conducted on ice and after processing, each specimen was isolated in an Eppendorf®, labelled and returned to -80 °C to minimise metabolism of sugars post-collection. Parasitoids were identified to genus where possible, and in some cases, species. Parasitoid trophic function (primary parasitoid of a phytophage, hyperparasitoid, or primary parasitoid of an arthropod predator) was determined, and the primary host identified. Where it was not possible to identify to genus, the parasitoid was identified to family. For these specimens, neither trophic function nor primary host were identified, and they were classified as 'other' parasitoids. The head width (taken as the distance from eye to eye) of each individual was measured using an ocular graticule, calibrated using a stage micrometre. The head width of leaf miner, *P. citrella,* was not measured as adults use a long proboscis to feed, so all nectar resources would be deemed accessible (Patt, Hamilton and Lashomb, 1997).

Figure 5.1 *Diaplazon* (Hymenoptera: Ichneumonidae), a parasitoid, forages for Syrphid larvae in a colony of aphids amongst budding orange blooms.

To investigate the presence of carbohydrates, all parasitoids and *P. citrella* were then tested with Anthrone reagent to determine whether they had recently fed on carbohydrates in the field (Heimpel *et al.*, 2004). All parasitoids and *P. citrella* were cleaned by vortexing each specimen separately in an Eppendorf® with ~60°C deionized water. Washed specimens were then transferred to a fresh Eppendorf®, re-labelled, and transferred back to the freezer. To test for the presence of carbohydrates, each washed insect specimen was placed on a separate microscope slide with 5 µl of the Anthrone reagent and crushed whole under a cover slip to release the gut contents. When carbohydrate had been consumed by the specimen and was still present, a green-blue colour developed around the crushed insect (Lee and Heimpel, 2003; Segoli and Rosenheim, 2013; Kishinevsky, Cohen, *et al.*, 2017). The colour was allowed to develop for up-to one hour at room temperature, during which it was important to remain vigilant, as in some specimens the green-blue colour developed and then faded. This process was always conducted with at least two observers in order to verify the colour change, which could be difficult to detect in smaller specimens. Each slide was photographed on a white background once the green-blue colour had become visible or after the one-hour period, allowing for cross-referencing or further verification if necessary. All specimens were then classified as either recently carbohydrate fed or not recently carbohydrate fed.

Nectar accessibility was determined in all the flower genera identified during the carbohydrate resource surveys. Individual flowers were dissected longitudinally and the corolla width at the narrowest point was measured under stereomicroscopy using a calibrated ocular graticule. Flowers with trap openings, such as *Psoralea bituminosa* were not measured as they are not accessible to parasitoids (Vattala *et al.*, 2006). Flowers with exposed nectaries and plants with extra floral nectaries were assigned an arbitrary corolla width of 3mm. This width was selected as it was greater than the widest parasitoid head measurement and it was assumed that all parasitoids could feed without limitation on extra-floral nectar.

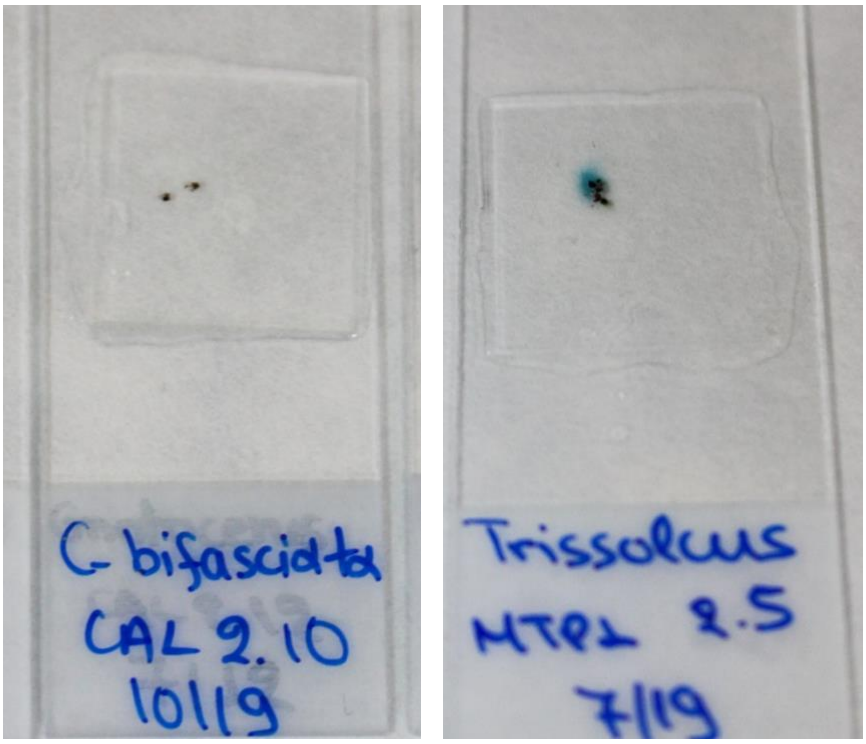

Figure 5.2 Anthrone reagent turned green-blue (right) in the presence of carbohydrate after being released from a parasitoid's gut when pressed under a microscope slide. Contrasted with no colour change observed (left) in the absence of carbohydrate. Nectar accessibility for arthropods.

5.2.8. Data analysis

All statistical analysis were performed using R studio (RStudio Team, 2015) Version 1.3.1056 for R version 4.0.2 (R Core Team, 2019). Data manipulation was carried out using the tidyr and dplyr packages (Wickham, 2020; Wickham *et al.*, 2020).

5.2.8.1. The influence of alleyway treatment on the abundance of carbohydrate resources in the alleyways and in the canopy

To determine if alleyway treatment influences the total abundance of carbohydrate resource available in the alleyway or the citrus tree canopy, values of carbohydrate resource units (number of floral units and/or honeydew producer colonies) according to treatment were analysed using negative binomial generalised linear models (GLMM) (Bates *et al.*, 2015). A separate model was fitted for each of the three seasons sampled, spring, summer, and autumn. Values of carbohydrate resource units were regressed against alleyway treatment (control, standard management wildflower treatment, and active management wildflower treatment). A negative binomial error structure was selected to account for zero inflated count data. To account for the replicate block design, orchard block was included as a random factor. Goodness of fit was verified by plotting the residuals (y) against the estimated responses (x), observing equal distribution about y = 0, and by plotting a quantile-quantile (QQ) plot to check for dispersion. Type II Walds chi-square tests were then conducted using the CAR package (Fox and Weisberg, 2019). The Multcomp package (v1.4-13) was used to perform Tukey's pairwise comparisons between alleyway treatments within each model (Hothorn, Bretz and Westfall, 2008).

To determine if alleyway treatment influenced abundances of the different nectar and/or honeydew sources in the alleyways or citrus tree canopy, values of carbohydrate resource units per species/genera according to treatment were analysed using the manyglm function in the mvabund package (v3.9.2) in R (Wang *et al.*, 2019). Negative binomial multivariate generalised linear models were fitted for zero-inflated count data. *P*-values were estimated using Monte Carlo bootstrapping with 999 resample runs (Warton, Thibaut and Wang, 2017). To account for the replicate block design, permutations for resampling were restricted to within orchard block using Permute (Simpson, 2019). For carbohydrate resource in the alleyways, the response variable was a 29-vector matrix composed of counts of floral units per plant species and honeydew-producer colony per species from each quadrat taken along the alleyway. For carbohydrate resource in the tree canopy, the response variable was a five-vector matrix composed of counts of honeydew-producer colonies per species from each quadrat taken from within the canopy. A separate model was fitted for each of the three seasons (spring, summer, and autumn). Goodness of fit was verified as described in section 5.2.8.1. The manyglm function fits multiple univariate GLMs to each vector of the multivariate response matrix which were then explored to identify which species infer most variance within the models (Wang *et al.*, 2012). A resampling-based implementation of Holm's step-down method was used within the manyglm function to make pairwise contrasts between significant treatments within each model (Wang *et al.*, 2012).

5.2.8.2. The influence of alleyway treatment on the carbohydrate feeding in parasitoids and a citrus pest, Phyllocnistis citrella

For each of the three sampling seasons, the proportion of parasitoids and *P. citrella* that were recently fed on carbohydrates was modelled against alleyway treatment using binomial GLMM. The same random effect structure was applied as described in section 5.2.8.1. Goodness of fit of the binomial models were verified by comparing the null deviance and residual deviances. ANOVA tables were generated by type II Walds chi-square tests were then conducted using the CAR package (Fox and Weisberg, 2019). Pairwise comparisons of significant variables were then investigated using Tukey-test in the multcomp package (v1.4-13) (Hothorn, Bretz and Westfall, 2008).

5.2.8.3. The influence of alleyway treatment on the abundance on the abundance of trophic functional traits

Differences in abundance of the five trophic functional traits (three parasitoid functional traits, one phytophage pest and one group for parasitoids ranked to family) for each season were explored using the manyglm function in the mvabund package (v3.9.2) (Wang *et al.*, 2019). The randomized block design was again accounted for by restricting permutations as described in section 5.2.8.1. Negative binomial multivariate generalised linear models were fitted to account for zero inflated count data, and Monte-Carlo bootstrapping resampled 999 times was used to estimate *P*-values (Warton, Thibaut and Wang, 2017). Univariate GLMs fitted to each response vector were then explored to infer the effect of alleyway treatment on each functional trait. Pairwise contrasts between treatments were investigated as previously described (5.2.8.1.).

5.2.8.4. Predicting carbohydrate feeding in parasitoids based on parasitoids traits and orchard characteristics

The parasitoid phenotypic and functional traits were defined as head width, functional group, and honeydew production in the primary host. Orchard characteristics were defined as the number of floral units in the alleyways, number of honeydew producer colonies in the canopy, mean corolla width of flowers per alleyway, and alleyway habitat structural heterogeneity. In the case of carbohydrate resource units from the alleyway and the canopy, mean counts per 25cm^2 quadrat were calculated for each alleyway to have a representative value of each alleyway.

To explore which factors were most significant in determining parasitoid carbohydrate feeding in the field, carbohydrate feeding was modelled against orchard characteristics and parasitoid phenotypic and functional traits using generalised linear mixed effects models using lme4 (V1.1-21) in R (Bates *et al.*, 2015). The maximal model was generated using knowledge of parasitoid biology and behaviour and expected ecological interactions. The response variable was a single binary vector composed of each insect's response to the Anthrone reagent, as previously described. Treatment was nested within farm as random factors and random intercepts also defined for each sample season. Parasitoid family, parasitoid trophic function, parasitoid head width, honeydew production in the primary host, mean corolla width of flowers present within the quadrat, median number of honeydew-producing colonies per quadrat in the canopy, median number of carbohydrate units per quadrat in the alleyway, and heterogeneity of alleyway habitat structure were considered fixed effects. Interaction terms were included between mean corolla width and parasitoid head width, median number of honeydew-producing colonies in the canopy and honeydew production in the primary host, and median carbohydrate units in the alleyway and honeydew production in the primary host. The modes of the random effects were extracted and the variance plotted to ensure their inclusion within the model was justified (Bates *et al.*, 2015).

Initially a subset of the data was used, which included only parasitoids ranked to genus or species (*n* = 661), which permitted trophic function and honeydew production in primary host to be included in the model. Automated model selection was then carried out with the 'Dredge' function of the MuMIn package (v1.43.17) (Barton, 2020), in which repeated evaluations of all possible subsets of the maximal model were conducted. Models were selected on the basis of second-order Akaike information criterion (AICc) corrected for small sample size, ΔAICc, and model weights. Models with the lowest AICc were considered to be most parsimonious, explaining the largest proportion of variation in the response variable;

models where ΔAICc was <2 are considered to be reasonable (Burnham and Anderson, 2002). As neither trophic function nor honeydew production in the primary host were included in the selected models, these terms were dropped. As such, the whole dataset of parasitoids, included those ranked to family (*n* = 763), could be modelled.

The final GLMM included the same terms and random effects as previously described, though with trophic function and honeydew production in the primary host dropped. Two-way interactions were included between the mean carbohydrate resources units in alleyway and the mean carbohydrate resource units in canopy, mean carbohydrate resources units in alleyway and habitat structural heterogeneity, mean carbohydrate resource units in canopy and habitat structural heterogeneity, mean carbohydrate resources units in alleyway and parasitoid head width, and mean corolla width and parasitoid head width. Once the model was fitted, the modes of the random effects were then extracted and the variance plotted to ensure their inclusion within the model was justified (Bates *et al.*, 2015). Automated model selection was then carried out (Barton, 2020) and models selected using ΔAICc and model weights, as described.

5.3. Results

5.3.1. The accessibility of floral nectar present in the alleyways to the parasitoid community sampled

In spring (year three), 12 nectar producing flowers were present in the alleyway treatments (Figure **5.4**a). Five of these were sown plant species. Of which, *Achillea millefolium*, *Cichorium intybus* and *Marrubium vulgare* were recorded only from the standard management wildflower treatment, and *Salvia verbenaca* was recorded from both wildflower treatments. Seven species recorded were unsown, five of which were *Crepis* sp.2, *Andryala* sp., *Malva sylvestris, Daucus* sp. and *Capsella bursa-pastoris,* were only recorded from the standard management wildflower treatment. One species, *Malva parviflora,* was recorded only in the control treatment, while *Crepis* sp.1 was recorded from both the standard management wildflower and the control treatments. No unsown species were recorded from the active management wildflower treatment.

Irrespective of alleyway treatment, 50% of the 231 parasitoids recorded from the canopies in spring had a head width measuring 0.388mm or less, 75% of parasitoid heads measuring 0.464mm or less and 95% of parasitoid heads measuring 0.599mm or less (Figure **5.3**a). Nectar accessibility for parasitoids varied between species, with *C. bursa-pastoris, Daucus* species, *S. verbenaca, M*. *parviflora,* and *M. sylvestris* permitting more than 95% of parasitoids to feed based on parasitoid morphology. *Cichorium intybus, Andryala* sp. and *M. vulgare* permitted between 75 and 95% of parasitoids to feed, while *Crepis* sp. 1 and 2 and *A. millefolium*, permitted between 50 to 75% of parasitoids to feed.

In summer, nectar producing flowers were present in all three treatments (Figure **5.4**b). Four sown species were recorded, *S. verbenaca, Marrubium vulgare, Anchusa azurea* and *C. intybus,* all from the standard management wildflower treatment. Three unsown species were recorded: *Malva sylvestris* was recorded from the standard management wildflower treatment, *Dittrichia graveolens* from both the standard management wildflower and control treatments, and *Polygonum aviculare* from the active management wildflower and control treatments. *Calendula arvensis*, *Senecio* sp., *Lythrum junceum*, and *Euphorbia chamaesyce* were recorded only from control treatment.

Irrespective of treatment, 50% of the 298 parasitoids recorded in summer measured 0.349mm or less across the head, 75% measured 0.493mm or less, and 95% of parasitoids measured 0.678mm or less (Figure **5.3**b). *Salvia verbenaca, M. sylvestris, P. aviculare* and *E. chamaesyce* permitted nectar access to more than 95% of parasitoid species. *Marrubium vulgare* permitted access to 75 to 95%. *Cichorium intybus, C. arvensis, Senecio* and *L. junceum* permitted access to 50 to 75%. *Dittrichia graveolens* permitted access to less than 50%.

In autumn, two sown species were recorded, of which *Mentha suaveolens* was in both the standard and active management wildflower treatments, while *S. verbenaca* was recorded only from the active management wildflower treatment. Four unsown species were recorded, of which *Heliotropium europaeum* was recorded from only the standard management wildflower treatment. *Dittrichia graveolens* and *P. aviculare* were recorded from only the active management wildflower treatment. *Solanum nigrum* was recorded both from the standard management wildflower and control treatments (Figure **5.4**c).

Irrespective of treatment, 50% of the 239 parasitoids recorded in autumn had a head width measuring 0.430 mm or less, 75% of measuring 0.462mm or less, and 95% measuring 0.616mm or less (Figure **5.3**c). Nectar was accessible in all three treatments. More than 95% or parasitoid species were able to access nectar from *M. suaveolens, Heliotropium europaeum, S. nigrum,* and *P. aviculare,* based on parasitoid morphology. As in summer, *D. graveolens* permitted less than 50% of parasitoid species to access nectar.

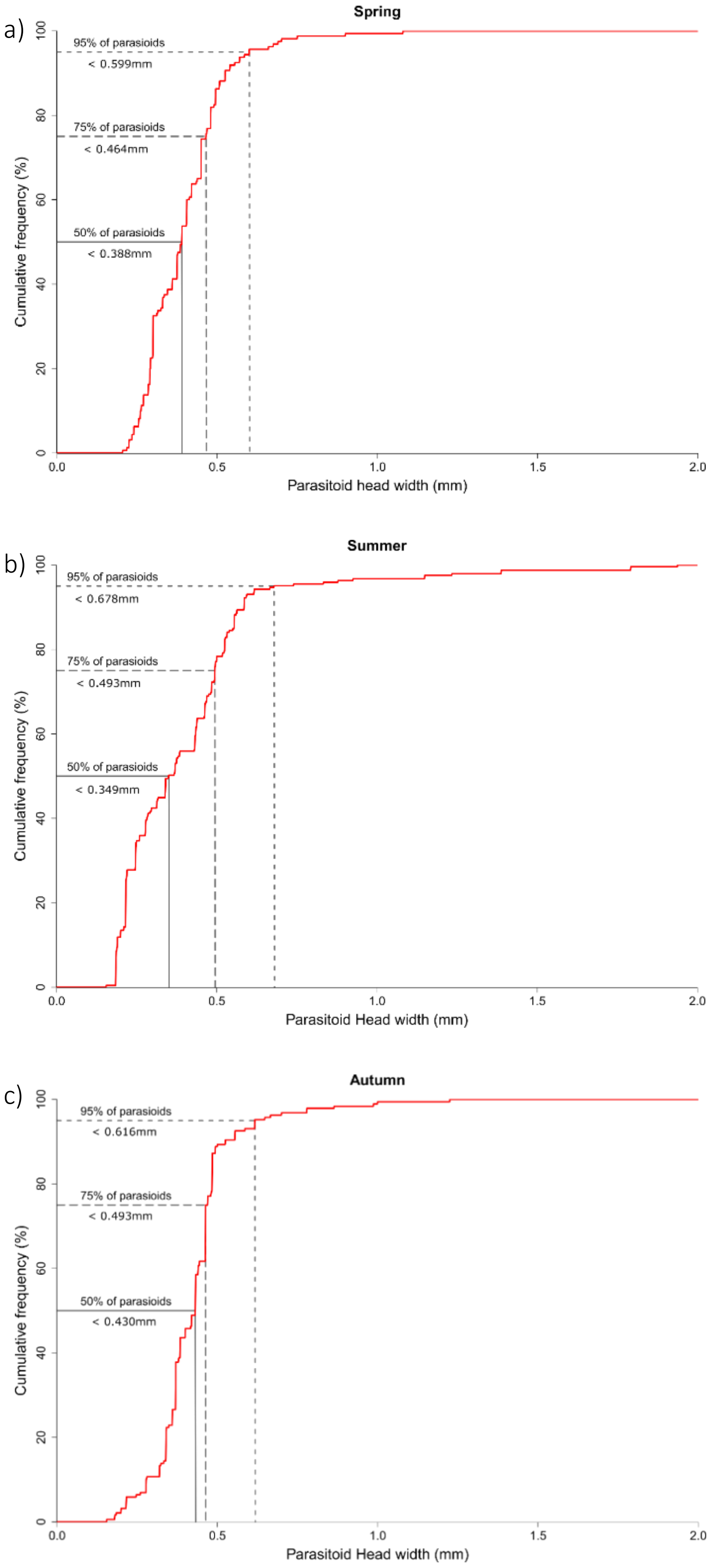

Figure 5.3 Cumulative percentage of parasitoids of each head width class of 0.001mm for spring a), summer b), and autumn c) are depicted by the red lines. Head width size of 50%, 75% and 95% of sampled parasitoids are shown by the solid, dashed, and dotted grey lines, respectively.

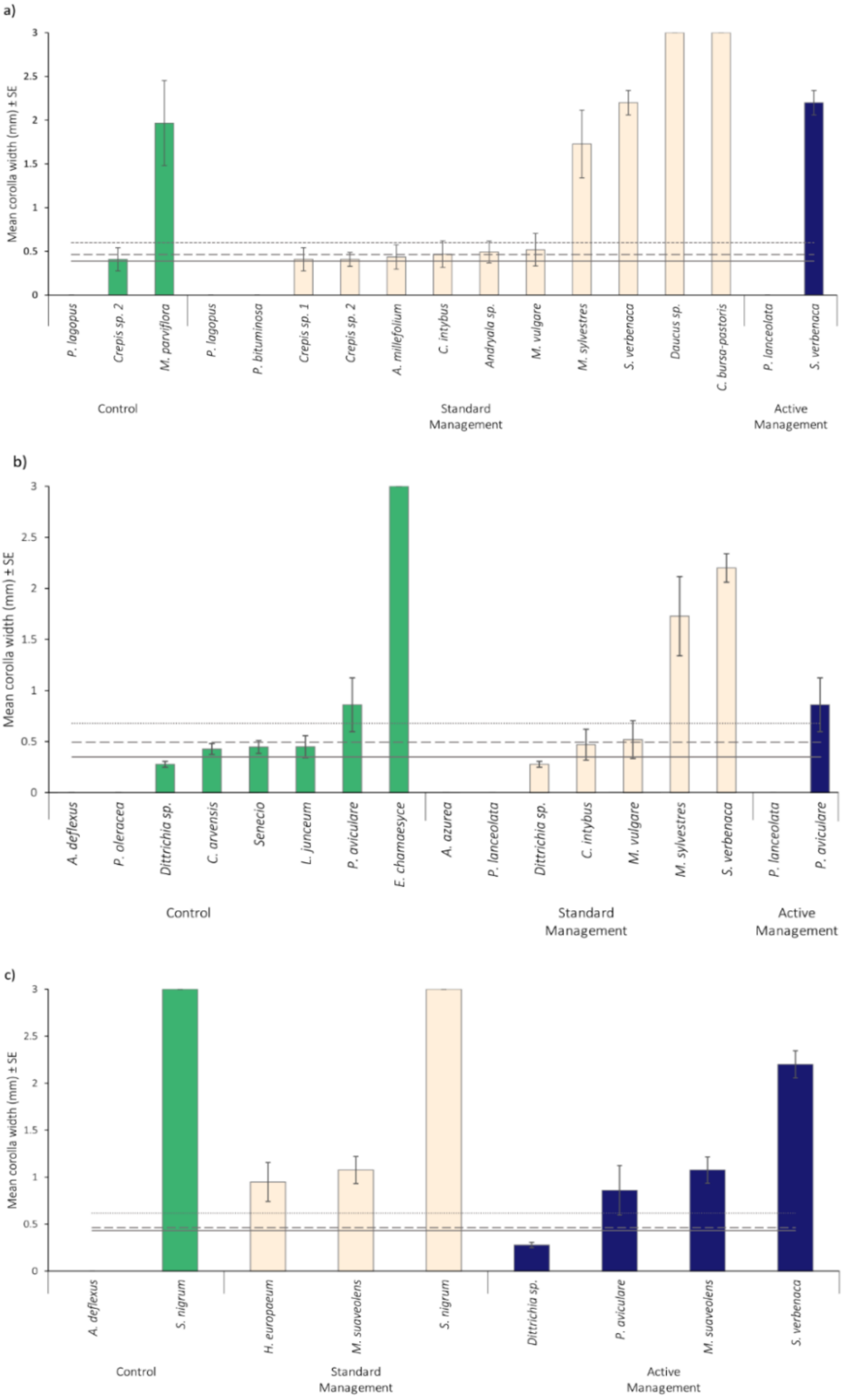

Figure 5.4 Corolla width measurements of the floral species present in spring a), summer b), and autumn c), for each of the three treatments, control (green bars), standard management wildflower (cream bars), and active management wildflower (blue bars). Error bars represent ± 1 SEM. The parasitoid head width of 50%, 75%, and 95% of sampled parasitoid community are represented by the solid, dashed, and dotted lines, respectively.

5.3.2. The influence of alleyway treatment on the abundance of carbohydrate resources in the alleyways

In all three seasons, the abundance of accessible carbohydrate resources in alleyways was found to be significantly affected by alleyway treatment (spring, GLMM: $\chi^2 = 44.21$, df = 2, $P < 0.001$; summer, GLMM: $\chi^2 = 31.6$, df = 2, $P < 0.001$; autumn, GLMM: $\chi^2 = 9.678$, df = 2, $P = 0.008$, $n = 60$) (Figure **5.5**). In spring, the abundance of carbohydrate resource units in the standard management wildflower treatment was 15 times greater than in the control plots. However, there was no significant difference in the amount available between the active management and the control treatment, nor the standard management and active management treatment (Appendix 3.1). In summer, both standard management and control treatments provisioned five times more carbohydrate resource than the active management treatment (Figure **5.5**). The standard management treatment and the control treatments provided a similar amount of carbohydrate resource (Appendix 3.1). In autumn, the standard management provided three times more resources compared to the control (Figure **5.5**). The active management wildflower treatment provisioned a similar amount of resource as both the standard management wildflower and control treatments (Appendix 3.1).

As well as total abundance of alleyway carbohydrate resource, the treatment applied to the orchard alleyways also affected the abundances of the different nectar producing genera and honeydew producing species (carbohydrate sources) present across all seasons (manyglm spring: LRT = 50.96, df = 2, $P = 0.001$, summer: LRT = 78.51, df = 2, $P = 0.001$, and autumn: LRT = 31.97, df = 2, $P = 0.001$) (Table **5.1** and Appendix 3.2). Across all seasons, the greatest differences in the abundance of the different alleyway carbohydrate sources (nectar and honeydew producing species) were observed between the standard management treatment and the other two treatments (control and active management wildflower treatments) (Appendix 3.2). In spring and autumn, the abundance of the different sources of carbohydrate were not different between the control and active management treatments (Table **5.1** and Appendix 3.2). In contrast, in summer, abundances of the different carbohydrate sources were also different between the control and active management treatments (Table **5.1** and Appendix 3.2).

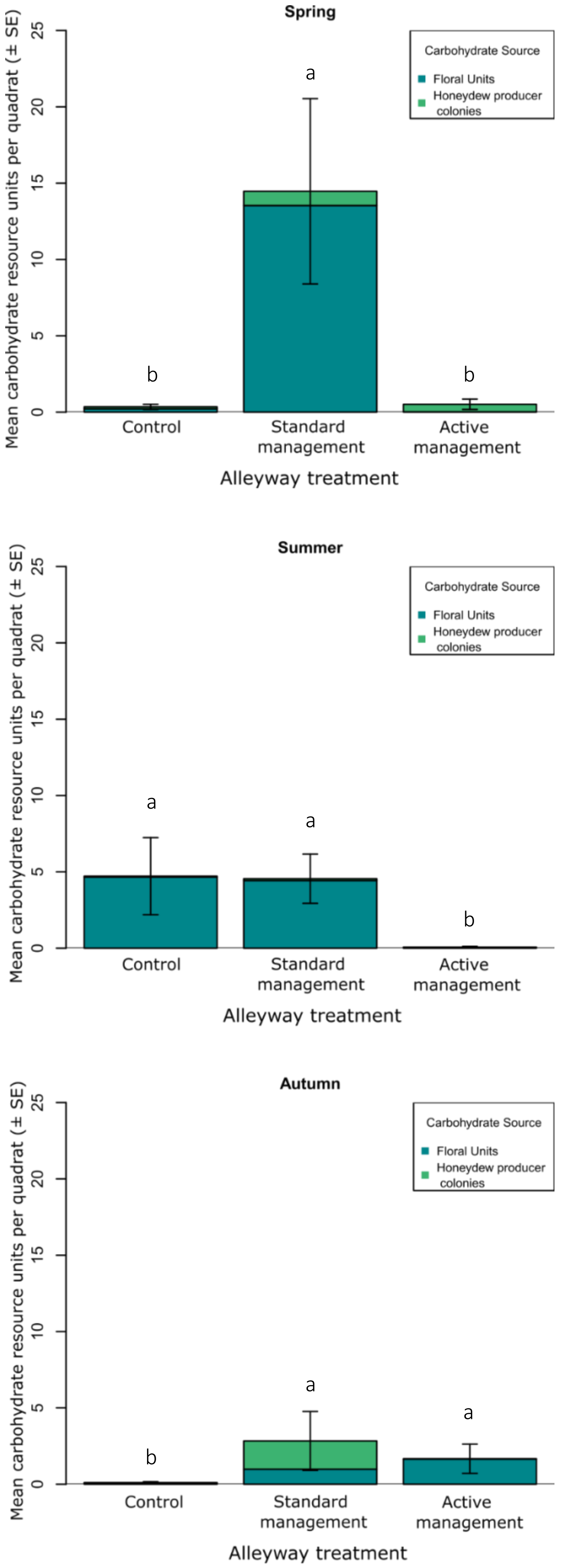

Figure 5.5 The abundance of carbohydrate resource available in the alleyways of each treatment; control, standard management wildflower and active management wildflower, at the three sampling seasons, spring, summer and autumn, represented as the mean number of floral units (turquoise bars) and mean number of honeydew-producing hemipteran colonies (green bars) per quadrat (±SE). Carbohydrate resource units were defined as the number of floral units and/or honeydew-producing colonies. The abundance of carbohydrate resource between treatments with different letters are significantly different (Tukey's pairwise contrasts; $P < 0.05$).

In spring, six carbohydrate sources in the alleyway explained 62.1% of the variation in the model. Five of which were nectar sources, *C. intybus, Daucus* sp., *M. vulgare, Crepis* sp.1, and *C. bursa-pastoris,* and one of which, aphid colonies, was a honeydew source. All the different carbohydrate sources were more abundant in the standard management wildflower treatment than the control or active management treatments in spring (Table 5.1). In summer, four alleyway carbohydrate sources explained 51.8% of the variation in the model, all of which were nectar sources. *Marrubium vulgare, M. sylvestris, C. intybus* and *D. graveolens* were all more abundant in the standard management treatment than the control or active management treatments (Table 5.1). In autumn, five different sources of carbohydrate were responsible for 72.3% of the variation in the model, four of which were nectar sources and one of which was a source of honeydew. *Polygonum aviculare* and *D. graveolens* (nectar sources) were most abundant in the active management treatment*, S. nigrum* (nectar and extrafloral nectar source) was most abundant in the control, and *P. citri* (honeydew source) and *M. suaveolens* (nectar source) most abundant in the standard management treatment (Table 5.1).

Table 5.1 Alleyway carbohydrate sources explaining at least 50% of the variation in the spring, summer, and autumn models where the abundances of the different alleyway carbohydrate sources were compared between the three alleyway treatments, control, standard management of wildflower habitat and active management of wildflower habitat. The mean number of carbohydrate resource units per quadrate (± SE) are presented, as well as the percentage contribution to the total treatment effect of the manyglm model. Superscript plus symbol (+) denotes forbs species which were sown.

Carbohydrate source	Carbohydrate type	Mean carbohydrate units per quadrate (± SE)			Variation within the model (%)
		Control	Standard management	Active management	
Spring					
Aphids	Honeydew	0.12 (± 0.06)	0.9 (± 0.59)	0.5 (± 0.34)	13.86
Cichorium intybus^{+}	Nectar	-	0.38 (± 0.32)	-	13.16
Daucus sp.	Nectar	-	4.32 (± 3.17)	-	8.76
Marrubium vulgare^{+}	Nectar	-	3.9 (± 2.97)	-	8.76
Crepis sp.1	Nectar	-	0.17 (± 0.12)	-	8.76
Capsella bursa-pastoris	Nectar	-	0.15 (± 0.11)	-	8.76
Summer					
Marrubium vulgare^{+}	Nectar	-	3.2 (± 1.48)	-	26.69
Malva sylvestris	Nectar	-	0.12 (± 0.08)	-	8.56
Cichorium intybus^{+}	Nectar	-	0.12 (± 0.09)	-	8.52
Dittrichia graveolens	Nectar	0.27 (± 0.18)	0.67 (± 0.4)	-	8.03
Autumn					
Polygonum aviculare	Nectar	-	0 (± 0)	1.28 (± 0.95)	21.08
Dittrichia graveolens	Nectar	-	0 (± 0)	0.08 (± 0.06)	13.96
Planococcus citri	Honeydew	-	0.18 (± 0.17)	-	13.89
Solanum nigrum	Nectar	0.10 (± 0.06)	0.03 (± 0.03)	-	12.49
Mentha suaveolens^{+}	Nectar	-	0.55 (± 0.52)	0.23 (± 0.16)	10.92

5.3.3. The influence of alleyway treatment on the abundance of carbohydrate resources in the tree canopy

No floral resource was recorded from the citrus tree canopy across any of the seasons or treatments. As such, all carbohydrate resource units from the canopy were composed of colonies of honeydew-producing Hemiptera. In spring and autumn, the abundance of carbohydrate resource in the canopy was affected by treatment (spring, GLMM: $\chi^2 = 9.2821$, df = 2, $P = 0.010$; autumn, GLMM: $\chi^2 = 12.649$, df = 2, $P = 0.002$; Figure 5.6). In contrast, no significant difference in carbohydrate resource abundance was found in the canopy in summer (GLMM: $\chi^2 = 5.225$, df = 2, $P = 0.073$) (Figure 5.6 and Appendix 3.1). In spring, colonies of honeydew producers were twice as abundant in tree canopies adjacent to alleyways with the standard management wildflower treatment compared to the control and active management wildflower treatment. No difference was found between the control and the active management wildflower alleyways (Appendix 3.1). In autumn, colonies of honeydew producers were three times more abundant in standard management alleyways than in the control alleyways and were twice as abundant in the standard management alleyways as in the active management alleyways (Appendix 3.1).

As well as total abundance of canopy carbohydrate resource, the treatment applied to the orchard alleyways also affected the abundances of the different honeydew producing species (carbohydrate sources) present in the canopy across summer and autumn (manyglm summer: LRT = 20.31, df = 2, $P = 0.023$, and autumn: LRT = 31.27, df = 2, $P = 0.001$) (Table **5.2** and Appendix 3.2). Alleyway treatment did not affect the abundances of different canopy carbohydrate sources in spring, however (manyglm spring: LRT = 13.36, df = 2, $P = 0.215$).

In summer, the abundances of the different carbohydrate sources (honeydew-producing hemipteran species) were different between the standard and the active management treatments (Table **5.2** and Appendix 3.2). However, their abundances did not differ between the control and standard management treatments or the control and the active management treatments (Table **5.2** and Appendix 3.2). The abundance of colonies of three honeydew-producing species (*Icerya purchasi*, *Aleurothrixus floccosus* and *Planococcus citri*) were responsible for more than 90% of the variation within the model. *Icerya purchasi,* which was responsible for 73.7% of the treatment effect, was present in the canopy of all treatments. However, it was more than four times more abundant in the active management treatment than the control or standard management treatments (Table **5.2**). *Aleurothrixus floccosus* was only present in the standard management treatment but was very rare (0.02 colonies per plot). *Planococcus citri* was much more ubiquitous and was present across all treatments, though most abundant in the canopy of the standard management treatments (Table **5.2**).

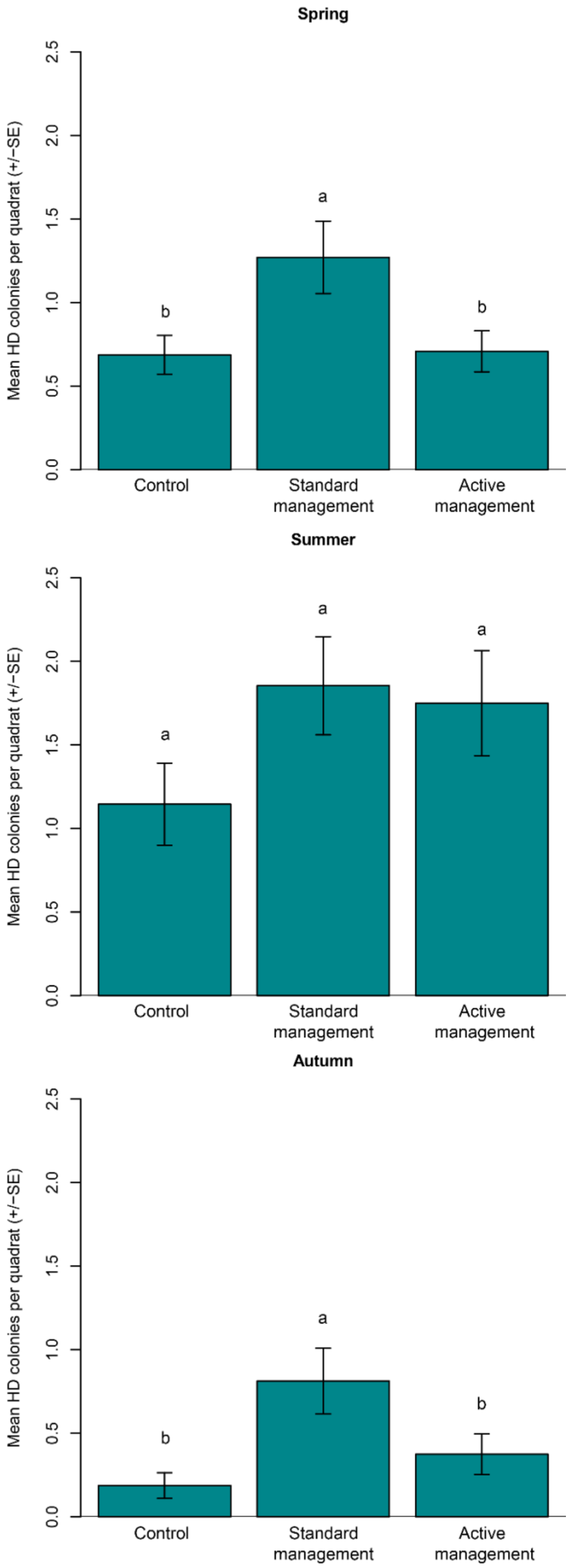

Figure 5.6 The abundance of carbohydrate resource available in the canopy of each treatment; control, standard management wildflower, and active management wildflower, during the three sampling seasons; spring, summer, and autumn, represented as the mean number of honeydew-producing Hemiptera colonies from each hoop (± SE).

Table 5.2 Sources of canopy carbohydrate resource explaining at least 90% of the variation in the summer and autumn models (in spring the abundances of different canopy carbohydrate sources were not different between treatment). The mean number of carbohydrate resource units per species per 0.5m x 0.5m quadrat (± SE) are presented, as well as the percentage contribution to the total treatment effect of the manyglm model, as calculated from the LR test statistic.

Carbohydrate source	Mean carbohydrate units per quadrate (± SE)			Variation within the model (%)
	Control	Standard management	Active management	
Summer				
Icerya purchasi	0.08 (± 0.04)	0.06 (± 0.04)	0.44 (± 0.12)	73.7
Aleurothrixus floccosus	0 (± 0)	0.02 (± 0.02)	0 (± 0)	10.82
Planococcus citri	0.77 (± 0.24)	1.29 (± 0.3)	1 (± 0.29)	6.4
Autumn				
Aleurothrixus floccosus	0 (± 0)	0.19 (± 0.08)	0 (± 0)	51.2
Aphis spiraecola	0.04 (± 0.03)	0.15 (± 0.08)	0.21 (± 0.08)	22.75
Planococcus citri	0 (± 0)	0.18 (± 0.17)	0 (± 0)	10.18
Icerya purchasi	0.1 (± 0.06)	0.03 (± 0.03)	0 (± 0)	8.83

In autumn, unlike in summer, the greatest differences in the abundance of the different sources of carbohydrate were between the standard and control treatments and active and standard management treatments (Table 5.2 and Appendix 3.2). The abundances of the different carbohydrate sources did not differ between the control and the active management treatments. The abundance of four honeydew-producing species were responsible for almost 93% of the variation within the model (Table 5.2). *Aleurothrixus floccosus* and *P. citri,* were only present in the standard management treatment at a rate of 0.19 and 0.18 colonies per quadrat, respectively. *Aphis spiraecola* was present in all treatments, though colonies were at least three times more abundant in the canopy of the active and standard management treatments than in the canopy of the control plots. Colonies of *I. purchasi* were most abundant in the control (Table 5.2).

5.3.4. The influence of alleyway treatment on carbohydrate feeding in parasitoids and a citrus pest, Phyllocnistis citrella

In spring, the proportion of parasitoids which had recently fed on carbohydrate did not differ according to alleyway treatment (GLMM with binomial error structure: $\chi^2 = 4.696$, df = 2, $P = 0.096$) (Figure **5.7** and Appendix 3.3). Irrespective of alleyway treatment, more than 75% of all parasitoids sampled had recently fed on a carbohydrate source.

In summer, the alleyway treatment was shown to influence the proportion of parasitoids recorded as having recently fed on carbohydrates (GLMM: $\chi^2 = 7.367$, df = 2, $P = 0.025$) (Figure **5.7**). The proportion of parasitoids was over 20% greater in standard management than in active management plots, but not significantly different from the control (Figure **5.7**). In summer, the Lepidopteran pest species *P. citrella* was also captured and tested for carbohydrate feeding. The mean proportion of *P. citrella* recently fed on carbohydrates in the control treatment was 6% (±6), in the standard management was 41% (±9), and in the active management treatment was 27% (±12). However, no significant differences were found between alleyway treatments (GLMM: $\chi^2 = 5.108$, df = 2, $P = 0.078$).

In autumn, the proportion of parasitoids which had recently fed on carbohydrates was also affected by the treatment applied to the orchard alleyways (GLMM: $\chi^2 = 17.802$, df = 2 $P < 0.001$) (Figure **5.7** and Appendix 3.3). The proportion of parasitoids which had recently fed on carbohydrates in the standard management wildflower treatment was almost double that of the active management wildflower treatment (Figure **5.7**).

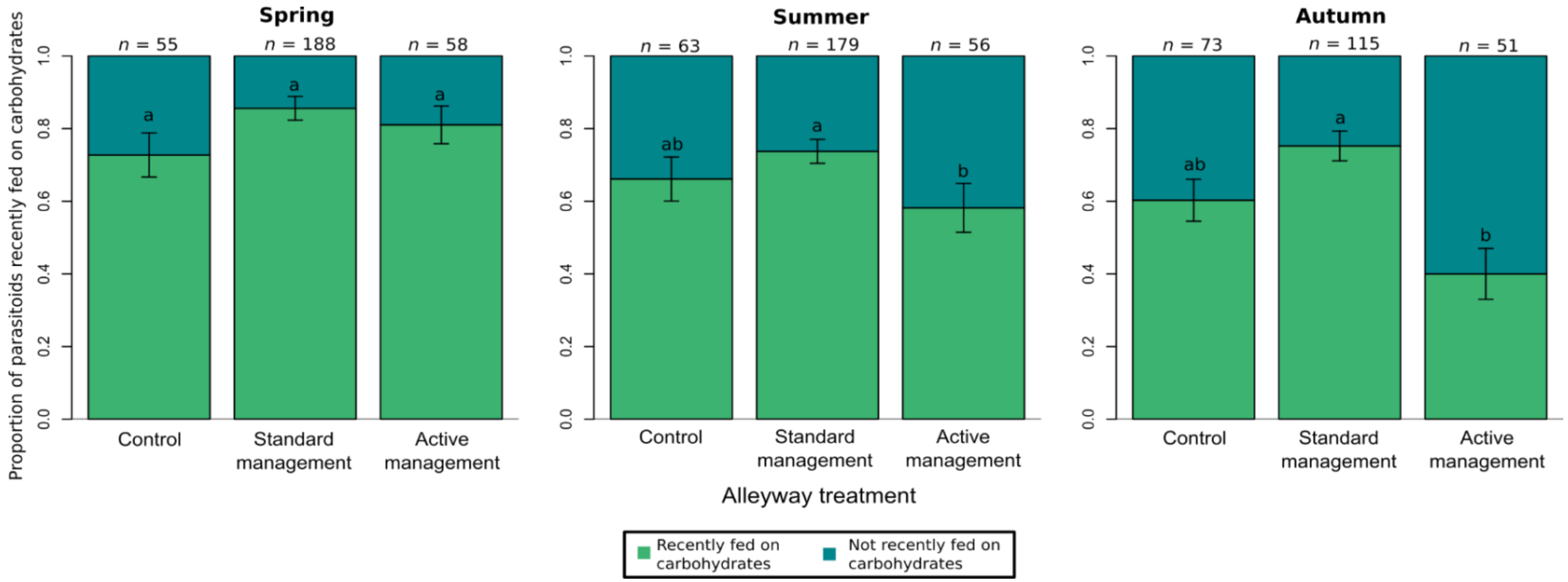

Figure 5.7 The proportion of parasitoids recently fed on carbohydrates (green bars) and carbohydrate negative (turquoise bars) between alleyway management treatments, control, standard management of wildflower habitat and active management of wildflower strips across the three sampling seasons; spring, summer, and autumn. The proportion of recently carbohydrate-fed parasitoids between treatments with different letters are significantly different (Tukey's pairwise contrasts; $P < 0.05$).

5.3.5. *Differences in trophic function across treatments*

Significant differences in the abundances of the five trophic functional groups (primary parasitoids, hyperparasitoids, parasitoids of predators, other parasitoids, and a phytophage) were found between treatments for each season (spring: $\chi^2 = 37.71$, df = 2, $P = 0.001$, summer: $\chi^2 = 46.37$, df = 2, $P = 0.001$; autumn: $\chi^2 = 33.84$, df = 2, $P = 0.002$) (Figure 5.8 and Appendix 3.4).

In spring, the greatest differences in abundance of different trophic functional groups were observed between the standard management and control treatments and the standard management and the active management treatments, though differences in abundance were also observed between active management and the control treatment (Figure 5.8 and Appendix 3.5). All functional groups were more abundant in the standard management treatment than either the active management or control treatments. The greatest variation in the model, as observed through the test statistic of the univariate GLMs, was due to the greater abundance of other parasitoids in the standard management treatment (1.21 ± 0.31 individuals per sample), which were more than twice as abundant as in the control (0.5 ± 0.14 per sample) or active management treatments (0.29 ± 0.09 per sample). The abundance of parasitoids of arthropod predators were also more abundant in the standard (0.75 ± 0.22 per sample) and active management (0.5 ± 0.16 per sample) treatments than in the control (0.08 ± 0.06 per sample), from which they were almost absent. Similarly, primary parasitoids were more than twice as abundant in the standard management (1.96 ± 0.33 per sample) than the control (0.88 ± 0.16 per sample) and 50% more abundant in the active management than the control treatment (1.25 ± 0.24 per sample) (Appendix 3.4).

In summer, the greatest differences in the abundances of the different functional groups were observed between the standard management and the two other treatments (Figure 5.8 and Appendix 3.4). These differences between treatments were driven by the differences in abundance of primary parasitoids. Primary parasitoids were three times more abundant in the standard management treatment (2.9 ± 0.48 per sample) than the control (0.98 ± 0.19 per sample) and more than four times abundant in the standard management than the active management treatment (0.67 ± 0.14 per sample) (Appendix 3.4).

Similarly, in autumn as in summer, the greatest differences in the abundances of different functional groups were observed between the standard management and the two other groups (Figure 5.8 and Appendix 3.5). Primary parasitoids were more than twice as abundant in the standard management treatment (1.54 ± 0.29 per sample) than the control and active management treatments (0.71 ± 0.16 and 0.71 ± 0.15 per sample, respectively. However, the variation between treatments in autumn was due not only to differences in the abundance of primary parasitoids, but also differences in abundance parasitoids of predators and hyperparasitoids between the different treatments (Figure 5.8 and Appendix 3.4). Though the abundance of parasitoids of predators was low in the standard management treatment (0.1 ± 0.05 per sample), they were absent from the control and the active management treatment. Hyperparasitoids were almost twice as abundant in the control treatment (0.6 ± 0.17 per sample) than in the standard management treatment (0.33 ± 0.11 per sample) and more than four times abundant than the active management treatment (0.13 ± 0.06 per sample) (Appendix 3.4).

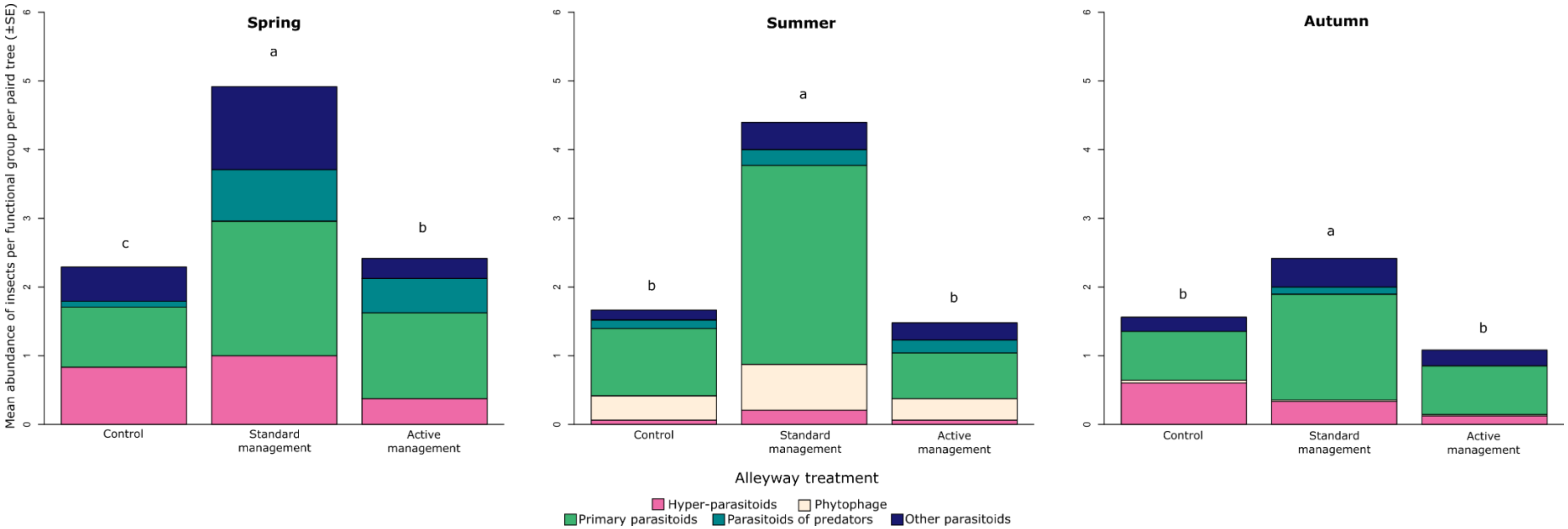

Figure 5.8 Abundance of different trophic functional traits between treatments; control, standard management wildflower, and active management wildflower, and across the three seasons; spring, summer, and autumn. Generalised multivariate linear models were used to infer treatment affects. Treatments with different letters are significantly different (Holm's step-down method for pairwise contrasts; *P* < 0.05).

5.3.6. Predicting carbohydrate feeding in parasitoids based on parasitoids traits and orchard characteristics

The most parsimonious hierarchical GLMM from the subset of data which only included parasitoids which had been identified to genus, as selected by dredging in MuMIn, included the following fixed effects: mean carbohydrate resources units in alleyway, parasitoid family, sward heterogeneity, and parasitoid head width. There were no interaction terms. Treatment was nested within orchard as random effects and further random intercepts were applied for each sample date applied. Parasitoid function was dropped from all but one model where $\Delta AIC_c < 2$, and such the models can be considered feasible. While honeydew production in the primary host was dropped from all models where $\Delta AIC_c < 2$ (Appendix 3.6).

The most parsimonious hierarchical GLMM from all parasitoid data, as selected by dredging in MuMIn, included the following fixed effects, mean carbohydrate resources units in alleyway, parasitoid family, mean corolla width, sward heterogeneity, parasitoid head width, and a two-way interaction between mean corolla width and parasitoid head width. Treatment was nested within orchard as random effects and further random intercepts were applied for each sample date applied (Table 5.3).

The more heterogeneous the alleyway habitat structure and the larger the parasitoid head width, the greater the proportion of carbohydrate fed parasitoids. The proportion of carbohydrate fed individuals was different between the 14 parasitoid families identified. Megaspilidae, Ceraphronidae and Platygastridae showed the highest proportion of carbohydrate individuals (Figure 5.9 and Table 5.4).

Table 5.3 Overview of the model selection predicting carbohydrate feeding in parasitoids in citrus orchards. The model was fit using the whole parasitoid dataset. Model selection was performed using the dredge function of the MuMIn package in R. The number of parameters in the model is indicated by df, AIC$_c$ is the corrected AIC for small sample sizes, Δ is the difference in AIC$_c$ with the model with the lowest AIC$_c$ value, and Akaike weights (Weight) represent the posterior probability of the model. Based on these parameters, only the ten best models are presented, although any model where $\Delta < 2$ is considered plausible. Terms included in the model are represented by the plus symbol (+) while terms dropped form the model are represented by the minus symbol (-).

	Model ranked according to weight							
	1	2	3	4	5	6	7	8
Mean abundance of carbohydrate resources units in alleyway	+	+	-	-	+	+	+	+
Family	+	+	+	+	+	+	+	+
Mean corolla width	-	-	+	-	-	+	+	+
Mean abundance of carbohydrate resource units in canopy	-	-	-	-	-	-	-	-
Sward heterogeneity	+	+	+	+	+	+	+	+
Parasitoid head width	+	+	+	+	-	+	+	+
Mean carbohydrate resources units in alleyway * Mean carbohydrate resource units in canopy	-	-	-	-	-	-	-	-
Mean carbohydrate resources units in alleyway * Sward heterogeneity	-	-	-	-	-	-	-	+
Mean carbohydrate resources units in alleyway * Head width	-	-	-	-	-	-	+	-
Mean corolla width * Parasitoid head width	+	-	+	-	-	-	+	+
Mean carbohydrate resource units in canopy * Sward heterogeneity	-	-	-	-	-	-	-	-
df	22	20	21	19	19	21	23	23
AIC$_C$	857.4	857.9	858.3	858.6	859.2	859.3	859.4	859.5
Δ AIC$_c$	0	0.498	0.947	1.222	1.781	1.927	2.04	2.08
Weight	0.162	0.126	0.101	0.088	0.066	0.062	0.058	0.057

Table 5.4 Model estimates and 95% confidence intervals (on a natural logarithm scale) of the most parsimonious model based parasitoids trait and environmental variable. The model has an AIC of 856.0 (AICc = 857.4) and a binominal error distribution.

Predictors	Odds Ratios	Lower 95% CI upper	Lower 95% CI upper	*P*-value
(Intercept)	0.40	0.14	1.12	0.081
Mean carbohydrate resources units in alleyway	1.01	0.99	1.03	0.349
Braconidae	0.63	0.36	1.08	0.092
Ceraphronidae	3.97	1.37	11.49	**0.011***
Chalcididae	0.36	0.03	3.96	0.407
Diapriidae	0.50	0.16	1.55	0.229
Encyrtidae	0.72	0.44	1.19	0.199
Eulophidae	1.20	0.69	2.11	0.517
Figitidae	1.16	0.63	2.16	0.633
Ichneumonidae	0.41	0.15	1.12	0.081
Megaspilidae	5.99	1.47	24.44	**0.013***
Mymaridae	0.84	0.44	1.61	0.594
Platygastridae	2.63	1.49	4.64	**0.001****
Pteromalidae	1.13	0.60	2.14	0.709
Trichogrammatidae	0.95	0.15	6.13	0.958
Mean corolla width	1.43	0.97	2.11	0.072
Sward heterogeneity	9.93	2.49	39.59	**0.001****
Parasitoid head width	5.04	1.34	18.96	**0.017***
Mean corolla width * Parasitoid head width	0.46	0.21	1.00	0.050

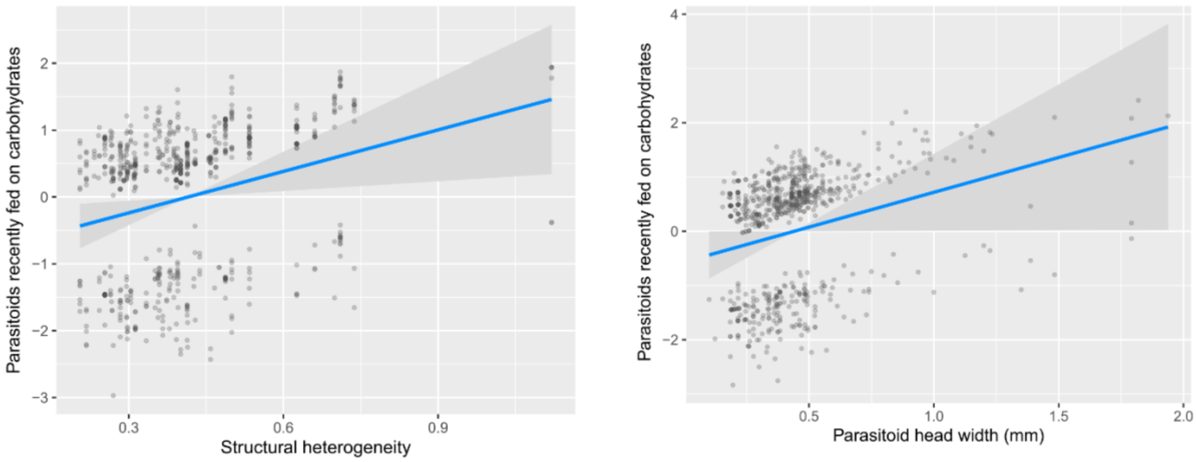

Figure 5.9 The effect of each significant independent variable and interaction from the optimal GLMM model on parasitoid carbohydrate feeding in field-captured parasitoids (see Table **5.3** for model selection and Table **5.4** for included terms). The fitted lines were predicted from the optimal generalized mixed effects model, the points represent partial residuals, and the grey bands depict confidence intervals. Plots were generated using the visreg and ggplot2 packages in R.

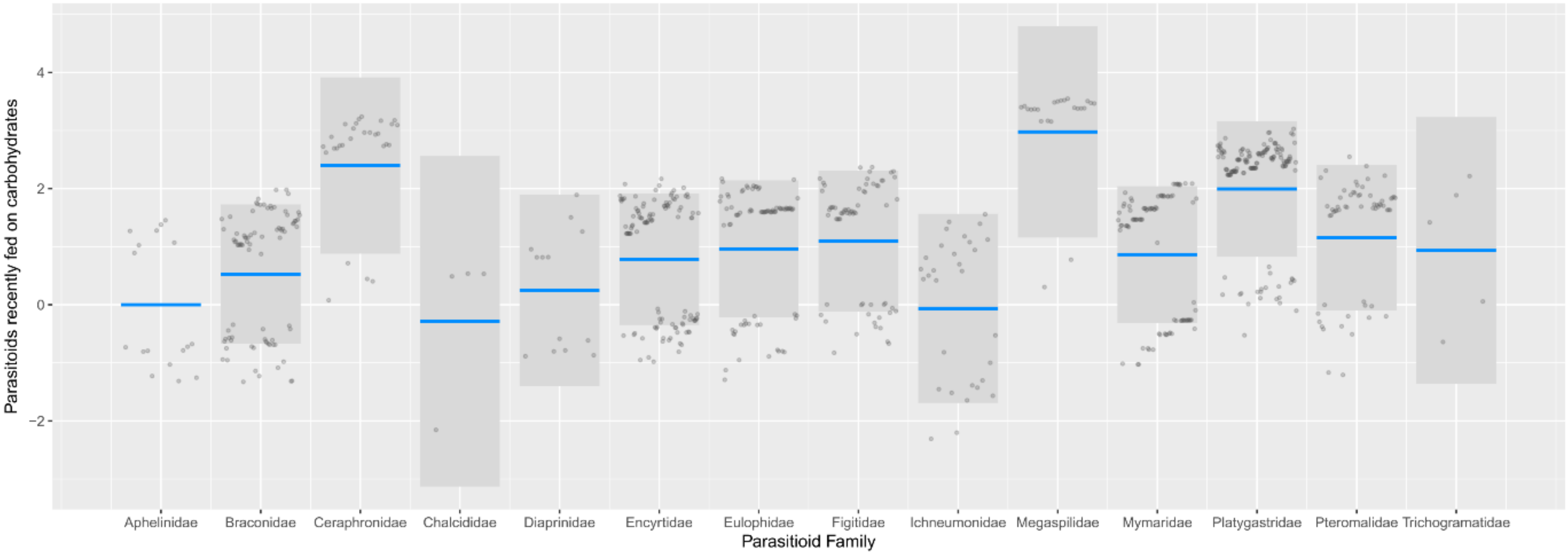

Figure 5.9 Continued, blue lines represent the medians, and the grey areas represent ± 1 SEM. Plots were generated using the visreg and ggplot2 packages in R.

5.4. Discussion

The provision of carbohydrate resources for parasitoids in orange orchards was increased by creating diverse wildflower habitat in alleyways between rows of trees that were allowed to grow tall and complex under the standard management wildflower treatment. This response was driven by a greater number of floral units possessing accessible nectar for parasitoids and the greater number of plant species providing these resources, compared to wildflower alleyways managed with cutting three times a year (active management), and alleyways managed conventionally (control). The standard management of the wildflower strip in alleyways also supported the greatest proportion of parasitoids recently fed on carbohydrates across all three seasons. Species included in the wildflower seed mix were selected to provide a range of floral resources throughout the year, whilst unsown species in the standard management treatment are also expected to benefit parasitoids. However, regular cutting of this wildflower habitat was associated with a reduction in plant diversity (Chapter three), and also prevented plants from flowering. Frequent cutting removed inflorescences and hence the abundance of available nectar to parasitoids.

This study has identified ten different plant species which possess nectar that is accessible to 95% of the parasitoid community present across the three citrus orchards. Two species were sown, and eight were unsown. Five of these provided nectar for parasitoids in late spring (*Malva parviflora*, *Malva sylvestris*, *Salvia verbenaca*, *Daucus* sp. and *Capsella bursa-pastors*), compared to three in summer (*Polygonum aviculare, Euphorbia chamaesyce, M. sylvestris* and *S. verbenaca*), and five in autumn (*Solanum nigrum, Heliotropium europaeum, Mentha suaveolens, P. aviculare* and *S. verbenaca*). The sown species, *Salvia verbenaca,* was the only species present throughout all three seasons and provided accessible resource for 95% of parasitoids. *Heliotropium europaeum* and the sown species *M. suaveolens* provided accessible resource for parasitoids during two seasons, including the autumn which is a critical period when carbohydrate availability is typically limiting for parasitoids (Tena *et al.,* 2013a). *Mentha suaveolens* was present both in the standard management as well as the active management regime and was recorded in two of the three sites surveyed. Similarly, *H. europaeum* was found at two of the three sites sampled in this study, but only recorded from the standard management treatment. As such, these two sown and one unsown forb species can be recommended for inclusion in wildflower seed mixes designed for feeding parasitoids.

One of the unsown species which permitted access to 95% of the parasitoid community, *P. aviculare,* is a highly competitive annual and is able to suppress establishment of other species (Alsaadawi and Rice, 1982). This species is associated with high disturbance (Costea and Tardif, 2005) and was most common in the two treatments characterised by regular cutting, the control and active management. As such, it would not be recommended to sow this species, and any management applied should aim to limit its establishment and prevent it outcompeting sown species (Radicetti, Mancinelli and Campiglia, 2012). Despite providing nectar and extrafloral nectar in response to damage (Gentry, 2003), *S. nigrum* is associated with diverse acarifauna and can harbour economically important citrus pest species, such as *Tetranychus urticae* Koch (Acari: Tetranychidae) (Ferreira and Sousa, 2011; Celepci *et al.*, 2017). However, it can also support populations of mite predators that might move into the citrus canopy (Pereira *et al.*, 2014). *Malva* species have been found to host the citrus pest *Aphis gossypii* Glover (Hemiptera: Aphididae) in clementine orchards, as well as non-pest aphid species (Gómez-Marco, Urbaneja and Tena, 2016), as such it was not included in the initial seed mix. This plant species might facilitate early season colonisation of citrus by aphids (Norris and Kogan, 2000) but may also provide alternative hosts and honeydew as food for parasitoids when these are scarce in the canopy during winter. The same occurred with *P. bituminosa* and *A. millefolium*, two plant species that did not have accessible nectar for all parasitoid species but hosted honeydew-producing species during autumn. The roots of these two plant species harboured several large colonies of *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) and *Coccus hesperidium* L. (Hemiptera: Coccidae), respectively. These plant species in the alleyway may have facilitated spill-over onto the crop. *Coccus hesperidium* in spring, for example, was only recorded from the two wildflower treatments where these plant species were present, and *P. citri* in autumn was only recoded from the standard management wildflower treatment. During these two seasons, total number of honeydew-producing hemipteran colonies were increased in the citrus canopy with the standard management wildflower treatment. It is unlikely that this was due to tending behaviour of ants, as ant abundance was not increased with the standard management treatments relative to the control or active management wildflower treatment (Chapter four,

Figure 4.6). Though these Hemiptera excrete honeydew of high quality for parasitoid fitness, they can be economically important pests of citrus (Tena, Llácer and Urbaneja, 2013).

The proportion of recently fed parasitoids differed according to season and importantly, the trend across seasons altered between alleyway management treatments. In late spring, most of the parasitoids collected (~80%) had recently fed on carbohydrates, suggesting carbohydrate is not limiting during spring, and hence pest regulation services are not limited. Tena *et al.* (2013a) found a comparable proportion of recently fed *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) during the spring in a citrus orchard which had been sown solely with grass species in the alleyways. The proportion of recently fed *A. melinus* decreased throughout the year as resources became limiting (Tena *et al.*, 2013). The same observation was made in this study in the control and the active management treatments, but not it the standard management treatment. Under the standard management wildflower treatment, parasitoids were maintained across year as the seed mix was designed to provide resource throughout the length of the year. As carbohydrate feeding in the field can boost parasitoid populations and increases their pest regulation capacity, carbohydrate limitation towards the end of summer and into autumn can lead to a reduction in pest regulation services (Tena *et al.*, 2015). The ability of the standard management regime to disrupt this seasonal trend could lead to enhanced pest regulation services at a time which is typically associated with reduced parasitism levels (Sorribas, Rodríguez and Garcia-Mari, 2010). A key finding is that whilst the active management wildflower treatment was aimed at increasing the movement of natural enemies into the adjacent trees to enhance pest regulation services, this practice was associated with the dramatic loss of carbohydrate resources. Therefore, to support parasitoids in orange orchards to boost pest regulation services, the standard management wildflower treatment is recommended.

Not only did the standard management of the wildflower strip increase carbohydrate feeding, but it also influenced the abundance of different functional groups. As hyperparasitoids in citrus limit biological control (Gómez-Marco *et al.*, 2015), other authors have expressed caution of feeding hyperparasitoids through the provision of carbohydrate resource (Tougeron and Tena, 2019). The four parasitoid functional groups had similar capability to feed on carbohydrate resource, but it was only the primary parasitoids which displayed a sustained trend across the treatments and season; primary parasitoid abundance was boosted in association with standard management wildflower alleyways. This demonstrates that other resources provided by the wildflower habitat under standard management, such as alternative hosts, refuge, and shelter against adverse climatic conditions, are differentially favouring primary parasitoids, perhaps synergistically (Rand, van Veen and Tscharntke, 2012).

The study has also demonstrated the importance of promoting structural heterogeneity in alleyway habitats to boost carbohydrate feeding in parasitoids, which can be achieved by sowing a greater range of species. Greater structural heterogeneity can be an indicator of accessibility into the sward and facilitates foraging within otherwise dense habitats (Blake *et al.*, 2013), and is positively related to the abundance of predatory arthropods (Woodcock *et al.*, 2008; Blake *et al.*, 2013). Furthermore, greater heterogeneity can reduce competition between predatory species due to enhanced niche structure (Chesson, 2000). Increased structural heterogeneity might therefore present more foraging opportunities for parasitoids under relaxed competition.

5.5. Conclusions

Based on their accessibility to a high proportion of parasitoids (>95%) and ubiquity across seasons, three plant species, *S. verbenaca*, *M. suaveolens*, and *H. europaeum,* are recommended for wildflower seed mixes aimed at feeding parasitoids in citrus under Mediterranean conditions. Four more species, *P. bituminosa, A. millefolium* and *M. sylvestris,* have desirable characteristics such as accessible nectar or host honeydew-producing Hemiptera, but will require further investigation to confirm their inclusion for citrus seed mixes for parasitoid feeding. This study has therefore also highlighted the complexities of parasitoid feeding ecology in a community context and the importance of studying both the provision of resources as well as the potential for plant species to host crop pests. Such plant species act as a double edged 'sward', with the potential to harbour economically significant pest species, but provisioning vital floral and extra-floral nectar and honeydew resource for parasitoids in an otherwise carbohydrate limiting environment. Above all, structural heterogeneity in wildflower habitat has been found to be key for foraging parasitoids. This finding can be extrapolated to other systems in which wildflower habitat can be managed to increase structural heterogeneity in order to maximise carbohydrate feeding in parasitoids.

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Chapter 6

General discussion

6.1. Effect of the wildflower habitat and its management

In Spain, the management of orchard floor vegetation has traditionally focused on controlling soil erosion by maintaining naturally occurring vegetation in the orchard alleyways or sowing leguminous seed mixes (Arenas Arenas, Hervalejo García and de Luna Armenteros, 2015). Although not specifically designed for this purpose, both these practices are known to support a greater abundance of natural enemies compared with the conventional approach of maintaining bare soil (Silva *et al.*, 2010). Such an approach is now encouraged by the Rural Development agri-environmental scheme (AES) in Spain. Indeed, all the orchards used in this study were already maintained with naturally occurring vegetation in the alleyways, cut four to five times annually. Over the past decade there has been increased research into the use of ecological interventions for pest regulation services in Spanish citrus. Alleyways sown with *Schedonorus arundinaceus* support greater abundances of Phytoseiid predators which facilitates their spill-over onto the citrus crop and promotes management of thrips and mites (Aguilar-Fenollosa *et al.*, 2011a; Jacas and Aguilar-Fenollosa, 2013). However, these alleyway strips only enhance the regulation of other pest species (aphids) when unsown forb species are present (Gómez-Marco, Urbaneja and Tena, 2016). This suggests that responses could be very site specific due to compositional differences in the naturally occurring floral communities. To overcome the issue of relying on unsown and beneficial plant species being present, species rich seed mixes can be sown to support a greater diversity of beneficial insects (Rouabah *et al.*, 2015; Balzan, Bocci and Moonen, 2016; Campbell *et al.*, 2017). Numerous studies have demonstrated the benefits of sowing wildflower habitat designed to maximise both resource provision across the year and diversify functional diversity (McKerchar *et al.*, 2020), however this had not yet been investigated in Spanish citrus.

For the first time, the provision of species rich wildflower habitat in Mediterranean citrus has been shown to support greater abundance and richness of natural enemies than the recommended practice encouraged by Spanish AES of leaving the naturally occurring vegetation in the alleyways. The wildflower habitat, when left to grow throughout the year under the standard management treatment, supported a plant community distinct from the naturally occurring orchard vegetation, which boosted plant richness and enhanced the abundance of resources for natural enemies. Furthermore, this translated into an increased incidence of carbohydrate fed parasitoids, which is known to optimise foraging and increase parasitism rates (Tena *et al.*, 2015). The provision of wildflower habitat also enhanced pest regulation services (depletion of sentinel prey) during late spring and early summer, by as much as 78.1% in June of year three. Of all the arthropod functional groups recorded, the abundance of primary parasitoids was most strongly enhanced by the presence of the wildflower habitat and were more than twice as abundant (2.57 times) in the standard management wildflower treatment than the control treatment. The cutting regime, however, played a key role and under the active management treatment, the wildflower habitat were not associated with an increase abundance of primary parasitoids relative to the control. Cutting the wildflower habitat removed fundamental carbohydrate resource (nectar and honeydew) and due to the arid climate conditions, these resources were unable to recover. As such, differences in primary parasitoid abundance between treatments were greatest during the summer and early autumn months, when parasitoids are typically deficient in carbohydrates (Tena *et al.*, 2013).

The key predictor of carbohydrate feeding in parasitoids was high structural heterogeneity within the alleyway habitat driven primarily by the presence of wildflower and tussock-forming grass species and the management treatments applied. During the hottest and most arid months of the year (July and August) (IFAPA, 2021), the abundance of primary parasitoids and predators was increased in the crop canopy adjacent to the standard management wildflower habitat, compared to the control alleyways and active management wildflower habitat. Habitats with high structural heterogeneity are characterised by high plant species and trait diversity (such as height, woodiness, and leaf breadth) (Londe *et al.*, 2020). The wider niche breadth provided by structurally heterogeneous swards can support a greater diversity of arthropod phenotypic and functional traits, such as size, developmental stage, and sex, which may have discrete and very different resource requirements (Cherrill and Brown, 1990). Variance in the sward structure is expected to increase accessibility into otherwise structurally dense swards allowing access to resources within, such as alternative hosts, hemipteran honeydew, refuge, and microclimate shelter (Woodcock *et al.*, 2007, 2008; Blake *et al.*, 2013). Carbohydrates (nectar, honeydew, and guttation), protein (pollen and prey/host), and shelter as provided by highly heterogeneous habitats form the cornerstones of habitat management for pest regulations services (Landis, Wratten and Gurr, 2000). As has been demonstrated here, when provided, they facilitate optimum carbohydrate feeding in parasitoids and enhance their abundance. Furthermore, these resources are associated with increased natural enemy diversity, and the delivery of ecosystem services (Campbell *et al.*, 2017; Cresswell *et al.*, 2018).

The standard management wildflower habitat, designed to provide a variety of different plant traits and supported the highest plant richness, enhanced the abundance of natural enemies and boosted pest regulations services on the crop. In year three, the abundance of Coccinellidae in citrus canopies with the standard management wildflower habitat was more than double that of the control alleyways (102.4% greater) and almost double that of the active management wildflower treatment (96.4% greater). The Coccinellidae are economically important predators in Mediterranean citrus systems, implicit in the regulation of (Aphidoidea), mealybugs (Hemiptera: Pseudococcidae), Diaspidids such as *A. aurantii* Maskell (Hemiptera: Diaspididae), Coccids (Hemiptera: Coccidae), *Icerya purchasi* (Hemiptera: Monophlebidae), and spider mites (Acari: Tetranychidae) (Magro and Hemptinne, 1999; Mendel *et al.*, 1999; Michaud, 2001; Michaud and Harwood, 2012; Gómez-Marco, Urbaneja and Tena, 2016; Bouvet *et al.*, 2019). Other studies have demonstrated how creating wildflower habitat designed to provision resource for natural enemies, as in this study, can augment Coccinellid populations within the swards themselves (Tschumi *et al.*, 2014, 2016), which can increase populations in the adjacent crop and enhance pest regulation services (Blaauw and Isaacs, 2015). Some shorter studies, however, did not record a spill-over effect (Silva *et al.*, 2010; Tschumi *et al.*, 2016), which highlights the importance of conducting studies over at least three years. With the active management wildflower treatment pest regulation services were not different from, or even lower than, those recorded with control treatment and the timed cuts had no observed benefit on the crop. This may be expected from the reduction in arthropod richness and abundance in the active management wildflower. Though cutting, as in the active management treatment, is typically associated with an increase in plant diversity (Blake *et al.*, 2011; Mazalová *et al.*, 2015), cutting in the arid conditions of summer prevents habitat from recovering and hence the arthropod communities which would typically be supported cannot recover either, for lack of resources (Morris, 2000). Cutting dramatically reduced resource availability in addition to floral diversity and sward structure, which is likely to have reduced the abundance and diversity of invertebrates within the habitat (Vickery *et al.*, 2001). The control alleyways were also regularly cut but carbohydrate feeding in parasitoids and predations rates were significantly lower in the crop canopy with the active management wildflower treatment during certain times during the study. This might be due to the control alleyways being a poorer quality resource to start with; low plant richness, lacking tussock-forming grasses for refuge and shelter, and fewer carbohydrate resource throughout the year. Though the intermediate resource of the active management treatment may have provided greater resource than the control, this may act as a 'honeypot', attracting natural enemies away from the crop canopy, but with insufficient resource to augment their populations and facilitate spill-over, as in the standard management treatment (Herz *et al.*, 2019). Furthermore, cutting releases volatile blends similar to those released when under phytophage attack (Khan *et al.*, 2008), which may act as a 'call for help' attracting natural enemies into the alleyway habitat (Dicke, van Loon and Soler, 2009). Any possible short-term increases in natural enemy abundance in the citrus crop achieved by cutting were outweighed by the long-term effects the disturbance had on both the plant community in the alleyway and the arthropod natural enemies in the crop.

For natural enemy populations in the sward to recover following cutting, it must be timed so that the sward is also able to recover, such as during the spring and summer (Morris, 2000; Vickery *et al.*, 2001), cut to greater height, such as 20 cm (Mateos-Fierro *et al.*, 2021), or only part of the wildflower habitat cut so that some remains intact (Woodcock *et al.*, 2008; Vercher *et al.*, 2012). Such strategies might mitigate the trade-offs between the removal of vital resource which cannot recover and realising the desired pest regulation services in the canopy by enforced spill-over onto the crop by cutting. Mateos-Fierro *et al.* (2021) reported increased natural enemy richness and abundance and enhanced pest regulation services associated with wildflower habitat which was managed with regular cutting (two or three times a month) to a height of 20 cm. Furthermore, the number of floral units provisioned were not negatively impacted by this treatment (Mateos-Fierro *et al.*, 2021), suggesting ample resource for natural enemies was still provided. This strategy in Mediterranean citrus, timed to coincide with the most susceptible instars of target pests might be sufficient disturbance to move natural enemies onto the crop, but allow swards to recover and provision resource for natural enemies. Partial scarification of species poor habitats retains the structural complexity in the intact sections and increases sward heterogeneity overall, and hence in turn enhances natural enemy abundance (Woodcock *et al.*, 2008; Blake *et al.*, 2013). The same principal of applied to cutting (i.e., cutting just part of the habitat) could be used to retain resource provisioned by the wildflower habitat (carbohydrates, protein, alternative hosts, and shelter), increase structural heterogeneity of the sward (Westbury *et al.*, 2008; Blake *et al.*, 2011), while helping to promote natural enemy spill-over from the area cut.

Due to the continued use of PPPs, it was not possible to adequately monitor pest population dynamics in the canopy adjacent to the different alleyway treatments. Instead, sentinel prey cards, baited with *Ephestia kuehniella* Zeller (Lepidoptera: Phycitidae) eggs, were used to assess pest regulation services as it was not possible to use *A. aurantii* infested fruit over concerns of introducing a key pest to plots where it was absent. However, the guild of natural enemies which feed on the immobile Lepidoptera eggs is likely quite different to those which feed on citrus key pests (Zou *et al.*, 2017). Furthermore, complex inter- and intra-guild interactions exist in the field which can be altered by the provision of alternative resource (Pina *et al.*, 2012; Vanaclocha *et al.*, 2013; Calabuig, Garcia-Marí and Pekas, 2014). For example, ants are considered both predators and mutualists of many economically important hemipteran crop pests (Way, 1963). While their presence in citrus canopies increases both honeydew-producing and non-honeydew-producing pest incidence (Calabuig, Garcia-Marí and Pekas, 2014), as scavengers they would likely deplete sentinel prey cards. Furthermore, ambush or hunting predators, such as Salticidae (Araneae), are unlikely to feed on static prey such as *E*. *kuehniella* eggs (Forster, 1977) and parasitism could not be measured. However, using an alternative sentinel prey, such as fruit baited with *A. aurantii*, would not reveal parasitism rates either, as the parasitoids would likely be absent from the plots as when the sentinel prey was removed from the field their hosts would be absent. Nonetheless, the use of *E. kuehniella* eggs are frequently used as sentinel prey to quantify predation rates in field, laboratory and glasshouse trials and as such are a well-established proxy for evaluating pest regulation services (Campbell *et al.*, 2017; McHugh *et al.*, 2020).

A key success of this study was achieving high establishment rates of the sown species which bolstered plant richness in the alleyway habitat and provided a distinct plant community compared with the control alleyways. The plant provided resources formed the foundations for boosting pest regulation services on the crop. Of the 14 species sown in the seed mix, 13 were recorded in the wildflower habitat during the three-year study; only *Helichrysum stoechas* (Asteraceae) was not recorded. *Helichrysum stoechas* has proven difficult to establish in other Mediterranean field trials (Oliveira *et al.*, 2012). *Ononis natrix* (Fabaceae), despite being initially abundant during the establishment year (year one), failed to persist in either of the wildflower treatments (standard or active management). Nevertheless, the establishment and persistence of 85.7% of the sown species within the wildflower habitat makes this seed mix highly successful compared with other studies (Tinsley, Simmons and Windhager, 2006).

This study has demonstrated for the first time that establishing a wildflower habitat targeted for the natural enemies in citrus can support a greater abundance and richness than conventional methods. Furthermore, the way in which the habitat is managed strongly influences the provision of resources and the delivery or pest regulation services. Allowing the wildflower habitat to grow taller and more complex as in the standard management treated enhance natural enemy abundance, increased carbohydrate feeding, and boosted pest regulation services in the crop canopy. Such findings are imperative for guiding growers and decision makers when striving for more sustainable approaches to food production.

6.2. Integrated pest management and agri-environmental schemes in Spain

As of 2014, the use of integrated pest management techniques has been mandatory across Europe, including Spain (Lefebvre, Langrell and Gomez-y-Paloma, 2014). In addition to adopting IPM, one of the key objectives outlined in the 2014-2020 agri-environmental scheme for climate and the environment in Spain is the application of Integrated Production practices (Anonymous, 2020). Integrated Production includes the maintenance of ecological infrastructure, such as hedgerows and banks, the conservation of orchard vegetation, and the sowing of wildflower habitats in all perennial crops, such as citrus (Anonymous, 2014). In Andalucía, citrus growers may be supplemented up to €31,200 over a five-year period for implementing integrated production within their orchards (Anonymous, 2016). This is granted as a maximum of €11,700 in year one, €9,100 in year two, €6,500 in year three,

€2,600 in year four, and €1,300 in year five. However, there is a requirement for a minimum hectarage managed under Integrated Production of 125 ha and a maximum of 250 ha (Anonymous, 2016), which creates a barrier to uptake. Of the 81,000 hectares under citrus production in Andalusia (MAPA, 2020), 8.164 ha is under organic production (certified or in conversion) (CAGPDS, 2020), and only 5,000 ha of citrus is produced under these Integrated Production techniques (Anonymous, 2015).

Despite the availably of grants for Integrated Production, the conditions of the grants fail to encourage the sowing of wildflower habitats. The implementation cost, and therefore associated risk to the growers, of Integrated Production and the creation of ecological infrastructure can be a barrier to adoption (Espinosa-Goded, Barreiro-Hurlé and Dupraz, 2013). For example, with relatively low per hectare incentives (€24.96) for implementing such practices in Andalusia, producers typically opt for the most economically viable and least complex options, such as allowing naturally occurring vegetation to grow instead of sowing a seed mix (Barreiro-Hurlé, Espinosa-Goded and Dupraz, 2010). Nonetheless, the characteristics of citriculture in Andalucía, such as large intensively farmed orchards, encourage the uptake of agri-environmental schemes (Lastra-Bravo *et al.*, 2015; Pavlis *et al.*, 2016). As such, to enhance the sustainability of food production, more support is needed to encourage growers to make the shift towards ecological approaches which harness the specific ecosystem services required at a regional or community scale as well as supporting biodiversity in general (Barreiro-Hurlé, Espinosa-Goded and Dupraz, 2010; Ekroos *et al.*, 2014). As one of the strongest drivers for adoption is prior participation in agri-environmental schemes and the greatest barriers being technical factors of the scheme, adoption of agri-environmental schemes in Spain might be facilitated by defining entry-level schemes followed by mid- and higher-tier programmes which could focus on targeted approaches for specific ecosystem services, including tailored wildflower habitats (Barreiro-Hurlé, Espinosa-Goded and Dupraz, 2010).

6.3. Study limitations

The study was conducted in commercial orange orchards which brings associated challenges, such as the continued use of plant protection products (PPPs) which can disrupt pest regulation services (McKerchar *et al.*, 2020) and the trampling of the wildflower habitat during harvest (early spring) which can also affect the establishment and persistence of sown species (Whitecotton *et al.*, 2000). However, for the study to be directly relevant to citrus growers, it was essential that the research was carried out in commercial orchards.

The study was also affected by the poor establishment of wildflowers in two of the replicate blocks which had to be abandoned due to poor drainage leading to pools of standing water in the winter after sowing. A further replicate block was lost in the third year when the grower regrafted the trees to change the variety, which involved the removal of the whole citrus canopy. As such, in years one and two of the study, four replicate blocks were studied and in year three, three replicate blocks were used (except for alleyway plant studies which were conducted in all four blocks. A lower number of replicated blocks can introduce more variance due to unexplained differences between sites (Dutilleul, 1993).

6.4. Recommendations for growers

Based on the findings of this study, the sown wildflower habitats managed under the standard cutting treatment are recommended for use is Mediterranean citrus. Of the 14 species sown, only the ten which successfully established could be recommended (the two tussock-forming grasses, *Dactylis glomerata* and *Schedonorus (Festuca) arundinaceus;* and the eight forbs, *Anchusa azurea, Salvia verbenaca, Psoralea bituminosa, Mentha suaveolens, Plantago lanceolata, Marrubium vulgare, Achillea millefolium, and Cichorium intybus*) (Chapter 3three). Furthermore, two of these species were associated with citrus pests in autumn, *Cichorium intybus* with *Planococcus citri* Risso (Hemiptera: Pseudococcidae) and *Achillea millefolium* with *Coccus hesperidium* L. (Hemiptera: Coccidae). As such, until further research has investigated the possible impact, these two plant species would not be recommended for inclusion. The floral architecture of an unsown species, *Heliotropium europaeum* (Boraginaceae), enabled access to more than 95% of the parasitoid community sampled from the orange orchards and has a long bloom period (March until November), which includes the summer months when carbohydrate resource is typically limited (Blamey and Grey-Wilson, 2004). Furthermore, *H. europaeum* was recorded at two out of three orchards, suggesting it would establish well across different sites. Based on establishment success and associations with beneficial arthropods, a total of nine plant species are therefore recommended for use in future wildflower seed mixes aimed at enhancing pest regulation services in Mediterranean citrus (). A sowing rate of 5 to 6 gm^{-2} is recommended for successful establishment, with *H. europaeum* sown at the same rate as *Marrubium vulgare* and the two grass species. If the wildflower habitat is to be established specifically to support parasitoids, the sowing rate of *Salvia verbenaca*, *H. europaeum* and *Mentha suaveolens* could be increased, as these species were found to provide accessible nectar to almost all of the parasitoid community recorded (>95%). If the wildflower strips were to target predators, the sowing rate of *Marrubium vulgare* could be increased as it serves as a reservoir for alternative hosts and prey (Terrón-Sierra *et al.*, 2011) () and *Anchusa azurea*, which provides high quality pollen for hoverflies and other natural enemies (Pinheiro *et al.*, 2013a). The unsown species *Crepis vesicaria,* which can increase hoverfly longevity (Pinheiro *et al.*, 2013b), could also be included. The genus *Crepis* was identified during the study but with highest abundance in the control alleyways. Inclusion in the seed mix could help boost its abundance in the wildflower habitats to provide additional high value pollen for predators. Although *Plantago lanceolata* is a key source of pollen for predatory natural enemies (Morgado *et al.*, 2014) and alternative prey/hosts (Nieminen and Vikberg, 2015), it established well with high abundance in all wildflower treatments at the current rate, and so would not require increasing.

Table 6.1 Species and sowing rates (expressed as the percentage weight of seeds and the actual number of seeds sown per m^2 per species) recommended to enhance pest regulation services in Mediterranean citrus, sown at a total rate of 5 to 6 gm^{-2}.

Scientific name	Common name	Sowing rate (% by weight)	Actual sowing rate (seeds m^2)
Anchusa azurea	Bugloss	2.08	3-4
Salvia verbenaca	Wild clary	2.84	36-43
Psoralea bituminosa	Arabian pea	4.59	9-11
Mentha suaveolens	Yellow restharrow	13.64	8184-9621
Plantago lanceolata	Ribwort plantain	15.28	191-229
Dactylis glomerata	Orchard grass	15.39	770-923
Schedonorus (Festuca) arundinaceus	Tall fescue	15.39	693-831
Marrubium vulgare	White horehound	15.39	385-462
Heliotropium europaeum	European turn-sole	15.39	462-554
Total		100.00	10731-12878

To harness the full benefits of the wildflower habitat, after the establishment year, one cut a year in spring (early May) to a height of 5-10 cm is recommended to increase the competitive advantage of the sown species against naturally occurring annual species (Woodcock *et al.*, 2005). Ideally, cuttings should be collected (Nowakowski and Pywell, 2016), however this is not always possible due to lack of farm equipment. In this case, and as in this study, cuttings can be left *in situ.* The wildflower habitat should be left to grow throughout the rest of the year in order to provision resource for natural enemies and enhance their abundance and pest regulation services in the crop, as in the standard management wildflower treatment. If cutting to a shorter height is desired to target observed pest incidence, this should not be done during the summer months so that the sward is able to recover and continue to support natural enemies. These management strategies could also be applied in a way which would increase structural heterogeneity, such as cutting only part of the habitat (Blake *et al.*, 2011).

Figure 6.1 *Marrubium vulgare* in the wildflower habitat supports a population of non-pest aphid species which provides a source of carbohydrate.

6.5. Further work

To overcome the issue of continued use of PPPs, further study is required to evaluate the role of wildflower habitat under organic production or at least reduced pesticide use in order to determine their true ecological benefit. Furthermore, there is some concern under the arid climate in Southern Spain, and other Mediterranean biomes, that wildflower habitats may compete with the crop for the limited supply of water (Herz *et al.*, 2019). Determining their potential impact would further help determine their suitability in other crops.

Different citrus species and growing regions are affected by distinct crop pest (Urbaneja, Grout, *et al.*, 2020). This study was conducted in sweet orange (*Citrus sinensis*), but further research should determine the suitability of these wildflower habitats in other citrus species and varieties across the Mediterranean citrus growing region. This might be particularly important in clementine orchards where pollination can increase seediness (Chao, 2005) and the provision of a wildflower resource is likely to boost pollinators . Furthermore, as the habitats were able to support a diverse range of generalist predators and increase abundance of other parasitoids, not only those implicit in the control of citrus pests, their use in a wider range of crops grown in the Mediterranean region, other than citrus, should also be studied.

The effect of wildflower habitats on biodiversity and other ecosystem services should also be investigated. This study has focused on biological control, however wildflower habitat can also provide others ecosystem services, such as soil protection, carbon storage, nitrogen fixation, weed control, and water resource management (Wratten *et al.*, 2012; Haddaway *et al.*, 2016). Furthermore, a key aim of habitat management moving forward should not only be to provide ecosystem services but also to support biodiversity within increasingly conflicted landscapes (Gurr *et al.*, 2017).

In hot and arid climates such as the Mediterranean, shelter from adverse temperatures is a key resource required by natural enemies (Diehl, Wolters and Birkhofer, 2012). Where the wildflower habitat was cut to 10 cm during the summer months in the active management wildflower treatment, abundance of natural enemies decreased and pest regulation services were diminished, which is likely due to the loss of this key resource, at least in part. Further studies should investigate the internal microclimate of the wildflower habitat and the crop in the Mediterranean and couple this with the diel directional movement of natural enemies between the canopy and the wildflower habitat to determine whether natural enemies do indeed migrate between wildflower habitats and the crop in response to climatic conditions. Furthermore, understanding when and why natural enemies migrate between the wildflower habitat and the crop habitat could further inform timing of the active management (by cutting) of the wildflower habitat. Further studies should investigate whether changing the timing of the cutting, altering the height at which the wildflower habitat is cut (see Mateos-fierro *et al.* 2021), and whether partial cutting of the habitat, as in Blake *et al.* (2011) may increase spill-over of natural enemies onto the adjacent crop canopy and enhance pest regulations services during pest critical periods.

Further studies should also investigate the mechanisms delivering pest regulations services onto the crop by studying natural enemy species traits and trait diversity. For example, dispersal capacity is a strong predictor of pest regulation services (Samaranayake and Costamagna, 2018). Understanding dispersal capacity of natural enemies supported by wildflower habitats would help inform decisions such as the appropriate proximity to the crop and whether active management via cutting might be suitable.

6.6. Conclusions

Perennial, native wildflower habitats can be successfully established in commercial Mediterranean citrus orchards. In turn, this wildflower habitat boosted natural enemy richness and abundance in the adjacent crop canopy and enhanced pest regulation services. However, how the wildflower habitat was managed by cutting had a significant effect on natural enemy richness and abundance and the associated pest regulation services. The standard management wildflower treatment most strongly enhanced the abundance of primary parasitoids in the adjacent canopy and supported the greatest proportion of carbohydrate fed individuals. This was especially significant at the end of summer and during autumn when parasitism rates are typically limited by carbohydrate availability. Based on the findings of this study, it is recommended to leave the wildflower strips to grow throughout the year, cutting just once in spring, as in the standard management wildflower treatment. This treatment provided a diverse range of plant resource, carbohydrates, protein, shelter, and alternative oviposition sites, throughout the length of the sampling season (April to October).

To support ecological intensification of agriculture and sustainably feed an increasing global population, the repertoire of ecologically sound methods for use in IPM and organic production must be augmented. Under global climate change, environmental pollution, land-use change, biodiversity loss, and increased invasions of alien species, agriculture systems must become more resilient whilst simultaneously reducing agrochemical inputs. This study provides another method for citrus growers in Spain to help reduce the environment footprint of pest control, with the potential to implement them in other regions and crops.

6.7. References

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Appendix 1

Appendix 1.1 Holm's stepdown pairwise comparisons of negative binomial models for the abundances of the different plant species/general recorded from the alleyways under the three treatments; control (C), standard management wildflower (SM) active management wildflower (AM), during years two and three when these treatments were applied.

Year	Pair	Test	Test statistic (LR)	*P*-value
Two (2018)	SM:C	Holm´s	244.22	0.001 ***
	AM:C	Holm´s	247.45	0.001 ***
	AM:SM	Holm´s	74.52	0.004 **
Three (2019)	SM:C	Holm´s	178.69	0.001 ***
	AM:C	Holm´s	171.36	0.001 ***
	AM:SM	Holm´s	64.69	0.005 **

Appendix 1.2 Mean percentage cover of the ten resources classes between the two alleyway treatments investigated in year one (2017): the control treatment and the establishing wildflower treatments. Subscripts represent significant differences between treatments ($P < 0.05$)

Cover Class	Mean % cover (± SE)	
	Control	Establishing wildflower treatments
2017		
Bare soil	28.75 (±4.06)^{a}	24.58 (±3.44)^{a}
Leaf litter	21.77 (±2.43)^{a}	13.75(±2.07)^{b}
Vegetative forbs	2.00 (±0.58)^{b}	17.75 (±3.06)^{a}
Vegetative grasses	2.82 (±0.97)^{a}	6.17 (±1.75)^{a}
Budding forbs	1.62 (±0.66)^{a}	4.24 (±1.12)^{a}
Budding grasses	1.32 (±0.57)^{a}	0.65 (±0.52)^{a}
Flowering forbs	11.72 (±2.46)^{a}	10.01(±0.56)^{a}
Flowering grasses	18.95 (±3.54)^{a}	17.11 (±3.48)^{a}
Seeding forbs	8.06 (±1.93)^{a}	0.74 (±0.37)^{b}
Seeding grasses	1.96 (±1.26)^{a}	3.78 (±2.14)^{a}

Appendix 1.3 Mean percentage cover of the ten resources classes between the three treatments investigated in years two (2018) and three (2019): control, standard management wildflower treatment (standard management), and active management wildflower treatment (active management). Subscripts represent significant differences between treatments ($P < 0.05$)

Cover class	Mean % cover (± SE)		
	Control	Standard management	Active management
2018			
Bare soil	37.81 (±4.02)^{a}	12.19 (±1.57)^{b}	15.52 (±2.49)^{b}
Leaf litter	17.92 (±1.81)^{a}	18.23 (±2.25)^{a}	14.38 (±1.54)^{a}
Vegetative forbs	4.01 (±0.85)^{b}	14.80 (±1.73)^{a}	17.09 (±2.27)^{a}
Vegetative grasses	3.11 (±0.92)^{a}	2.91 (±0.87)^{a}	8.59 (±2.62)^{a}
Budding forbs	2.16 (±0.79)^{b}	11.10 (±2.10)^{a}	10.57 (±1.77)^{a}
Budding grasses	1.45 (±0.56)^{b}	3.20 (±0.95)^{ab}	9.26 (±2.46)^{a}
Flowering forbs	1.16 (±0.38)^{b}	7.92(±1.63)^{a}	5.23 (±1.42)^{a}
Flowering grasses	23.25 (±2.83)^{a}	16.72 (±3.31)^{b}	7.97 (±1.83)^{c}
Seeding forbs	1.11 (±0.47)^{a}	0.75 (±2.29)^{a}	2.84 (±1.90)^{a}
Seeding grasses	7.14 (±1.86)^{a}	2.69 (± 0.98)^{a}	4.13 (±1.65)^{a}
2019			
Bare soil	38.85 (±3.51)^{a}	23.13 (±2.50)^{b}	28.75(±2.58)^{ab}
Leaf litter	38.13 (±3.62)^{a}	3.08 (±0.675)^{a}	2.65 (±2.50)^{a}
Vegetative forbs	4.95 (±1.20)^{b}	13.04 (±2.48)^{a}	16.63 (±2.32)^{a}
Vegetative grasses	8.72 (±1.69)^{b}	8.45 (±1.92)^{b}	16.45 (±2.99)^{a}
Budding forbs	5.52 (±1.33)^{b}	14.34 (±2.77)^{a}	7.62 (±1.54)^{ab}
Budding grasses	3.11 (±0.84)^{b}	3.47 (±0.96)^{b}	10.34 (±1.99)^{a}
Flowering forbs	4.21 (±1.27)^{a}	8.24 (±1.87)^{a}	3.73 (±1.00)^{a}
Flowering grasses	10.83 (±1.81)^{a}	17.14 (±2.73)^{a}	12.32 (±1.63)^{a}
Seeding forbs	1.62 (±0.65)^{a}	5.22 (±1.31)^{a}	3.53 (±1.11)^{a}
Seeding grasses	1.25 (±0.60)^{b}	11.47 (±2.24)^{a}	3.91 (±0.94)^{b}

Appendix 2

Appendix 2.1 Mean abundance of different natural enemy families recorded in year one (2017) in the canopies with the two alleyway treatments: control and the establishing wildflower treatments.

Family	Order	Functional group	Control	Wildflower strips
15-May				
Salticidae	Araneae	Predator	0.16 (±0.07)	0.06 (±0.04)
Araneidae	Araneae	Predator	-	0.03 (± 0.03)
Clubionidae	Araneae	Predator	-	-
Cheiracanthiidae	Araneae	Predator	-	-
Sparassidae	Araneae	Predator	-	-
Lycosidae	Araneae	Predator	-	0.03 (±0.03)
Oxyopidae	Araneae	Predator	-	-
Thomisidae	Araneae	Predator	0.13 (±0.06)	-
Other Araneae	Araneae	Predator	1.19 (±0.32)	1.53 (±0.35)
Coccinellidae	Coleoptera	Predator	0.28 (±0.09)	0.22 (±0.09)
Carabidae	Coleoptera	Predator	-	-
Staphylinidae	Coleoptera	Predator	-	-
Nabidae	Hemiptera	Predator	-	-
Reduviidae	Hemiptera	Predator	-	-
Anthocoridae	Hemiptera	Predator	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-
Braconidae	Hymenoptera	Parasitoid	-	0.13 (±0.07)
Encyrtidae	Hymenoptera	Parasitoid	-	0.06 (±0.06)
Eulophidae	Hymenoptera	Parasitoid	-	0.03 (±0.03)
Pteromalidae	Hymenoptera	Parasitoid	-	-
Scelionidae	Hymenoptera	Parasitoid	-	0.06 (±0.04)
Chalcididae	Hymenoptera	Parasitoid	-	-
Chrysopidae	Neuroptera	Predator	0.63 (±0.15)	1.19 (±0.30)
Hemerobiidae	Neuroptera	Predator	-	0.06 (±0.04)
Myrmeleontidae	Neuroptera	Predator	-	-
Coniopterygidae	Neuroptera	Predator	0.03 (±0.03)	0.03 (±0.03)
Syrphidae	Diptera	Predator	-	-
Formicidae	Hymenoptera	Antagonist	0.97 (±0.24)	0.59 (±0.16)
12-Jun				
Salticidae	Araneae	Predator	0.03 (±0.03)	0.09 (±0.07)
Araneidae	Araneae	Predator	(± 0.04)	0.22 (±0.10)
Clubionidae	Araneae	Predator	-	-
Cheiracanthiidae	Araneae	Predator	-	-
Sparassidae	Araneae	Predator	-	-
Lycosidae	Araneae	Predator	-	-
Oxyopidae	Araneae	Predator	0.13 (±0.06)	0.06 (±0.04)
Thomisidae	Araneae	Predator	0.06 (±0.04)	0.06 (±0.04)

Family	Order	Functional group	Control	Wildflower strips
Other Araneae	Araneae	Predator	0.88 (±0.15)	0.59 (±0.15)
Coccinellidae	Coleoptera	Predator	0.06 (±0.04)	0.16 (±0.08)
Carabidae	Coleoptera	Predator	-	-
Staphylinidae	Coleoptera	Predator	-	-
Nabidae	Hemiptera	Predator	-	-
Reduviidae	Hemiptera	Predator	-	-
Anthocoridae	Hemiptera	Predator	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-
Braconidae	Hymenoptera	Parasitoid	-	-
Encyrtidae	Hymenoptera	Parasitoid	0.19 (±0.09)	0.22 (±0.10)
Eulophidae	Hymenoptera	Parasitoid	0.06 (±0.04)	-
Pteromalidae	Hymenoptera	Parasitoid	-	-
Scelionidae	Hymenoptera	Parasitoid	0.53 (±0.02)	0.06 (±0.04)
Chalcididae	Hymenoptera	Parasitoid		
Chrysopidae	Neuroptera	Predator	0.22 (±0.07)	0.66 (±0.12)
Hemerobiidae	Neuroptera	Predator	-	-
Myrmeleontidae	Neuroptera	Predator	-	-
Coniopterygidae	Neuroptera	Predator	-	-
Syrphidae	Diptera	Predator	-	-
Formicidae	Hymenoptera	Antagonist	0.75 (±0.23)	0.25 (±0.16)
10-July				
Salticidae	Araneae	Predator	0.25 (±0.09)	0.28 (±0.09)
Araneidae	Araneae	Predator	0.09 (±0.05)	-
Clubionidae	Araneae	Predator	-	-
Cheiracanthiidae	Araneae	Predator	-	-
Sparassidae	Araneae	Predator	-	-
Lycosidae	Araneae	Predator	-	-
Oxyopidae	Araneae	Predator	0.09 (±0.05)	0.06 (±0.04)
Thomisidae	Araneae	Predator	0.03 (±0.03)	-
Other Araneae	Araneae	Predator	0.44 (±0.13)	0.16 (±0.07)
Coccinellidae	Coleoptera	Predator	0.03 (±0.03)	0.16 (±0.07)
Carabidae	Coleoptera	Predator	-	-
Staphylinidae	Coleoptera	Predator	-	-
Nabidae	Hemiptera	Predator	-	-
Reduviidae	Hemiptera	Predator	-	-
Anthocoridae	Hemiptera	Predator	-	0.03 (±0.03)
Aphelinidae	Hymenoptera	Parasitoid	-	-
Braconidae	Hymenoptera	Parasitoid	0.09 (±0.05)	-
Encyrtidae	Hymenoptera	Parasitoid	0.03 (±0.03)	-
Eulophidae	Hymenoptera	Parasitoid	0.41 (±0.15)	0.34 (±0.12)
Pteromalidae	Hymenoptera	Parasitoid	-	-
Scelionidae	Hymenoptera	Parasitoid	0.06 (±0.04)	0.16 (±0.07)
Chalcididae	Hymenoptera	Parasitoid	-	-
Chrysopidae	Neuroptera	Predator	0.09 (±0.05)	0.19 (±0.08)
Hemerobiidae	Neuroptera	Predator	-	-

Family	Order	Functional group	Control	Wildflower strips
Myrmeleontidae	Neuroptera	Predator	-	-
Coniopterygidae	Neuroptera	Predator	-	-
Syrphidae	Diptera	Predator	-	-
Formicidae	Hymenoptera	Antagonist	0.16 (±0.07)	0.34 (±0.12)
04-Sep				
Salticidae	Araneae	Predator	0.09 (±0.05)	0.09 (±0.05)
Araneidae	Araneae	Predator	-	-
Clubionidae	Araneae	Predator	-	-
Cheiracanthiidae	Araneae	Predator	-	-
Sparassidae	Araneae	Predator	-	-
Lycosidae	Araneae	Predator	-	0.03 (±0.03)
Oxyopidae	Araneae	Predator	0.03 (±0.03)	-
Thomisidae	Araneae	Predator	-	0.03 (±0.03)
Other Araneae	Araneae	Predator	0.19 (±0.07)	0.09 (±0.05)
Coccinellidae	Coleoptera	Predator	0.09 (±0.05)	0.03 (±0.03)
Carabidae	Coleoptera	Predator	-	-
Staphylinidae	Coleoptera	Predator	-	-
Nabidae	Hemiptera	Predator	-	-
Reduviidae	Hemiptera	Predator	-	-
Anthocoridae	Hemiptera	Predator	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-
Braconidae	Hymenoptera	Parasitoid	-	0.03 (±0.03)
Encyrtidae	Hymenoptera	Parasitoid	-	0.03 (±0.03)
Eulophidae	Hymenoptera	Parasitoid	0.13 (±0.07)	0.16 (±0.07)
Pteromalidae	Hymenoptera	Parasitoid	-	-
Scelionidae	Hymenoptera	Parasitoid	0.06 (±0.04)	0.09 (±0.05)
Chalcididae	Hymenoptera	Parasitoid	-	-
Chrysopidae	Neuroptera	Predator	0.09 (±0.07)	0.06 (±0.04)
Hemerobiidae	Neuroptera	Predator	-	-
Myrmeleontidae	Neuroptera	Predator	-	-
Coniopterygidae	Neuroptera	Predator	-	-
Syrphidae	Diptera	Predator	0.06 (±0.04)	0.03 (±0.03)
Formicidae	Hymenoptera	Antagonist	0.34 (±0.20)	0.06 (±0.04)
02-Oct				
Salticidae	Araneae	Predator	0.25 (±0.10)	0.25 (±0.10)
Araneidae	Araneae	Predator	-	-
Clubionidae	Araneae	Predator	-	0.06 (±0.04)
Cheiracanthiidae	Araneae	Predator	0.03 (±0.03)	-
Sparassidae	Araneae	Predator	-	-
Lycosidae	Araneae	Predator	-	-
Oxyopidae	Araneae	Predator	0.03 (±0.03)	-
Thomisidae	Araneae	Predator	0.03 (±0.03)	-
Other Araneae	Araneae	Predator	0.19 (±0.07)	0.25 (±0.09)
Coccinellidae	Coleoptera	Predator	0.66 (±0.15)	0.19 (±0.07)

Family	Order	Functional group	Control	Wildflower strips
Carabidae	Coleoptera	Predator	-	-
Staphylinidae	Coleoptera	Predator	-	-
Nabidae	Hemiptera	Predator	-	-
Reduviidae	Hemiptera	Predator	-	-
Anthocoridae	Hemiptera	Predator	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-
Braconidae	Hymenoptera	Parasitoid	-	-
Encyrtidae	Hymenoptera	Parasitoid	0.06 (±0.04)	0.09 (±0.05)
Eulophidae	Hymenoptera	Parasitoid	0.16 (±0.07)	0.09 (±0.05)
Pteromalidae	Hymenoptera	Parasitoid	-	-
Scelionidae	Hymenoptera	Parasitoid	-	0.03 (±0.03)
Chalcididae	Hymenoptera	Parasitoid	-	-
Chrysopidae	Neuroptera	Predator	0.91 (±0.19)	1.13 (±0.21)
Hemerobiidae	Neuroptera	Predator	-	0.03 (±0.03)
Myrmeleontidae	Neuroptera	Predator	-	-
Coniopterygidae	Neuroptera	Predator	-	-
Syrphidae	Diptera	Predator	-	-
Formicidae	Hymenoptera	Antagonist	0.22 (±0.12)	0.44 (±0.19)

Appendix 2.2 Mean abundance of different natural enemy families recorded in year two (2018) in the canopies with the two alleyway treatments: control and the establishing wildflower treatments.

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
23-April					
Salticidae	Araneae	Predator	0.09 (±0.05)	0.19 (±0.07)	0.34 (±0.11)
Araneidae	Araneae	Predator	-	0.13 (±0.06)	-
Clubionidae	Araneae	Predator	0.06 (±0.04)	-	-
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	0.09 (±0.05)	-	0.03 (±0.03)
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	-	-
Thomisidae	Araneae	Predator	0.09 (±0.05)	-	-
Other Araneae	Araneae	Predator	0.19 (±0.07)	0.19 (±0.09)	0.28 (±0.13)
Coccinellidae	Coleoptera	Predator	0.22 (±0.07)	0.28 (±0.09)	0.25 (±0.09)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	0.03 (±0.03)	0.13 (±0.06)	0.03 (±0.03)
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-	-

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Braconidae	Hymenoptera	Parasitoid	2.56 (±0.56)	2.75 (±0.52)	2.56 (±0.48)
Encyrtidae	Hymenoptera	Parasitoid	0.09 (±0.07)	-	-
Eulophidae	Hymenoptera	Parasitoid	0.03 (±0.03)	-	0.03 (±0.03)
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.13 (±0.06)	0.28 (±0.10)	0.19 (±0.08)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.09 (±0.07)	0.13 (±0.07)	0.19 (±0.08)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	-	-	-
Syrphidae	Diptera	Predator	-	0.03 (±0.03)	0.06 (±0.04)
Formicidae	Hymenoptera	Antagonist	0.06 (±0.04)	0.94 (±0.45)	0.91 (±0.35)
21-May					
Salticidae	Araneae	Predator	-	0.03 (±0.03)	0.13 (±0.07)
Araneidae	Araneae	Predator	-	-	-
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	-	0.06 (±0.04)	0.03 (±0.03)
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	0.03 (±0.03)	-
Thomisidae	Araneae	Predator	-	0.13 (±0.06)	0.06 (±0.04)
Other Araneae	Araneae	Predator	0.81 (±0.20)	0.66 (±0.18)	0.81 (±0.12)
Coccinellidae	Coleoptera	Predator	0.34 (±0.12)	0.25 (±0.10)	0.56 (±0.16)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	-	-	0.09 (±0.05)
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-	-
Braconidae	Hymenoptera	Parasitoid	0.34 (±0.12)	0.28 (±0.09)	0.34 (±0.11)
Encyrtidae	Hymenoptera	Parasitoid	0.06 (±0.06)	0.31 (±0.16)	0.09 (±0.07)
Eulophidae	Hymenoptera	Parasitoid	-	-	-
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.19 (±0.08)	0.06 (±0.04)	0.13 (±0.07)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.16 (±0.08)	0.25 (±0.09)	0.47 (±0.13)
Hemerobiidae	Neuroptera	Predator	0.03 (±0.03)	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	0.04 (±0.04)	0.19 (±0.09)	0.04 (±0.04)
Syrphidae	Diptera	Predator	-	0.06 (±0.06)	0.03 (±0.03)
Formicidae	Hymenoptera	Antagonist	0.81 (±0.27)	0.47 (±0.15)	1.03 (±0.31)
18-June					
Salticidae	Araneae	Predator	0.09 (±0.05)	0.41 (±0.17)	0.06 (±0.04)
Araneidae	Araneae	Predator	0.16 (±0.11)	0.09 (±0.07)	-

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Clubionidae	Araneae	Predator	-	-	0.09 (±0.05)
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	0.03 (±0.03)	0.03 (±0.03)	-
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	-	-
Thomisidae	Araneae	Predator	1.28 (±0.34)	0.56 (±0.13)	0.53 (±0.15)
Other Araneae	Araneae	Predator	3.19 (±1.06)	1.94 (±0.64)	1.28 (±0.20)
Coccinellidae	Coleoptera	Predator	0.28 (±0.08)	0.38 (±0.11)	0.41 (±0.13)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	0.03 (±0.03)	-	-
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	0.03 (±0.03)	-	0.13 (±0.07)
Aphelinidae	Hymenoptera	Parasitoid	-	0.03 (±0.03)	-
Braconidae	Hymenoptera	Parasitoid	0.06 (±0.04)	0.06 (±0.04)	0.03 (±0.03)
Encyrtidae	Hymenoptera	Parasitoid	0.06 (±0.06)	0.19 (±0.13)	-
Eulophidae	Hymenoptera	Parasitoid	-	0.03 (±0.03)	0.03 (±0.03)
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.03 (±0.03)	0.06 (±0.04)	0.34 (±0.16)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.69 (±0.22)	0.84 (±0.19)	0.28 (±0.08)
Hemerobiidae	Neuroptera	Predator	-	-	0.03 (±0.03)
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	0.03 (±0.03)	0.03 (±0.03)	0.06 (±0.06)
Syrphidae	Diptera	Predator	0.06 (±0.04)	0.03 (±0.03)	-
Formicidae	Hymenoptera	Antagonist	2.69 (±0.63)	1.41 (±0.25)	0.63 (±0.24)
16-July					
Salticidae	Araneae	Predator	0.56 (±0.12)	0.31 (±0.09)	0.72 (±0.19)
Araneidae	Araneae	Predator	0.13 (±0.07)	0.19 (±0.09)	0.06 (±0.04)
Clubionidae	Araneae	Predator	-	0.06 (±0.04)	0.09 (±0.05)
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	0.13 (±0.06)	0.22 (±0.16)	0.13 (±0.07)
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	0.06 (±0.04)	-	0.09 (±0.04)
Thomisidae	Araneae	Predator	0.19 (±0.08)	0.19 (±0.08)	0.28 (±0.14)
Other Araneae	Araneae	Predator	0.47 (±0.10)	0.75 (±0.20)	0.69 (±0.15)
Coccinellidae	Coleoptera	Predator	0.34 (±0.10)	0.44 (±0.13)	0.41 (±0.13)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	-	-	-
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-	-
Braconidae	Hymenoptera	Parasitoid	-	-	-
Encyrtidae	Hymenoptera	Parasitoid	-	0.03 (±0.03)	-

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Eulophidae	Hymenoptera	Parasitoid	0.03 (±0.03)	0.06 (±0.04)	-
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.09 (±0.07)	0.06 (±0.04)	0.06 (±0.04)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.13 (±0.07)	0.06 (±0.04)	0.16 (±0.07)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	0.09 (±0.07)	-	-
Syrphidae	Diptera	Predator	-	0.03 (±0.03)	-
Formicidae	Hymenoptera	Antagonist	7.91 (±2.81)	4.22 (±1.12)	1.63 (±0.52)
13-August					
Salticidae	Araneae	Predator	0.28 (±0.09)	0.78 (±0.18)	0.56 (±0.13)
Araneidae	Araneae	Predator	0.06 (±0.04)	-	-
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	0.09 (±0.05)	-	0.06 (±0.04)
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	-	-
Thomisidae	Araneae	Predator	0.03 (±0.03)	0.16 (±0.08)	0.13 (±0.06)
Other Araneae	Araneae	Predator	0.41 (±0.10)	0.84 (±0.14)	0.69 (±0.21)
Coccinellidae	Coleoptera	Predator	0.16 (±0.08)	0.28 (±0.09)	0.63 (±0.15)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	-	-	-
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-	-
Braconidae	Hymenoptera	Parasitoid	-	-	-
Encyrtidae	Hymenoptera	Parasitoid	0.06 (±0.04)	-	-
Eulophidae	Hymenoptera	Parasitoid	0.06 (±0.04)	0.06 (±0.04)	0.09 (±0.05)
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.09 (±0.05)	0.34 (±0.11)	0.13 (±0.06)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.16 (±0.07)	0.13 (±0.06)	0.06 (±0.04)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	-	-	-
Syrphidae	Diptera	Predator	-	-	-
Formicidae	Hymenoptera	Antagonist	2.34 (±0.81)	2.59 (±0.70)	1.28 (±0.60)
17-September					
Salticidae	Araneae	Predator	0.66 (±0.19)	0.38 (±0.09)	0.34 (±0.11)
Araneidae	Araneae	Predator	0.03 (±0.03)	-	-
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	-	-	-

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Sparassidae	Araneae	Predator	0.03 (±0.03)	0.06 (±0.04)	0.03 (±0.03)
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	0.03 (±0.03)	-
Thomisidae	Araneae	Predator	0.06 (±0.04)	0.25 (±0.10)	0.19 (±0.07)
Other Araneae	Araneae	Predator	0.75 (±0.16)	0.47 (±0.13)	0.34 (±0.11)
Coccinellidae	Coleoptera	Predator	0.19 (±0.08)	0.31 (±0.19)	0.19 (±0.07)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	0.03 (±0.03)	-	0.06 (±0.06)
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	-	0.03 (±0.03)	-
Aphelinidae	Hymenoptera	Parasitoid	-	-	-
Braconidae	Hymenoptera	Parasitoid	-	-	0.06 (±0.04)
Encyrtidae	Hymenoptera	Parasitoid	0.16 (±0.08)	0.28 (±0.12)	0.34 (±0.14)
Eulophidae	Hymenoptera	Parasitoid	0.22 (±0.09)	0.53 (±0.12)	0.34 (±0.11)
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.31 (±0.12)	0.72 (±0.15)	0.13 (±0.06)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.03 (±0.03)	0.47 (±0.13)	0.19 (±0.08)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	-	-	-
Syrphidae	Diptera	Predator	-	-	-
Formicidae	Hymenoptera	Antagonist	0.69 (±0.26)	1.50 (±0.72)	1.09 (±0.40)
15-October					
Salticidae	Araneae	Predator	0.28 (±0.11)	0.41 (±0.13)	0.44 (±0.16)
Araneidae	Araneae	Predator	-	0.03 (±0.03)	-
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	-	0.06 (±0.04)	0.09 (±0.07)
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	0.03 (±0.03)	0.03 (±0.03)
Thomisidae	Araneae	Predator	0.03 (±0.03)	0.03 (±0.03)	0.16 (±0.08)
Other Araneae	Araneae	Predator	0.28 (±0.10)	0.34 (±0.12)	0.44 (±0.12)
Coccinellidae	Coleoptera	Predator	0.13 (±0.06)	0.25 (±0.10)	0.19 (±0.07)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	0.09 (±0.07)	0.34 (±0.11)	0.19 (±0.09)
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	0.03 (±0.03)	0.03 (±0.03)
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	0.03 (±0.03)	0.03 (±0.03)
Braconidae	Hymenoptera	Parasitoid	0.06 (±0.04)	0.13 (±0.07)	0.03 (±0.03)
Encyrtidae	Hymenoptera	Parasitoid	0.38 (±0.15)	1.19 (±0.25)	0.25 (±0.10)
Eulophidae	Hymenoptera	Parasitoid	0.66 (±0.23)	0.84 (±0.28)	1.50 (±0.49)
Pteromalidae	Hymenoptera	Parasitoid	-	-	-

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Scelionidae	Hymenoptera	Parasitoid	0.09 (±0.05)	0.31 (±0.09)	0.25 (±0.16)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.22 (±0.09)	0.41 (±0.12)	0.41 (±0.13)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	-	-	-
Syrphidae	Diptera	Predator	-	0.03 (±0.03)	0.03 (±0.03)
Formicidae	Hymenoptera	Antagonist	0.06 (±0.06)	0.31 (±0.10)	0.13 (±0.07)

Appendix 2.3 Mean abundance of different natural enemy families recorded in year three (2019) in the canopies with the two alleyway treatments: control and the establishing wildflower treatments

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
08-April					
Salticidae	Araneae	Predator	0.17 (±0.10)	0.42 (±0.15)	0.38 (±0.15)
Araneidae	Araneae	Predator	-	-	-
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	-	0.21 (±0.08)	0.13(±0.07)
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	0.08 (±0.06)	0.04(±0.04)
Thomisidae	Araneae	Predator	0.17 (±0.08)	0.13(±0.09)	0.58 (±0.24)
Other Araneae	Araneae	Predator	0.58 (±0.22)	0.63 (±0.15)	0.63 (±0.17)
Coccinellidae	Coleoptera	Predator	0.21 (±0.10)	0.58 (±0.17)	0.33 (±0.12)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	0.21 (±0.13)	0.17 (±0.10)	0.08 (±0.06)
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	0.04 (±0.04)	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	0.04 (±0.04)	-
Braconidae	Hymenoptera	Parasitoid	2.17 (±0.49)	3.58 (±0.87)	2.92 (±0.48)
Encyrtidae	Hymenoptera	Parasitoid	-	-	-
Eulophidae	Hymenoptera	Parasitoid	0.04 (±0.04)	0.21 (±0.08)	0.29 (±0.19)
Pteromalidae	Hymenoptera	Parasitoid	0.08 (±0.08)	0.29 (±0.14)	0.17 (±0.08)
Scelionidae	Hymenoptera	Parasitoid	0.04 (±0.04)	0.08 (±0.08)	0.04 (±0.04)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.63 (±0.25)	0.46 (±0.16)	0.71 (±0.18)

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Hemerobiidae	Neuroptera	Predator	-	0.04 (±0.04)	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	0.08 (±0.06)	0.04 (±0.04)	0.08 (±0.06)
Syrphidae	Diptera	Predator	-	--	
Formicidae	Hymenoptera	Antagonist	0.08 (0.06)	0.33 (±0.16)	0.13 (±0.13)
06-May					
Salticidae	Araneae	Predator	0.13 (±0.09)	0.21 (±0.10)	0.17 (±0.10)
Araneidae	Araneae	Predator	-	-	-
Clubionidae	Araneae	Predator	0.04 (±0.04)	-	-
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	0.04 (±0.04)	0.08 (±0.06)	-
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	-	0.04 (±0.04)
Thomisidae	Araneae	Predator	0.21 (±0.12)	0.79 (±0.26)	0.83 (±0.29)
Other Araneae	Araneae	Predator	1.50 (±0.28)	2.67 (±0.29)	1.83 (±0.34)
Coccinellidae	Coleoptera	Predator	0.71 (±0.16)	3.17 (±0.74)	1.08 (±0.41)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator-	-	0.04 (±0.04)	0.04 (±0.04)
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	0.04 (±0.04)
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	0.08 (±0.06)	-
Braconidae	Hymenoptera	Parasitoid	0.13 (±0.07)	0.21 (±0.10)	0.08 (0.06)
Encyrtidae	Hymenoptera	Parasitoid	0.25 (±0.11)	0.71 (±0.21)	0.33 (±0.12)
Eulophidae	Hymenoptera	Parasitoid	-	0.04 (±0.04)	-
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.17 (±0.08)	0.96 (±0.29)	0.67 (±0.21)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.54 (±0.23)	1.00 (±0.24)	0.42 (±0.13)
Hemerobiidae	Neuroptera	Predator	-	-	0.04 (±0.04)
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	0.08 (0.08)	0.04 (±0.04)	0.21 (±0.10)
Syrphidae	Diptera	Predator	-	-	0.04 (±0.04)
Formicidae	Hymenoptera	Antagonist	1.42 (±0.52)	2.08 (±0.76)	0.54 (±0.17)
03-June					
Salticidae	Araneae	Predator	0.13 (±0.17)	0.83 (±0.23)	0.25 (±0.11)
Araneidae	Araneae	Predator	0.71 (±0.25)	0.33 (±0.17)	0.21 (±0.12)
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	-	0.08 (0.06)	-
Sparassidae	Araneae	Predator	-	-	-
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	-	-
Thomisidae	Araneae	Predator	2.08 (±0.52)	1.67 (±0.55)	1.42 (±0.35)
Other Araneae	Araneae	Predator	1.46 (±0.46)	0.50 (±0.22)	1.25 (±0.31)

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Coccinellidae	Coleoptera	Predator	1.17 (±0.32)	1.46 (±0.47)	0.54 (±0.17)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	-	-	-
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	0.08 (±0.06)	0.04 (±0.04)	-
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-	-
Braconidae	Hymenoptera	Parasitoid	-	-	-
Encyrtidae	Hymenoptera	Parasitoid	0.17 (±0.10)	0.71 (±0.22)	0.08 (±0.06)
Eulophidae	Hymenoptera	Parasitoid	0.04(±0.04)	-	-
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.13 (±0.07)	0.83 (±0.30)	0.79 (±0.32)
Chalcididae	Hymenoptera	Parasitoid	0.08 (±0.06)	-	-
Chrysopidae	Neuroptera	Predator	0.67 (±0.17)	0.33 (±0.14)	0.42 (±0.12)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	0.08 (±0.06)	0.04 (±0.04)	0.04 (±0.04)
Syrphidae	Diptera	Predator	-	-	-
Formicidae	Hymenoptera	Antagonist	2.04 (±0.66)	2.42 (±0.74)	1.92 (±0.87)
01-July					
Salticidae	Araneae	Predator	0.63 (±0.15)	0.96 (±0.27)	0.71 (±0.24)
Araneidae	Araneae	Predator	0.06 (±0.06)	0.17 (±0.12)	0.08 (±0.08)
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	-	0.13 (±0.09)	-
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	0.04 (±0.04)	0.13 (±0.09)	0.04 (±0.04)
Thomisidae	Araneae	Predator	0.42 (±0.16)	0.58 (±0.26)	0.46 (±0.12)
Other Araneae	Araneae	Predator	0.83 (±0.23)	1.25 (±0.25)	1.29 (±0.29)
Coccinellidae	Coleoptera	Predator	0.83 (±0.21)	1.50 (±0.32)	1.00 (±0.26)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	-	-	0.04 (±0.04)
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	0.13 (±0.09)	-
Braconidae	Hymenoptera	Parasitoid	0.08 (±0.06)	0.04 (±0.04)	0.04 (±0.04)
Encyrtidae	Hymenoptera	Parasitoid	0.17 (±0.10)	1.04 (±0.29)	0.38 (±0.16)
Eulophidae	Hymenoptera	Parasitoid	0.21 (±0.08)	0.13 (±0.07)	0.08 (±0.06)
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.46 (±0.12)	0.79 (±0.19)	0.50 (±0.16)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.58 (±0.22)	0.75 (±0.24)	0.63 (±0.20)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Coniopterygidae	Neuroptera	Predator	0.13 (±0.09)	0.13 (±0.07)	0.33 (±0.14)
Syrphidae	Diptera	Predator	-	-	-
Formicidae	Hymenoptera	Antagonist	1.42 (±0.60)	1.08 (±0.36)	0.50 (±0.17)
29-July					
Salticidae	Araneae	Predator	0.83 (±0.22)	1.33 (±0.30)	0.88 (±0.21)
Araneidae	Araneae	Predator	-	0.04 (±0.04)	-
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	0.21 (±0.12)	0.08 (±0.06)	0.38 (±0.12)
Sparassidae	Araneae	Predator	-	-	-
Lycosidae	Araneae	Predator	0.33 (±0.14)	0.50 (±0.17)	0.17 (±0.13)
Oxyopidae	Araneae	Predator	0.13 (±0.07)	0.38 (±0.13)	0.33 (±0.14)
Thomisidae	Araneae	Predator	0.50 (±0.16)	0.38 (±0.17)	0.38 (±0.16)
Other Araneae	Araneae	Predator	0.29 (±0.09)	1.17 (±0.27)	0.92 (±0.27)
Coccinellidae	Coleoptera	Predator	1.17 (±0.22)	1.33 (±0.25)	0.92 (±0.27)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	-	-	-
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	0.04 (±0.04)	0.04 (±0.04)
Anthocoridae	Hemiptera	Predator	-	0.04 (±0.04)	-
Aphelinidae	Hymenoptera	Parasitoid	0.08 (±0.08)	0.04 (±0.04)	0.04 (±0.04)
Braconidae	Hymenoptera	Parasitoid	-	0.04 (±0.04)	-
Encyrtidae	Hymenoptera	Parasitoid	1.17 (±0.52)	2.54 (±0.52)	1.46 (±0.47)
Eulophidae	Hymenoptera	Parasitoid	0.25 (±0.09)	0.33 (±0.16)	0.58 (±0.21)
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.33 (±0.14)	0.63 (±0.20)	0.17 (±.0.08)
Chalcididae	Hymenoptera	Parasitoid	0.04 (±0.04)	0.08 (±0.06)	-
Chrysopidae	Neuroptera	Predator	0.71 (±0.22)	0.71 (±0.20)	0.63 (±0.18)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	0.04 (±0.04)	-
Coniopterygidae	Neuroptera	Predator	2.42 (±0.71)	2.25 (±0.62)	2.25 (±0.74)
Syrphidae	Diptera	Predator	-	-	-
Formicidae	Hymenoptera	Antagonist	1.75 (±0.85)	1.79 (±0.63)	1.29 (±0.77)
26-August					
Salticidae	Araneae	Predator	0.88 (±0.19)	0.79 (±0.18)	0.63 (±0.17)
Araneidae	Araneae	Predator	-	-	0.08 (±0.06)
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	0.08 (±0.06)	0.38 (±0.12)	0.17 (±0.08)
Sparassidae	Araneae	Predator	-	-	-
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	0.46 (±0.16)	0.17 (±0.08)	0.21 (±0.08)
Thomisidae	Araneae	Predator	0.42 (±0.16)	0.13 (±0.07)	0.58 (±0.18)
Other Araneae	Araneae	Predator	0.33 (±0.12)	0.29 (±0.11)	0.33 (±0.10)
Coccinellidae	Coleoptera	Predator	0.63 (±0.18)	1.50 (±0.32)	0.75 (±0.19)
Carabidae	Coleoptera	Predator	-	-	-

Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Staphylinidae	Coleoptera	Predator	-	-	-
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	-	0.04 (±0.04)	-
Aphelinidae	Hymenoptera	Parasitoid	-	0.08 (±0.06)	0.08 (±0.08)
Braconidae	Hymenoptera	Parasitoid	0.08 (±0.08)	0.04 (±0.04)	0.08 (±0.08)
Encyrtidae	Hymenoptera	Parasitoid	0.67 (±0.27)	0.88 (±0.23)	0.63 (±0.23)
Eulophidae	Hymenoptera	Parasitoid	0.46 (±0.18)	0.50 (±0.20)	0.33 (±0.16)
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	1.33 (±0.30)	0.63 (±0.27)	0.92 (±0.19)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.46 (±0.18)	0.83 (±0.26)	0.25 (±0.12)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	0.38 (±0.12)	0.25 (±0.11)	0.96 (±0.38)
Syrphidae	Diptera	Predator	-	-	-
Formicidae	Hymenoptera	Antagonist	1.08 (±0.32)	2.79 (±1.22)	2.75 (±1.41)
23-September					
Salticidae	Araneae	Predator	0.50 (±0.17)	0.58 (±0.17)	0.71 (±0.19)
Araneidae	Araneae	Predator	0.04 (±0.04)	-	-
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	0.21 (±0.10)	0.08 (±0.06)	0.33 (±0.13)
Sparassidae	Araneae	Predator	-	-	-
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	0.17 (±0.13)	0.08 (±0.06)	0.04 (±0.04)
Thomisidae	Araneae	Predator	0.21 (±0.10)	0.21 (±0.08)	0.25 (±0.12)
Other Araneae	Araneae	Predator	0.33 (±0.18)	0.25 (±0.12)	0.33 (±0.13)
Coccinellidae	Coleoptera	Predator	0.29 (±0.11)	0.71 (±0.18)	0.46 (±0.15)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	-	-	-
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	0.04 (±0.04)	-	-
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	-	-	0.04 (±0.04)
Braconidae	Hymenoptera	Parasitoid	0.08 (±0.06)	0.13 (±0.07)	-
Encyrtidae	Hymenoptera	Parasitoid	1.00 (±0.20)	0.88 (±0.26)	0.42 (±0.15)
Eulophidae	Hymenoptera	Parasitoid	0.17 (±0.10)	0.25 (±0.11)	0.33 (±0.17)
Pteromalidae	Hymenoptera	Parasitoid	-	-	-
Scelionidae	Hymenoptera	Parasitoid	0.71 (±0.21)	0.33 (±0.14)	0.17 (±0.10)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.63 (±0.17)	0.79 (±0.25)	0.46 (±0.16)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	0.29 (±0.11)	0.63 (±0.25)	0.42 (±0.16)
Syrphidae	Diptera	Predator	-	-	-

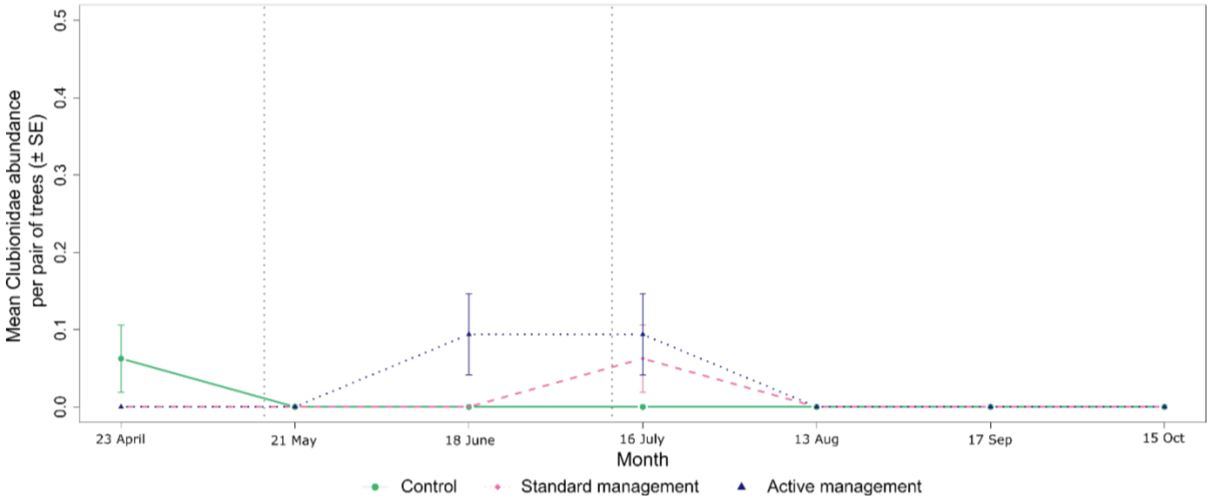
Family	Order	Functional group	Control	Standard wildflower management	Active wildflower management
Formicidae	Hymenoptera	Antagonist	2.17 (±1.28)	1.88 (±0.89)	0.96 (±0.35)
21-October					
Salticidae	Araneae	Predator	0.08 (±0.06)	0.13 (±0.07)	0.29 (±0.15)
Araneidae	Araneae	Predator	-	-	-
Clubionidae	Araneae	Predator	-	-	-
Cheiracanthiidae	Araneae	Predator	-	-	-
Sparassidae	Araneae	Predator	0.04 (±0.04)	-	-
Lycosidae	Araneae	Predator	-	-	-
Oxyopidae	Araneae	Predator	-	-	-
Thomisidae	Araneae	Predator	0.25 (±0.12)	0.13 (±0.07)	0.04 (±0.04)
Other Araneae	Araneae	Predator	0.71 (±0.26)	0.63 (±0.18)	0.50 (±0.17)
Coccinellidae	Coleoptera	Predator	0.21 (±0.10)	0.29 (±0.09)	0.29 (±0.11)
Carabidae	Coleoptera	Predator	-	-	-
Staphylinidae	Coleoptera	Predator	0.04 (±0.04)	0.04 (±0.04)	0.13 (±0.09)
Nabidae	Hemiptera	Predator	-	-	-
Reduviidae	Hemiptera	Predator	-	-	-
Anthocoridae	Hemiptera	Predator	-	-	-
Aphelinidae	Hymenoptera	Parasitoid	0.04 (±0.04)	-	-
Braconidae	Hymenoptera	Parasitoid	-	0.04 (±0.04)	0.04 (±0.04)
Encyrtidae	Hymenoptera	Parasitoid	0.25 (±0.11)	0.17 (±0.08)	0.17 (±0.08)
Eulophidae	Hymenoptera	Parasitoid	-	0.04 (±0.04)	-
Pteromalidae	Hymenoptera	Parasitoid	0.04 (±0.04)	-	-
Scelionidae	Hymenoptera	Parasitoid	-	0.04 (±0.04)	0.04 (±0.04)
Chalcididae	Hymenoptera	Parasitoid	-	-	-
Chrysopidae	Neuroptera	Predator	0.29 (±0.14)	0.50 (±0.16)	0.42 (±0.17)
Hemerobiidae	Neuroptera	Predator	-	-	-
Myrmeleontidae	Neuroptera	Predator	-	-	-
Coniopterygidae	Neuroptera	Predator	0.42 (±0.12)	0.63 (±0.21)	0.83 (±0.32)
Syrphidae	Diptera	Predator	0.08 (±0.06)	0.04 (±0.04)	-
Formicidae	Hymenoptera	Antagonist	0.21 (±0.10)	0.50 (±0.30)	1.00 (±0.26)

Appendix 2.4 Pairwise comparison in abundance of natural enemy families recorded from the citrus canopy with the three difference treatments in the alleyways: control (C), standard management wildflower treatment (SMW), and active management wildflower treatment (AMW), during years two and three when these treatments were applied.

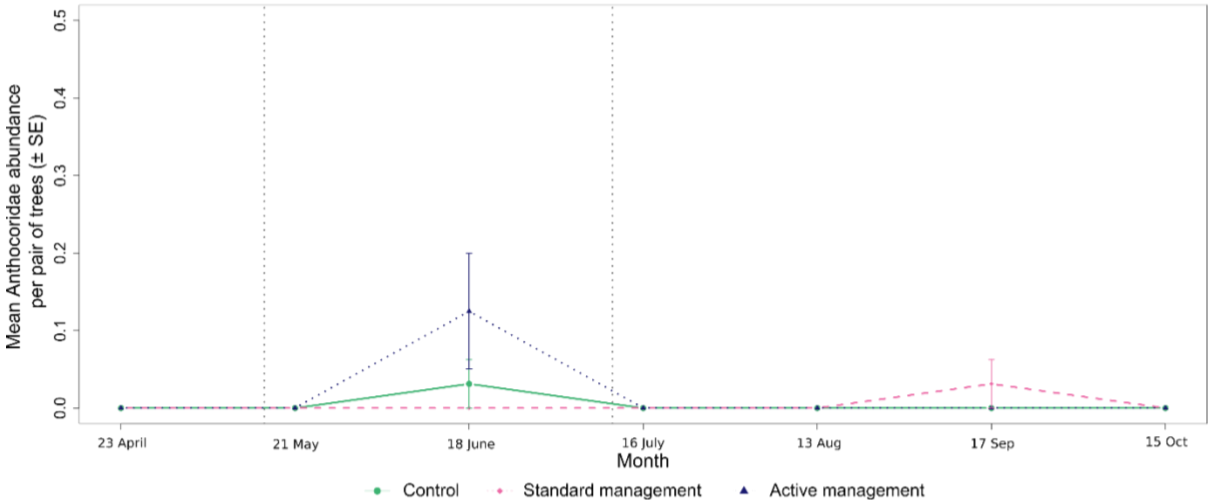
Year	Pair	Test	Test statistic (LR)	*P*-value
Two (2018)	SMW:C	Holm´s	31.60	0.001
	AMW:C	Holm´s	25.61	0.003
	AMW:SMW	Holm´s	10.20	0.155
Three (2019)	SMW:C	Holm´s	57.54	0.001
	AMW:C	Holm´s	12.52	0.075
	AMW:SMW	Holm´s	57.90	0.001

Appendix 2.5 Pairwise comparison in abundance of natural enemy families recorded from the citrus canopy with the three difference treatments in the alleyways: control (C), standard management wildflower treatment (SMW), and active management wildflower treatment (AMW), during years two and three when these treatments were applied.

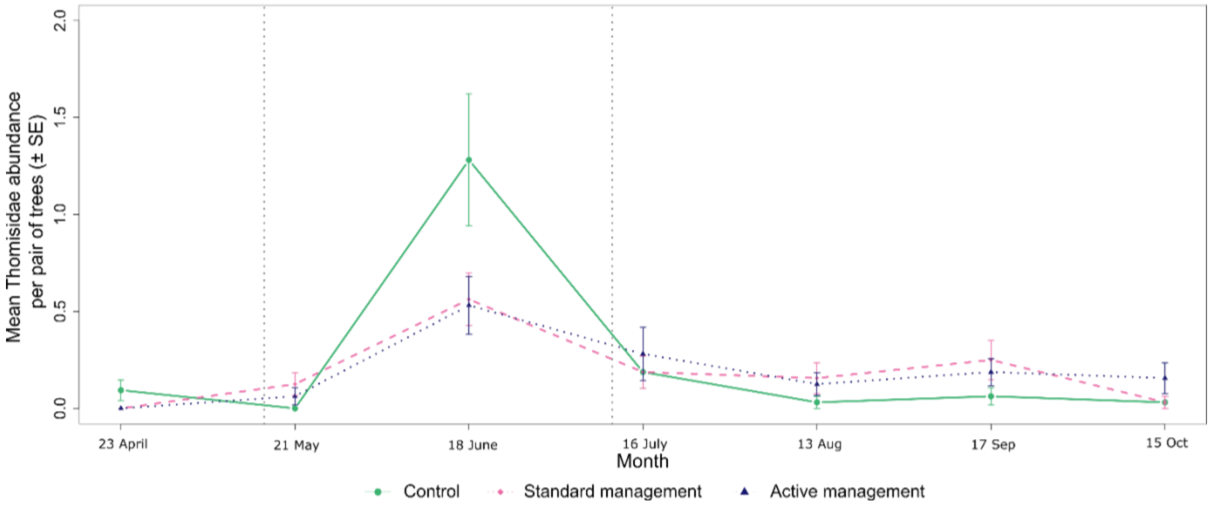
Year	Pair	Test	Test statistic (LR)	*P*-value
Two (2018)	SMW:C	Holm´s	41.10	0.047
	AMW:C	Holm´s	38.24	0.060
	AMW:SMW	Holm´s	36.23	0.060
Three (2019)	SMW:C	Holm´s	51.84	0.001
	AMW:C	Holm´s	11.02	0.883
	AMW:SMW	Holm´s	43.65	0.004



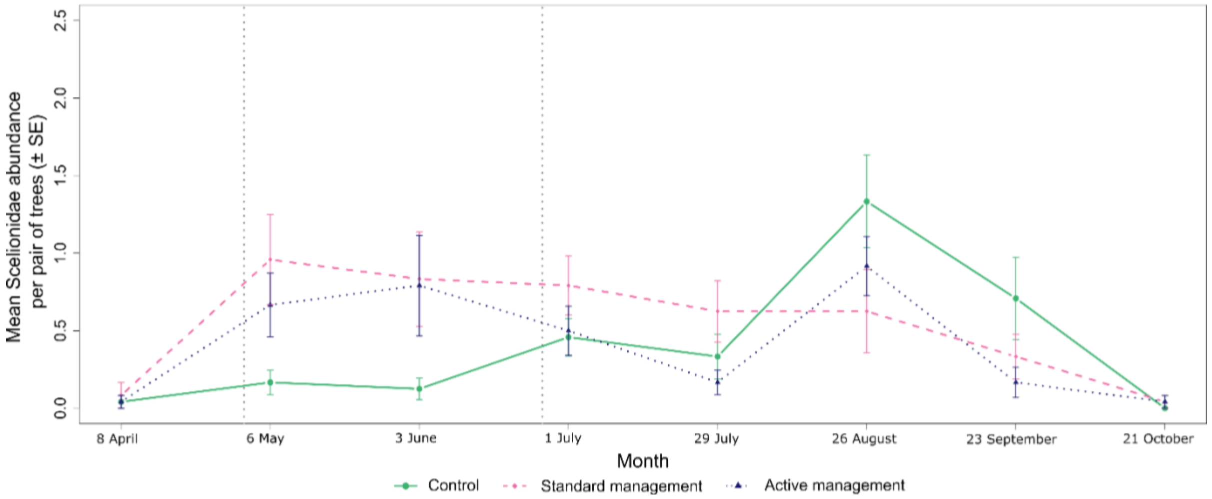
Appendix 2.6 Mean monthly abundance of the Clubionidae between treatments; control (green), standard management (pink), and active management (blue), during the year two (2018). effect (overall treatment and treatment * month interaction) within the 2018 model. Error bars represent ± 1 SEM.



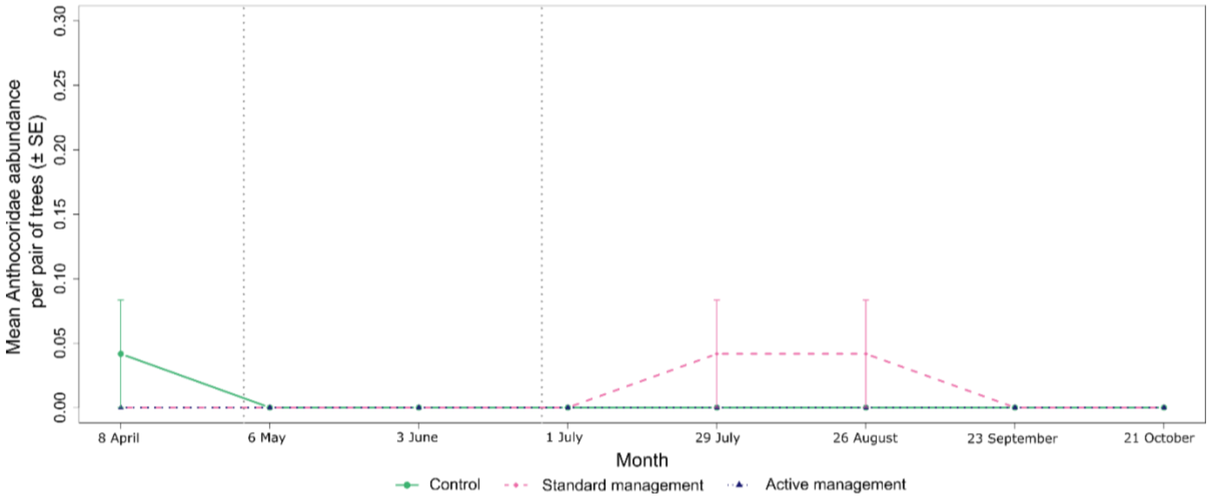
Appendix 2.7 Mean monthly abundance of the Anthocoridae between treatments; control (green), standard management (pink), and active management (blue), during the year two (2018). Error bars represent ± 1 SEM.



Appendix 2.8 Mean monthly trends in abundance of the Thomisidae between treatments; control (green), standard management (pink), and active management (blue), during the year two (2018). Error bars represent ± 1 SEM.



Appendix 2.9 Mean monthly trends in abundance of the Scelionidae between treatments; control (green), standard management (pink), and active management (blue), during year three (2019.) Error bars represent ± 1 SEM.



Appendix 2.10 Mean monthly trends in abundance of the Anthocoridae between treatments; control (green), standard management (pink), and active management (blue), during year three (2019.) Error bars represent ± 1 SEM

Appendix 3

Appendix 3.1 Tukey’s pairwise contrasts of carbohydrate resource abundance between treatments, control (C), standard management (SM), and active management (AM). Only significant explanatory variables from within GLMM of total abundance of carbohydrate resource

Carbohydrate resource	Season	Pair	Test	Test statistic (Z)	*P*-value
Alleyway	Spring	SM:C	Tukey´s	5.903	< 0.001 ***
		AM:C	Tukey´s	0.595	0.823
		AM:SM	Tukey´s	-5.390	< 0.001 ***
Alleyway	Summer	SM:C	Tukey´s	-0.055	0.998
		AM:C	Tukey´s	-5.177	< 0.001 ***
		AM:SM	Tukey´s	-5.133	< 0.001 ***
Alleyway	Autumn	SM:C	Tukey´s	2.959	0.009 **
		AM:C	Tukey´s	2.477	0.035*
		AM:SM	Tukey´s	-0.499	0.872
Canopy	Spring	SM:C	Tukey´s	2.592	0.026*
		AM:C	Tukey´s	0.138	0.99
		AM:SM	Tukey´s	-2.458	0.037*
Canopy	Summer	SM:C	Tukey´s	2.238	0.065
		AM:C	Tukey´s	1.766	0.181
		AM:SM	Tukey´s	-0.482	0.880
Canopy	Autumn	SM:C	Tukey´s	3.348	0.002**
		AM:C	Tukey´s	1.205	0.448
		AM:SM	Tukey´s	-2.364	0.047*

Appendix 3.2 Holm’s stepdown pairwise comparisons of negative binomial models for the multispecies abundances of the different sources of alleyway and canopy carbohydrate resource between treatment. Log-likelihood ratio tests were used to compute χ^2 and P-values were estimated from 999 bootstrapped permutations.

Carbohydrate resource	Season	Independent variable	Pair	Test	Test statistic (Free Stepdown)	*P*-value
Alleyway	Summer	Treatment	SM:C	Holm’s	32.10	0.011 **
			AM:SM	Holm’s	28.05	0.019 *
			AM:C	Holm’s	10.16	0.088
Alleyway	Summer	Treatment	SM:C	Holm’s	45.80	0.001 ***
			AM:SM	Holm’s	34.20	0.001 ***
			AM:C	Holm’s	24.81	0.008 **
Alleyway	Autumn	Treatment	SM:C	Holm’s	9.1	0.109
			AM:SM	Holm’s	17.08	0.048 *
			AM:C	Holm’s	16.97	0.048*
Canopy	Spring	Treatment	SM:C	Holm’s	NA	NA
			AM:SM	Holm’s	NA	NA
			AM:C	Holm’s	NA	NA
Canopy	Summer	Treatment	SM:C	Holm’s	4.12	0.501
			AM:SM	Holm’s	14.10	0.035*
			AM:C	Holm’s	9.74	0.107
Canopy	Autumn	Treatment	SM:C	Holm’s	18.24	0.004
			AM:SM	Holm’s	17.08	0.004
			AM:C	Holm’s	7.35	0.087

Appendix 3.3 Tukey's pairwise comparisons of the proportion of parasitoids recently fed on carbohydrates between each of the three treatments. Comparisons of means were only conducted from summer and autumn when treatment was found to be significant within the binomial GLMM.

Season	Pair	Test	Test statistic (Z)	*P*-value
Spring	SM: C	Tukey´s	NA	NA
	AM:C	Tukey´s	NA	NA
	AM:SM	Tukey´s	NA	NA
Summer	SM: C	Tukey´s	1.747	0.186
	AM:C	Tukey´s	-0.664	0.783
	AM:SM	Tukey´s	-2.517	0.031*
Autumn	SM: C	Tukey´s	0.324	0.081
	AM:C	Tukey´s	0.375	0.072
	AM:SM	Tukey´s	0.362	<0.001

Appendix 3.4 Functional group traits of the parasitoids and phytophage *P. citrella* the variation in the spring, summer, and autumn models where the abundances of the different functional traits were compared between the three alleyway treatments, control, standard management, and active management. The mean number of each function group G-vac suction sample (± SE) are presented, as well as the percentage contribution to the total treatment effect of the manyglm model, as calculated from the LRT test statistic.

Carbohydrate source	Mean number of individuals collected per sample (± SE)			Variation within the model (%)
	Control	Standard management	Active management	
Spring				
Primary parasitoids	0.88 (± 0.16)	1.96 (± 0.33)	1.25 (± 0.24)	24.55
Parasitoids of predators	0.08 (± 0.06)	0.75 (± 0.22)	0.50 (± 0.16)	29.61
Hyperparasitoids	0.83 (± 0.22)	1.00 (± 0.26)	0.38 (± 0.13)	13.18
Other parasitoids	0.50 (± 0.14)	1.21 (± 0.31)	0.29 (± 0.09)	32.66
Phytophage	NA	NA	NA	NA
Summer				
Primary parasitoids	0.98 (± 0.19)	2.9 (± 0.48)	0.67 (± 0.14)	68.82
Parasitoids of predators	0.13 (± 0.06)	0.23 (± 0.07)	0.19 (± 0.06)	2.85
Hyperparasitoids	0.06 (± 0.04)	0.21 (± 0.07)	0.06 (± 0.04)	12.1
Other parasitoids	0.15 (± 0.06)	0.39 (± 0.1)	0.25 (± 0.07)	10.1
Phytophage	0.35 (± 0.12)	0.67 (± 0.29)	0.31 (± 0.11)	5.85
Autumn				
Primary parasitoids	0.71 (± 0.16)	1.54 (± 0.29)	0.71 (± 0.15)	27.15
Parasitoids of predators	0 (± 0)	0.1 (± 0.05)	0 (± 0)	32.47
Hyperparasitoids	0.6 (± 0.17)	0.33 (± 0.11)	0.13 (± 0.06)	26.56
Other parasitoids	0.21 (± 0.06)	0.42 (± 0.09)	0.23 (± 0.06)	12.44
Phytophage	0.04 (± 0.03)	0.02 (± 0.02)	0.02 (± 0.02)	1.39

Appendix 3.5 Holm's stepdown pairwise comparisons of negative binomial models for the abundances of the different functional traits collected from the citrus canopy, conducted using mvabund. Log likelihood ratio tests were used to compute χ^2 and P-values were estimated from 999 bootstrapped permutations.

Season	Pair	Test	Test statistic (Z)	*P*-value
Spring	SM:C	Holm´s	24.16	0.001 ***
	AM:C	Holm´s	12.87	0.021 *
	AM:SM	Holm´s	18.84	0.003 **
Summer	SM:C	Holm´s	27.59	0.001 ***
	AM:C	Holm´s	3.51	0.639
	AM:SM	Holm´s	34.59	0.001 ***
Autumn	SM:C	Holm´s	18.763	0.004 **
	AM:C	Holm´s	8.912	0.086
	AM:SM	Holm´s	18.878	0.004 **

Appendix 3.6 Overview of the model selection predicting carbohydrate feeding in parasitoids in citrus orchards. The initial model was fitted using the subset of the parasitoid dataset which included only parasitoids identified to genus. Model selection was performed using the dredge function of the MuMIn package in R. The number of parameters in the model is indicated by df, AIC_c is the corrected AIC for small sample sizes, Δ is the difference in AIC_c with the model with the lowest AIC_c value, and Akaike weights (Weight) represent the posterior probability of the model. Based on these parameters, only the ten best models are presented, although any model where $\Delta < 2$ is considered plausible. Terms included in the model are represented by the plus symbol (+) while terms dropped form the model are represented by the minus symbol (-).

	Model ranked according to weight									
	1	2	3	4	5	6	7	8	9	10
Mean carbohydrate resources units in alleyway	+	+	-	+	-	+	+	-	+	+
Family	+	+	+	+	+	+	+	+	+	+
Functional group	-	-	-	-	-	-	+	-	-	-
Honeydew production in primary host	-	-	-	-	-	-	-	-	-	-
Mean corolla width	-	-	-	+	+	+	-	+	+	+
Mean carbohydrate resource units in canopy	-	+	-	-	-	-	-	-	+	+
Sward heterogeneity	+	+	+	+	+	+	+	+	+	+
Parasitoid head width	+	+	+	+	+	+	+	+	+	+
Mean carbohydrate resources units in alleyway * Honeydew production in primary host	-	-	-	-	-	-	-	-	-	-
Honeydew production in primary host * Mean carbohydrate resource units in canopy	-	-	-	-	-	-	-	-	-	-
Mean corolla width * Parasitoid head width	-	-	-	-	-	+	-	+	-	+
df	28	29	217	29	28	30	30	29	30	31
AIC_C	734.7	735.3	735.6	736.3	736.5	736.6	736.6	736.6	736.6	736.6
Δ AIC_c	0	0.571	0.830	1.589	1.807	1.820	1.842	1.872	1.876	1.890
Weight	0.140	0.105	0.092	0.063	0.057	0.0562	0.056	0.055	0.055	0.054