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On the floral rewards and flower-visitor assemblages of annual urban flower meadow seed mixes

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Declaration

This thesis was composed by me and it is the result of my own work: It contains no work done by others or in collaboration with others, except where clearly stated otherwise. The data collected as part of this project has not been submitted for any other degree or qualification, except in the following cases:

1) Data on meadow composition, floral rewards and insect visitation collected in 2012 were used by my field assistant Marianne Coulon in her MSc at Université Blaise Pascal, Clermont-Ferrand in 2012.

2) Data on meadow composition, floral rewards and insect visitation from 2013 were used by Fiona Plenderleith in her BSc Honours project at the University of Edinburgh. As part of her thesis Fiona Plenderleith quantified the mean volume of pollen in anthers and flowers of plant species to enable estimation of per-floral unit and per-unit area of meadow pollen reward provision. In Appendix A5.3 I include a figure (Fig. A5.3) created by Fiona Plenderleith using our shared data.

3) For some flowering species in meadows, data on floral nectar and pollen rewards were taken from Hicks *et al.*, 2016; whilst, for some species the estimates of floral unit longevity used to calculate floral pollen rewards per floral unit were taken from a MPhil thesis submitted by Maria Nikolitsi, University of Leeds, 2015.

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Abstract

Flower seed mixes are increasingly used to enhance the biodiversity and amenity values of urban green spaces. Urban or "pictorial" flower seed mixes are often used because they are designed using cultivars and non-native species to provide more colourful and longer-lasting flower displays. Although these seed mixes are effective in providing a high density of large colourful flowers, over an extended season, their value for biodiversity, and in particular the floral rewards they provide for flower-visitors, is largely unknown. The overall aim of my thesis was to assess and improve the value of these new urban habitats as forage resources for flower-visiting insects.

My approach was to quantify and compare floral reward provision and insect visitation between meadows grown from three exemplar commercial pictorial flower meadow seed mixes (called Marmalade Annual, Short Annual and Cornfield Annual). I also compared these standard commercial mixes with corresponding 'nectar-enriched' formulations, which were designed by increasing the proportional seed weight contribution of selected species predicted to produce high quantities of nectar within each mix. To compare floral rewards and visitation between meadows grown from these seed mixes, I set up a field experiment in Sheffield, UK, using a complete randomised block design with six replicate blocks, each with six 25 m^2 plots sown with one of the six seed mix treatments.

My first objective was to quantify the floral nectar and pollen rewards provided by each flowering species recorded in the meadows (on the scale of a single flower or inflorescence). My second objective was to use these data to quantify the floral rewards provided per unit area by replicate meadows of different seed mix treatments, testing whether enrichment of seed mixes is

an effective method of increasing floral nectar sugar rewards. My third objective was to corroborate/correct my morphology-based flower-visitor identifications using DNA barcoding to screen for misidentifications and morphologically cryptic species. I then used these DNA barcode-based identifications to assess whether there are systematic biases in the structure of flower-visitor networks constructed using molecular taxon identifications compared to traditional morphology-based taxon identifications. My fourth objective was to quantify patterns of insect visitation to meadows, testing whether meadows of different seed mix types attract different flower-visitor assemblages.

Meadow floral composition surveys revealed that contamination by unintended horticultural species was widespread across replicate seed mix treatments, with contaminants likely germinating from a seed bank laid down during a failed attempt at this experiment the previous year. Contamination particularly affected Marmalade mixes, mainly because the common contaminant species were often also components of the Short and Cornfield mixes. For example, contaminants contributed on average about a third of nectar sugar mass or pollen volume per unit area in Marmalade mix meadows. Hence, contamination fundamentally undermined the internal validity of seed mix treatments, reducing the ability to directly attribute meadow level patterns in floral rewards or flower-visitors to seed mixes. As result, examination of patterns of floral resource provision and insect visitation were more informative at a species scale.

In terms of patterns of insect visitation, *Centaurea cyanus* received 91% of bumblebee visits, 88% of honeybee visits and 29% of hoverfly visits, whilst *T. inodorum* received 27% of hoverfly visits. Patterns of bumblebee and

honeybee visitation indicated preferential visitation to floral units of *Centaurea cyanus.* Although this species produced high quantities of nectar sugar mass and pollen volume, this did not differentiate it from other Asteraceae, such as *Glebionis segetum*, *Rudbeckia hirta* and *Coreopsis tinctoria*, which all produced high quantities of both floral rewards. Hence, it is likely that floral traits not measured in this study, such as nectar accessibility ('nectar-holder depth') or concentration/volume characteristics (which can affect accessibility due to constraints imposed by feeding morphology), drove patterns of preferential visitation in bumblebees and honeybees to *C. cyanus*. Given that in the absence of contamination there would have been very few bumblebee or honeybee visitors to Marmalade mix meadows, aesthetically designed pictorial meadows can fail to jointly provide benefits for people and some important flower-visiting insect taxa.

DNA barcoding did not change specimen identifications for most morphotaxa. However, splitting and/or lumping processes affected almost one third of morphotaxa, with lumping of morphotaxa the most common type of change. This was in part because males and females from sexually dimorphic species were often separated by morphological identification. These DNA barcode-based changes to visitor taxonomy resulted in consistent minor changes in network size and structure across replicate networks. Lumping of morphotaxa decreased taxon richness, reducing the number of unique links and interaction diversity (the effective number of links). Lumping also increased flower-visitor generality, reducing plant vulnerability and increasing overall network connectance. However, taxonomic changes had no effect on interaction evenness or network specialisation. Thus, for this well-studied fauna, DNA barcode-based flower-visitor networks were systematically biased toward fewer taxa and links,

with more generalist visitors and specialist plants. Given that many tropical faunas have more species and are less described than in Britain this pattern may not be replicated in other studies. Further studies in contrasting plant-pollinator communities are required before generalisations can be made about systematic biases between networks constructed using morphological versus molecular data.

Overall, meadows grown from annual pictorial flower meadow seed mixes provide abundant floral units per unit area of meadow and are a valuable alternative to traditional horticultural flower beds or amenity grasslands in high profile urban contexts. Nevertheless, care must be taken during design of seed mixes and selection of mixes for planting to ensure that species in the mix provide suitable floral resources for an array of flower-visitors, including bees. This would be aided by the integration of informative measures for candidate species of floral rewards or visitor types and visitation rates during seed mix design.

Lay Summary

Flower seed mixes are increasingly used to enhance the biodiversity and amenity values of urban parks. “Pictorial” flower seed mixes are often used because they are designed using cultivars and non-native species to provide colourful and long-lasting flower displays. Although these seed mixes are effective in providing a high density of large colourful flowers, over an extended season, their value for biodiversity, and in particular the floral pollen and nectar rewards they provide for flower-visitors, is largely unknown. The overall aim of my thesis was to assess and improve the value of these new urban habitats as forage resources for flower-visiting insects.

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My first objective was to quantify the floral nectar and pollen rewards provided by each flowering species recorded in the meadows (on the scale of a single flower or composite flowerhead). My second objective was to use these data to quantify the floral rewards provided per unit area by replicate meadows of different seed mix treatments, testing whether enrichment of

seed mixes is an effective method of increasing floral nectar sugar rewards. My third objective was to corroborate/correct my morphology-based flower-visitor identifications using DNA sequence-based identification to screen for misidentifications and morphologically cryptic species. I then used these DNA sequence-based identifications to assess whether there are systematic differences in the structure of flower-insect food webs constructed using molecular taxon identifications compared to traditional morphology-based taxon identifications. My fourth objective was to quantify patterns of insect visitation to meadows, testing whether meadows of different seed mix types attract different flower-visiting insects.

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plant vulnerability (the average number insect species visiting a plant species) and increasing overall food web connectance (a measure of the density of interactions between species). Thus, for this well-studied fauna, DNA barcode-based flower-visitor food webs were systematically biased toward fewer taxa and unique interactions, with more generalist visitors and specialist plants. Given that many tropical faunas have more species and are less described than in Britain this pattern may not be replicated in other studies. Further studies in contrasting plant-pollinator communities are required before generalisations can be made about systematic biases between food webs constructed using morphological versus molecular data.

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Acknowledgements

The research presented in this thesis would not have been possible without the help and support of many people, to whom I am grateful and whom I would like to thank.

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Contents

Chapter 1: General introduction

1.1 Flower-visiting insects and pollination

Flower-visiting insects such as bees, butterflies and hoverflies obtain food from flowers in the form of pollen and/or nectar (Proctor *et al.* 1996; Rotheray & Gilbert 2011; Willmer 2011). In the process they may facilitate fertilization, fruiting and seed set through the transfer of pollen within or between flowers (Proctor *et al.* 1996; Willmer 2011). This pollination service is critically important to the production of food and animal fodder, with almost a third of estimated global food mass produced in 2004 dependent to some extent on animal-mediated pollination (Klein *et al.* 2007). The economic value of this pollination service to global human food production has been estimated to be around €153 billion or 9.5% of the value of food produced in 2005 (Gallai *et al.* 2009). Flower-visitors and their pollination services are also integral to the maintenance of many terrestrial ecosystems, with an estimated 87.5% of the world's flowering plants dependent on/benefitting from animal-meditated pollination for their reproduction (Ollerton *et al.* 2011).

1.2 Pollinator declines

Given that human societies depend on pollination and numerous other ecosystem services provided by flowering plants, recently reported widespread declines of both managed (Potts *et al.* 2010b; vanEngelsdorp *et al.* 2010; Seitz *et al.* 2015) and wild insect pollinators (Biesmeijer *et al.* 2006; Goulson *et al.* 2008; Cameron *et al.* 2011; Goulson *et al.* 2015) provide considerable cause for concern (Allen-Wardell *et al.* 1998; Potts *et al.* 2016a; Potts *et al.* 2016b). Although the scale of impacts from pollinator declines continues to be debated (Ghazoul 2005a, 2005b; Steffan-Dewenter *et al.* 2005; Potts *et al.* 2010b), pollinators can underpin food diversity and human

nutrition (Klein *et al.* 2007; Ellis *et al.* 2015; Smith *et al.* 2015) and that in recent decades pollinator assemblages have undergone significant changes (Biesmeijer *et al.* 2006; Carvalheiro *et al.* 2013; Senapathi *et al.* 2015). For example, using landscape-scale data of bee and hoverfly records from Britain, Biesmeijer *et al.* (2006) reported that wild bee diversity declined in 52%, and increased in only 10%, of eighty-one 10x10 km squares compared for their pre- and post-1980 pollinator assemblages. Bee diversity also declined in an equivalent Dutch dataset, although no consistent shifts in hoverfly diversity were found between the two countries (Biesmeijer *et al.* 2006). Among the drivers of these reported declines in pollinators are the loss, degradation and fragmentation of habitats and the increasing use of agro-chemicals, such as herbicides and pesticides (Kearns & Inouye 1993; Potts *et al.* 2010a; Potts *et al.* 2010b; Vanbergen *et al.* 2013; Goulson *et al.* 2015). In Britain and continental Europe, these drivers are mainly associated with changes in land use and farming practices that occurred over the latter half of the 20th century (Robinson & Sutherland 2002; Gerard *et al.* 2010), which simplified the rural landscape and reduced opportunities for nesting, egg-laying and foraging (Carvell *et al.* 2006; Goulson *et al.* 2008).

1.3 Green space management and flower seed mixes

Given the threats facing pollinators, and many of the plants they visit, there is a growing need to both raise public awareness of their ecological role and importance, and to take active steps to support their populations. One way of doing this is to enhance urban environments, which are growing rapidly (e.g Gerard *et al.* 2010; Turrini & Knop 2015), as habitats for pollinators (Aronson *et al.* 2017; Hall *et al.* 2017). Studies to date show that although some city habitats support low pollinator diversity (Sirohi *et al.* 2015), others can be rich in species or individuals (Fortel *et al.* 2014; Baldock

et al. 2015; Sirohi *et al.* 2015). A recent analysis as part of the UK Insect Pollinators initiative showed that cities can be surprisingly rich in pollinators compared to nature reserves and farmland, supporting higher bee species richness, but lower hoverfly abundance (Baldock *et al.* 2015).

Multiple factors likely limit pollinator populations – particularly the abundance and quality of nest sites or substrates, and the availability of food resources (Carvell *et al.* 2006; Müller *et al.* 2006; Vanbergen *et al.* 2014; Goulson *et al.* 2015; Gill *et al.* 2016). It is generally true that the more flowers there are, the more flower-visiting insects will be present (Ahrne *et al.* 2009; Gunnarsson & Federsel 2014; Lowenstein *et al.* 2014). This suggests that enriching urban habitats with more flowers or qualitatively better food resources for pollinators could increase urban pollinator populations, which could then possibly contribute to pollination services in surrounding, non-urban environments (Gill *et al.* 2016; Hall *et al.* 2017). One way of improving food availability is to plant urban habitats with plants selected for the rewards they provide to flower-visiting insects (Gunnarsson & Federsel 2014; Bretzel *et al.* 2016; Aronson *et al.* 2017).

In recent years, seed mixes have started to be designed explicitly for use in urban landscapes, creating extensive flowering borders or so-called 'pictorial' meadows (Hitchmough & Woudstra 1999; Hitchmough 2000; Hitchmough & Dunnett 2004). Prof James Hitchmough and Dr Nigel Dunnett of the Department of Landscape at Sheffield University are at the forefront of seed mix design, trialling some of the first pictorial meadows and setting up a company of the same name to develop and market them (Hitchmough 2004; Hitchmough & Dunnett 2004; Hitchmough 2008, 2010, 2011; Köppler & Hitchmough 2015). This is now part owned and operated by

Green Estate, my CASE studentship sponsor, a social enterprise that continues to develop seed mixes for urban plantings. Urban or pictorial flower seed mixes often combine cultivars of native and exotic species to increase the size, range of colours and flowering period of flowers in the resulting meadows. The ultimate aim of the flower seed mixes emerging from seed mix designers with backgrounds in landscape architecture is to enhance human quality of life by improving the amenity value of urban green spaces (Ahern & Boughton 1994; Chiesura 2004; Scott 2008; Hoyle *et al.* 2017a; Southon *et al.* 2017). Seed mixes designed or tested by ecologists (particularly those containing native cornfield species) are typically designed to enhance the biodiversity value of urban areas, and are often inspired by the planting of pollinator strips around arable field margins (e.g. Kells *et al.* 2001; Carvell *et al.* 2004; Pywell *et al.* 2011; Blaauw & Isaacs 2014). However, due to real and perceived aesthetic constraints, and often-cited public complaints, parks managers and city councils often prefer to plant aesthetically designed seed mixes (Dunnett & Hitchmough 2004; Hoyle *et al.* 2017a; Hoyle *et al.* 2017b). Urban flower seed mixes include non-native species and cultivars to increase the available colour palette and extend the flowering season, and exclude grasses to reduce competition and improve reliability, ensuring the pictorial seed mixes provide meadows with a high visual impact (Hitchmough & Woudstra 1999; Hitchmough & Dunnett 2004).

Although not designed for pollinators, these flower-rich urban meadows likely provide more flowers and nectar and pollen resources than the frequently-mowed amenity grasslands which they often replace. However, this assumption has seldom been tested (but see Blackmore & Goulson 2014; Hicks *et al.* 2016). Recent studies show that urban meadow plantings are visited by pollinators at higher rates than unplanted

comparison plots (Blackmore & Goulson 2014), and that they provide an order of magnitude more pollen and nectar resources (Hicks *et al.* 2016). However, for most plant species/cultivars used in flower seed mixes the nectar and pollen rewards they provide remain unquantified. In particular, we do not know the relative nectar and pollen resources provided per flower by different urban meadow species and cultivars, nor do we know the amount of resource provided per unit area of different annual seed mixes or how the availability of these resources may change through the season.

Without this information, seed merchants cannot design seed mixes for both aesthetic amenity and resource provision for pollinators. Given that the quantity, quality and seasonal timing of floral resources are all important for pollinator populations (Bowers 1986; Cartar & Dill 1991; Potts *et al.* 2003; Potts *et al.* 2004), seed mixes designed to support pollinators should provide pollen and nectar throughout the season, without seasonal gaps in resource availability that could limit pollinator populations (Roulston & Goodell 2011; Schellhorn *et al.* 2015).

1.4 Aims and approach of this thesis

The overarching aim of my thesis was to assess and improve the value of three exemplar annual urban flower-meadow seed mixes as habitat for flowering-visiting insects. These were the Marmalade Annual, Short Annual and Cornfield Annual mixes, designed by Pictorial Meadows Ltd. My aims were to:

(1) quantify the floral resources provided by three commercially-available, exemplar urban flower meadow seed mixes;

(2) assess the impact on floral resource provision of enriching these seed mixes by increasing the abundance of selected component species predicted to produce lots of nectar;

and (3) quantify patterns of insect visitation to the meadows that result when these standard commercial and nectar-enriched seed mixes are grown in the field.

My main objectives, in the order they are addressed in my thesis, were:

(1) to validate the treatment structure of my experiment by comparing the floral composition of replicate meadows, examining whether meadows grown from flower seed mixes constitute distinct floral communities that are representative of initial seed mix treatments.

(2) to quantify the floral rewards provided by each flowering species recorded in meadows (including weeds), and to use these data to quantify the floral rewards provided by meadows of different seed mixes; testing the effectiveness of enriching seed mixes by increasing the floral abundance of species predicted to produce high quantities of nectar.

And (3) to quantify patterns of insect visitation to meadows grown from urban flower seed mixes, testing whether meadows of different seed mix types attract different flower-visitor assemblages.

The experimental approach I adopted stemmed from the observation that the meadows that develop when seed mixes are sown are the result of an array of different factors, including the date of sowing, weather during germination and establishment, soil conditions, microclimate, ecological interactions, including competition for resources, and germination of unintended species such as weeds (Prentis & Norton 1992; Highways Agency 1993; Aldrich 2002; Hitchmough 2017). Moreover, the composition of meadows varies over time, therefore perceptions of meadow floral species composition will depend on the period surveyed.

Hence, to assess differences in floral composition and resources between meadows of different seed mix treatments requires a blocked experimental design to reduce the effect of uncontrolled environmental variation between seed mix treatment differences in floral composition. Hence, I used a randomised complete-block (RCB) design, in which blocks were composed of one replicate of each of six seed mix treatments (standard versus enriched formulations of 3 seed mix types). Furthermore, I sampled meadows multiple times during the season to account for seasonal variation in meadow floral composition.

Seed mix types (which are described in detail in Chapters 2 and 3) were selected based on multiple criteria. The approach was not to perform an exhaustive assessment of alternative urban flower meadow seed mixes, which would be prohibitively challenging to do, but to illustrate the impact that different urban flower seed mixes designs can have on floral resource

provision and patterns of insect visitation. Hence, seed mixes were selected to provide contrasts in species richness and floral morphological diversity, ranging from species poor and low in floral morphological diversity (the Marmalade Annual mix) to species rich and high in floral morphological diversity (the Short Annual mix). Similarly, the Cornfield annual mix was selected to provide a contrast between a mix that was species poor and low in floral morphological diversity (the Marmalade Annual mix) and a mix that was species poor and high in floral morphological diversity (the Cornfield Annual mix). Furthermore, Cornfield annual mixes are the most common flower seed mixes on the market in the UK and are often considered and marketed as native flower seed mixes. The Cornfield mix used in this experiment contained no grasses and only 4 traditional herbaceous Cornfield species (*Agrostemma githago*, *Centaurea cyanus*, *Tripleurospermum inodorum* and *Papaver rhoeas*); therefore, it should be considered an urban flower seed mix, rather than a wildflower mix. Nevertheless, inclusion of a mix containing Cornfield species provides a useful reference point for comparison with designed urban meadows containing non-native species. Hence, seed mixes were intentionally selected, from a potentially enormous array of seed mixes with subtly different species compositions, to provide contrasting exemplars to inform future design and use of urban flower meadow seed mixes.

Surveys of the meadows revealed that meadows grown from sown seed mixes often included two additional categories of plants derived from the soil seed bank: non-mix horticultural contaminants (resulting from previous experiments and horticultural trials by Green Estate) and weeds (unintended ruderal and arable species). In quantifying floral abundance, floral rewards and insect visitation in meadows, I therefore distinguished between three floral categories: intended seed mix treatment species,

horticultural contaminants, and weeds (described in more detail in Chapters 2 and 3). Given that annual meadows sown in urban areas are likely to contain garden escapes or to be re-sown on the same plots year after year, these three floral categories are likely to be a common feature of meadow resources whenever seed mixes are sown. Therefore understanding their contribution to meadow floral resources and patterns of flower visitation is valuable. However, the extent to which patterns of floral reward provision and insect visitation at a meadow level can be attributed to seed mixes is contingent on the degree to which contaminants and weeds affect the overall floral composition of meadows (an issue I address in Chapter 3).

Assessing patterns of insect visitation to meadows required identification of a diversity of flower-visiting insects. Identification of flower-visiting insects to species is challenging for many insect groups, even for experienced taxonomists. Moreover, professional taxonomists may not have time, sufficient incentives or an inclination to work outwith their own projects. This creates a 'taxonomic impediment' for projects in which a large number of specimens need to be identified. One way of circumventing this problem is to identify specimens using DNA sequence-based approaches based on the concept of DNA 'barcodes'. To corroborate or correct taxonomic identifications made on the basis of morphology, I applied this approach to identification of the flower-visiting insects I collected during urban flower meadow surveys. My aim was to explore the extent to which DNA-based approaches change morphology-based taxon definitions, testing whether use of DNA sequence-based identification changes the perceived composition of flower-visitor assemblages in a way that alters flower-visitor network structure.

1.5 Thesis outline

This thesis is comprised of three data chapters:

Chapter 3: Flowering performance and floral enrichment of annual urban flower seed mixes.

In Chapter 3, I quantify the floral composition of meadows that resulted when six seed mix treatments (standard versus enriched formulations of three seed mix types) were sown in a randomised complete block design in Sheffield, UK, during spring and summer 2013. I describe overall patterns of floral abundance, floral richness, floral diversity and floral composition for all species in the meadows combined, as well as for species classified into treatment, contaminant or weed floral categories. I then test whether manipulating the proportional seed weight contribution of species in seed mixes is an effective approach to increasing the floral abundance of those species at a meadow level. My analyses have the following specific objectives and associated questions:

Objective 3.1: to characterise and compare the flowering performance of different annual urban flower seed mixes in terms of user's horticultural expectations.

Objective 3.2: to characterise and compare the flowering performance of different annual urban flower seed mixes as contrasting plant communities.

Objective 3.3: to test whether experimental enrichment for particular species was effective in increasing the floral abundance of target species.

Chapter 4: The impact of molecular taxonomic analysis on the composition of flower-visiting insect assemblages and the structure of flower-visitor interaction networks.

In Chapter 4, I apply DNA sequence-based barcoding to identification of insects sampled from meadows grown from seed mix treatments. I compare DNA-sequence based taxon definitions with morphological taxon definitions based on traditional Linnaean morphological taxonomy. My analyses have the following specific objectives and associated questions:

Objective 4.1: to assess the impact of using molecular information to delineate insect taxa on the perceived composition of flower-visiting insect assemblages.

Objective 4.2: to examine the extent to which use of molecular taxon designations alters the structure of flower-visitor networks compared to morphology-based networks.

Chapter 5: Floral resources and patterns of insect visitation in urban flower meadows.

In Chapter 5, I quantify the amount of nectar sugar (mass/day) and pollen (volume/day) provided per floral unit by species found flowering in meadows of each seed mix treatment, including associated contaminants and weeds. I rank species by their floral pollen and nectar rewards, highlighting the most rewarding species in each seed mix type, and examining whether the species selected for enrichment were correctly predicted to rank highly for floral nectar rewards. Individual species values are then used to estimate floral reward provision per unit area of meadow. I compare floral reward provision between mix types, examine the relative contribution of different floral categories (treatment, contaminants and weeds), and test whether the

enrichment treatment affected floral resource provision at a meadow level. I then use meadow level resource values to test whether bumblebee or hoverfly abundance are correlated with meadow floral abundance, floral nectar sugar mass or floral pollen volume. Finally I examine overall patterns of insect visitation to the floral species that composed meadows surveyed in this study. My analysis has the following specific aims:

Objective 5.1: To quantify and compare rewards per floral unit for each flowering species and the floral rewards provided per unit area by each meadow treatment.

Objective 5.2. To examine patterns and floral resource correlates of insect visitation to planted meadows.

Objective 5.3. To identify which plant species are most visited by bees and hoverflies.

Chapter 2: General methods

2.1 Introduction

This Chapter introduces my study system, experimental design, seed mix treatments, and field survey methods. Chapter-specific methods such as insect identification methods (Chapter 4) and floral reward quantification methods (Chapter 5) are presented in the relevant chapters.

2.2 Materials and methods

2.2.1 Stud

y system and field site location

This thesis focuses on the floral resources and flower-visiting insect assemblages of planted meadows grown from annual pictorial flower seed mixes. These aesthetically designed mixes are designed using careful plant selection to provide colourful and visually attractive meadows, which are often used as a cost-effective method of improving the visual amenity and biodiversity value of urban parks. Pictorial meadows typically do not contain grasses but incorporate horticultural cultivars and non-native species, which can have larger, more colourful flowers and longer flowering seasons compared to comparable native forb species.

Fieldwork for this research was performed on an 11.2 ha area of urban green space at Sheffield Manor Lodge, Sheffield, UK (OS Grid: SK 376 868). The site was located among residential housing and amenity parks and contained a diversity of habitat and vegetation types including: woodland, heathland, grazed pasture, allotments, horticultural trial beds, flower meadows, and rank and amenity grasslands.

2.2.2 Research approach

To compare patterns for floral resource provision and insect visitation between meadows grown from different seed mix treatments I used a field experiment set up using a randomized complete block design. This had the advantage of minimizing the effect of environmental variation on between-treatment differences in meadow floral composition, although as flower-visitors are mobile and may spill over from attractive to unattractive meadows, differences between meadows may be conservative.

I set up an initial experiment in 2012 with six blocks (A-F), each comprising six seed mixes (described below), each of which was sown randomly into one of six 25 m^2 plots, which were arranged in either a 3x2 or 6x1 array. Two blocks were sown during good weather on 20th April 2012 (A and B), but an exceptionally wet spring prevented sowing of the remaining blocks until 29th May (C, D, E and F), which was followed by a month of unusually high rainfall. As a result, many sown species failed to germinate and few flowers were produced in 4/6 blocks during 2012 (Fig. 2.1). Although limited data was collected during this season, sampling intensity was higher in 2013 therefore data from 2012 are not shown for comparison.

I repeated this experiment in 2013 (as described below), using similar but not identical block positions. Blocks A, B, D and E were located in consistent sites, but blocks C and F moved position to accommodate other horticultural experiments on site (Fig. 2.2). Plots within blocks (and blocks themselves) were not marked out using permanent inter-annual markers in 2012 (and hence blocks and plots could not be precisely relocated), therefore I re-randomised seed mix treatments in 2013.

Figure 2.1 (below and overleaf): Photographs taken between 15th-17th August 2012, showing 'peak flowering' in six blocks (A, B, C, D, E and F). Although blocks A and B produced abundant flowers, blocks C, D, E and F often produced very few.


(D)
(E)
(F)

Figure 2.2: Locations of experimental blocks A-F in 2012 and 2013 on the field site at Sheffield Manor Lodge, Sheffield, showing change in the locations of blocks C and F. Scale bar measures 100 m. Block symbols not to scale.

2.2.3 Seed mix treatments

Seed mix treatments consisted of 2 formulations (standard vs. enriched) of each of 3 commercially available flower seed mixes (Marmalade Annual, Short Annual, and Cornfield Annual; Table 2.1 and 2.2), which were provided by my CASE partner organisation (Pictorial Meadows Ltd., Sheffield, UK). These seed mixes were chosen to allow comparison of a common flower seed mix comprising traditional corn field species (Cornfield Annual, n=4 species), with two exemplar pictorial mixes selected to contrast in species and flower-shape richness. The Marmalade mix (n=6 species) was selected as an exemplar of a species-poor and flower shape-poor mix, mainly comprised of species with open- or disk-shaped flowers (families Asteraceae, Papaveraceae, and Linaceae). The Short mix (n=13 species) was selected as an exemplar of a species-rich and flower shape-rich mix and included a greater diversity of floral morphologies (families Asteraceae, Papaveraceae, Linaceae, Brassicaceae, Caryophyllaceae, Convolvulaceae, Ranunculaceae, Scrophulariaceae). The Cornfield mix was selected as an exemplar of a seed mix based on traditional annual arable 'wildflowers' (families Asteraceae, Papaveraceae, and Caryophyllaceae).

Table 2.1: Species composition and species percentage seed weight in 'standard' and 'enriched' formulations of Marmalade, Short and Cornfield annual flower seed mixes. ǂLinum grandiflorum subspecies/cultivars not distinguished in analyses.

Mix	Species	Common name	Cultivar	Family	Standard % seed weight	Enriched % seed weight	% change	Change
Marmalade	*Coreopsis tinctoria*	Coreopsis	'Tall Mixture'	Asteraceae	20	30	+10	**Increased**
	Eschscholzia californica	California Poppy	'Single Mixed'	Papaveraceae	20	10	-10	Decreased
	Glebionis segetum	Corn Marigold	'Mixed'	Asteraceae	10	10	0	
	Ismelia carinata	Painted Daisy	'Merry Mixture'	Asteraceae	10	10	0	
	Linum grandiflorum	Red Flax	'rubrum'	Linaceae	20	10	-10	Decreased
	Rudbeckia hirta	Black-eyed Susan	'My Joy'	Asteraceae	20	30	+10	**Increased**
Short	*Centaurea cyanus*	Cornflower	'Polka Dot'	Asteraceae	10	15	+5	**Increased**
	Convolvulus tricolor	Convolvulus	'Mixed'	Convolvulaceae	5	15	+10	**Increased**
	Coreopsis tinctoria	Coreopsis	'Dwarf Mixed'	Asteraceae	30	20	-10	Decreased
	Crepis rubra	Pink Hawks-beard	'Select'	Asteraceae	3	3	0	
	Dimorphotheca sinuata	African daisy	'Mixed hybrids'	Asteraceae	3	3	0	
	Gypsophila elegans	Baby's Breath	'Covent Garden'	Caryophyllaceae	10	5	-5	Decreased
	Iberis umbellata	Candy Tuft	'Dwarf Fairy'	Brassicaceae	5	15	+10	**Increased**
	Linaria maroccana	Fairy Toadflax	'Fairy Bouquet'	Plantaginaceae	10	10	0	
	*Linum grandiflorum*ǂ	Red Flax	'rubrum'	Linaceae	4	4	0	
	*Linum grandiflorum*ǂ	Flowering flax	'Bright eyes'	Linaceae	4	4	0	
	Linum usitatissimum	Blue Flax	'Sutton's Blue'	Linaceae	5	5	0	
	Nigella damascena	Love-in-a-Mist	'Persian Violet'	Ranunculaceae	3	3	0	
	Papaver rhoeas	Shirley Poppy	'Shirley hybrids'	Papaveraceae	3	3	0	
	Silene armeria	Catchfly	'Electra'	Caryophyllaceae	5	5	0	
Cornfield	*Agrostemma githago*	Corncockle	-	Caryophyllaceae	40	30	-10	Decreased
	Centaurea cyanus	Cornflower	'Polka Dot'	Asteraceae	20	15	-5	Decreased
	Papaver rhoeas	Field Poppy	-	Papaveraceae	15	15	0	
	T. inodorum	Scentless Mayweed	-	Asteraceae	25	20	-5	Decreased
	Glebionis segetum	Corn Marigold	-	Asteraceae	0	*15*	+15	**added**
	Echium vulgare	Viper's Bugloss	'Blue Bedder'	Boraginaceae	0	5	+5	**added**

Table 2.2: Seed mix compositions with flower types, floral unit definitions, information on value to bees from a selection of grey literature, and approximate flowering periods. N = nectar provider; P = pollen provider. Bee plant = visited by bees.

Mix type	Species	Flower type	Floral unit definition	RHS[1]	Bee Plants[2]	Plants and Honeybees[3]	Plants for Bees[4]	Flowering period[4,5]							
								A	M	J	J	A	S	O	N
Marmalade Annual	*Coreopsis tinctoria*	composite	composite	Y						■	■	■	■		
	Eschscholzia californica	open	single	Y	N/P bee plant		P bee plant				■	■	■		
	Glebionis segetum	composite	composite	Y						■	■	■	■	■	
	Ismelia carinata	composite	composite							■	■	■	■		
	Linum grandiflorum	trumpet	single							■	■	■			
	Rudbeckia hirta	composite	composite	Y	bee plant		bee plant				■	■	■	■	
Short Annual	*Centaurea cyanus*	composite	composite	Y	N/P good bee plant	N/P	N/P bee plant			■	■	■	■		
	Convolvulus tricolor	trumpet	single	Y	N/P bee plant						■	■	■		
	Coreopsis tinctoria	composite	composite	Y						■	■	■	■		
	Crepis rubra	composite	composite							■	■	■	■		
	Dimorphotheca sinuata	composite	composite												
	Gypsophila elegans	open	single	Y	N bee plant				■	■	■	■	■		
	Iberis umbellata	umbel	cluster‡		N/P bee plant		bee plant				■	■	■	■	
	Linaria maroccana	nectar spur	single	Y						■	■	■	■	■	
	Linum grandiflorum¥	trumpet	single							■	■	■			
	Linum usitatissimum	open	single		good bee plant		N bee plant		■	■	■	■	■		
	Nigella damascena	composite	composite	Y	N/P bee plant		N/P bee plant		■	■	■	■	■		
	Papaver rhoeas	open	single	Y	P bee plant	P	P bee plant		■	■	■	■	■	■	
	Silene armeria	narrow tube	cluster‡							■	■	■	■	■	
Cornfield Annual	*Agrostemma githago*	narrow tube	single	Y	P bee plant					■	■	■	■		
	Centaurea cyanus	composite	composite	Y	N/P good bee plant	N/P				■	■	■	■		
	Papaver rhoeas	open	single	Y	P bee plant	P	P bee plant		■	■	■	■	■	■	
	Tripleurospermum inodorum	composite	composite								■	■	■		
	Glebionis segetum	composite	composite	Y						■	■	■	■	■	
	Echium vulgare	wide tube	single	Y*	N/P bee plant*	N/P*	N/P bee plant*			■	■	■	■		

Note: flower types for descriptive purposes only; approximate flowering periods based predominantly on references 1 and 2 where information was available.

¥*Linum grandiflorum* subspecies/cultivars not distinguished in analyses;

‡For *Iberis umbellata* and *Silene armeria*, floral units were defined as a cluster of flowers, with the average number of flowers per cluster counted and then averaged over all recorded clusters;

*Information refers to related biennial *E. vulgare*.

1. RHS (2017) Perfect for Pollinators plant lists. The Royal Horticultural Society, London, U.K.

URL: https://www.rhs.org.uk/science/conservation-biodiversity/wildlife/the-importance-of-insects/plants-for-pollinators?lnk=12 – last accessed 29th Feb. 2017.

2. Crawford, M. (2000) Bee Plants. 2nd Edition. Agroforestry Research Trust, Totnes, Devon, U.K.

3. Aston, D. and Bucknall, S. (2009) Plants and Honey Bees: their relationships. Northern Bee Books, Mytholmroyd, West Yorkeshire, U.K.

4. Kirk, W.D.J. and F.N. Howes (2012) Plants for Bees: A Guide to the Plants that Benefit the Bees of the British Isles. IBRA, Cardiff, U.K.

5. Rose, F. and O'Reilly C. (2006) The Wildflower Key. Penguin Books, London, U.K.

Enriched seed mix formulations were designed to maintain commercially desirable characteristics, including reliability, efficient use of seed and aesthetic character (i.e. colour, height), while increasing floral nectar rewards for flower-visiting insects. This was achieved by taking baseline seed mixtures, provided by standard seed mix formulations, and, for each mix type, increasing the proportional seed weight of selected species that were predicted to provide high-quantities of floral nectar sugar per floral unit (Table 2.1). Species were selected for enrichment with reference to grey literature on whether they attracted bees and butterflies (Crawford 2000; Hooper & Taylor 2006; IBRA 2008; RHS 2011; The Xerces Society 2011; Kirk & Howes 2012). For each mix type, the species selected for enrichment included at least one species with a short corolla tube and another with a long corolla tube to increase floral rewards for both short- and long-tongued bees. However, this was not possible for the Marmalade mix, which was composed mainly of species with either large singleton flowers (poppies) or composite inflorescences (capitula), comprising numerous flowers with short corollas (Asteraceae).

For the Short mix, enrichment involved increasing the proportional seed weights of *Iberis umbellata* (Brassicaceae), *Centaurea cyanus* (Asteraceae) and *Convolvulus tricolor* (Convolvulaceae). For the Marmalade mix, there was only one constituent species with a long trumpet-shaped corolla (*Linum grandiflorum*; Linaceae): all other species were either composites (Asteraceae) or poppies (Papaveraceae). There was no evidence in the grey literature examined that *Linum grandiflorum* was attractive to flower-visitors; therefore, *Coreopsis tinctoria* and *Rudbeckia hirta* (Asteraceae) were enriched as the two species in the Marmalade mix most frequently reported as attractive to bees (Crawford 2000; RHS 2011; Kirk & Howes 2012). Enrichment of the Cornfield

mix involved adding *Glebionis segetum* (syn. *Chrysanthemum segetum*; Corn Marigold; family Asteraceae) and *Echium vulgare* 'Blue Bedder' (syn. *Echium plantagineum*; annual Viper's Bugloss; family Boraginaceae; here after *E. vulgare*). Thus, the standard Cornfield mix comprised 4 species (from families Asteraceae, and Papaveraceae, Caryophyllaceae) and the enriched Cornfield mix comprised 6 species (from families Asteraceae, Papaveraceae, Caryophyllaceae, and Boraginaceae).

Enriched mixes were created by Pictorial Meadows Ltd., using standard seed mix design practices to optimise the proportional seed weight contribution of each species in the mix. This required reduction of the proportional seed weight contribution of some 'non-target' species within enriched formulations of each seed mix type (Table 2.1). Each of the six seed mix treatments was sown into a 5x5 m plot. Blocks were prepared and sown in pairs on 26 April (C & F), 29 April (A & B) and 3 May 2013 (D & E). Blocks were separated by an average nearest-neighbour distance of approximately 100 m (108 ±26 mean ±SD).

2.2.4 Meadow floral composition and flower-visitor surveys

Meadows were surveyed for their floral composition and flower-visitor assemblages at three monthly time-points during the flowering season in 2013 (hereafter, 'survey rounds'): in late July (30 July-3 Aug.), late August (27 Aug.-2 Sept.), and late September (20-27 Sept.). The first two survey-rounds approximately corresponded to peak flowering across meadows in 2013.

Flower-visiting insects were surveyed by walking a 5 m-long transect along the diagonal axis of each 25 m^2 plot in each block, catching by handnet all Hymenoptera, Diptera, Lepidoptera and Coleoptera seen to be contacting

the reproductive parts of a flower, up to 1 m either side of the transect line. For each insect captured, the flower species visited was recorded and the insect was killed with ethyl acetate for identification and to prevent resampling (thereby ensuing observations are independent). To increase flower-visitor sample sizes each transect was sampled twice per survey round, with the two samples collected on the same day and with at least 30 minutes in-between. All insect surveys were conducted between 10.00 and 18:00 hrs in warm, dry weather, with temperature in the shade greater than 15 °C and wind speed lower than a moderate breeze (4 on the Beaufort scale).

Floral composition surveys were performed on blocks A, B, D, and E. Data from blocks C and F were excluded from further study because the seed mixes failed to establish due to overwhelming competition from unintended species (as described below; Fig. 2.3 pictures 'c' and 'f'). Floral composition surveys were performed within 48 h of corresponding flower-visitor surveys for the same survey round. Floral composition was quantified by counting the number of floral units of each species, including weeds, in five 1 m^2 quadrats in each 5x5 m plot. As in most community-scale studies on floral resources and flower-visitor interactions (e.g. Baldock *et al.* 2015; Baude *et al.* 2016; Hicks *et al.* 2016) floral units were defined as a flower or group of flowers that a honeybee-sized flower-visitor could walk within, but from which it must fly, rather than walk, to reach another equivalent floral unit (Dicks *et al.* 2002). Quadrats were located contiguously on alternating sides of the flower-visitor sampling transect, with random right/left placement of a first quadrat, followed by alternate left/right placement of subsequent quadrats.

2.2.5 Classification of floral units

Surveyed meadows contained floral units from an array of species, including species sown intentionally as part of seed mix treatments as well as unintentionally-occurring contaminants and weeds. To examine the extent to which the floral composition of each meadow reflected the species composition of the respective seed mix treatment, each floral species in each field plot was classified into one of three horticultural categories: 'treatment', 'contaminant' or 'weed' (hereafter, collectively: 'floral categories'). Weeds were naturally-occurring or unintentionally-propagated ruderal or arable species that were not sown as part of any of the seed mixes. Treatment and contaminant floral categories consisted of cultivated species that were sown in at least one of the seed mixes. Sown species were classified as treatment or contaminant based on whether they were an intended (treatment) or unintended (contaminant) component of the respective sown seed mix for a given meadow.

This floral classification enabled quantification of the floral abundance and richness of unexpected floral species in meadows of each seed mix treatment. For treatment floral units, plants were assumed to have germinated from seed applied as part of seed mix treatment. For contaminant floral units, plants were assumed to have germinated from seed that was laid down in the seed bank in 2012. For treatment floral units in a given field plot, no distinction could be made between plants that germinated from the seed mix applied to the field plot and plants that germinated from the seed bank laid down in 2012. All recorded floral units of sown species (species listed as present in one or more seed mix treatments) were assumed to have germinated from seed mixes. This was unlikely to have been the case for *Tripleurospermum inodorum* (Scentless Mayweed;

Asteraceae), which was widely present as a weed outwith field plots. However, because there was no practical way to distinguish in the field between floral units of wild *T. inodorum* (a weed) and those of the sown cultivar, all floral units of *T. inodorum* were classified as either treatment (in meadows of Cornfield mixes) or contaminant floral units (in meadows of Marmalade and Short mixes).

Figure 2.3: Photographs taken on 14th August 2013, before survey round 2, of the 6 replicate blocks of meadows (A, B, C, D, E and F). Note that block C was destroyed by contractors attempting to control a dominant weed, whilst block F was dominated by contaminant *Glebionis segetum* and *Echium vulgare* 'Blue Bedder'.


(C)
(D)


(E)
(F)

2.3 Justification for the exclusion of blocks 'C' and 'F'

Blocks 'C' and 'F' were excluded from further analysis in this thesis. For Block C, no data was collected as the meadow species were outcompeted by a dominant weed (Charlock; *Sinapis arvensis*). The treatments were eventually completely destroyed in an attempt by grounds workers to control this weed species (Fig 2.3 C). For block F, contamination was particularly pervasive, with each plot containing a high density of floral units from two contaminant species: *Echium vulgare* 'Blue Bedder' (Boraginaceae) and an unknown cultivar of *Glebionis segetum* (Asteraceae). In block F, *Echium vulgare* was present in all plots regardless of which seed mix treatment was sown. Moreover, *E. vulgare* produced on average 13.9 ±19.3% of floral units recorded over the season in each meadow in block F (mean±SD across plots), whereas no floral units of *E. vulgare* were observed in blocks A, B, D or E.

Similarly, *Glebionis segetum* was abundant in all plots in block F, regardless of seed mix treatment, and composed on average 59.8 ±29.9% of floral units recorded over the season in each meadow. In contrast, in blocks A, B, D and E, the proportion of floral units of *G. segetum* in each meadow ranged from 2.2 ±2.5% in block 'E' to 5.0 ±4.7% in block A (see Figure A2.1 for bar plots of the proportion of floral units of *E. vulgare* and *G. segetum* recorded in each field plot).

Given that meadows in block F contained exceptionally high densities of *E. vulgar*e and *G. segetum*, which originated from the seed bank rather than sown seed mix treatments, meadows in this block were considered to be unrepresentative of seed mix treatments and are excluded from further analyses in this chapter and in subsequent chapters of this thesis.

Chapter 3: Flowering performance and floral enrichment of annual urban flower seed mixes.

3.1 Introduction

Commercial flower seed mixes are increasingly used in urban areas as a cost-effective approach to improving the aesthetic amenity value of urban green spaces (Hitchmough & Dunnett 2004; Scott 2008; Bretzel *et al*. 2016). Given that funds for park maintenance are in decline (Dunnett *et al*. 2002; Barber 2007; Lambert 2014), and there is increasing evidence and awareness that urban areas can provide valuable habitat for biodiversity (Baldock *et al*. 2015; Aronson *et al*. 2017; Hall *et al*. 2017), flower seed mixes can help park managers meet their responsibilities to manage for both human amenity and biodiversity (Hitchmough & Dunnett 2004; Hitchmough 2010). Such joint considerations are actively encouraged by civic schemes, such as 'Britain in Bloom' (https://www.rhs.org.uk/communities/campaigns/britain-in-bloom) and the 'Green Flag Awards' (http://www.greenflagaward.org), and can be required by statute (as with the 'biodiversity duty' in the Nature Conservation (Scotland) Act 2004, and the Natural Environment and Rural Communities Act 2006). However, a challenge for parks managers is making evidence-based decisions on which seed mixes best deliver benefits for both people and biodiversity.

Most annual 'wildflower' seed mixes in Britain and northern Europe are based on mixtures of flowering herbaceous plants that have co-evolved over thousands of years with arable crops (Preston *et al.* 2004). These mixtures of 'native cornfield annuals' are the most commonly available type of flower seed mix on the market in the UK (Dunnett 2008). They are comprised of hardy annuals that have evolved to germinate readily in

disturbed soil and to set seed before crops are harvested in late summer or early autumn (Preston *et al.* 2004). As such, the flowering season of cornfield annuals is typically short, which can lead to dissatisfaction among users of commercial versions of cornfield annual seed mixes (Dunnett 2008). This has resulted in the development of annual seed mixes designed for their visual appeal to people (known as urban or 'pictorial' flower meadow seed mixes; Hitchmough 2004; Heatherington & Sargeant 2005; Hitchmough 2008, 2011). These pictorial seed mixes are designed to produce a high density of visually attractive flowers over an extended flowering (Hitchmough 2004, 2017). To be commercially viable, they must also perform reliably and require minimal maintenance (both of which can be enhanced by the suppression of weeds; Hitchmough 2010; Köppler & Hitchmough 2015). Design of appropriate seed mixes involves using horticultural knowledge of plant traits to meet the following criteria:

- Reliability: plants are selected to have similarly high germination and growth rates (Hitchmough 2010; Köppler & Hitchmough 2015).
- Low-maintenance: selection of species, site preparation and sowing regimes are intended to exclude invasion by (and survive competition with) weeds and grasses (Aldrich 2002; Hitchmough 2010; Köppler & Hitchmough 2015).
- Visual attractiveness: species are selected based on colour, often with desirable colour themes in mind. Favoured species often have large flowers, low vegetative mass, discrete and erect growth form enabling a high density of flowers (Dunnett 2008; Hitchmough 2011).
- Long flowering season: species are selected to combine early and late flowering species, creating a seasonal succession of flowers. Non-

native species are often incorporated to increase the range of colours available and to extend the flowering season (Dunnett 2008; Hitchmough 2008, 2011)

Within these general design criteria, pictorial meadows seed mixes vary substantially in floral species richness and composition, and in architectural diversity based on floral traits such as height and colour. Thus, the choice of which flower seed mix is used for an urban planting is likely to affect resource provision for flower-visitors. However, although potential biodiversity benefits are sometimes highlighted in marketing, pictorial meadows are primarily designed to provide a dramatic visual display. The resources that they provide for flower-visitors have, until recently, been little studied. There is substantial 'grey literature' of accumulated anecdotal or informal observations on which species are attractive to flower visiting insects (Crawford 2000; Hooper & Taylor 2006; IBRA 2008; RHS 2011; The Xerces Society 2011; Kirk & Howes 2012), but only a few studies have performed cross-species comparisons of species contained in urban flower seed mixes (Blackmore & Goulson 2014; Baude *et al*. 2016; Hicks *et al*. 2016). Flower-visiting insect communities are comprised of many species with varying resource requirements. For example, bees and hoverflies require nectar sugar for energy and pollen for protein, which supports body maintenance and the development of eggs and offspring (Haslett 1989; Nicolson 2011; Rotheray & Gilbert 2011; Vaudo *et al*. 2015). In contrast, adult butterflies and moths primarily feed on nectar for sugars, obtaining most of the protein and amino acids that they require when foraging as larvae (Dennis 1992; Dennis *et al*. 2006). Flower visitor taxa also differ in their ability to access or efficiently exploit resources from different floral morphologies: for example, the depth of nectaries within flower corollas correlates with the

'tongue' length of visiting bees (Ranta & Lundberg 1980a; Harder 1985) and hoverflies (Gilbert 1981; Branquart & Hemptinne 2000), and provides an upper limit on nectar accessibility for butterflies (Corbet 2000). Plants with deep flowers tend to produce relatively high volumes of dilute nectar (Plowright 1987; Ackermann & Weigend 2006), which long-tongued insects require for efficient nectar uptake (Harder 1986; Kim *et al*. 2011). In contrast, flower visiting flies can consume highly concentrated and almost crystalline nectar from nectaries in very shallow flowers (Woodcock *et al*. 2014), such as the on the umbel inflorescences of Umbelliferae (e.g. Willmer 1983 for visitors to hogweed, Heracleum). Both the characteristics of floral resources (Potts *et al*. 2003; Potts *et al*. 2004; Ackermann & Weigend 2006) and their accessibility within floral morphologies (Harder 1985; Branquart & Hemptinne 2000) are thus key determinants of the nutritional value of flowers to visiting insects (Ranta & Lundberg 1980b; Harder 1986; Kim *et al*. 2011; Balfour *et al*. 2013). A diversity of flower shapes and floral resource traits are therefore required to support a diverse flower-visiting insect community (Potts *et al*. 2004). Moreover, appropriate resources must be available throughout the year to support visitors active at specific times of year or throughout the season, such as many solitary (Oertli *et al*. 2005)) or eusocial bees, respectively (Westphal *et al*. 2009; Rundlof *et al*. 2014; Schellhorn *et al*. 2015).

Some of the design criteria for pictorial meadows – such as the production of a high density of flowers over an extended flowering season – are thus also desirable for supporting flower-visiting insects. However, pictorial meadows seed mixes are often composed of hardy annual Asteraceae and Papaveraceae, which (per flower or flower head) typically provide low to very low amounts of often viscous or crystalline nectar (Hicks

et al. 2016). Hence, although pictorial meadows provide abundant pollen, and nectar sources that are accessible to a subset flower visitors (such as hoverflies and short-tongued solitary bees), they often provide unsuitable nectar sources for larger, long-tongued insects, such as bumblebees.

3.1.1 Objectives

In this chapter, I examine the impact of seed mix choice on the composition and abundance of flowers recorded in planted meadows. I used a field experiment to compare 3 commercially-available annual flower seed mixes that differed in species and flower-shape richness (see Methods). Mixes were explicitly chosen to provide contrasting exemplars from an array of seed mix compositions, and to contain annual species commonly used in pictorial mixes, such as Cornflower (*Centaurea cyanus*), California poppy (*Escholozia californica*), and Corn poppy (*Papaver rhoeas*).

To examine whether floral resource provision could be improved whilst maintaining these aesthetic designs, I also devised a nectar enrichment treatment intended to increase nectar sugar mass the per unit area in meadows. The aim was to increase the seed contribution (and hence floral abundance) of species selected for enrichment, whilst maintaining the aesthetic purpose of meadows and ensuring the efficient use of seed.

In this chapter, I examine the flowering performance of seed mixes with three objectives.

Objective 3.1. ***To characterise and compare flowering performance of different pictorial meadows seed mixes in terms of users horticultural expectations.***

I first describe total-season floral richness and floral abundance in planted meadows, examining whether floral richness matches expectations based on input seed mixes. I also highlight species that flower inconsistently or do not flower at all, which can inform future seed mix design.

Objective 3.2. ***To characterise and compare the flowering performance of different pictorial meadows seed mixes as contrasting plant communities.***

I then characterise and compare floral diversity, abundance and composition between meadows of different seed mix types. The aim here was to examine whether observed meadows represent contrasting plant communities and hence valid seed mix treatments on which to base subsequent analyses.

Objective 3.3. ***To test whether experimental enrichment for particular species was effective in increasing the floral abundance of target species.***

Finally, I test the effectiveness of my enrichment treatment, examining whether enriched seed mixes produce meadows with more floral units of enriched species.

3.2 Materials and methods

The methodology used here corresponds to the experimental design and seed mix treatments outlined for year 2013 in Chapter 2 General Methods.

3.2.1 Study system and field experiment

Meadow floral composition data were collected from a field experiment, set up using a randomised complete block design, with 6 replicate blocks (A, B, C, D, E and F). Each block was sown with six annual seed mix treatments, consisting of 2 formulations (standard vs. enriched) of each of 3 commercially available flower seed mixes (Marmalade Annual, Short Annual, and Cornfield Annual), which were provided by my CASE partner organisation (Pictorial Meadows Ltd., Sheffield, UK; see Chapter 2 Table 2.1 for seed mix compositions). These seed mixes were chosen to allow comparison of a common flower seed mix comprising traditional corn field species (Cornfield Annual, n=4 species), with two exemplar pictorial mixes selected to contrast in species and flower-shape richness. The Marmalade mix (n=6 species) was selected as an exemplar of a species-poor and flower shape-poor mix. The Short mix (n=13 species) was selected as an exemplar of a species-rich and flower shape-rich mix. The Cornfield mix was selected as an exemplar of a seed mix based on traditional annual arable 'wildflowers'. For full details of mix compositions see Chapter 2.

Enriched seed mix formulations were designed to maintain commercially desirable characteristics, including reliability, efficient use of seed and aesthetic character (i.e. colour, height), while increasing floral nectar rewards for flower-visiting insects. This was achieved by taking baseline seed mixtures, provided by standard seed mix formulations, and,

for each mix type, increasing the proportional seed weight of selected species that were predicted to provide high-quantities of floral nectar sugar per floral unit. Full details of the species selected for the nectar enrichment treatment are provided in Chapter 2.

Each of the six seed mix treatments was sown into a 5x5 m plot. Blocks were prepared and sown in pairs on 26 April (C & F), 29 April (A & B) and 3 May 2013 (D & E). Blocks were separated by an average nearest-neighbour distance of approximately 100 m (108 ±26 mean ±SD).

3.2.2 Meadow floral composition surveys

Meadows were surveyed for their floral composition and flower-visitor assemblages at three monthly time-points during the flowering season (hereafter, 'survey rounds'): in late July (30 July-3 Aug.), late August (27 Aug.-2 Sept.), and late September (20-27 Sept.).

Floral composition surveys were performed on blocks A, B, D, and E. Data from blocks C and F were not included in this study because the seed mixes failed to establish due to overwhelming competition from unintended species (see Chapter 2). Floral composition surveys were performed within 48 h of corresponding flower-visitor surveys for the same survey round (see Chapter 5 for analyses of visitation). Floral composition was quantified by counting the number of floral units of each species, including weeds, in five 1 m^2 quadrats in each 5x5 m plot. Quadrats were located contiguously on alternating sides of the flower-visitor sampling transect, with random right/left placement of a first quadrat, followed by alternate left/right placement of subsequent quadrats.

3.2.3 Classification of floral units

Each floral species in each field plot was classified into one of three horticultural categories: 'treatment', 'contaminant' or 'weed' (hereafter, collectively: 'floral categories'). Sown species were classified as treatment or contaminant based on whether they were an intended (treatment) or unintended (contaminant) component of the respective sown seed mix for a given meadow. Weeds were naturally-occurring or unintentionally-propagated ruderal or arable species that were not sown as part of any of the seed mixes. This floral classification enabled quantification of the floral abundance and richness of unexpected floral species in meadows of each seed mix treatment.

3.2.4 Data analysis

Statistical analyses

This experiment was designed to compare meadow floral resources, floral rewards and insect flower-visitors between meadows of different seed mix types and formulations. A randomised complete-block (RCB) design was used to reduce extraneous variation between seed mix treatments caused by uncontrolled environmental variation. To account for seasonal variation in meadow floral composition, meadows were sampled multiple times during the season. Hence, data are spatially and temporally grouped (non-independent).

To account for this non-independence, data were analysed using linear mixed models (LMMs) and generalised linear mixed models (GLMMs) constructed using package 'lme4' (Bates *et al.* 2015) in R v. 3.3.2 (R Development Core Team 2016). These allow dependencies within data to be accounted for by specifying grouping variables as varying-intercept random

effects. All models were tested for heteroscedasticity and, where relevant, overdispersion and normality.

To model spatial and temporal effects, I considered multiple alternative random effects structures. Statisticians typically advise against specifying terms with fewer than 5 levels as random effects since estimates of their variance are unreliable (Bolker 2008; Zuur *et al.* 2009), although this does not in itself preclude the use of mixed effects models (Gelman & Hill 2007). In this study, there were 4 experimental blocks (with meadows representative of seed mix treatments) and 3 survey rounds, suggesting that both spatial and temporal effects should be fitted as fixed effects. However, given that this dataset consists of only 72 observations and that random effects are more efficient in their use of degrees of freedom, I examined the effects of alternative random effect structures on model coefficient estimates.

Given that blocks were independent, more numerous than survey rounds, and were not expected *a priori* to have strong directional main effects or treatment-level interactions, I fitted block as a varying-intercept random effect in all models. In contrast, there were fewer survey rounds, which were ordered by substantial directional seasonal change that was expected to have strong directional main effects on the floral characteristics of meadows, with potential treatment-level interactions.

To account for uncertainty regarding the impact of alternative model structures on model coefficient estimates, I examined coefficient estimates and inferences of models in which survey round was fit as either a fixed or random effect. Thus, I compared 'fixed round' models in which 'round' was specified as a fixed effect (and block as a random effect), with 'random round' models in which 'round' and 'block' were specified as crossed

varying-intercept random effects. This provided a pragmatic approach to assess the impact of a potential trade-off between over-fitted models (fixed round models) and models with unreliable estimates of random effect variances (random round models). Despite this potential trade-off, coefficient estimates and inferences regarding main treatment effects (mix type and mix formulation) were unaffected by these alternative model structures. Therefore, I present results from 'fixed round' models in the main text and provide 'random round' model summaries in appendices.

'Seed mix type' and 'seed mix formulation' (enrichment status) were specified and maintained in all models as fixed effects. Additional covariates were added to models where appropriate to account for known systematic sources of variation such as sampling effort. Given the limited sample size and risk of overfitting models, interactions were tested only when this corresponded to a direct *a priori* hypothesis test or when, due to the inherent hierarchical structure of the data, omission of an interaction may have led to misleading inferences regarding main effects.

Single-term deletion log-likelihood ratio tests (LRTs) of nested models were used for omnibus tests of the significance of fixed effects. Post-hoc pairwise contrasts were used to test for differences in estimated marginal means between each level of factors found to have a significant effect on response variables. For significant interactions, conditional pairwise contrasts were used to test for differences between the estimated marginal means of each level of each factor, within each level of a corresponding interacting factor. Pairwise contrasts were performed using the R package 'lsmeans' (Lenth 2016).

P-values for all pairwise contrasts were adjusted to control for elevated Type 1 error rates due to multiple comparisons. Adjustments were made to ensure a family-wise Type 1 error rate of α=0.05, using either Tukey's HSD or the 'mvt' method in 'lsmeans'. This method performs a one-step adjustment (similar to classical Bonferroni) that adjusts the critical value used to calculate confidence intervals and *p*-values using a multivariate *t* distribution for *k* pairwise contrasts (Lenth 2016). For LMMs, 'lsmeans' uses Satterthwaite approximations to estimate degrees of freedom with which to perform tests and calculate confidence intervals. For GLMMs, degrees of freedom are not available; therefore, lsmeans performs asymptotic tests and calculates asymptotic intervals (Lenth 2016). Tests and confidence intervals therefore assume large samples sizes.

Data summaries presented throughout this chapter are means ± 1 standard error of the mean (SE), unless otherwise stated. Values presented in text, tables or figures are calculated from raw data, except as follows. All figures showing pairwise contrasts between meadows of different seed mix treatments, and all text descriptions of % differences in the magnitude of the response variables between treatments, are based on estimated marginal means from linear models.

(i) Objective 3.1. *Does observed floral species richness in meadows of differences seed mix types match expectations based on respective input seed mix species lists?*

To characterise and compare the flowering performance of seed mixes, I first examined seasonal floral species richness and floral abundance as simple descriptive measures of seed mix performance. Given that users of flower seed mixes expect them to produce meadows containing abundant

'flowers' from a diversity of species, these are crude but valid baseline measures of seed mix performance.

To disentangle the effect of sown seed mixes from contamination and weeds, I examined seasonal floral species richness and abundance separately for treatment, contaminant and weed floral units, as well as collectively for all flowering species.

(ii) Objective 3.2. *Do observed meadows comprise distinct floral communities concordant with expectations based on input seed mix treatments?*

(a) *Does floral species richness or floral diversity differ between meadows of different seed mix types?*

Estimates of species richness are sensitive to sampling effort, with more species likely to be detected with more sampling (Gotelli & Colwell 2001). In community-level surveys, quadrat sampling methods may be used to standardise sampling effort; however, densities of individuals typically vary between communities, resulting in uneven sampling effort at the level of individuals. This can lead to inaccurate conclusions when comparing species richness estimates between communities.

To accurately compare species richness between communities requires estimation of asymptotic species richness or estimation of species richness for a standardised level of sampling effort. The asymptotic species richness of a community can either be estimated by: (a) sampling enough of each community for species accumulation/rarefaction curves to asymptote; or (b) using an extrapolation procedure that estimates asymptotic richness (Gotelli and Colwell, 2011; Chao and Chiu, 2012, 2016a). Alternatively, species richness can be compared for a standardised sampling effort. This can be

done by: (c) estimating richness from rarefaction curves for a standard sample size equivalent to the smallest sample size among communities (Gotelli & Colwell 2001); or (d) estimating richness from combined rarefaction/extrapolation curves, which rarefy or extrapolate estimates for different communities to a common sampling effort (number of individuals, sample area or sample coverage; Chao & Jost 2012a; Colwell *et al.* 2012; Chao *et al.* 2014).

To assess and account for differences in the number of individuals sampled in each replicate meadow, I compared estimates of species richness from empirical data with estimated richness from an asymptotic species richness estimator (Chao2; Chao 1987; 'b') and estimated species richness based on a projected doubling of sampling effort ('d'). The Chao2 estimator is a non-parametric 'asymptotic' estimator of species richness for incidence-based (quadrat) data, which was derived to provide a lower bound estimate of 'true' (asymptotic) species richness (Chao 1987). In contrast, extrapolation of incidence-based sampling effort enabled estimation of expected species richness in 10 m^2 rather than 5 m^2 of meadow (Colwell *et al.* 2012).

To crudely assess the degree to which empirical species richness approached asymptotic richness, I also calculated sample coverage for each community using an analytical formula for expected sample coverage (Chao and Chiu, 2016b). Sample coverage essentially provides an estimate of the location of a sample on a species accumulation curve and is defined as the proportion of the total number of individuals in the community that belongs to species in the sample (Chao and Chiu, 2016b). It provides a measure of sample completeness, which can be estimated accurately from a reference sample, assuming sample sizes are large (Chao and Chiu, 2016b).

Overall, estimated sample coverage was high in meadows of each seed mix treatment (see Appendix Table A3.3.1). However, Chao2 estimated species richness per meadow per round (5 m^2/round) was higher than extrapolation-based estimates, which were in turn higher than empirical estimates (see Appendix Figure A3.3.1 and Table A3.3.1). Most of these differences were due to variation associated with weeds, with only minor differences between alternative richness estimates for treatment and contaminant floral units (see Figure A3.3.2 and Table 3.3.2). Consequently, the sampling effort adopted in this study (5 m^2 in each meadow per round) was enough to accurately represent treatment and contaminant floral components of communities, but not enough to accurately characterise the weed component of communities.

To assess the impact of alternative methods of species richness estimation on community comparisons, I compared models of empirical richness, extrapolated richness and Chao2 richness. Models of extrapolated richness and Chao2 richness were constructed using Gamma GLMMs with log-link functions, which contained fixed effects of 'mix type', 'mix formulation' and 'round', with 'block' as a random effect (see Appendix Table A3.4.1). Empirical species richness was modelled using a Poisson GLMM with a log-link function, containing the same fixed and random structure as other models but with 'total floral abundance' as an additional covariate to control for variation in sampling effort.

Results of models were not consistent, with significant effects of mix formulation and round detected in models of extrapolated richness and Chao2 richness (see Table 3.1). However, simulation studies have shown that, as is likely in this study, use of estimators of species richness can lead to

elevated Type 1 error rates in comparative experiments when sample sizes and species-abundance distributions vary between communities (Gwinn *et al.* 2016).

Given that sample coverage for treatment and contaminant floral units was high and that variation in the floral composition of weeds was associated with blocks rather than seed mix treatments (this chapter), variation in species richness estimates due to undersampling of weeds is unlikely to systematically affect empirical species richness estimates and comparisons between mix types. Moreover, given that models of empirical species richness explicitly modelled the processes that gave rise to the data (Poisson counts of species from samples of varying numbers of individuals (sampling effort)), and that model diagnostic plots suggested a better model fit, only results from empirical species richness models are presented. Full results of models of extrapolated richness and Chao2 richness are presented in appendices (Table A3.4.1).

To examine and compare diversity between meadows of different seed mix treatments, I calculated exponential Shannon's entropy (hereafter 'Shannon diversity') and the inverse of Simpson's concentration (hereafter 'Simpson's diversity'; Chao & Jost 2012b). Shannon diversity weights species in proportion to their floral abundance and can be interpreted as the number of 'common' species in the community. In contrast, Simpson's diversity down weights rare species and can be interpreted as the number of 'highly abundant' species in the community (Chao *et al.* 2014). All estimates of floral species richness and diversity indices were performed using EstimateS v.9.1 (Colwell 2013; Colwell & Elsensohn 2014).

Table 3.1: Results of 'fixed round' and 'random round' models of floral species richness. Empirical floral richness was modelled using a log-link Poisson GLMM. Extrapolated richness and Chao2 richness were modelled using log-link Gamma. All fixed round models contained fixed effects of 'mix type', 'formulation' and 'round', with 'block' as a random effect. Random round models were identical except that round was fitted as a random effect. Models for empirical richness also contained 'total floral abundance' as an additional covariate to control for variation in sampling effort. Results show single term deletion log-likelihood ratio tests. For Chao2 richness, the random round model did not converge so results are not shown.

		Model structure							
		Models with 'round' as fixed				**Models with 'round' as random**			
Response	**Predictors (fixed effects)**	**df**	**AIC**	**χ^2**	**p-value**	**df**	**AIC**	**χ^2**	**p-value**
Species richness	Full model AIC		388.06				381.54		
	log(Floral abundance+1)	1	380.04	1.20	0.27	1	383.80	4.26	**0.0389**
	Mix type	2	392.62	15.78	**0.0004**	2	393.08	15.54	**0.0004**
	Formulation	1	380.89	2.06	0.15	1	381.51	1.97	0.16
	Round	2	379.54	2.70	0.26	-	-	-	-
Extra-polated richness	Full model AIC		405.97				408.42		
	Mix type	2	416.11	14.13	**0.0009**	2	418.38	13.95	**0.0009**
	Formulation	1	407.97	3.99	**0.0456**	1	410.29	3.86	**0.0493**
	Round	2	415.45	13.48	**0.0012**	-	-	-	-
Chao2 richness	Full model AIC		450.04			-	-	-	-
	Mix type	2	456.16	10.11	**0.0063**	-	-	-	-
	Formulation	1	452.19	4.14	**0.042**	-	-	-	-
	Round	2	453.79	7.75	**0.0208**	-	-	-	-

To compare Shannon and Simpson's floral diversity between meadows of different mix types, I used LMMs with fixed effects of mix type, formulation and round, with block as a random effect.

In addition to comparison of total empirical floral species richness and diversity between meadows of different seed mix types, I compared floral species richness of treatment, contaminant and weed floral categories between meadows of different mix types. For this I used a Poisson GLMM with log-link, testing fixed effects of mix type, formulation, floral category and round, with block as a random effect. To compare floral categories

within and between mix types, I fitted an interaction between floral category and mix type and used conditional pairwise contrasts to test for differences between the estimated marginal means of each level of each factor, within each level of the corresponding interacting factor. P-values were adjusted to account for 18 simultaneous tests using the 'mvt' method in R package 'lsmeans' (Lenth 2016), as explained above.

(b) *Does floral abundance or seasonal pattern of flowering differ between seed mix treatments?*

To test for differences in total floral abundance between meadows of different mix types, I used a Poisson GLMM with log-link function. Fixed effects were mix type, formulation and round; with an interaction between mix type and round; and block as a random effect. A random effect of observation ID (for n=72 observations) was fitted to account for overdispersion (following Elston *et al.* 2001; Harrison 2014; Hayward *et al.* 2015). Overdispersion likely resulted from floral contaminants, which were unevenly distributed across plots and ensuring this model effectively had a missing variable of floral category (which was accounted for in the model described below, which was not overdispersed). Alternative approaches to modelling floral abundance either foundered due to lack of convergence (negative binomial GLMM) or yielded the same results (LMM on log-transformed floral counts).

To compare abundance of floral units from different floral categories, I used a negative binomial GLMM using function 'glmer.nb' in the R package 'lme4' (Bates *et al.* 2015), testing for differences in floral abundance between treatment, contaminant and weed floral categories. Fixed effects were mix

type, formulation, floral category and round, with an interaction between mix type and floral category. Block was fitted as a random effect.

To compare floral abundance of difference floral categories within and between mix types, I used conditional pairwise contrasts to test for differences in estimated marginal means between each level of each factor, within each level of the corresponding interacting factor. *P*-values were adjusted to account for multiple tests using the 'mvt' method in the R package 'lsmeans' (Lenth 2016).

(c) *Do meadows of different seed mix treatments differ in floral composition? (Do meadows represent distinct floral communities?)*

To compare seasonal floral composition between meadows of different seed mix treatments, I first assessed pairwise Bray-Curtis community dissimilarities using non-metric multidimensional scaling (NMDS) ordinations, and then tested the effects of seed mix type and formulation on these pairwise dissimilarities using permutational analysis of variance (PERMANOVA; Anderson 2001).

NMDS is an ordination method that enables graphical representation of a matrix of pairwise community dissimilarities (Clarke & Warwick 2001). In this study, communities were meadows in different field plots and pairwise dissimilarities between field plots were calculated for species floral abundance data. Dissimilarities were calculated for raw seasonal-totals of species floral abundance using Bray-Curtis dissimilarity, a dissimilarity measure which incorporates both species composition (presence of species in one or both communities, while ignoring joint absences) and relative abundance (relative abundance of each species in each community; Bray & Curtis 1957; Anderson *et al.* 2011). Bray-Curtis dissimilarity takes values

between 0 and 1, with low values indicating low dissimilarity (high similarity) and high values indicating high dissimilarity (low similarity). Pairwise dissimilarities were calculated and NMDS ordinations performed using functions 'vegdist' and 'metaMDS' in the R package Vegan v. 2.4-2 (Oksanen *et al.* 2017).

NMDS ordination ranks the dissimilarities between communities and creates a graphical representation, in a user-defined number of dimensions, of the relationships between communities, which preserves the rank order of dissimilarities, rather than their relative metric values. Hence, NMDS ordinations simply organise communities into a 'map' in which more similar communities are located closer together and more dissimilar communities further apart. The accuracy of this map in reflecting the rank-order dissimilarities between communities can be evaluated by a measure of 'stress' (values between 0 and 1), which is a function of the sum of squared residuals of a monotonic regression between ranked pairwise dissimilarities and ranked pairwise distances in ordination space. Thus, lower stress values indicate more reliable graphical representation of community relations, with non-zero values indicating some degree of distortion. Stress values of <0.1 indicate a reliable configuration, while values between 0.1-0.2 indicate specific relationships are unreliable, although broad patterns may be representative (Clarke & Warwick 2001).

In this study, three NMDS ordinations were performed to compare composition and relative abundance of floral units of (i) all flowering species, (ii) flowering weed species and (iii) 'sown seed mix species' between all 24 replicate field plots sown with different seed mix treatments. NMDS scores were calculated for 2-dimensional representations of relations between

communities. Stress values ranged from low to high (0.07, 0.11, 0.16) indicating potentially misleading distortion in some NMDS ordinations. Ordinations were therefore repeated allowing communities to be configured in 3 dimensions, increasing the flexibility of NMDS to configure communities correctly, but making 2D depiction more complex. Although 3D NMDS ordination provided more accurate depictions of the relationships between communities, this did not qualitatively change inferences regarding factors affecting floral composition of meadows. Therefore, I present the results of 2D NDMS ordinations.

To directly test the effect of seed mix type and seed mix formulation on floral composition of meadows (for each of the 'i-iii' floral datasets), I used PERMANOVA as implemented in function 'adonis' in the R package Vegan v. 2.4-2 (Oksanen *et al.* 2017). The 'adonis' function partitions variation in a pairwise dissimilarity matrix into sources of variation and fits a linear model to this variation, testing each term in the model sequentially, using permutation procedures to generate a null distribution for the test statistic (a pseudo F-ratio) calculated for each model term (Oksanen *et al.* 2017).

Conceptually, for a simple one term (one-way) model, total variation in pairwise dissimilarities (total sum of squared dissimilarities: SS_T) is partitioned into 'within group variation' (the sums of squared dissimilarities within groups: SS_W) and 'between group variation' ($SS_T - SS_W = SS_B$). A pseudo F-ratio test statistic is then calculated in the form of the ratio of 'between group variation' to 'within group variation' (SS_B/SS_W), with a higher F-value indicating more variation between groups than within groups. Statistical tests of this F-value provide an omnibus test of whether groups of differ in location.

For each term in the model, statistical significance is assessed by comparing the observed F-value to a null distribution of F-values, generated from n=999 permutations of pairwise dissimilarities among an appropriate subset of communities. *P*-values are then calculated as the proportion of values in the null distribution that are greater than or equal to the observed F-value.

The fundamental assumption of PERMANOVA is that permuted data are 'exchangeable' under a true null hypothesis. Thus, to test effects of seed mix type and formulation, I stratified all permutations by block, ensuring that no dissimilarities were permuted between blocks. This is analogous to fitting block (the effect of which is not directly tested) as a random effect. To test the effect of seed mix type on meadow floral composition, pairwise dissimilarities were freely randomised between all pairwise combinations of communities within each block. To test the effect of seed mix formulation, pairwise dissimilarities were randomised between standard and enriched formulations within each seed mix type within each block.

PERMANOVA provides an omnibus test of whether locations of groups differ in multivariate space (floral species dissimilarity); however, it has been shown to confound location and dispersion effects (Warton *et al.* 2012). Thus, for groups with identical centroid locations but substantially different dispersions, PERMANOVA may detect statistical differences, risking misattribution of the cause of differences and misinterpretation of treatment effects. Therefore, the null hypothesis tested is that there are no differences in location and/or spread in multivariate space between the compared groups.

(iii) Objective 3.3. *Do enriched seed mixes produce meadows with more floral units of enriched species?*

To determine whether the enrichment manipulation was effective in increasing the floral abundance of enriched species, I first examined, for each mix type, the net effect of enrichment on total seasonal floral abundance of species in three categories (hereafter 'amendment categories'). These were defined, with respect to the changes made to seed weights of species in enriched seed mix formulations, as: (i) species for which the proportion of seed was 'increased'; (ii) species for which the proportion of seed was 'decreased'; and (iii) species for which the proportion of seed was 'not changed'. Thus, for each seed mix type, species that composed seed amendment categories ('increased', 'decreased' and 'not changed') were the same set of species classified as producing 'treatment floral units' in meadows of the respective mix type.

To determine whether the enrichment manipulation was effective in increasing the floral abundance of individual species in the 'increased' or 'decreased' floral amendment categories, I then examined the net effect of enrichment on the floral abundance of individual species within amendment categories.

I considered several approaches to testing the effect of enrichment on the floral abundance of species in seed amendment categories. GLMMs directly testing the effect of seed amendment categories were prohibitively complex to specify for the full dataset. Conversely, separate models testing for an interaction between seed mix formulation (enrichment) and amendment category, within each mix type, did not have the power to detect an effect. Therefore, for each mix type, I calculated the mean difference,

between meadows of standard and enriched seed mixes, in the total number of floral units produced over the season by all species in each amendment category ('increased', 'decreased' or 'not changed'). Differences were calculated between meadows of standard versus enriched mixes paired within n=4 blocks, which was not enough replication for a Wilcoxon sign-rank test. Hence, I calculated confidence intervals to test whether differences were significantly different from zero.

Confidence intervals were corrected for small sample sizes (n=4). This was done by selecting a critical value for a two-tailed test at a significance level of 0.05, from a t-distribution with 3 degrees of freedom (critical value=3.182). Confidence intervals were then adjusted for multiple comparisons using a simple Bonferroni-correction equivalent to 0.05/k, where k=9 simultaneous comparisons (yielding a final critical value of 7.453). This corrected for 9 simultaneous tests which were conducted for differences in floral abundance of 'increased', 'decreased' and 'not changed' amendment categories in Marmalade, Short and Cornfield mix types. Resulting confidence intervals were equivalent to individual 99.5% confidence intervals with a family-wise Type 1 error rate of 0.05.

For tests of an effect of enrichment on individual floral species, differences in floral abundance were calculated for individual species between meadows of standard versus enriched mixes paired within blocks. Confidence intervals were corrected for sample size and for the number of species-level tests performed within the respective mix type. Although this was anti-conservative, more stringent procedures were not required and did not affect inferences.

3.3 Results

3.3.1 Objective 3.1: Comparing flowering performance of seed mixes with expectations based on seed mix composition

Overall, for four replicate blocks (A, B, D, and E), 71,777 floral units from 50 plant species were recorded in the 360 m^2 of meadows surveyed over all treatments, blocks and survey rounds.

The 6 seed mix treatments were composed of 20 plant species, of which five were present in multiple seed mix treatments (see Chapter 2 Table 2.1). Of these 20 sown seed mix species, 19 produced floral units in at least one plot over the course of the season (Table 3.2). The exception was *Echium vulgare*, which, although an annual cultivar of Viper's Bugloss (RHS 2011; Pictorial Meadows Ltd., personal communication), did not flower in any meadows.

Table 3.2: List of species recorded in at least one meadow, along with their assumed horticultural origin. Horticultural origins were defined based on whether species were listed as part of seed mixes or not.

Species	Family	Horticultural origin
Centaurea cyanus	Asteraceae	Sown species
Coreopsis tinctoria	Asteraceae	Sown species
Crepis rubra	Asteraceae	Sown species
Dimorphotheca sinuata	Asteraceae	Sown species
Glebionis segetum	Asteraceae	Sown species
Ismelia carinata	Asteraceae	Sown species
Nigella damascena	Asteraceae	Sown species
Rudbeckia hirta	Asteraceae	Sown species
Tripleurospermum inodorum	Asteraceae	Sown species
Iberis umbellata	Brassicaceae	Sown species
Agrostemma githago	Caryophyllaceae	Sown species
Gypsophila elegans	Caryophyllaceae	Sown species
Silene armeria	Caryophyllaceae	Sown species
Convolvulus tricolor	Convolvulaceae	Sown species
Linum grandiflorum	Linaceae	Sown species
Linum usitatissimum	Linaceae	Sown species
Eschscholzia californica	Papaveraceae	Sown species
Papaver rhoeas	Papaveraceae	Sown species
Linaria maroccana	Plantaginaceae	Sown species
Achillea millefolium	Asteraceae	Weed species

Cirsium arvense	Asteraceae	Weed species
Hypochaeris radicata	Asteraceae	Weed species
Lactuca serriola	Asteraceae	Weed species
Lapsana communis	Asteraceae	Weed species
Matricaria discoidea	Asteraceae	Weed species
Scorzoneroides autumnalis	Asteraceae	Weed species
Senecio sylvaticus	Asteraceae	Weed species
Sonchus asper	Asteraceae	Weed species
Sonchus oleraceus	Asteraceae	Weed species
Myosotis arvensis	Boraginaceae	Weed species
Brassica rapa	Brassicaceae	Weed species
Capsella bursa-pastoris	Brassicaceae	Weed species
Lepidium sativum	Brassicaceae	Weed species
Sinapis arvensis	Brassicaceae	Weed species
Sisymbrium officinale	Brassicaceae	Weed species
Cerastium fontanum	Caryophyllaceae	Weed species
Spergula arvensis	Caryophyllaceae	Weed species
Stellaria media	Caryophyllaceae	Weed species
Medicago lupulina	Fabaceae	Weed species
Trifolium pratense	Fabaceae	Weed species
Lamium album	Lamiaceae	Weed species
Epilobium hirsutum	Onagraceae	Weed species
Epilobium montanum	Onagraceae	Weed species
Veronica persica	Plantaginaceae	Weed species
Gilia achilleifolia	Polemoniaceae	Weed species
Fallopia convolvulus	Polygonaceae	Weed species
Persicaria lapathifolia	Polygonaceae	Weed species
Persicaria maculosa	Polygonaceae	Weed species
Polygonum aviculare	Polygonaceae	Weed species
Galium aparine	Rubiaceae	Weed species

These 19 species comprised most (78%) of all floral units recorded during meadow surveys (56,144 floral units). Of these, most were classified as 'treatment floral units' (44,488 floral units; 79%), while the remaining floral units of these sown species were classified as 'contaminant floral units' (11,656 floral units; 21%). The remaining 22% of all floral units (15,633 floral units), were from 31 'weed' species (Table 3.2), which produced large numbers of comparatively smaller floral units.

On a plot scale, seasonal totals of floral richness and abundance varied within and between meadows of different seed mix treatments (Tables 3.3 and 3.4). Seasonal floral richness was lowest in meadows of standard Cornfield treatments, but highest in standard Short (Standard Cornfield: 16.5 ±2.4; Standard Short 23.8 ±2.8 species; Table 3.4). The opposite was true for

seasonal floral abundance, with standard Short treatments having the lowest and standard Cornfield the highest numbers of floral units over the season (Standard Short: 2,675 ± 276; Standard Cornfield: 3,678 ±1,205; Table 3.3). Despite an inverse pattern between these two specific treatments, overall, there was no tight relationship between total seasonal floral richness and abundance (Fig. 3.1).

An inverse pattern was likely observed between these two treatments due to a combination of (i) intrinsic differences in richness between these mix types (Short and Cornfield mixes having the highest and lowest species richnesses, respectively); (ii) the enrichment of enriched Short mixes for *Centaurea cyanus* (an abundant and dominant species); and (iii) atypically high floral abundance in meadows of Cornfield mixes in block B, which produced thousands more floral units than any other replicate plot (Fig. 3.1). For both Cornfield mixes in block B, meadows were dominated by *Tripleurospermum inodorum,* with this species comprising over 80% of all floral units recorded in both of these replicate meadows. With the exception of these outliers, seasonal floral species richness and floral abundance were roughly similar between meadows of different treatments. However, the proportional contributions of treatment, contaminant and weed floral categories varied substantially between seed mix types (Tables 3.3 and 3.4).

Table 3.3: The abundance of floral units recorded in meadows of six different seed mix treatments over the season. Values are means ±SE for n=4 replicates of each treatment. Values are for surveys of 5 m^2 per replicate meadow in each of 3 survey rounds (15 m^2 per replicate in total). Percentages are calculated per plot and then averaged.

Seed mix treatment combination		Treatment floral units		Contaminant floral units		Weed floral units		Total abundance of floral units
Seed mix type	**Seed mix formulation**	**Abundance**	**%**	**Abundance**	**%**	**Abundance**	**%**	
Marmalade	Standard	724±103	27±5	1232±516	42±13	780±271	31±1	2735±299
Marmalade	Enriched	829±293	32±13	1175±621	39±21	797±270	29±1	2800±220
Short	Standard	1777±287	66±8	245±94	9±3	653±219	25±9	2675±276
Short	Enriched	2028±408	71±4	164±65	8±5	641±212	21±7	2832±503
Cornfield	Standard	3116±1206	81±7	55±14	2±1	507±155	17±7	3678±1205
Cornfield	Enriched	2648±1011	75±1	45±10	2±1	532±100	23±9	3224±991

Table 3.4: Expected and observed richness of flowering species recorded in meadows of six different seed mix treatments over the season. Values are means ±SE for n=4 replicates of each treatment. Values are for surveys of 5 m^2 per replicate meadow in each of 3 survey rounds (15 m^2 per replicate in total). Percentages are calculated per plot and then averaged.

Seed mix treatment combination		No. of species in seed mix species list	Treatment floral units		Contaminant floral units		Weed floral units		Total richness of floral species
Seed mix type	Seed mix formulation		Species richness	%	Species richness	%	Species richness	%	
Marmalade	Standard	6	5.8±0.3	30.3±3.3	4.5±0.3	23.6±2.1	9.3±1.7	46.2±4.8	19.5±1.7
Marmalade	Enriched	6	5.3±0.5	31.0±5.4	4.5±1.0	24.8±4.3	8.0±1.5	44.3±5.0	17.8±1.9
Short	Standard	13	11.8±1.3	49.9±3.4	2.8±0.5	11.9±2.2	9.3±1.7	38.2±2.8	23.8±2.8
Short	Enriched	13	11.0±0.9	55.5±1.7	2.3±0.8	11.5±4.0	6.8±1.4	33.0±4.0	20.0±2.1
Cornfield	Standard	4	4.0±0.0	26.3±4.7	4.5±0.6	28.1±3.6	8.0±2.1	45.6±7.0	16.5±2.4
Cornfield	Enriched	6	5.0±0.0	30.7±4.6	4.5±1.9	23.1±5.9	8.0±1.1	46.3±3.2	17.5±2.8

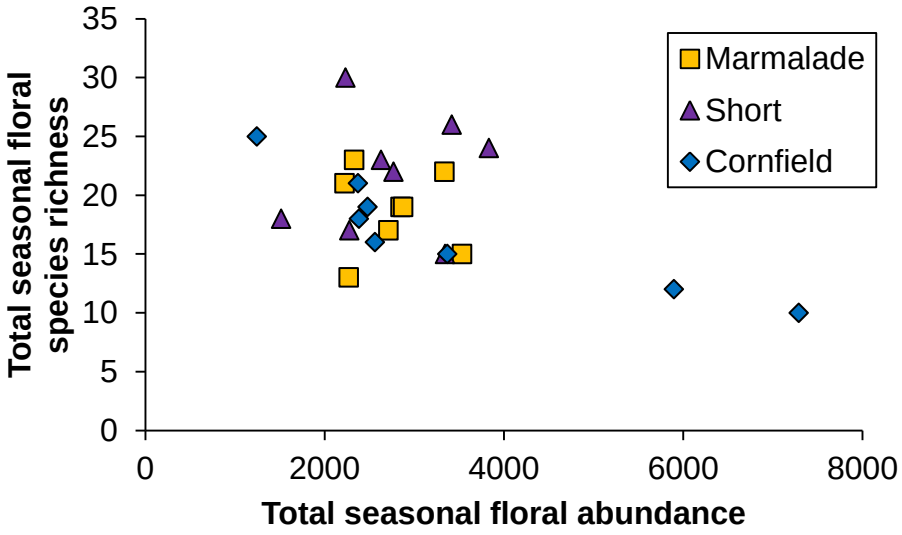


Figure 3.1: The relationship between total seasonal floral abundance and total seasonal floral species richness in 24 replicate meadow plots. Coloured symbols indicate the seed mix type applied to each of the 24 plots (Marmalade, Short or Cornfield). Seed mix formulation (enrichment status) is not shown. Values are totals from 3 surveys, each of 5 m^2 per replicate plot, performed monthly during 2013 (in late July, late August and late Sept.). Outliers are meadows of standard and enriched Cornfield in block B.

Weed seasonal richness and floral abundance were roughly similar across seed mix treatments (Tables 3.3 and 3.4); however, the species composition of weed flora varied across seed mix treatments and blocks (see Tables A3.1.1 & A3.1.2). Weed floral abundance was highly skewed, with 6 species accounting for 93% of all weed floral units. These were: *Polygonum aviculare* (54%), *Persicaria lapathifolia* (35%), *Persicaria maculosa* (26%), *Capsella bursa-pastoris* (4%), *Sisymbrium officinale* (4%), and *Stellaria media* (2%; Fig. A3.2.1c). Hence, most weed species were relatively rare.

For treatment floral units, the floral species richness in meadows closely matched expectations based on input seed mixes (Table 3.4). Floral richness of treatment species in meadows of enriched Cornfield mixes was lower than expected due to the absence of *Echium vulgare*. However, floral

richness of treatment floral units was higher in enriched compared to standard Cornfield mixes because *Glebionis segetum* floral units, which were present in almost all meadows of Cornfield mixes, were classified as treatment floral units in meadows of enriched Cornfield but as contaminants in meadows of standard Cornfield mixes (Table 3.4). For meadows of Marmalade and Short mixes, richness of treatment floral units was lower than expected due to the recurrent absence of certain species, which failed to establish and flower in some plots (Marmalade: *Ismelia carinata* and *Linum grandiflorum*; Short: *Convolvulus tricolor*, *Iberis umbellata*, *Linum grandiflorum*, *Nigella damascena*, *Papaver rhoeas*; see Table A3.1.1).

Contamination was widespread, with at least one contaminant recorded in each plot during the season (Table A3.2.1). This ubiquity may partly be because the most abundant and widespread species, *Tripleurospermum inodorum*, is also a native weed species, but all floral units were classified as either treatment or contaminant floral units, since floral units of the sown cultivar could not be reliably distinguished from native *T. inodorum* (a weed). Nevertheless, contamination by other species was common, with contaminant floral units of *Centaurea cyanus*, *Linaria maroccana*, *Glebionis segetum*, *Silene armeria* and *Coreopsis tinctoria* both widespread and numerous (Table A3.1.1; Fig. A3.2.1(b); Fig. 3.3a-c).

Contaminant floral richness was similar in meadows of Marmalade and Cornfield mixes (Table 3.4). This was because floral units of widespread species (germinating in part from the seed bank) were either classified as contaminants for meadows of both of these mixes (*Linaria maroccana, Linum usitatissimum, and Silene armeria*), or they were reciprocally classified as treatment for one and contaminant for the other (*Centaurea cyanus, Coreopsis*

tinctoria, Linum grandiflorum, and T. inodorum). For example, floral units of *Centaurea cyanus* were classified as 'treatment' in meadows of Cornfield mixes but 'contaminants' for meadows of Marmalade mixes, whilst floral units of *Coreopsis tinctoria* were classified as 'treatment' in meadows of Marmalade mixes but 'contaminants' for meadows of Cornfield mixes; Table A3.1.1). Contaminant floral richness was lowest in meadows grown from Short mixes. This was because most of the common and widespread species present in the seed bank (i.e. *Centaurea cyanus, Coreopsis tinctoria, Linaria maroccana, Linum grandiflorum, Linum usitatissimum,* and *Silene armeria*) were classified as treatment floral units in this mix.

Contaminant floral abundance was higher in meadows of Marmalade mixes compared to Short or Cornfield, and higher in Short compared to Cornfield (Table 3.3 and Fig. 3.2(a)). This was primarily due to variation in the classification of two abundant and widespread species: *Tripleurospermum inodorum* and *Centaurea cyanus*, which together accounted for 49.9% of all 71,777 floral units recorded in this study (see Table A3.1.1 for plot-scale species lists showing the spatial spread of species, and Figure A3.1.1 for rank abundance diagrams showing the floral abundance of each species). Floral units of these species were classified as contaminants in meadows of Marmalade mixes, but as either treatment (*C. cyanus*) or contaminants (*T. inodorum*) in Short mixes, or as treatment floral units in Cornfield mixes (both species). Conversely, treatment floral abundance was higher in Cornfield mixes compared to Short and Marmalade, and higher in Short compared to Marmalade (Table 3.3 and Fig. 3.2(a)). At a species level, treatment floral abundance was highly skewed, with six species accounting for 93% of all treatment floral units (Fig. 3.3).

Contamination (and to a lesser extent the presence of weeds) led to substantial qualitative overlap in species composition between meadows of different seed mix treatments, with 13 out of 19 sown seed mix species and 9 out of 31 weed species recorded in one or more of the four replicates of each seed mix treatment (Table A3.1.1). Hence, meadows of different mix types and formulations were qualitatively more similar in floral composition than expected based on input seed mix species lists.

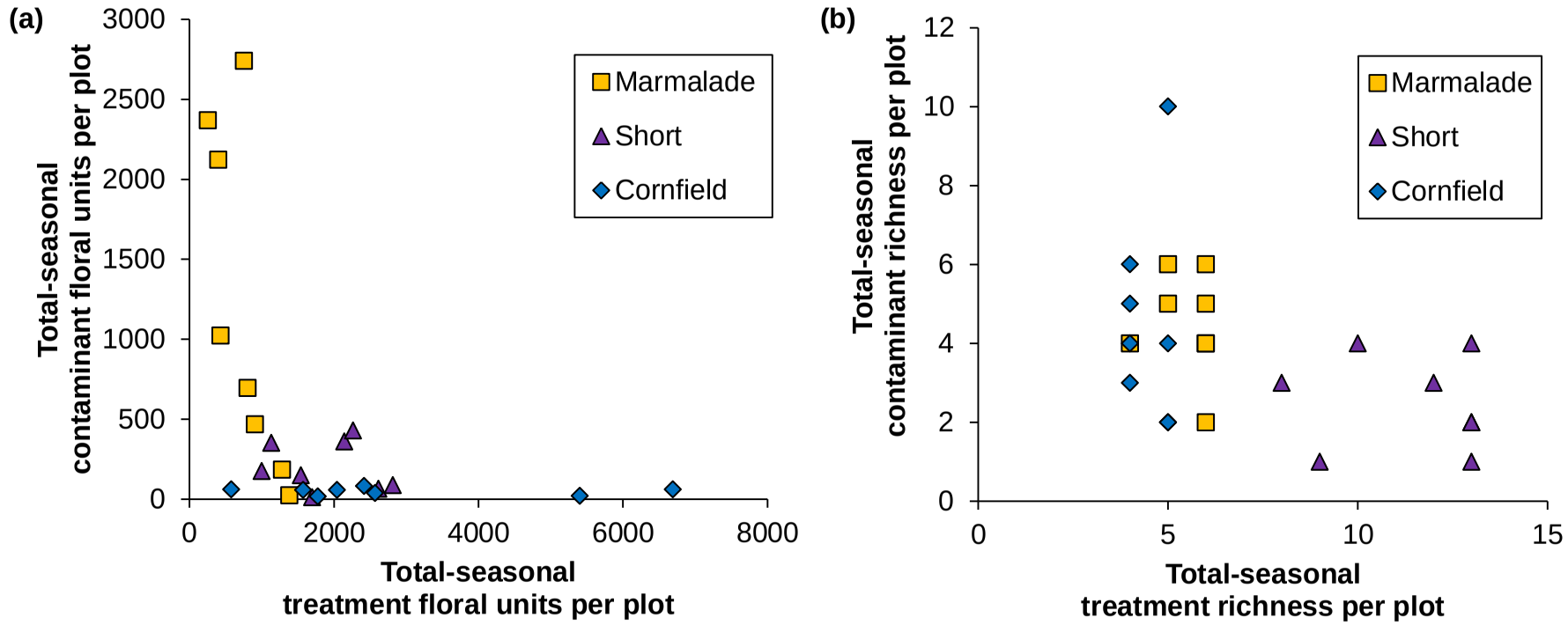


Figure: 3.2: The relationship between total seasonal treatment and contaminant floral abundance or richness in 24 replicate meadow plots, showing: (a) treatment vs. contaminant seasonal floral abundance; and (b) treatment vs. contaminant seasonal floral richness. Coloured symbols indicate seed mix type (Marmalade, Short or Cornfield). The seed mix formulation applied to plots (enrichment status) is not shown.

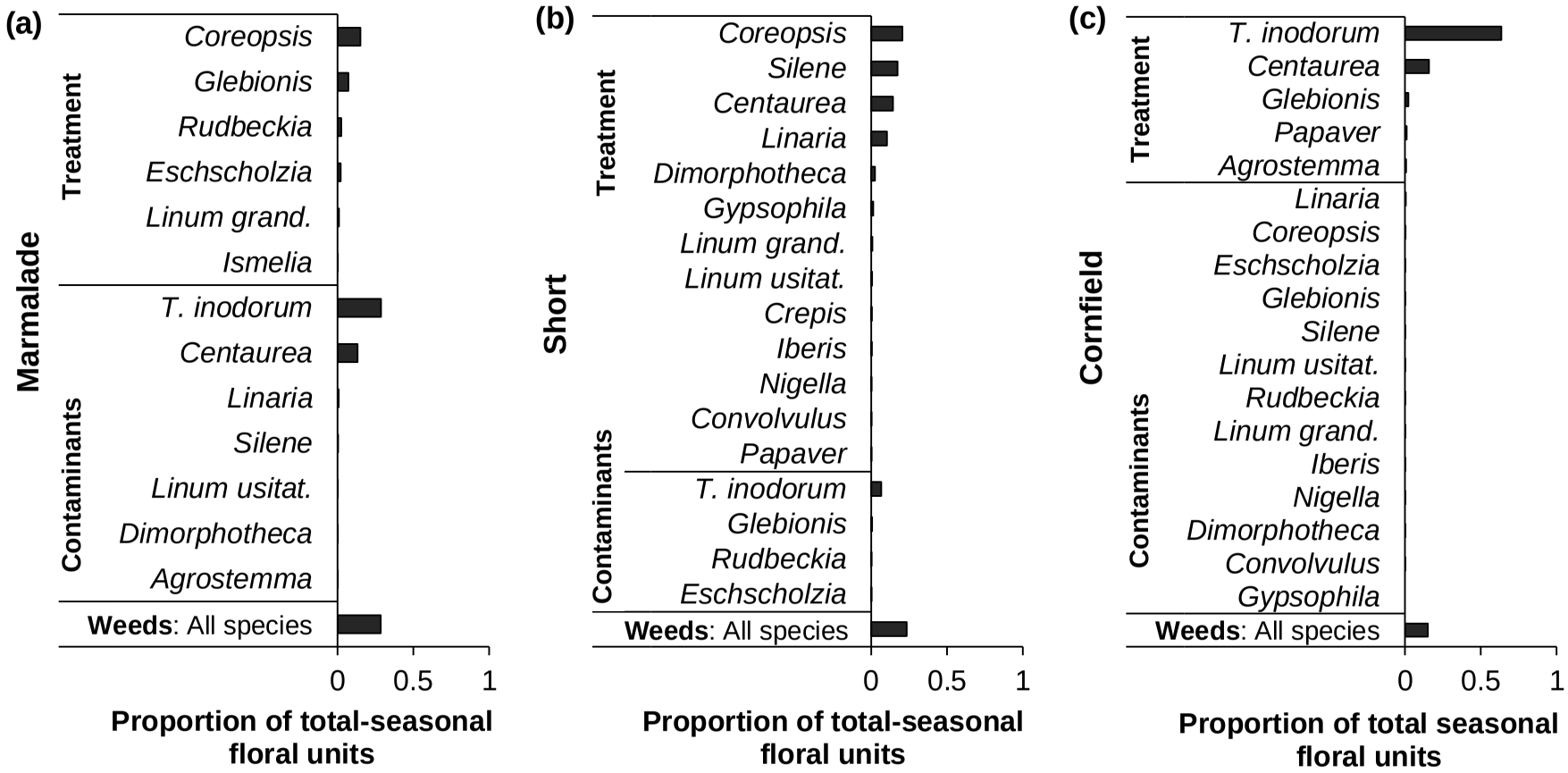


Figure 3.3: Proportion of floral units recorded during the season contributed by individual sown species within each of 3 seed mix types: (a) Marmalade; (b) Short; and (c) Cornfield. Values are calculated from data summed across seed mix formulations and blocks. Weed species are not distinguished.

3.3.2 Objective 3.2: Comparing floral communities between meadows of different seed mix treatments

(a) *Does floral species richness or floral diversity differ between meadows of different seed mix types?*

Floral richness per meadow per round was 24% higher in meadows of Short mixes than in Marmalade mixes (Short: 15.5 ±1.9 species; Marmalade: 12.4 ±1.0; contrast: z=2.8, p<0.02; Table 3.5), and 35% higher than in Cornfield mixes (11.4 ±1.3; z=3.8, p<0.001; Tables 3.5 and A3.4.3). Meadows of Short mixes were also the most diverse, containing more 'common' floral species (contrast: z=3.98 p<0.001; Table 3.5) and more 'highly abundant' floral species than meadows of Cornfield mixes (contrast: z=3.37, p=0.0036; Table 3.5). There were no main effects of seed mix formulation (Log-likelihood ratio test (LRT): χ^2=2.1; p=0.15) or of survey round (LRT: χ^2=2.7; p= 0.26) on overall floral richness or diversity (Tables 3.5 and A3.4.1)

The relative contribution of different floral categories to floral richness of meadows varied across mix types (LRT: χ^2=56.6; p<0.001; Table 3.5). Treatment floral richness in meadows of Short mixes was 80% higher than in Marmalade mixes (Short: 9.3 ±0.9; Marmalade: 4.2 ±0.2; z=4.75; p<0.001), and over twice as high as richness in meadows of Cornfield mixes (Cornfield: 4.0 ±0.2; z=7.13, p<0.001; Fig. 3.4b; Table A3.4.12; Table 3.5). Reciprocally, contaminant floral richness was lower in meadows of Short compared to Cornfield mixes (Short: 1.7 ±0.2; Cornfield: 2.7 ±0.4; z=3.28, p=0.0165; Table 3.5). In contrast, weed floral richness per meadow per round did not differ between mix types, with meadows of each mix type containing approximately 5 weed species in each round (Table 3.5).

Table 3.5: Results of linear models testing for differences in floral species richness and diversity between meadows of different mix types and between different survey rounds. Significant results from log-likelihood ratio tests are in bold. Models for species richness were constructed either ignoring or distinguishing floral categories. GLMMs were used to model species richness, either ignoring (Tables A3.4.1-A3.4.3) or distinguishing floral categories (Tables A3.4.10-A3.5.12). LMMs were used to model Shannon and Simpson's diversities (Tables A3.4.6-A3.4.7 and A3.4.8-A3.4.9). See Methods section 3.2.4 for model details. For mix types, means ±SE are from raw data and are averaged across rounds, mix formulations, and then blocks (n=4). For survey rounds, means ±SE are averaged across mix formulations, mix types and then blocks (n=4). Diff. indicates the direction of significant pairwise differences.

Descriptive index and floral level (total vs. categories)	**Mean species richness or diversity ±SE per meadow per survey round (5 m²/round)**			**Effect of seed mix type**		**Post-hoc pairwise comparison**	
	Seed mix type			χ^2	***p-value***	**diff.**	***p-value***
	Marmalade	**Short**	**Cornfield**				
Species richness							
Total	12.4 ± 1.0	15.5 ± 1.9	11.4 ± 1.3	15.8	**<0.001**	S>M&C	**<0.001**
Treatment	4.2 ± 0.2	9.3 ± 0.9	4.0 ± 0.2	Interaction between floral category and mix type: χ^2 =56.6; *p* **<0.001** (see Tab. A3.4.12 & Fig. 3.4)			
Contaminant	3.3 ± 0.3	1.7 ± 0.2	2.7 ± 0.4				
Weed	5.0 ± 0.7	4.5 ± 1.0	4.7 ± 0.9				
Shannon diversity							
Total	5.3 ± 0.7	6.1 ± 0.8	4.1 ± 0.7	15.3	**<0.001**	S>C	**<0.001**
Treatment	2.3 ± 0.2	4.0 ± 0.3	2.3 ± 0.2	-	-	-	-
Contaminant	1.9 ± 0.2	1.3 ± 0.1	2.1 ± 0.3				
Weed	2.5 ± 0.2	2.3 ± 0.3	2.1 ± 0.3				
Simpson's diversity							
Total	4.1 ± 0.6	4.6 ± 0.6	3.1 ± 0.5	11.9	**0.003**	S>C	**0.004**
Treatment	2.0 ± 0.2	3.1 ± 0.3	1.9 ± 0.2	-	-	-	-
Contaminant	1.7 ± 0.2	1.2 ± 0.1	1.9 ± 0.3				
Weed	2.0 ± 0.1	1.9 ± 0.2	1.8 ± 0.2				
Floral level	**Mean species richness ±SE per meadow (5 m²)**			χ^2	***p-value***	**diff.**	***p-value***
	Survey round						
	Round 1	**Round 2**	**Round 3**				
Species richness							
Total	14.3 ±1.0	13.8 ± 1.8	11.2 ± 1.5	2.7	0.26	-	-

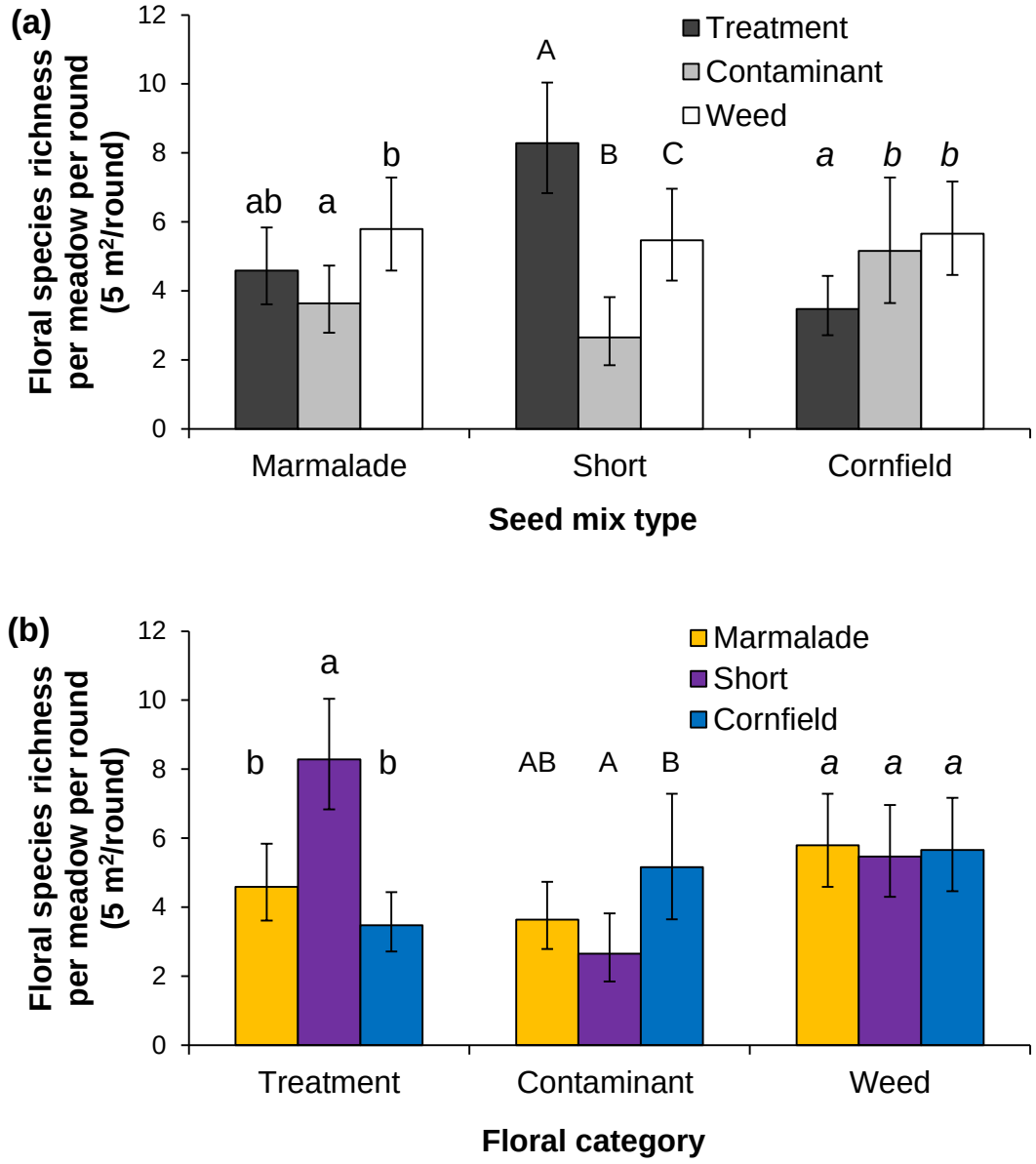


Figure 3.4: Mean (±95% CI) floral species richness per meadow per round (for 5 m^2/round), showing tests for: (a) differences in richness between floral categories within mix types; and (b) differences in richness of floral categories between mix types. Values are predicted marginal means from a GLMM, which are averaged over levels of enrichment and round and back-transformed onto the data scale (Tables 3.5 and A3.4.10-A3.4.12). Significant contrasts are indicated by different letters, with contrasts only valid within x-axis categories (indicated by font and case).

(b) *Does floral abundance or seasonal pattern of flowering in meadows differ between seed mix treatments?*

Overall, meadows of different seed mix types produced roughly the same number of floral units per meadow per round (LRT: χ^2=0.36; p=0.83; Table 3.6). The enrichment treatment (mix formulation) had no effect on overall floral abundance (LRT: χ^2= 0.209; p=0.65; Table A3.5.1). There was a strong seasonal effect on floral abundance, with total floral abundance relatively stable between late July and late August (Rounds 1 and 2; z=1.17, p=0.47), but declining by over 50% by late September (Round 3; z=6.31, p<0.001; Fig. 3.5).

Table 3.6: Results of linear models testing for differences in floral abundance between meadows of different mix types and between different survey rounds. Significant results from log-likelihood ratio tests are highlighted in bold. Models for species richness were constructed either ignoring or distinguishing floral categories. GLMMs were used to model species richness, either ignoring (Tables A3.5.1-A3.5.3) or distinguishing floral categories (Tables A3.5.4-A3.5.6). See Methods section 3.2.4 for model details. GLMMs were used to test effects of mix type and round on floral abundance, both ignoring (Tables A3.5.1-A3.5.3) and distinguishing floral categories (Tables A3.5.4-A3.5.6 & Fig. 3.6). For seed mix types, means ±SE are from raw data and are averaged across rounds, mix formulations, and then blocks (n=4). For survey rounds, means ±SE are averaged across mix formulations, mix types and then blocks (n=4). Diff. indicates direction of significant pairwise differences.

Descriptive index and floral level (total vs. categories)	**Mean floral abundance ±SE per meadow per survey round (5 m²/round)**			**Effect of seed mix type**		**Post-hoc pairwise contrasts**	
	Seed mix type			χ^2	***p-value***	**diff.**	***p-value***
	Marmalade	**Short**	**Cornfield**				
Floral abundance							
Total	922.5±61.6	917.8±107.1	1150.3±356.0	0.36	0.83	-	-
Treatment	258.9±61.9	634.1±88.1	960.7±360.2	Interaction between floral category and mix type: χ^2 =87.2; p **<0.001** (see Tab. A3.5.4 & Fig. 3.6)			
Contaminant	401.0±179	68.0±24.4	16.6±2.3				
Weed	262.7±90	215.7±71.5	173.0±41.9				
Floral level	**Mean floral abundance ±SE per meadow (in 5 m²)**			χ^2	***p-value***	**diff.**	***p-value***
	Survey round						
	Round 1	**Round 2**	**Round 3**				
Floral abundance							
Total	1312.7±203.7	1234.8±334.1	443.3±77.5	45.5	**<0.001**	R1&R2 >R3	**<0.001**

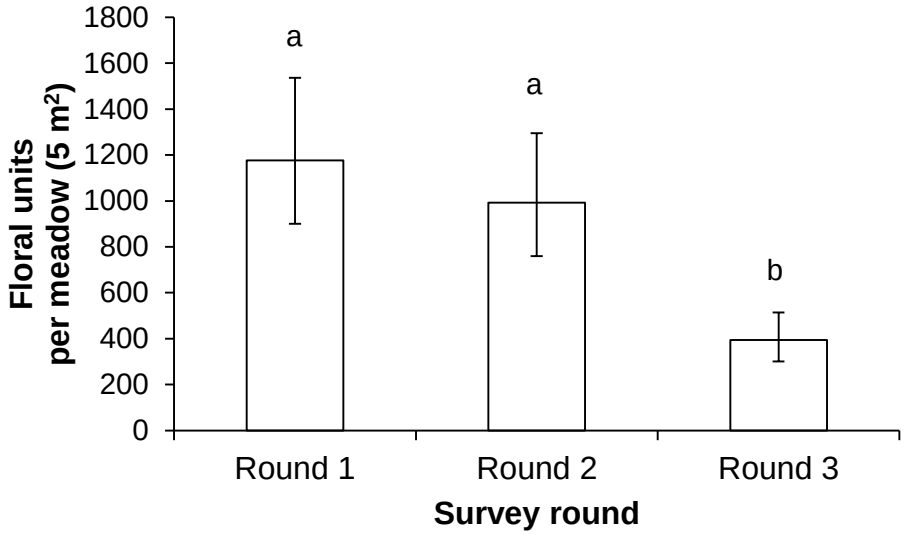


Figure 3.5: Mean (±95% CI) floral abundance per meadow in each survey round. Values are predicted marginal means from a GLMM (Tables 3.6 and A3.5.1-A3.5.3), which are averaged over levels of mix type and formulation and back-transformed to the data scale. Significant contrasts are indicated by different letters.

Although total floral abundance did not differ between meadows of different seed mix types, the relative contributions of treatment, contaminant and weed floral categories varied substantially between mix types (LRT: χ^2 =87.2, p<0.001; Table 3.5; Fig. 3.6). Within meadows of Marmalade mixes, treatment, contaminant and weed floral units were roughly equally abundant (Fig. 3.6a; Table A3.5.6b), whereas within meadows of Short and Cornfield mixes, most floral units were treatment floral units, with comparably few contaminants or weeds (Fig. 3.6a).

Weed floral abundance did not differ between meadows of different seed mix types, with each meadow typically containing hundreds of weed floral units (Fig. 3.6b). Most of these were produced by *Polygonum aviculare* and *Persicaria maculosa*, which were the most abundant flowering weeds across all treatments (Figs. 3.7a-f). In contrast, treatment and contaminant

floral abundance varied substantially between meadows of different mix types.

Treatment floral abundance was 173% higher in meadows of Cornfield mixes than in meadows of Marmalade mixes (Contrast: z=3.566, p=0.006; Fig. 3.6b; Table A3.5.6). For meadows of Short mixes, treatment floral abundance was intermediate to and did not differ from Marmalade or Cornfield mixes (Fig. 3.6b). In contrast, for meadows of Marmalade mixes, contaminant floral abundance was over 5 times higher than in Short mixes (z=6.29, p<0.0001; Fig. 3.6b) and over 24 times higher than in Cornfield mixes (z=9.68, p<0.0001 Fig. 3.6b). For meadows of Short mixes, contaminant floral abundance was also 4 times higher than in Cornfield mixes (z=3.67, p<0.0041). These patterns within survey rounds mirrored those found for seasonal floral abundance, with variation in contamination mainly a function of the classification of two common species (*T. inodorum* and *C. cyanus*).

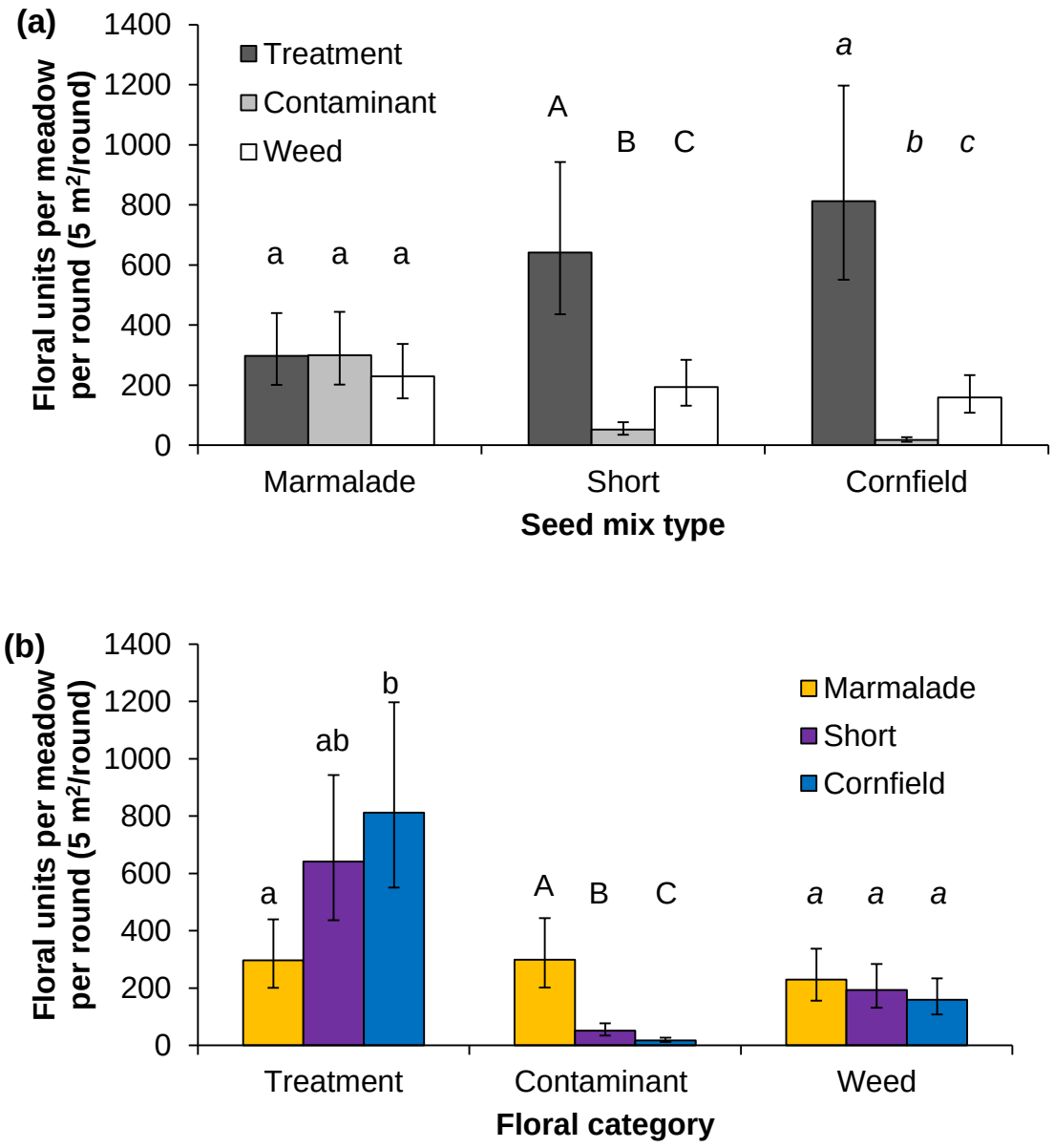
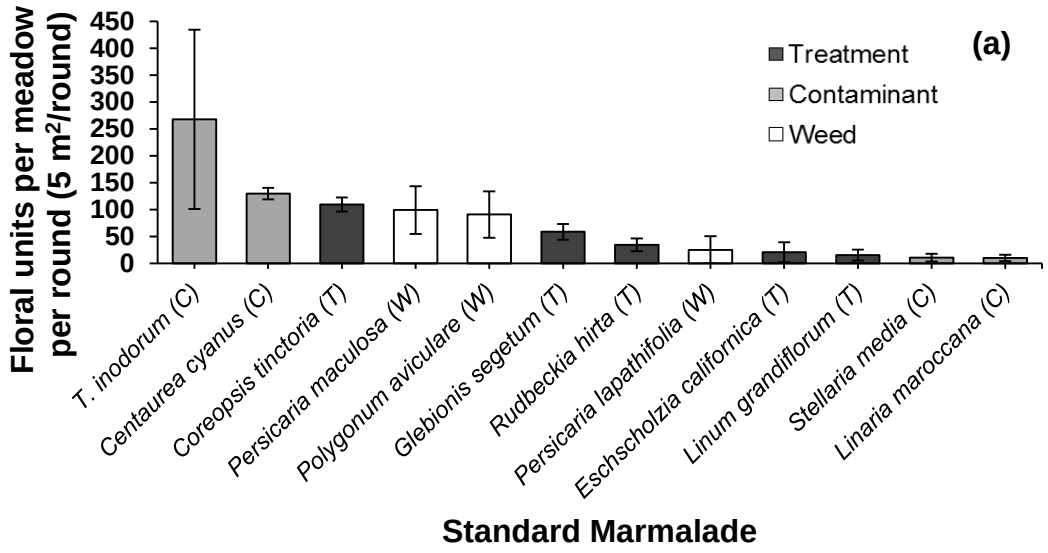
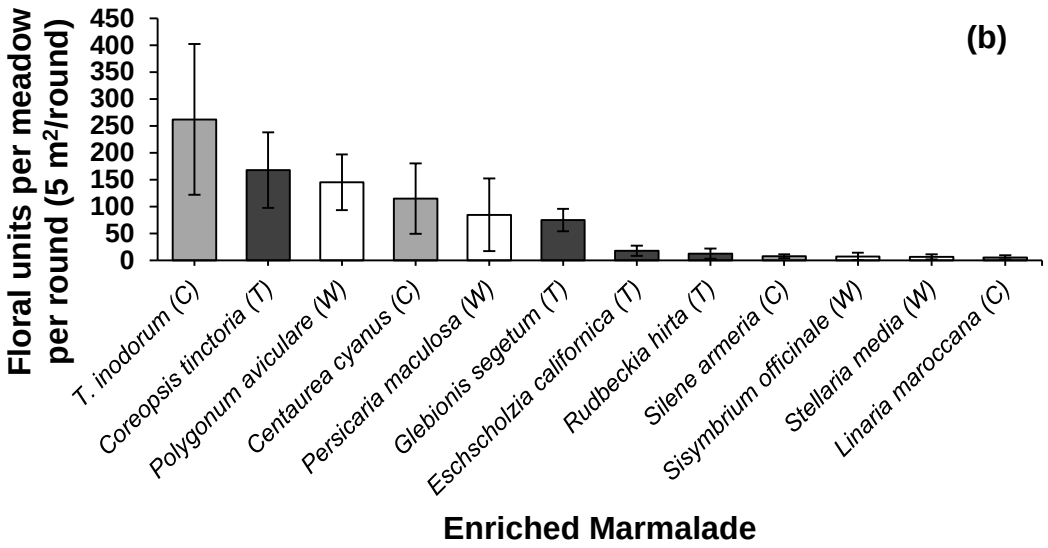
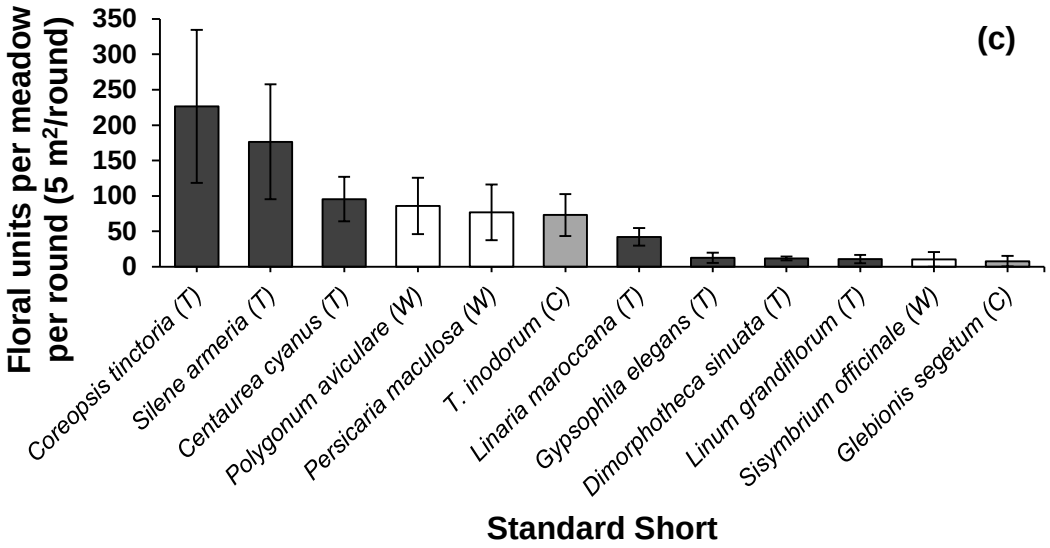


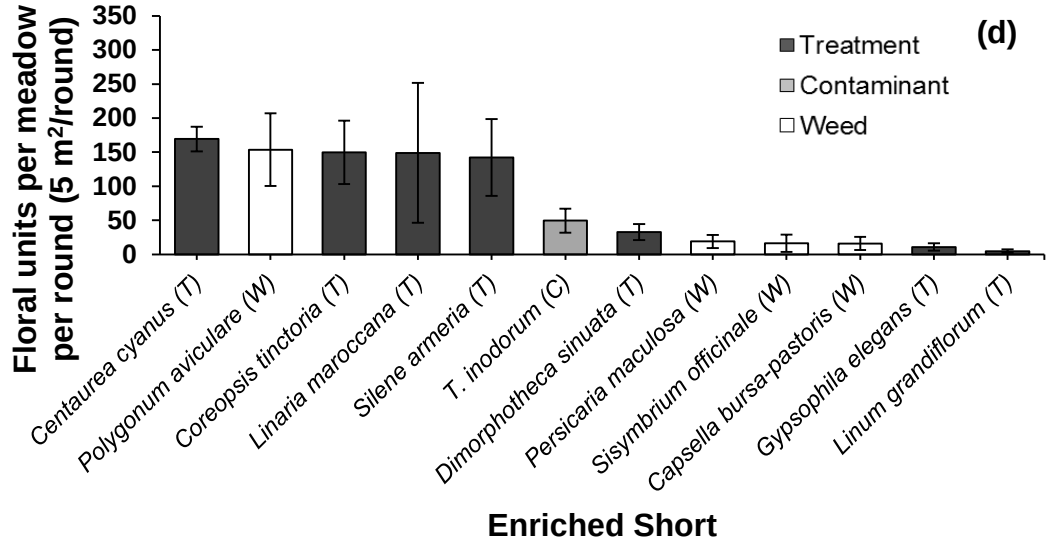
Figure 3.6: Mean (±95% CI) floral abundance per meadow per round (5 m^2 /round), showing tests for: (a) differences in abundance between floral categories within mix types; and (b) differences in abundance of floral categories between mix types. Values are predicted marginal means from a GLMM, which are averaged over levels of enrichment and round and back-transformed to the data scale (A3.5.4-A3.5.6). Significant contrasts are indicated by different letters, with contrasts only valid within x-axis categories (indicated by font and case).

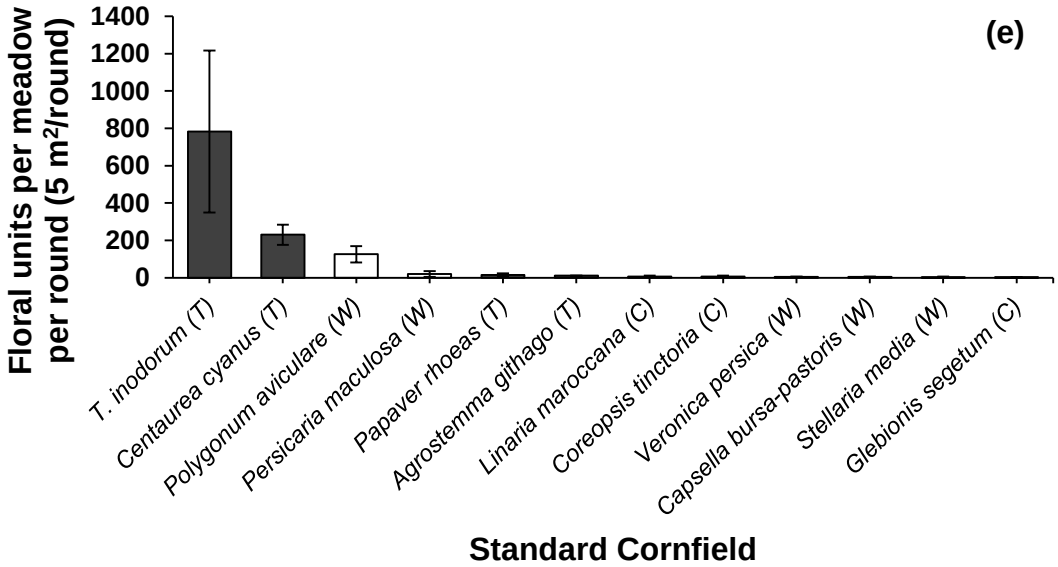
Figure 3.7 (on following pages): Rank abundance diagrams showing the mean ±SE number of floral units per species per meadow per round (5 m^2/round), for (a) Standard Marmalade, (b) Enriched Marmalade, (c) Standard Short, (d) Enriched Short, (e) Standard Cornfield, and (f) Enriched Cornfield. For each mix type, floral units of each species are classified as treatment floral units (dark grey/T), contaminants (light grey/C) or weeds (white/W). *T. inodorum* indicates *Tripleurospermum inodorum*.

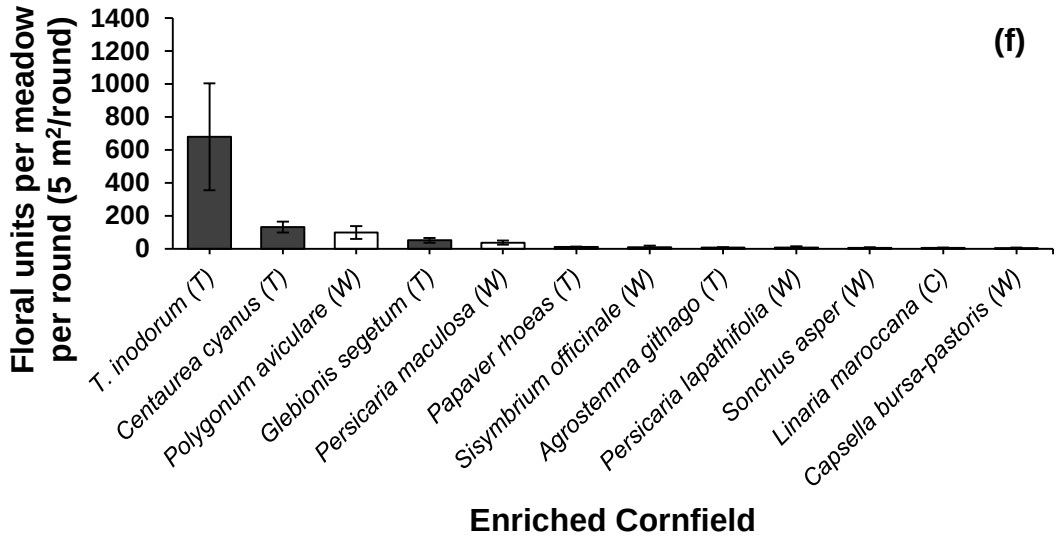





(c)
Floral units per meadow per round (5 m²/round)
350
300
250
200
150
100
50
0
Coreopsis tinctoria (T)
Silene armeria (T)
Centaurea cyanus (T)
Polygonum aviculare (W)
Persicaria maculosa (W)
T. inodorum (C)
Linaria maroccana (T)
Gypsophila elegans (T)
Dimorphotheca sinuata (T)
Linum grandiflorum (T)
Sisymbrium officinale (W)
Glebionis segetum (C)
Standard Short


(d)
Treatment
Contaminant
Weed
Floral units per meadow per round (5 m²/round)
350
300
250
200
150
100
50
0
Centaurea cyanus (T)
Polygonum aviculare (W)
Coreopsis tinctoria (T)
Linaria maroccana (T)
Silene armeria (T)
T. inodorum (C)
Dimorphotheca sinuata (T)
Persicaria maculosa (W)
Sisymbrium officinale (W)
Capsella bursa-pastoris (W)
Gypsophila elegans (T)
Linum grandiflorum (T)
Enriched Short


(e)
Floral units per meadow per round (5 m²/round)
1400
1200
1000
800
600
400
200
0
T. inodorum (T)
Centaurea cyanus (T)
Polygonum aviculare (W)
Persicaria maculosa (W)
Papaver rhoeas (T)
Agrostemma githago (T)
Linaria maroccana (C)
Coreopsis tinctoria (C)
Veronica persica (W)
Capsella bursa-pastoris (W)
Stellaria media (W)
Glebionis segetum (C)
Standard Cornfield



(c) *Do meadows of different seed mix treatments differ in floral composition?*

NDMS ordinations for all flowering species recorded in meadows suggest that, despite high levels of contamination and the presence of numerous weeds, seed mix type was an important factor structuring variation in the floral composition of meadows (Fig. 3.8(i)a). This was confirmed by PERMANOVA (F=4.85, df=2 ,p=0.001; R^2=0.32; Fig. 3.8(i)a, Table 3.7). Seed mix type accounted for a substantial proportion of variation in floral composition (R^2=0.32). However, meadows did not form discrete mix type-based clusters on NMDS ordinations. Meadows of different seed mix types often had more similar floral compositions than meadows of the same seed mix type (Fig. 3.8(i)a), due to overlapping seed mix species lists, contamination and weeds. Although block was not directly tested, there was no strong evidence of an effect of block on overall floral composition of meadows (Fig. 3.8(ii)b).

To disentangle the effect of weed species from shared seed mix species and contaminants, an NMDS ordination and PERMANOVA model were performed for weed species only. NMDS ordination and PERMANOVA provided no evidence for an effect of seed mix type on the floral composition of weeds (F=0.46, df=2, p=0.17; R^2=0.04; Table 3.7; Fig. 3.8(ii)a). However, there was a strong effect of block (spatial location of field plots) on weed floral composition, with floral composition in block D markedly different from all other blocks. Block A also appeared to differ subtly from block B and E (Fig. 3.8(ii)b). Exclusion of data for weeds made no qualitative difference to inferences drawn from the analysis of full community floral composition, and seed mix type retained a significant effect on the meadow floral composition of sown species (F=6.76, df=2, p= 0.002; R^2=0.41). Exclusion of weeds reduced spatial variation in floral composition, enabling more accurate representation of pairwise community dissimilarities in 2 dimensions (i.e. stress decreased from 0.16 to 0.11). The proportion of variation explained by mix type also increased substantially (Full model R^2= 0.32; Sown seed only model R^2=0.41). Visual analysis of the NMDS ordination for sown species confirmed mix type had a strong effect on the location of plots in multivariate space (Fig. 3.8(ii)a). However, meadows of different seed mix types did not form discrete clusters, rather, some meadows of each seed mix type were more similar in floral composition to meadows of different seed mix types than they were to replicates of the same mix type. Thus, as a result of overlapping seed-mix species lists and high levels of species contamination, meadows of different seed mix types did not represent distinct floral communities. Block was not directly tested, but there was no evidence of an effect of block on the floral composition of sown seed mix species in meadows (Fig. 3.8(iii)b).

PERMANOVA models for both (i) all flowering species and (iii) sown species indicated there was a significant but very small (in terms of R^2 - the proportion of variance explained) effect of seed mix formulation (standard vs. enriched) on the floral composition of meadows within each seed mix type (All flowering species model: F=0.6, df=3, p=0.021; R^2=0.06; Sown species model: F=0.58, df=3, p=0.041; R^2=0.05; Table 3.7). However, visual examination of ordinations, comparing dispersion between replicate seed mix formulations within seed mix types (Fig. 3.8(i)a & (iii)a), suggests that this effect may be a function of differences in dispersion rather than differences in location.

Table 3.7: Results for PERMANOVA of pairwise dissimilarities in seasonal floral abundance between meadows of different seed mix treatments, for: (i) All 50 flowering species; (ii) 31 weed species; (iii) 19 sown seed mix species. For each term, models test for differences between groups in location and/or spread in multivariate space (floral composition).

NMDS model	Term	df	Sums of Squares	Mean Squares	F-value	R^2	*P*-value
(i) All 50 flowering species	Mix type	2	1.4551	0.72757	4.845	**0.33**	**0.001**
	Formulation	3	0.2722	0.09072	0.604	**0.06**	**0.021**
	Residuals	18	2.7030	0.15017	0.61		
	Total	23	4.4303	1.00000			
(ii) 31 weed species	Mix type	2	0.2335	0.11677	0.457	0.04	0.17
	Formulation	3	0.4902	0.16339	0.639	0.09	0.18
	Residuals	18	4.6017	0.25565	0.864		
	Total	23	5.3254	1.00000			
(iii) 19 sown seed mix species	Mix type	2	1.9139	0.95696	6.76	**0.41**	**0.002**
	Formulation	3	0.2449	0.08163	0.577	**0.05**	**0.041**
	Residuals	18	2.5480	0.14155	0.541		
	Total	23	4.7068	1.00000			

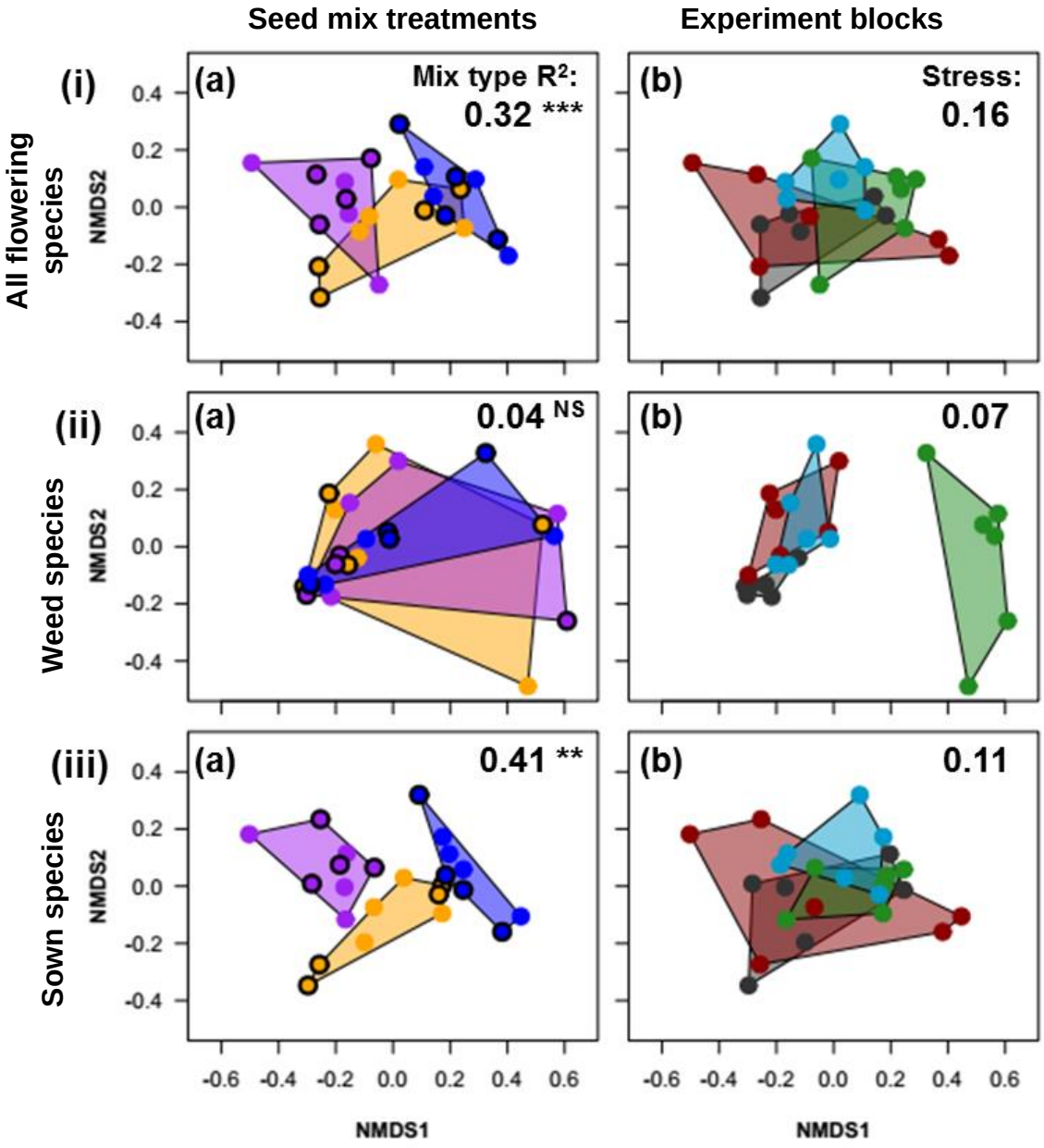


Figure 3.8: NMDS ordinations showing relative Bray-Curtis dissimilarities in seasonal floral composition between 24 meadow plots. Three 2D NMDS ordinations (i-iii) show the relative similarity/dissimilarity in seasonal floral composition between meadows of different seed mixes, for (i) 50 flowering species, (ii) 31 weed species and (iii) 19 sown species. For each ordination, panel (a) highlights seed mix treatments, with colours indicating Marmalade (●), Short (●) or Cornfield mixes (●), with outlines indicating enriched formulations (●●●). Panel (b) highlights the spatial location of meadows, with colours indicating meadows in blocks A(●), B(●), D(●) and E(●).

3.3.3 Objective 3.3: Assessing the impact of changes to proportional seed weights on floral abundance in meadows

There was no statistical evidence that changing the proportional contributions of species in seed mixes had any effect on their floral abundance in meadows. For each mix type and floral amendment category, confidence limits for differences in floral abundance between meadows of enriched vs. standard seed mixes included zero (Fig. 3.9). Confidence limits included zero regardless of whether or not they were corrected for sample size and multiple comparisons (see Methods 3.2.4 part (iii)). There was also no evidence of an effect on the floral abundance of individual species (Fig. 3.10).

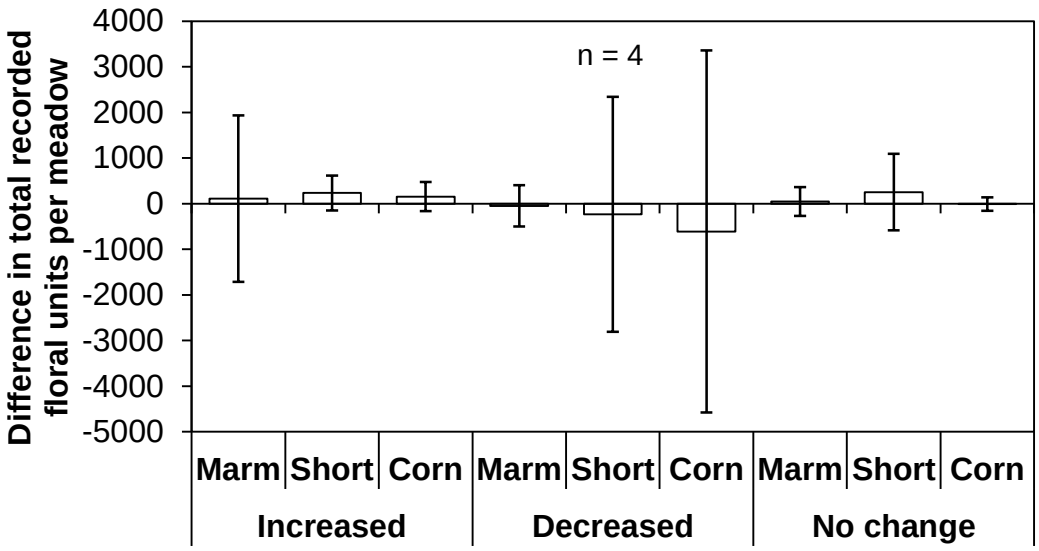
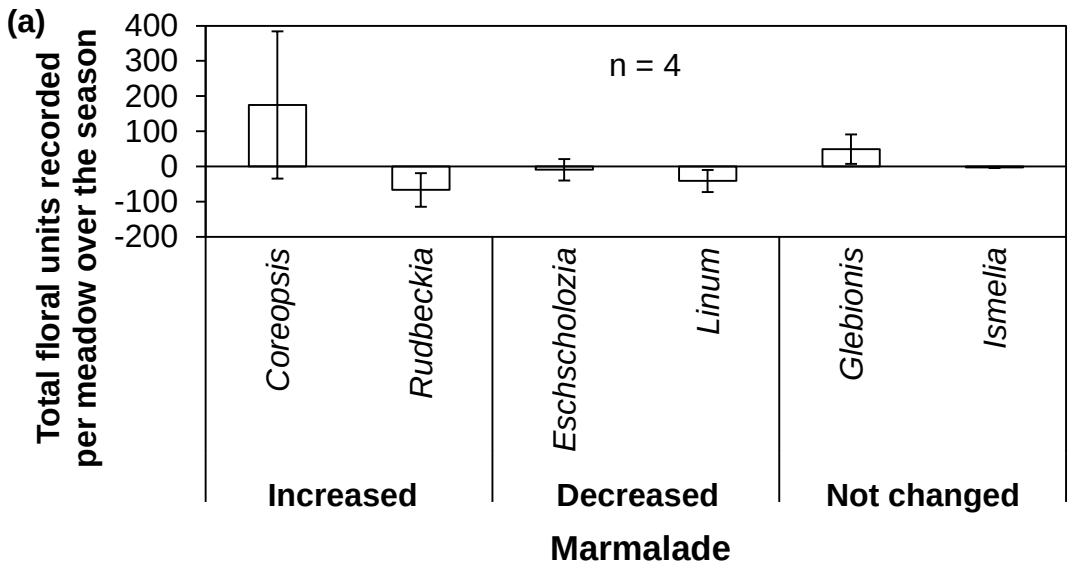
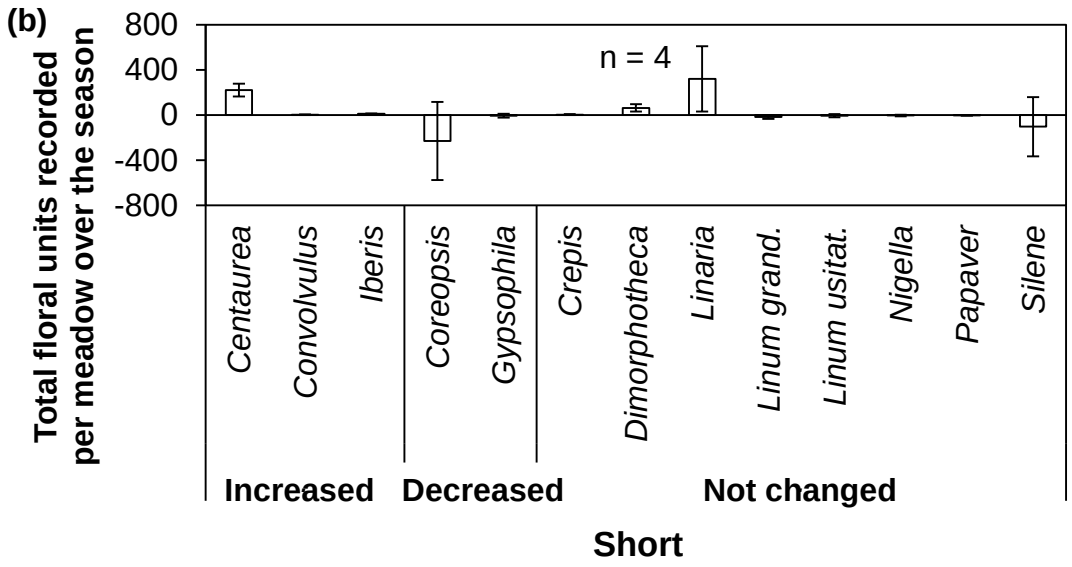
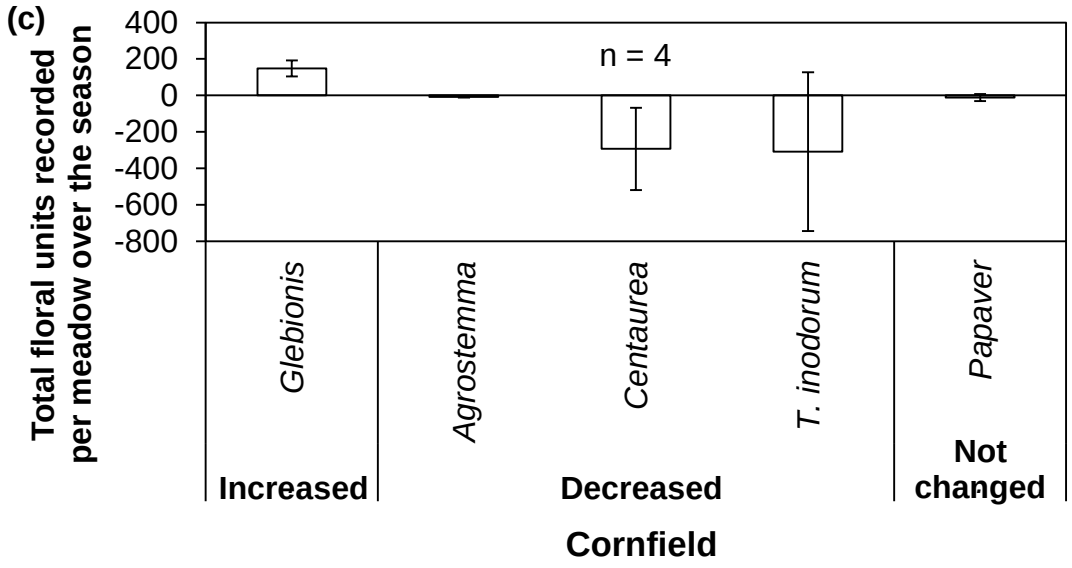


Figure 3.9: Mean differences (±99.5% CI) between meadows of standard and enriched seed mixes in the total number of floral units, recorded over the season, for species whose proportionate seed weight was either 'increased', 'decreased' or 'not changed' (n=4). 'Marm', 'Short' and 'Corn' correspond to Marmalade, Short and Cornfield seed mix types, respectively.

Figure 3.10 (on following page): Mean difference (±SE) between meadows of standard vs. enriched seed mix formulations in the total number of floral units recorded for individual sown species in (a) Marmalade mixes, (b) Short mixes, and (c) Cornfield mixes (n=4). Species names listed in order without repetition are: *Coreopsis tinctoria*, *Rudbeckia hirta*, *Eschscholzia californica*, *Linum grandiflorum 'rubrum'*, *Glebionis segetum*, *Ismelia carinatum, Centaurea cyanus*, *Convolvulus tricolor*, *Iberis umbellata*, *Gypsophila elegans*, *Crepis rubris*, *Dimorphotheca sinuata*, *Linaria maroccana*, *Linum usitatissimum*, *Nigella damescena*, *Papaver rhoeas*, *Silene armeria*; *Agrostemma githago*, *Tripleurospermum inodorum*.


(a)
Total floral units recorded per meadow over the season
400
300
200
100
0
-100
-200
n = 4
Coreopsis
Rudbeckia
Eschscholozia
Linum
Glebionis
Ismelia
Increased
Decreased
Not changed
Marmalade


(b)
Total floral units recorded per meadow over the season
800
400
0
-400
-800
n = 4
Centaurea
Convolvulus
Iberis
Coreopsis
Gypsophila
Crepis
Dimorphotheca
Linaria
Linum grand.
Linum usitat.
Nigella
Papaver
Silene
Increased
Decreased
Not changed
Short


(c)
Total floral units recorded per meadow over the season
400
200
0
-200
-400
-600
-800
n = 4
Glebionis
Agrostemma
Centaurea
T. inodorum
Papaver
Increased
Decreased
Not changed
Cornfield

3.4 Discussion

The aims of this study were to compare the flowering performance of different annual pictorial meadows seed mixes, and to assess the extent to which the floral composition of meadows accurately reflected seed mix treatments. Results demonstrated that all meadows contained a mixture of intended and unintended flowering species. In the following discussion, I examine the factors that influence the floral composition of meadows grown from seed mixes, and consider the implications of this study for subsequent analyses of this dataset and for future studies and formulations of flower seed mixes.

3.4.1 Factors influencing the floral composition of planted meadows.

The floral composition of a planted urban meadow is a result of multiple processes, including (a) the species composition of the seed mix that is sown, (b) the species composition of the soil seed bank, and (c) the many factors that influence germination, establishment, growth and flowering of each seed mix or seed bank species (Aldrich 2002; Glover 2014; Long *et al.* 2015).

The species composition of a seed mix is dependent on general and specific aspects of seed mix design and on which seed mix is chosen for a given urban planting scheme. Pictorial meadows seed mixes are designed to produce reliable, low-maintenance, and visually attractive flower meadows for people. This is achieved by diligent ground preparation prior to sowing (including initial weed control using herbicides; Prentis & Norton 1992; Highways Agency 1993; Aldrich 2002) and careful seed mix design, involving judicious plant selection for fast growing, competitive herbaceous

annuals (Hitchmough 2004, 2010; Köppler & Hitchmough 2015). Species are collectively selected to exclude weeds by dominating resources (such as space and light; Hitchmough 2010; Köppler & Hitchmough 2015), whilst producing a dense display of large colourful flowers (whilst perhaps meeting other architectural criteria such as height or colour themes). Exotic species and cultivars are often used to increase the floral colour palette available and to extend the flowering season (Hitchmough & Woudstra 1999; Hitchmough 2008, 2011). In terms of seed mix design, reliability in floral composition is dependent on species selection (in relation to intended site conditions and other component species), on seed quality (since species may be lost due to low germination rates) and on seed purity (since unintended species may occur as contaminants; Hitchmough & Dunnett 2004; Köppler & Hitchmough 2015; Hitchmough 2017). Seed mix choice is determined by multiple factors, including relative costs of mixes, aesthetic preferences (for particular colour themes), or site constraints (such as short mixes required for visibility around roads).

The composition of the soil seed bank is determined by seed deposition and seed persistence over time (Baker 1989; Thompson *et al.* 2005; Long *et al.* 2015). Seed can be deposited by previous, current or adjacent vegetation (Thompson *et al.* 2005; Hopfensperger 2007; Albrecht *et al.* 2011); hence, the species composition of soil seed banks is determined by land use history (including past use of seed mixes), site vegetation prior to ground preparation, and past and current seed rain from adjacent vegetation. Soil seed bank composition is also influenced by different rates of seed persistence among species (Wang *et al.* 2013; Long *et al.* 2015), which is affected by seed size and shape (Thompson *et al.* 1993).

Multiple factors influence germination, establishment, growth and flowering of seed mix and seed bank species (Aldrich 2002; Long *et al.* 2015). Key among these are sowing season (autumn vs spring; Highways Agency 1993; Hitchmough *et al.* 2004; SNH 2017), planting date (Aldrich 2002), weather (especially during germination and establishment; Aldrich 2002), soil characteristics (especially pH and fertility; McCrea *et al.* 2001; McCrea *et al.* 2004), interactions between species (especially competition; Hitchmough & de la Fleur 2006), plot preparation (including rotovation, chemical removal of weeds and use of mulching materials; Aldrich 2002; Hitchmough *et al.* 2004; Long *et al.* 2015), sowing technique (soil tilth and seed-soil contact; Prentis & Norton 1992), sowing rate (Aldrich 2002) and management (irrigation during dry weather; Prentis & Norton 1992; Aldrich 2002).

3.4.2 Objectives 3.1 and 3.2: on the flowering performance of seed mixes

(a) Seed mix performance.

An important component of commercial seed mix reliability is the extent to which the floral composition of a planted meadow reflects the composition of the seed mix design sold to the customer. In commercial terms, deviation in composition of a meadow from a seed mix may result in deviation from the intended aesthetic character of the meadow (thereby affecting customer satisfaction), or it may imply inefficient use of seed and hence a reduction in potential profits. In an experimental setting, the ability to attribute patterns in meadow floral characteristics (or floral rewards and insect visitation) to seed mix treatments depends crucially on whether meadows are representative of seed mixes. This issue can be understood by reference to the experimental design concepts of internal and external

validity (Campbell & Stanley 1963; Brewer & Crano 2014). Internal validity refers to whether a study measured what it set out to measure, and whether it is able to establish that variation in an outcome or response variable is a result of changes in the level or intensity of an explanatory variable, rather than another factor (Grimes & Schulz 2002). External validity refers to the extent to which the results of an experiment can be generalised to other populations, settings, times or treatments (Steckler & McLeroy 2008). Ideally, experimental designs should maximise both internal and external validity so that results are valid and broadly applicable; however, in practice there is often a trade-off between tightly controlled experimentation and the extent to which experimental settings are representative of real world scenarios (Brewer & Crano 2014). An important aspect of this study was to assess the validity of treatments based on the extent to which the floral composition of meadows was representative of seed mix treatments.

In general, seed mix treatments produced meadows containing abundant flowers and most of the species expected. For example, the total seasonal richness of treatment species in each mix type closely matched expectations based on seed mix species lists. Only one species did not occur at all, although a number of species were recorded in only a subset of treatment replicates. *Echium vulgare* was not recorded or observed in any plots sown with the enriched Cornfield seed mix. This may have been because *E. vulgare,* although here an annual cultivar (syn. *E. plantagineum*), was a poor competitor compared to other more vigorous annual species; however, accidental omission during preparation of seed mixes cannot be ruled out. The species that flowered in only a subset of replicate treatments (for example, *Ismelia carinata, Linum grandiflorum*, *Convolvulus tricolor*, *Iberis umbellata*, *Linum grandiflorum*, *Nigella damascena*, and *Papaver rhoeas*; Table

A3.1.1) also produced relatively few flowers (Fig. A3.2.1). Hence, this pattern of absence may simply result from low sampling intensity. Given that the floral composition of meadows grown from seed mixes is likely to be inherently variable in space and time (due to varying environmental conditions), further studies are required to establish how frequently these species are rare or absent (i.e whether they are rare 'by design', or are unsuited to or uncompetitive under certain environmental conditions). Given commercial imperatives to use seeds cost-effectively and to design reliable flower seed mixes, species that consistently produce few or no flowers may be candidates for removal from seed mixes. However, this should be weighed against other potential benefits they may provide such as redundancy in floral unit provision under unusual growing conditions, or provision of floral resources for species, such as solitary bees, that may require comparatively few flowers to meet their resource requirements (Müller *et al.* 2006). From this perspective, even low numbers of *Echium vulgare* flowers can provide substantial volumes of nectar for long tongued bees (Corbet 1978).

In addition to intended treatment species, meadows also contained many unintended contaminants and weeds that germinated from the soil seed bank. Contaminants were widespread and abundant; however, contaminants were neither naturally occurring 'garden escapes' nor the residue of previous independent greenspace management, rather they resulted directly from a previous failed attempt to perform this experiment, and were thus a subset of the same suite of species that comprised seed mix treatments (as discussed below). Moreover, as a consequence of this past experiment, the composition of the contaminant seed bank likely varied across treatments within each block (as discussed below). This contamination

has important consequences for the internal and external validity of this study.

Although contaminants and weeds are likely a common feature of planted meadows, pervasive contamination inherently reduces the internal validity of treatments, since community-level variation in meadow characteristics (and hence floral rewards and insect visitation) cannot be attributed solely to seed mix treatments. Internal validity could be improved by mulching plots prior to sowing, which would prevent germination from the seed bank (Aldrich 2002). However, this would reduce external validity since for most large urban plantings mulching is likely to be prohibitively expensive; therefore, contaminants and weeds should be expected in urban plantings (Thompson *et al.* 2005; Albrecht *et al.* 2011). Ideally, an experimental design will maximise both internal and external validity. If this is not possible, a sequence of studies should demonstrate the efficacy of an intervention (maximising internal validity) before establishing its effectiveness across a broader range of conditions (maximising external validity; Flay 1986; Steckler & McLeroy 2008). However, these alternative approaches are often either prohibitively challenging or prohibitively expensive (Steckler & McLeroy 2008). Hence, internal validity is typically prioritised since it is fundamental to establishing causation (Campbell & Stanley 1963; Brewer & Crano 2014). In this study, contaminants affected meadow floral characteristics both directly and indirectly (by competing with treatment species). Thus, community level patterns cannot be reliably attributed to seed mix treatments. The full implications of this for subsequent analyses of this dataset are discussed below.

(b) Contamination.

Planted meadows contained a mixture of intended treatment and unintended contaminant or weed flowering species. As with previous studies of urban soil seed banks, the species that germinated from the seed bank included both common native weeds and cultivated species from previous horticultural activities (Thompson *et al.* 2005; Albrecht *et al.* 2011). However, in this study, each of the unintended horticulturally-cultivated species that emerged from the seed bank was also a component of at least one seed mix treatment. Hence, the presence of these species effectively resulted in cross-contamination of treatments.

In this study, contamination likely resulted from performing the experiment in consecutive years (2012 and 2013), using consistent sites for blocks but without marking out permanent plots (see Chapter 2 General Methods). In 2012, 4/6 blocks failed to produce flowering meadows due to exceptionally high rainfall in the months after seed mixes were sown. In 2013, blocks were rotovated during ground preparation and seed mix treatments were re-randomised among plots within blocks. Hence, most contaminants likely originated from seeds that failed to germinate in 2012. Notwithstanding the inherent unpredictability of extreme weather events, a failure to plan for a multiannual experiment, and thus to mark out permanent plots, precluded the independent rotovation of plots and the precise re-positioning of plots within blocks in the second year. The resultant contamination was exacerbated by the re-randomisation of seed mix treatments among plots within blocks. Additional possible pathways for contamination include the use of impure seed from suppliers, accidental

contamination during seed mix preparation, and accidental contamination during broadcast sowing.

Contaminants were widespread across field plots, although the degree of contamination varied between mix types. Meadows of Marmalade mixes were most affected by contamination, and contained an order of magnitude more contaminant floral units than Short or Cornfield mixes (Fig. 3.6b). Contaminant floral units were also 4 times more abundant in meadows of Short mixes than in Cornfield mixes (Fig. 3.6b). These patterns were mainly caused by two widespread and abundant species: *T. inodorum* and *C. cyanus*.

T. inodorum and *C. cyanus* together produced almost half of all floral units recorded in meadows during surveys, and comprised 92% of all contaminant floral units. These two species were present in almost all plots, with plants likely originating from a mixture of seed laid down in 2012 (*T. inodorum* and *C. cyanus*), seed mix treatments sown in 2013 (*T. inodorum* and *C. cyanus*) and the weed seed bank (*T. inodorum*). The classification of these widespread and abundant species changed across meadows of different seed mix types. In meadows of Marmalade mixes, both *C. cyanus* and *T. inodorum* were contaminants. In meadows of Short mixes, *C. cyanus* was a treatment species, whilst *T. inodorum* was a contaminant. In contrast, they were both classified as treatment species in meadows of Cornfield mixes. Hence, contrasting patterns of contamination between meadows of different mix types resulted from horticultural expectations rather than seed mix characteristics or ecological interactions.

Contamination by *T. inodorum* was likely overestimated since naturally-occurring weed floral units of *T. inodorum* could not be distinguished from the cultivar in the field. Hence, all *T. inodorum* plants

were assumed to have originated from seed mixes sown in 2013 and their floral units were classified as either treatment or contaminant floral units. Use of control plots may have given an indication of the impact of this bias. However, regardless of whether controls were rotovated or were unmanaged, the floral abundance of *T. inodorum* weeds in control plots would not have been directly comparable to their abundance in treatment plots. Regardless of whether some floral units of *T. inodorum* were weeds or not, floral units of *T. inodorum* and *C. cyanus* were unexpectedly present in large numbers across meadows, which reduced expected differences in floral composition between meadows of different seed mix types. As a result, meadows of different seed mix types were often more similar in floral composition than replicate meadows of the same mix type.

Overall, contamination resulted in meadows that were not a direct and accurate reflection of seed mix treatments. However, the impact of contaminants on floral reward provision and insect visitation at a meadow community scale is contingent on their absolute and relative levels of reward provision and visitor attractiveness, and their effect on the floral abundance of intended treatment species. If contaminant species offered no rewards, were not visited by insects and did not affect the floral abundance of treatment species then their impact on this study and subsequent studies would be negligible. However, given that plant competition is inherent to planted meadow communities, and that contaminant species do provide floral rewards and are visited by insects (as I demonstrate in Chapter 5 of this thesis), this is not the case. Contamination therefore undermines the ability of this experiment to attribute community level patterns in meadow floral traits, floral rewards and insect visitation to seed mix treatments using

standard statistical methods, such as analysis of variance or more complex types of linear models.

Given that horticulturally cultivated species and weeds are common in urban soil seed banks (e.g. Thompson *et al.* 2005; Albrecht *et al.* 2011), contaminants and weeds are likely to germinate from the soil seed bank whenever seed mixes are sown in urban areas. Putting their impact on experimental design to one side, these species can provide valuable resources for flower-visiting insects, especially if they occur early or late in the year when sown seed mix species have yet to flower or are post-flowering (Hicks *et al.* 2016). Hence, despite treatment validation issues, this study provides field realistic meadows from which to assess the biodiversity benefits of different floral categories in urban meadows, including contaminants and weeds. Moreover, this study provides valuable information on the biodiversity value of individual floral species, especially contaminants, since their attractiveness to flower-visiting insects can be assessed within multiple alternative floral communities.

3.4.3 Objective 3.3: Effectiveness of enrichment manipulation and implications for seed mix design

There was no evidence that manipulation of proportional seed weights of species in seed mixes increased or decreased floral abundance in meadows. However, due to a number of issues with this study, this does not imply that manipulation of the proportional seed weight of species in seed mixes is inherently ineffective.

Firstly, there were only 4 replicate blocks available from which to compare standard versus enriched treatments. This does not provide enough power for a formal statistical test, such as a sign test, which would require a

minimum of 6 replicates. Moreover, 4 replicate pairs do not provide enough power to perform a strong test using sample size- and multiplicity-corrected 95% confidence intervals.

Secondly, germination, establishment, growth and flowering of plants from a flower seed mix is likely to be highly stochastic and dependent on site conditions, soil characteristics and inter- and intra-species interactions, such as competition, which may vary depending on which species perform well. Given that these factors affect individual plants and will vary on the scale of a few metres, stochastic variation in floral abundance is likely to be highest at small rather than large scales (Harper *et al.* 1965; Oomes & Elberse 1976). In this study I surveyed 5 m^2 per meadow/round to characterise floral composition in meadows. Given that stochasticity in germination and plant performance is likely to be high on this scale (Harper *et al.* 1965; Oomes & Elberse 1976), and that there are few replicates of standard and enriched treatments, there is a high risk of a Type 2 error in testing whether change to the percentage seed weights of a species is an effective approach to increasing its floral abundance meadows.

Finally, these limitations are compounded by the effect of contamination, which directly affected the floral abundance of species (such as *T. inodorum*, *C. cyanus, G. segetum*, and *C. tinctoria*) targeted for enrichment or reduced to make way for enriched species. The impact of contamination on the apparent effectiveness of the enrichment treatment depends on the evenness of spread of contaminant flowers within blocks. Given that floral contaminants are unlikely to have been evenly distributed within blocks, contamination may have randomly increased or decreased differences in floral abundance between standard vs enriched mixes in different blocks.

Overall, this increase in random variation in contaminant floral abundance within plots is likely to have inflated the risk of failing to reject a false null hypothesis (Type 2 error).

Overall, this experiment provided an unreliable test of the effectiveness of manipulating the percentage seed weight contribution of species in seed mixes in order to change their floral abundance in meadows. There is a high possibility of failing to detect a true effect due to a lack of power. The effectiveness of enrichment in increasing the floral abundance of targeted species in large scale plantings cannot be ruled out.

3.4.4 Conclusions

The meadow that results when a flower seed mix is sown is contingent on an array of different factors, including sowing season, weather patterns, soil characteristics, soil seed bank size and composition, and ecological interactions between individual plants and species. These factors all affect rates of germination, growth and flowering of sown seed mix species. In this study, a substantial soil seed bank was built up during 2012 when extreme weather prevented germination of most sown species. This resulted in high levels of floral contamination in meadows when the experiment was repeated in 2013. Whether this contamination has an impact on community level comparisons of floral reward provision and insect visitation between meadows of different mix types is contingent on whether contaminants provide floral rewards, whether they are visited by insects, and whether they affect the floral abundance of treatment species. Given that plant competition is inherent to planted meadow communities, contamination directly and indirectly affects community level patterns of floral abundance and diversity. Hence, contamination inherently prevents

direct linking of meadow community floral characteristics to seed mix treatments using standard statistical approaches.

In Chapter 5 of this thesis, I assess the extent to which contaminants affect community level patterns of floral reward provision and insect visitation. Despite the impact of contamination, judicious examination of species level patterns of floral resource provision and insect visitation can, in combination with seed mix species lists, provide insights into the importance of seed mix choice and plant composition in providing floral resources for insects using annual flower meadow seed mixes.

Chapter 4: The impact of molecular taxonomic analysis on the composition of flower-visiting insect assemblages and the structure of flower-visitor interaction networks.

4.1 Introduction

Species interaction networks provide a tractable framework for quantifying and analysing ecological interactions within communities (Proulx *et al.* 2005; Memmott 2009; Tylianakis *et al.* 2010). For plant-pollinator communities, flower-visitor networks are widely used to quantify and visualise interactions between flowering plants and their flower-visitors (e.g. Memmott 1999; Dupont *et al.* 2003; Lopezaraiza-Mikel *et al.* 2007; Carvalheiro *et al.* 2011). Using this approach, flower-visitor interactions are quantified as a matrix with plant species and flower-visitor species listed on opposite axes, with interactions between them quantified as cell entries using either qualitative (presence-absence) or quantitative data (interaction frequencies; Dormann *et al.* 2009; Blüthgen 2010). These matrices can be visualised as 'bipartite' networks comprised of two trophic levels, with lower level 'nodes' representing individual plant species and upper level nodes individual flower-visitor species. Interactions between plants and flower-visitors are represented by links between upper and lower level nodes. These networks can describe both the topology and relative strength of interactions (by incorporating quantitative data on species abundances and interaction frequencies), which can be represented by node and link widths (Pocock *et al.* 2016). Interactions are typically recorded when flower-visitors are observed feeding on or contacting the reproductive parts of a flower, and are only rarely resolved to specific functional processes in plant reproduction, such as effective pollination (e.g. rates of pollen deposition; King *et al.* 2013;

Ballantyne *et al.* 2015). Nevertheless, flower-visitor networks provide a useful tool for describing which species interact and how often (Memmott 1999; Memmott 2009; Blüthgen 2010; Kaiser-Bunbury & Blüthgen 2015).

Numerous metrics have been derived to describe different aspects of network structure. These mainly describe different aspects of the richness, distribution and evenness of interactions for individual species, trophic groups, or the whole community (Bersier *et al.* 2002; Tylianakis *et al.* 2007; Dormann *et al.* 2009; Bascompte & Jordano 2016). Examining how these metrics differ between communities or change due to human intervention has facilitated important advances in our understanding of the impacts of species invasions (Morales & Aizen 2006; Gibson *et al.* 2013), species extinctions (Memmott *et al.* 2004), anthropogenic climate change (Memmott *et al.* 2007), habitat restoration (Forup *et al.* 2008) and conservation management interventions on plant-pollinator communities (Carvalheiro *et al.* 2008; Heleno *et al.* 2010).

Construction of flower-visitor networks that are truly representative of the plant and visitor communities under study requires appropriate sampling and accurate species identification. Numerous studies have shown that interactions between flowering plants and flower-visitors vary dynamically in space and time (e.g. Herrera 1988; Guitián *et al.* 1996; Dupont *et al.* 2009), with consequent changes to the structure of networks (Burkle & Alarcon 2011). For example, the structure of flower-visitor networks has been shown to vary annually (Alarcón *et al.* 2008; Dupont *et al.* 2009), seasonally (Basilio *et al.* 2006; Olesen *et al.* 2008), day-to-day (Olesen *et al.* 2008), diurnally (Baldock *et al.* 2011), and with sampling methodology (Gibson *et al.* 2011). Construction of informative flower-visitor networks therefore requires

appropriate sampling protocols for a given system and objective (Hegland *et al.* 2010; Burkle & Alarcon 2011; Gibson *et al.* 2011). However, given that interactions or links are defined by the presence of individual visitors on flowers, accurate identification of 'who is who' underpins accurate identification of 'who visits whom'.

Flower-visitor networks are typically constructed by sorting plants and visitors into Linnaean species based on morphological characters. This approach can be limited by the 'taxonomic impediment' (the inability to rapidly and reliably identify individual specimens to species; Wheeler *et al.* 2004; Cardoso *et al.* 2011), which is particularly pronounced for plant-pollinator communities since they comprise a wide diversity of taxonomic groups (Mayer *et al.* 2011; Packer *et al.* 2016). In geographic regions with relatively depauperate and well-described floras and faunas, such as Britain and Ireland, accurate and well-presented species-level keys based on morphological characters are available for many taxonomic groups (e.g. Stubbs & Falk 2002; Rose & O'Reilly 2006; Falk & Lewington 2015). However, for many other groups (and regional floras and faunas) accurate keys do not exist, whilst those that do can be difficult to access or to use (e.g. due to their presentation or use of microscopic or ambiguous characters; Packer *et al.* 2009; Packer *et al.* 2016). Furthermore, the best available keys for some groups do not resolve to the species level (Packer *et al.* 2009; Packer *et al.* 2016). For example, there are no comprehensive species keys for the important flower-visiting dipteran family Anthomyiidae in Britain (taxonomic revisions and keys for specific genera are listed by Chandler 2010; Barnard 2011). The best resource for this group, in one of the most studied faunas in the world, is a draft key that only identifies males to genera

or species, often using difficult to access genital characters (Ackland, unpublished).

Species level identifications are also often impossible even for well-described groups due to uncertainty over species status and/or lack of characters that reliably separate morphologically similar species. For example, hoverflies (Diptera: Syrphidae) are well-studied in Britain and Ireland but no species level keys are available to distinguish between female *Sphaerophoria* spp. or males of *Syrphus vitripennis* and *S. rectus* (Stubbs & Falk 2002; Ball & Morris 2013). Similarly, there are no known diagnostic characters that can reliably distinguish between the bumblebee species *Bombus lucorum*, *B. cryptarum* and *B. magnus* - three morphologically cryptic species formerly described as '*B. lucorum*', but recently distinguished due to sequence variation in their mitochondrial DNA (Murray *et al.* 2008; Waters *et al.* 2011; Carolan *et al.* 2012; Scriven *et al.* 2016). Workers of these species are also easily confused with workers of *B. terrestris* (Wolf *et al.* 2010), such that studies often sort these taxa into a single *B. lucorum*/*B. terrestris* taxon (e.g. Dicks *et al.* 2002; Forup *et al.* 2008). This is a specific example of a more general approach whereby specimens are often simply sorted to species surrogates (often called morphospecies or morphotypes) on the basis of morphological similarity/dissimilarity (e.g. Memmott 1999; Lopezaraiza-Mikel *et al.* 2007; Geslin *et al.* 2013).

The challenges of morphological taxonomy and the use of species surrogates will often lead to inaccuracies in specimen identifications, such that single species are erroneously split into multiple taxa, or multiple species are lumped within a single taxon (e.g. Kaartinen *et al.* 2010). For example, the inability to distinguish between morphologically cryptic

species, or the use of morphotypes to 'identify' under-studied taxonomic groups, may result in erroneous non-splitting (incorrect lumping) of individuals from different species. In contrast, the inability to unite the sexes of dimorphic species (due to a lack of characters to distinguish closely-related species in one sex), may result in erroneous over-splitting of sexes within a species (as well as erroneous over-lumping of the unidentified sex with closely-related species). Furthermore, misidentifications may lead to either erroneous splitting or lumping of species. These inaccuracies will affect the perceived composition and richness of flower-visitor assemblages, patterns of flower-visitation, and associated interaction network structure.

An increasingly common approach for coping with these limitations and inaccuracies is the use of additional molecular taxonomic information in the form of DNA barcodes (Valentini *et al.* 2009; Joly *et al.* 2014; Creer *et al.* 2016). DNA barcodes are short standardised regions of DNA that can be compared with reference sequence databases to identify unknown specimens to known species (Hebert *et al.* 2003a; Ratnasingham & Hebert 2007, 2013). The gene region used in DNA barcoding varies across taxa, but for animals it is the 658 bp Folmer region of the mitochondrial gene cytochrome c oxidase subunit 1 (CO1; Hebert *et al.* 2003a; Savolainen *et al.* 2005). Sequence variation in this region can be used for species identification (Hebert *et al.* 2003), species discovery (Smith *et al.* 2006; Kekkonen & Hebert 2014), and for delimiting clusters of sequences called molecular operational taxonomic units (MOTUs; Floyd *et al.* 2002; Blaxter 2004) which have been used as species surrogates in ecological studies (Floyd *et al.* 2009; Valentini *et al.* 2009; Blaxter 2016). Reliable DNA barcoding depends on a common empirical observation (Hebert *et al.* 2003b; Hebert *et al.* 2004b) and broader assumption that sequences of the barcode locus for a single Linnaean species are

monophyletic and that within-species sequence variation is lower than between-species sequence variation (Meyer & Paulay 2005; Acs *et al.* 2010). If this is the case there is a phylogenetic gap (the so-called 'barcoding gap') between intra-specific and inter-specific sequence variation (Fig. 4.1a; Acs *et al.* 2010), which can be used to identify specimens into species (if reference sequences exist) or MOTUs by clustering sequences using a threshold within this gap (initially proposed as between 2-3% sequence divergence; Hebert *et al.* 2003).

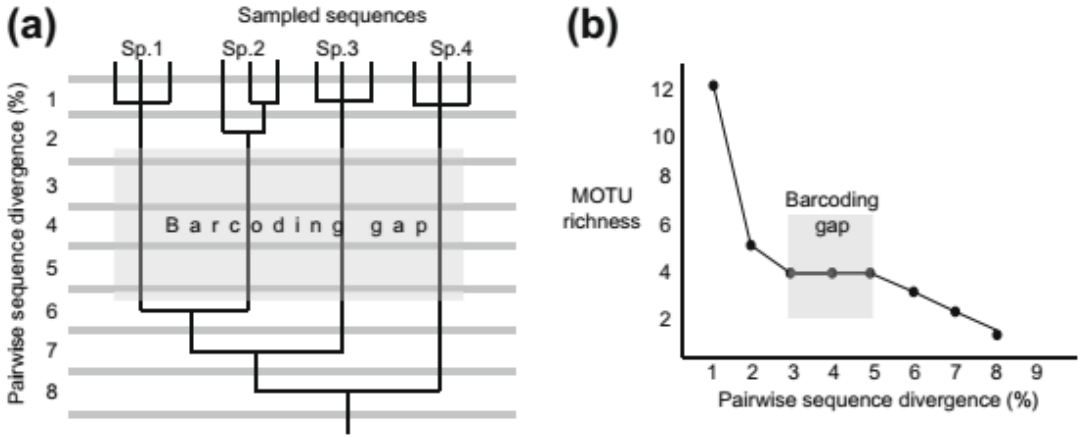


Figure 4.1: A diagrammatic representation of the barcoding gap, reproduced from Ács *et al.* (2010): (a) A visual illustration of the phylogenetic assumptions underlying single locus DNA barcoding, with a set of sample sequences from monophyletic species showing low pairwise sequence divergence within species and high pairwise sequence divergence between species. (b) For the same sample, the relationship between the sequence divergence threshold used to cluster sequences into MOTUs and the number of MOTUs defined in a given sample (MOTU richness). In this idealised example, the barcoding gap is revealed as a plateau in MOTU richness over a range of candidate sequence divergence clustering thresholds.

The presence of a barcoding gap within a set of sequences can be assessed visually by plotting the number of MOTUs defined for each of a series of candidate sequence divergence clustering thresholds (Fig. 4.1b). A barcoding gap is revealed as a plateau in MOTU richness over a range of candidate sequence divergence clustering thresholds. This plateau can be used to select an appropriate sequence divergence cut-off for clustering a set of sequences into MOTUs, with the lower limit rather than mean of the main plateau typically providing more accurate identifications (Meier *et al.* 2008).

Although commonly observed, barcoding gaps are not ubiquitous (e.g. Moritz & Cicero 2004; Meier *et al.* 2006; Wiemers & Fiedler 2007). Sequences from a single Linnaean species can be paraphyletic or polyphyletic for the barcode locus due to hybridisation/introgression or incomplete sorting of ancestral polymorphism (Funk & Omland 2003; Ballard & Whitlock 2004; Hurst & Jiggins 2005). Hence, use of DNA barcoding has been controversial, especially when used for species discovery or delineation of species surrogates (Moritz & Cicero 2004; Meyer & Paulay 2005; Wheeler 2005; Will *et al.* 2005). Nevertheless, barcodes have proved useful in (i) uniting males and females in sexually dimorphic species (e.g. Sheffield *et al.* 2009; Magnacca & Brown 2012); (ii) highlighting genetically discrete units that warrant further taxonomic investigation (Smith *et al.* 2006; González-Vaquero *et al.* 2016; Packer & Ruz 2016); (iii) distinguishing between known morphologically cryptic species (e.g. Danforth *et al.* 1998; Carolan *et al.* 2012); and (iv) providing MOTU analogues to morphology-based species surrogates such as morphospecies/morphotypes, which can enable ecological studies in under-studied fauna (Clare *et al.* 2013; Blaxter 2016). In this study I compared alternative techniques for identification of flower-visitors, assessing how DNA sequence-based MOTU identifications differ from

morphology-based identifications, and examining how use of molecular information changes perceived taxon richness and the structure of flower-visitor networks.

Relative to identifications based on morphology, MOTU-based specimen groupings may be fully concordant, yielding no change to specimen identifications or taxon richness. Alternatively, MOTU-based clustering may split or lump morphotaxa, creating new groupings and potentially changing taxon richness (e.g. Kaartinen *et al.* 2010). For example, sequence clustering may correctly split difficult to distinguish or morphologically cryptic species (such as bumblebees *B. terrestris*, *B. lucorum*, *B. magnus* and *B. cryptarum*), or correctly lump males and females from sexually dimorphic species for which available keys do not provide full species resolution. Additionally, MOTU delineation may result in more complex changes involving partial splitting and partial lumping of morphotaxa. For example, sequence clustering may split a morphotaxon into two or more MOTUs (such as for female hoverflies from multiple species identified to a single morphotaxon), with each subset lumped with individuals from another morphotaxon (such as male hoverflies of the respective species). This type of combined splitting and lumping may increase, decrease or not change taxon richness, which, along with simple re-assortment of specimens, may affect metrics of network structure. Finally, if sequences are compared to a database of references sequences, DNA barcoding should detect straightforward identification errors (without necessarily affecting specimen groupings).

To explore the network consequences of MOTU-based changes to insect taxonomy, I predicted how network metrics would change under four

idealised scenarios of MOTU-driven splitting or lumping of morphotaxa. I describe each of these scenarios in terms of their effect on visitor specialisation and generalisation (Waser *et al*. 1996; Johnson & Steiner 2000; Waser & Ollerton 2006), which in a network context refers to the number of floral partners (links) of visitor species and the relative strength of these interactions (link frequencies; Blüthgen *et al*. 2006; Blüthgen 2010). These simple scenarios, which are illustrated for a minimal number of taxa in Figure 4.2, are: (i) splitting of a single morphotaxon into two molecular taxa that both visit the same flower species, resulting in no change to the average level of specialisation of visitor taxa. (ii) splitting of a single morphotaxon into two molecular taxa that both visit fewer floral partners, resulting in an increase in the average level of specialisation. (iii) lumping of two morphotaxa that visit identical floral partners into a single molecular taxon, resulting in no change in the average level of generalisation of visitor taxa. (iv) lumping of morphotaxa that visit different floral partners, resulting in an increase in the average level of generalisation of visitor taxa.

My predictions of the impact of these changes on metrics describing different aspects of network structure for species (nodes), trophic levels (visitors or plants) or whole community are shown in Table 4.1. I predicted that splitting a morphotaxon will increase taxon richness, while lumping will decrease taxon richness, but that the impact of these changes will depend on how they impact the specialisation/generalisation of visitor taxa. For example, I predicted that splitting a morphotaxon into two MOTUs that do not overlap in their spectrum of floral partners, will increase the average specialisation of visitor taxa. However, this will not affect the number of unique links or their relative strength, and hence will not affect many quantitative metrics of network structure. This may occur when cryptic

species exhibit niche partitioning in their use of forage plants (e.g. Scriven *et al.* 2016). Conversely, splitting a morphotaxon into two MOTUs, which visit the same spectrum of floral partners, will not change the average specialisation of visitor taxa, but will increase the number of unique links and reduce their relative strength, which will be detected across multiple quantitative metrics of network structure. This may occur when similar-looking generalist species are sorted into the same morphotype due to limitations in morphological taxonomy.

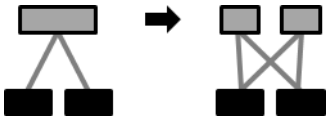
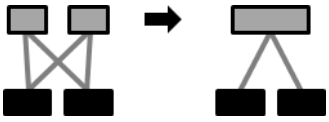
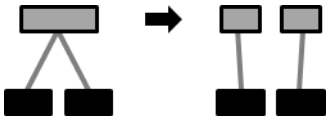
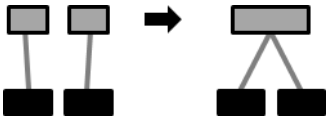
Network impact of molecular taxonomic analysis:		
Change in specialisation or generalisation of visitor taxa:	**A morphotaxon is split**	**Morphotaxa are lumped**
No change in specialisation / generalisation of visitor taxa	**(i)** 	**(iii)** 
Increase in specialisation (ii) & generalisation (iv) of visitor taxa	**(ii)** 	**(iv)** 

Figure 4.2: Four idealised scenarios representing extreme examples from a continuum of possible changes to insect taxon designations and flower-visitor interactions due to molecular taxonomic analysis. For each network, *upper bars* ( ) represent flower-visiting insect taxa and *lower bars* ( ) flowering plant species, while *links* ( ) between bars represent flower-visitor interactions. For each example, the transition from left- to right-hand networks represents the change from morphologically-defined insect taxa to molecular insect taxa. These simplified scenarios are defined based on whether molecular taxonomic analysis results in splitting or lumping of morphotaxa, and whether molecular taxa are equally or more specialist/generalist compared to morphologically-defined taxa.

Similarly, I predicted that lumping two morphotaxa that do not overlap in their spectrum of floral partners will increase the average generalisation of visitor taxa. However, this will not affect the number of unique links or their relative strength, and will not affect the value of many quantitative metrics. Conversely, lumping two morphotaxa that visit the same spectrum of floral partners will not change the average generalisation of visitor taxa. However, it will decrease the number of unique links and increase their relative strength, which will be detected across multiple quantitative metrics. This may occur when males and females of sexually dimorphic species are separated into separate morphotaxa due to a lack of keys or known characters with which to separate species of one sex (as described above).

These idealised scenarios were devised to enable exploration of the impact of MOTU-based changes to insect taxonomy on flower-visitor network structure. For real datasets, the impact of molecular taxonomic analysis will depend on the relative frequencies of splitting and lumping across networks, as well as on the characteristics of the morphotaxa affected, such as their level of specialisation/generalisation and their relative abundance. To my knowledge, this is the first study of plant-pollinator communities to use molecular information to corroborate visitor taxonomy and then examine the extent to which use of molecular taxonomic identification alters flower-visitor network structure.

4.1.1 Objectives

My main objectives in this study were two-fold:

Objective 4.1. ***To examine the impact of molecular taxonomic analysis on the perceived composition of flower-visiting insect assemblages.***

More specifically, I ask whether use of molecular information to delineate insect taxa changes the perceived number of taxa, or the distribution of individuals among taxa, compared to morphology-based taxonomy.

Objective 4.2. ***To examine the extent to which use of molecular taxon designations alters the structure of flower-visitor networks.***

Here, I ask whether there are any systematic biases between the structures of flower-visitor networks constructed solely using morphological taxonomy versus those constructed using molecular information to revise morphological identifications.

Table 4.1 (overleaf): Commonly-used metrics of network structure and how they are predicted to change when molecular data are used to delineate flower-visitor taxa. Four scenarios are defined based on whether use of molecular data results in splitting or lumping of insect morphotaxa, and whether this changes the specialisation/generalisation of molecular taxa compared to morphotaxa (see Fig. 4.2). Predicted increases in the values of metrics are indicated by a '**+**', decreases by a '**–**', and predictions of no change by a '**o**'. Brackets '**( )**' indicate a change that is possible but less probable than the alternative under the same scenario. Predictions are for the presence and direction (rather than magnitude) of change under specific defined scenarios. The magnitude of change will be dependent on the relative abundances/frequencies of affected taxa and interactions.

Network property	Definition	Mode of sensitivity to molecular taxonomic analysis:	Predicted impact of molecular taxonomic analysis on metric value assuming:			
			A morphotaxon is split		Morphotaxa are lumped	
			(i) No change in specialisation / generalisation of visitor taxa (molecular taxa share all floral partners)	**(ii) A generalist taxon is split into more specialist taxa** (molecular taxa do not share floral partners)	**(iii) No change in generalisation / specialisation of visitor taxa** (lumped taxa share all floral partners)	**(iv) Specialist taxa are lumped into a more generalist taxon** (lumped taxa do not share floral partners)
Insect visitor taxon richness (N)	The number of insect taxa in network	(i): splitting and lumping of morphotaxa	+	+	−	−
Number of unique interactions	The number of unique species-to-species links	(i) and (ii): degree of change in specialisation or generalisation	+	o	−	o
Weighted quantitative generality ($\mathbf{G_{qw}}$)	The mean effective number of interactions per visitor taxon, weighted by observed interactions per taxon	(i), (ii) and (iii): change to frequencies of insect taxa and links (i.e. taxon and link weightings in calculations)	o	−	o	+
Weighted quantitative vulnerability ($\mathbf{V_{qw}}$)	The mean effective number of interactions per plant taxon, weighted by observed interactions per taxon	(i), (ii) and (iii)	+	o	−	o
Weighted quantitative Connectance ($\mathbf{C_{qw}}$)	The mean effective number of interactions per taxon, weighted by observed interactions per taxon; then divided by the number of species in the network	(i), (ii) and (iii); however, dependent on relative directions and magnitudes of changes to G, V and N.	o	o (−)	o	(+) o
Interaction diversity (e^{H_2})	The effective number of interactions or links in the network	(i), (ii) and (iii)	+	o	−	o
Interaction evenness ($\mathbf{IE_S}$)	Shannon's evenness of interaction frequencies	(i), (ii) and (iii)	o −	o	+ o	o
Weighted quantitative network specialisation (H_2')	A network-level measure of the deviation of species interactions from a null expectation of neutral interactions in proportion to each species observation totals	Unclear	o	o	o	o

4.2 Materials and methods

4.2.1 Study system and field experiment

This study focuses on the flower-visiting insect assemblages associated with urban flower meadows grown in Sheffield, UK (see Chapter 2 General Methods). Floral density and flower-insect interaction data were collected from a field experiment composed of 4 replicate blocks, each sown with 2 formulations of each of 3 types of flower meadow seed mix (Pictorial Meadows Ltd., Sheffield, UK). These 6 seed mixes included a diverse array of floral morphologies likely to attract a range of flower-visiting insect taxa (see Table 2.1 Chapter 2 for seed mix compositions). For this study, data were amalgamated across treatments within blocks to remove treatment effects and increase the sample sizes of replicate flower-visitor networks. The total area of each block was 150 m^2, which comprised six 25 m^2 plantings laid out in either a 3x2 or 6x1 array. Blocks were separated by at least 80 metres, and were sown in pairs on 29 April and 3 May 2013.

4.2.2 Flower-visitor network surveys

Meadow floral composition and flower-visiting insect assemblages were surveyed at three time-points during the flowering season: late July (30 July-3 August), late August (27 August-2 Sept.), and late September (20-27 Sept.; hereafter, survey rounds '1', '2' and '3', respectively).

Flower-visiting insects were surveyed by walking a 5 m-long transect along the diagonal axis of each 25 m^2 plot in each block, catching by handnet all Hymenoptera, Diptera, Lepidoptera and Coleoptera seen to be contacting the reproductive parts of a flower, up to 1 m either side of the transect line. For each insect captured, the flower species visited was recorded and the insect was killed with ethyl acetate for identification and to prevent

resampling (thereby ensuing observations are independent). To increase flower-visitor sample sizes each transect was sampled twice per survey round, with the two samples collected on the same day and with at least 30 minutes in-between. All insect surveys were conducted between 10.00 and 18:00 hrs in warm, dry weather, with temperature in the shade greater than 15 °C and wind speed lower than a moderate breeze (4 on the Beaufort scale).

The methodology used for floral composition surveys is described in detail in Chapters 2 and 3. These surveys were performed within 48 hrs of flower-visitor surveys for the same survey round. Floral composition was quantified by counting the number of floral units of each species, including weeds, in five 1 m^2 quadrats in each 25 m^2 plot. Quadrats were located contiguously on alternating sides of the flower-visitor sampling transect, with random right/left placement of the first quadrat and alternating left/right placement of subsequent quadrats.

4.2.3 Morphological identification of insect specimens

All insect specimens (n=1570) were identified to morphotaxa at one of two levels of resolution: (1) to Linnaean species using taxon-specific keys (92% of specimens); or (2) to morphotype (8% of specimens). Specimens were identified to morphotype if limitations in current taxonomic keys or interpretation of morphological character states precluded identification to Linnaean species. To identify specimens to morphotype, individuals were first identified to family and then sorted into morphotaxa using external morphological characters identified as likely to be informative from taxon-appropriate keys.

4.2.4 Selection of specimens for DNA sequence-based taxon delineation

To efficiently screen flower-visitor networks for misidentified or morphologically cryptic insect species, I selected a subset of specimens (56% of n=1570 specimens) for DNA barcoding (Table A4.2). Resource and time limitations precluded generation of DNA barcode sequences for all specimens; therefore, subsampling was designed to target those taxa in which morphological identification was most challenging, either due to a lack of taxonomic resources, or due to the known existence of cryptic species that cannot reliably be separated using morphological characters. The sequenced subset was thus selected based on four criteria: (i) All individuals of groups known to contain morphologically cryptic or highly similar species, including solitary bees (Kuhlmann *et al.* 2007; Sheffield *et al.* 2009; Schmidt *et al.* 2015) and bumblebees (e.g. *Bombus terrestris* and *Bombus lucorum*; Wolf *et al.* 2010; Carolan *et al.* 2012; Scriven *et al.* 2016), or which cannot be fully resolved to species using current keys (e.g. hoverflies in genera *Sphaerophoria* and *Syrphus* (Diptera: Syrphidae); Stubbs & Falk 2002). (ii) All individuals of groups identified solely to morphotypes, including most Coleoptera and non-syrphid Diptera. To check the consistency of my morphological identifications (i.e. that individuals I identified as a single taxon were of the same species, and that individuals I identified as different species were actually different), I also sequenced: (iii) all individuals of Linnaean species with fewer than 10 individuals; and (iv) 10% of individuals, up to a maximum of 20, for Linnaean species with more than 10 individuals. If sequence analysis revealed discordance between morphological and molecular identifications, all individuals of the affected morphotaxon/morphotaxa were sequenced. This approach also allowed morphologically distinctive males and females, for which no species-level

keys were available, to be united into single taxa. For each morphotaxon in which only a subsample of individuals was sequenced, individuals were selected to cover the maximum possible range of planted meadow treatments, blocks and survey rounds.

4.2.5 Molecular methods

DNA was extracted from a single leg per specimen, which was incubated overnight at 37 °C in a mix of 40 µl of 5% chelex solution and 5 µl of 10 mg/ml Proteinase K. The Proteinase K was deactivated by incubation at 95 °C for 15 min. Undigested insect anatomy was removed by centrifugation for 2 min at 13,000 rpm, and the supernatant used for PCR.

All selected specimens were sequenced for part of the mitochondrial CO1 gene. All Hymenoptera, Diptera and Lepidoptera were sequenced for the standard animal DNA barcode region located at the 5′ end of CO1 (Folmer *et al.* 1994; Hebert *et al.* 2003a). This 658 bp DNA barcode region was sequenced using the forward/reverse primers: LepF1/LepR1 (Hebert *et al.* 2004a) or LCO/HCO (Folmer *et al.* 1994). If amplification was not successful, a shorter fragment within this region was sequenced using alternative combinations of mixed primers: MLepF1+HCO/LepR1 (407 bp; Hajibabaei *et al.* 2006) or LepF1/LCO+MLepR2 (307 bp; Hebert *et al.* 2013; see Table A4.3.1).

Beetles (Coleoptera) were sequenced using a two-locus strategy due to difficulties in the amplification of any single locus for all selected specimens. This approach involved sequencing Coleoptera for either the Folmer region, as defined above, or an adjacent 900 bp 3′ region of CO1, using a primer set developed for beetles (SJerryF/SPatR; Timmermans *et al.* 2010). As these regions do not overlap, a subset of specimens was sequenced for both

regions to enable subsequent cross-validation of analyses between regions (see Appendix A4.3 and Table A4.3.2).

PCRs for the Folmer region used 1 µl of DNA template added to a 19 µl mix of 13.6 µl distilled water, 2 µl BSA, 2 µl of 10x PCR buffer, 0.8 µl of 50mM $MgCl_2$, 0.2 µl of 20 µM solutions for each of the forward and reverse primers, 0.1 µl of 25 mM dNTPs and 0.1 µl of Taq (Bioline, London, UK). PCR conditions for amplifying this region were 94 °C for 2 min, followed by four repeated cycles of 94°C for 30 s, 45 °C for 40 s, and 72 °C for 40 s. These steps were followed by 34 repeated cycles of 94 °C for 30 s, 50 °C for 40 s, and 72 °C for 40 s, ending in a final step of 72 °C for 5 min and incubation at 10 °C. For PCR of the 900 bp 3' region, 1.2 µl of DNA template was added to a 18.8 µl mixture of 12.94 µl distilled water, 2 µl BSA, 2 µl of 10x PCR buffer, 1 µl of 50mM $MgCl_2$, 0.3 µl of 20 µM PCR primer, for each of the Sjerry_F and SpatR primers, 0.16 µl of 25 mM dNTPs and 0.1 µl of Taq (Bioline, London, UK). PCR conditions for these reactions were 94 °C for 2 min, followed by 34 repeated cycles of 94 °C for 30 s, 51 °C for 30 s, and 72 °C for 60 s, ending in a final step of 72 °C for 5 min and incubation at 10 °C.

Presence of a PCR product was checked by visualisation on a 2 % agarose gel stained with SYBR Safe™ DNA gel stain (Invitrogen). PCR products were purified to remove excess primers and oligonucleotides by incubation with 1 µl (1U) Shrimp Alkaline Phosphatase, 1.425 µl of its associated dilution buffer (VWR, UK), and 0.075 µl (1.5U) Exonuclease 1 solution (New England Biolabs, UK). Samples were incubated at 37 °C for 40 min, 94 °C for 15 min, and a final holding temperature of 10 °C. Samples were then sequenced on an ABI Prism 3730 Genetic Analyzer using ABI BigBye™ v3.1 Terminator sequencing chemistry (Applied Biosystems).

Sequencing reactions were adapted to account for variation in the amount of DNA in PCR products, with more DNA template added to sequencing reactions if samples contained little DNA (judged by the presence of weak bands on agarose gels). For bright bands, sequencing reactions used 4 µl DNA template, added to a 10 µl mix of 2.25 µl distilled water, 2 µl 5x sequencing buffer, 2 µl of a 3.2 µM solution for the reverse primer, and 0.75 µl BigDye Mix. For weak bands, sequencing reactions used 8 µl DNA template, added to a 10 µl mix of 0.55 µl 5x sequencing buffer, 1 µl of a 3.2 µM solution for the reverse primer, and 0.45 µl BigDye Mix. All samples were then incubated at 95 °C for 2 min and then for 25 repeated cycles of 95 °C for 10 s, 50 °C for 10 seconds, 60 °C for 75 s, and finally 10 °C until removed from the thermocycler.

Sequence chromatograms were edited and checked for an open reading frame to identify stop codons and errors in editing using Sequencher v5 (Gene Codes Corporation, Ann Arbor, USA).

4.2.6 MOTU delineation and morpho-molecular identification of insect specimens

Sequences were clustered into molecular operational taxonomic units (MOTUs; Floyd *et al.* 2002; Blaxter 2004; Blaxter *et al.* 2005) using two approaches: jMOTU (version 1.0.8; Jones *et al.* 2011) and ABGD (Automatic Barcode Gap Discovery; updated second release of Dec. 2011; Puillandre *et al.* 2012). Both approaches were highly concordant (Table A4.4.2); hence, only results from jMOTU are shown or used for network comparisons. The aim of these approaches is to avoid the arbitrary selection of a sequence divergence clustering threshold by examining a series of candidate thresholds for a given dataset and highlighting the presence of a 'barcoding gap', enabling data driven selection of a sequence clustering threshold.

For analysis, sequences were separated into six separate groups based on specimen taxonomy and the region of CO1 sequenced. Hence, sequences were analysed separately for: Hymenoptera, Lepidoptera, syrphid Diptera, non-syrphid Diptera, Coleoptera (5′ Folmer region) and Coleoptera (3′ non-Folmer).

jMOTU calculates the number of MOTUs delineated for a series of candidate pairwise sequence divergence clustering thresholds, with sequences clustered together if their pairwise sequence divergence is below a given threshold. For the relationship between candidate thresholds and MOTU richness, a barcoding gap is revealed when MOTU richness remains stable over a series of divergence values, which can be visualised as a plateau in MOTU richness bounded by change (Figure 1b). The lower limit of the main plateau was used as the sequence divergence clustering threshold, as this has been shown to yield more accurate identifications than the median distance (Meier *et al.* 2008). jMOTU analyses used a Low BLAST identify filter of 95%, and generated MOTU designations for variations of 0-10% of maximum length for each locus.

For ABGD analyses, settings were Pmin = 0.001, Pmax = 0.10, Steps = 30, X = 1.5, Number of bins = 30. I used simple phylogenetic distance rather than an evolutionary model following evidence favouring the simpler approach (Collins *et al.* 2012). ABGD requires a distance matrix to allocate sequences to MOTUs. As the data for syrphid and non-syrphid Diptera contained short non-overlapping sequences, I ran ABGD analyses twice, each time excluding short sequences from either the 5′ or the 3′ end of this sequence.

The results of jMOTU and ABGD analyses were fully concordant with the exception of one coleopteran and two small dipteran clades (see Tables A4.4.2 and A4.4.3). Given that these clades included short sequences and morphotaxa comprised of single individuals, and that ABGD is known to perform poorly with fewer than 3-5 samples per species (Puillandre *et al.* 2012), I base subsequent analyses on the results of jMOTU.

Given that for most morphotaxa only a subset of individuals were sequenced, construction of flower-visitor networks informed by molecular data required extrapolation of MOTU designations from the sequenced subset of specimens to the full specimen set. This was straightforward for my dataset because conflicts between morphotaxon and MOTU designations were only detected in groups for which all individuals were sequenced. For most morphotaxa (including those in which only a subset of individuals were sequenced), all individuals were allocated to the same MOTU. In each of these cases, I have assumed that all non-sequenced individuals of a morphotaxon can also be allocated to the single corresponding MOTU. Hence, in this study I compare flower-visitor networks constructed using morphologically-identified visitor taxa (morphotaxa) with equivalent networks constructed using visitor taxa identified using morphology supplemented by molecular taxonomic analysis (morpho-molecular taxa).

4.2.7 Comparison of morphology-based versus morpho-molecular flower-visitor networks

To assess the impact of molecular taxonomic analysis on network structure, I constructed flower-visitor networks for each of four replicate blocks in each of 3 survey rounds (n=12 networks), for datasets based on either morphological or molecular definitions of visitor taxa (i.e. n=12 pairs of networks).

For each network and dataset (n=24 networks in total), I calculated *visitor taxon richness*, the *number of unique interactions* and 6 metrics that describe different aspects of network structure and are widely used in comparative studies of plant-pollinator communities. These were (after Bersier *et al.* 2002; Blüthgen *et al.* 2006; Tylianakis *et al.* 2007; Blüthgen 2010):

(i) weighted quantitative *generality* (G_{qw} – the mean effective number of links per visitor taxon);

(ii) weighted quantitative *vulnerability* (V_{qw} – the mean effective number of links per floral taxon);

(iii) weighted quantitative *connectance* (C_{qw} – the mean effective number of links per taxon, divided by number of species in the network);

(iv) *Interaction diversity* (e^{H_2} – the effective number of links in the network);

(v) *Interaction evenness* (IE_S - the evenness of spread of interactions in the network);

(vi) weighted quantitative *network specialisation* (H_2' - a network-wide measure of the deviation of interactions from neutral expectations given species relative abundances).

All metrics were calculated using the package 'Bipartite'(version 2.06.1; Dormann *et al.* 2008; Dormann *et al.* 2009) in the R statistical environment (R Development Core Team 2016).

To quantify change in network structure, for each metric, I calculated the percentage difference in network structure between each morphology-based network and its paired morpho-molecular equivalent. To test whether these differences were significantly different from zero, I then used Wilcoxon signed rank tests. These were implemented using a normal approximation, since exact *P*-values from a permutation-based test were precluded by the number of 'ties' in the dataset (i.e. percentage differences between networks which were of equal magnitude for multiple pairs of networks; Depuy *et al.* 2005). To account for inflation of the family-wise Type 1 error rate due to multiple testing, I used a Bonferroni-corrected alpha of α/k (Abdi 2007), where the number of tests k was 8 (for 8 network metrics). Results were consistent regardless of whether percentage or absolute differences were analysed; thus, I present analyses based on percentage differences only.

This approach assumes that the 12 flower-visitor networks used are independent. Given that all flower visitors were caught and killed during sampling, there was no dispersal of individual flower visitors between blocks (spatial non-independence), or repeat sampling of the same visitor in two or more survey rounds (temporal non-independence). Hence, this is likely to have been the case.

To assess the robustness of changes in space and time and under different levels of sampling intensity, I examined change in network metrics for the same dataset at 4 different levels of aggregation. These were: (a) a single network containing all data for the field site over the season (n=1); (b) networks for each of 3 survey rounds (n = 3); (c) seasonal networks for each of four replicate experimental blocks (n = 4); (d) time-point specific networks for each of 4 replicate blocks surveyed for 3 survey rounds (n = 12; as above).

4.3 Results

4.3.1 Overview of the data and MOTU delineation

In total, 1,570 insects (82%) were caught from the 1,926 flower-visitor interactions observed during insect flower-visitor surveys. These consisted of 347 Hymenoptera, 1,003 Diptera, 46 Lepidoptera and 174 Coleoptera, equivalent to a capture rate of between 81-85% of all observed interactions for each insect order. From these, 888 sequences were recovered from 870 individuals, with 18 Coleoptera sequenced for two loci to unite MOTUs defined using separate regions of CO1 (Appendix A4.3). Sequences were recovered from 103 out of the 109 morphotaxa identified. Barcoding was not carried out for six highly distinctive morphotaxa (*Apis mellifera*, Bibionidae morphotype 1 (Diptera), *Paradelia* group A (Diptera), and *Aglais urticae*, *Maniola jurtina* and *Pieris brassicae* (all Lepidoptera). To enable clear and concise comparison of perceived taxon richness before and after molecular taxonomic analysis, these six taxa were included in taxon totals for both morphotaxa and MOTUs. This assumes that if sequences were available, each of these taxa would cluster into its own MOTU. Each of these morphotaxa, with the exception of honeybees, was rare, with all 5 (without honeybees) representing only 0.5% of the 1,570 specimens.

For each of the six taxon/locus-based sets of sequences clustered into MOTUs, jMOTU analysis revealed the barcoding gap as a plateau in MOTU richness over a range of candidate threshold sequence divergence values. These plateaus, ranked by length in bp, ranged from: 11-14 bp (1.7-2.2%) in non-syrphid Diptera; 6-12 bp (0.9-1.9%) in syrphid Diptera; 3-10 bp (0.5-1.6%) in Coleoptera sequenced for the Folmer region; 3-42 bp (0.5-6.6%) in Hymenoptera; 0-51 bp (0-7.8%) in Lepidoptera; and 3-94 bp (0.4-11.6%) in Coleoptera sequenced for 3′ CO1 (Appendix 4.4). For each set of sequences,

the lower limit of this range was used to cluster individuals into MOTUs. All selected thresholds were below 2%, ranging from 0% in Lepidoptera to 1.7% for non-syrphid Diptera (Table A4.4.2).

4.3.2 Objective 4.1: The impact of molecular taxonomic analysis on the richness and relative abundance of insect taxa

To quantify the number of observed changes to insect taxonomy, I defined five types of change based on the effect of molecular data on morphotaxa (i.e. split, lumped, or both) and the impact of these changes on taxon richness (i.e. increased, decreased, or not changed). These were 'full lumping', 'full splitting', and combined splitting and lumping resulting in either a net increase, a net decrease or no net change in taxon richness. Diagrammatic representations of these changes are shown in Table 4.2, with actual changes shown in Fig. 4.3.

There were 7 instances of full lumping, in which multiple morphotaxa were lumped into a single MOTU, and most of these affected Diptera (5/7 instances; Fig. 4.3). For example, three hoverfly morphotaxa in the genus *Sphaerophoria* (*Sphaerophoria* females, *S. interrupta* males, and *S. scripta* males) were lumped into a single MOTU (Fig. 4.3). Similarly, males comprising two morphotaxa in the dipteran family Anthomyiidae (*Botanophila* group D and *Delia* group C) were lumped into a single MOTU with two morphotypes comprising females (anthomyiid morphotypes '5' and '6'). There was a single instance of full splitting, in which one morphotaxon was split into multiple MOTUs, with *Helina reversio* individuals (Diptera: Muscidae) split into two MOTUs. There were also 4 instances of combined splitting and lumping with no change in taxon richness. For example, 12/15 *Bombus lucorum* individuals were reallocated to a MOTU with 45 *Bombus terrestris* (Hymenoptera: Apidae). For combined splitting and lumping, there was also a single

instance each of a net increase or decrease in taxon richness (Table 4.2). This included the lumping of *Syrphus vitripennis/rectus* males with *S. vitripennis* and *S. rectus* females (although one *S. rectus* female clustered with *Eupeodes latifasciatus*; Diptera: Syrphidae; Fig. 4.4).

Most morphotaxa (77 out of 109) were not changed by molecular taxonomic analysis. The net effect of observed changes was the reassortment of specimens from the remaining 32 morphotaxa into 22 MOTUs, yielding a net decrease in taxon richness of ~9% from 109 to 99 taxa (Table 4.3). Splitting of morphotaxa did increase the number of taxa by 2; however, this was outweighed by lumping of morphotaxa, which decreased taxon richness by 12 (Table 4.3). Taxon richness decreased in Hymenoptera, Diptera and Coleoptera, but stayed the same in Lepidoptera. Most changes were to Diptera, with specimens in 24 morphotaxa reassorted into 16 MOTUs, reducing Dipteran richness by 11%. For each of Hymenoptera and Coleoptera, specimens in 4 morphotaxa were reassorted into 3 MOTUs, reducing taxon richness in these orders by c. 6% and 9% respectively.

The magnitudes of changes to insect taxonomy (i.e. the number of individuals they affect) are an important component of the impact of molecular taxonomic analysis on quantitative metrics of flower-visitor network structure. However, quantifying the number of specimens affected by molecular taxonomic analysis (or change in the relative abundance of taxa) is challenging since the loss of taxa (due to lumping) or the generation of new taxa (due to splitting) creates MOTUs for which there are no analogous morphotaxa to serve as a baseline for comparison. Nevertheless, the number of individuals affected can be assessed qualitatively. For example, the 77 morphotaxa unchanged by molecular taxonomic analysis

contained only 43.6% of specimens (Table 4.3a), while the remaining 32 morphotaxa from which individuals were split or with which individuals were lumped contained most specimens (56.4%). However, the network impact of such changes can be minimal where the distribution of individuals among these changed taxa is highly skewed. For example, almost half of individuals (402/886) in the 32 affected morphotaxa belonged to the largest single morphotaxon, *Lucilia sericata* (Diptera: Calliphoridae), which was lumped with two specimens of *L. richardsii* (Fig. 4.3b).

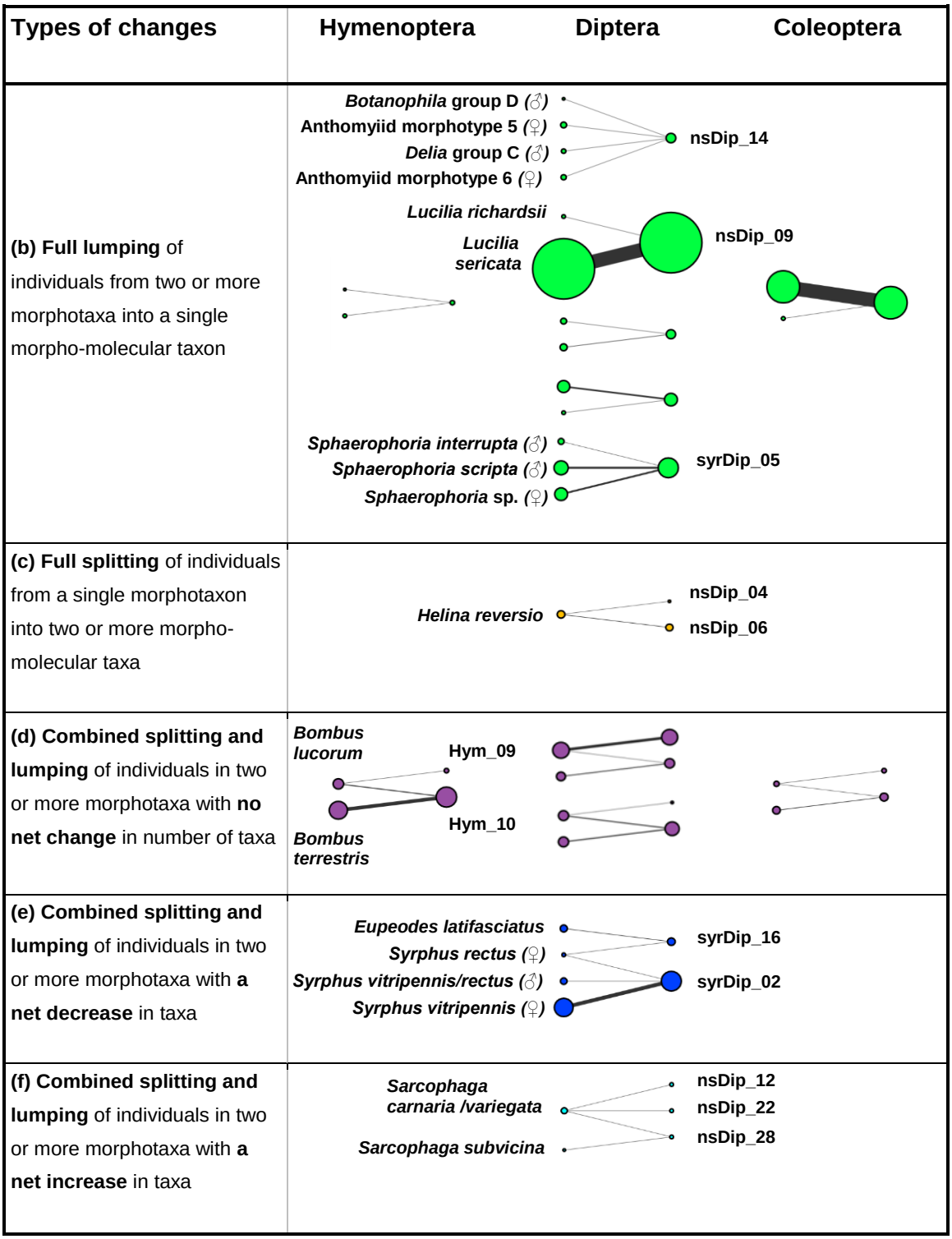


Figure 4.3: Diagrammatic representation of changes to insect taxonomy due to molecular taxonomic analysis (see Table 4.2). Circles represent taxa, with morphotaxa left and MOTUs right. Lines indicate reallocation of individuals. Circle area and line thickness are scaled independently by the relative abundance of individuals. Labels highlight selected exemplar taxa (full changes described in Table A4.4.5).

Table 4.2: Types of change to insect taxonomy due to molecular taxonomic analysis and the numbers of taxa affected. Five types of change were defined to describe the impact of molecular taxonomic analysis based on whether individuals in morphotaxa were split apart, lumped together or both and how these changes affected taxon richness. These types were: (i) full lumping of individuals from two or more morphotaxa into a single morpho-molecular taxon (green); (ii) full splitting of individuals in a single morphotaxon into two or more morpho-molecular taxa (orange); (iii) combined splitting and lumping of individuals in two or more morphotaxa with no net change in taxon richness (purple); (iv) combined splitting and lumping of individuals in two or more morphotaxa with a decrease in taxon richness (dark blue); (v) combined splitting and lumping of individuals in two or more morphotaxa with a net increase in taxon richness (light blue). Additionally, there was no change in the constituent individuals of a subset of morphotaxa (grey).

* Circles on the left of diagrams represent morphotaxa and circles on the right morpho-molecular taxa, with links indicating reallocation of constituent individuals.

ǂ Total number of individuals in the taxa affected by this type of change; the relative abundance of individuals gives a crude indication of the potential importance of changes. However, not all individuals are affected by changes (e.g. in taxa both split and lumped but with no change in richness, a subset of individuals are effectively reallocated) and the distribution of individuals among taxa is highly skewed (Figure 4.3).

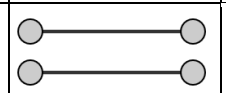
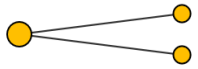
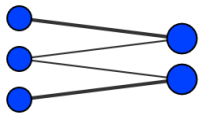
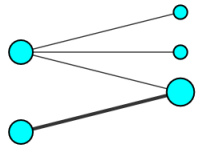
Types of changes to insect taxonomy due to molecular taxonomic analysis	Diagrammatic illustration of impact on number of taxa*	Effect on taxon richness	Number of times type of change observed	Hymenoptera			Diptera			Coleoptera			All taxa				
				Taxa	%	Change	Taxa	%	Change	Taxa	%	Change	Taxa	%	Change	Individuals‡	%
(a) No change to composition of morphotaxa		no change	no change	14	77.8	0	48	66.7	0	7	63.6	0	77	70.6	0	684	43.6
(b) Full lumping of individuals from two or more morphotaxa into a single morpho-molecular taxon		decrease	7	2	11.1	-1	13	18.1	-8	2	18.2	-1	17	15.6	-10	661	42.1
(c) Full splitting of individuals in a single morphotaxon into two or more morpho-molecular taxa		increase	1	0	0.0	0	1	1.4	+1	0	0.0	0	1	0.9	+1	8	0.5
(d) Combined splitting and lumping of individuals in two or more morphotaxa with **no net change** in number of taxa		no change	4	2	11.1	0	4	5.6	0	2	18.2	0	8	7.3	0	148	9.4
(e) Combined splitting and lumping of individuals in two or more morphotaxa with **a net decrease** in number of taxa		decrease	1	0	0.0	0	4	5.6	-2	0	0.0	0	4	3.7	-2	63	4.0
(f) Combined splitting and lumping of individuals in two or more morphotaxa with **a net increase** in number of taxa		increase	1	0	0.0	0	2	2.8	+1	0	0.0	0	2	1.8	+1	6	0.4
Total			14	18	100	-1	72	100	-8	11	100	-1	109	100	-10	1570	100

Table 4.3: Change to insect taxon richness when taxa are defined using morphology or morphology informed by molecular taxonomic information. The number of morphotaxa changed by molecular taxonomic analysis quantifies the number of morphotaxa for which the composition of individual specimens was altered by molecular information. This includes all morphotaxa for which constituent specimens were split apart or lumped together with specimens from another morphotaxa, including instances in which this reallocation of individuals causes no net change to taxon richness (see Table 4.2).

	Taxon richness when taxa defined using morphology	Taxon richness when taxa defined using morphological and molecular information	Number of morphotaxa changed by molecular taxonomic analysis	Reduction in taxa due to lumping of morphotaxa	Increase in taxa due to splitting of morphotaxa	Net Change	% Net Change
Hymenoptera	18	17	4	1	0	-1	-5.6
Diptera	72	64	24	10	2	-8	-11.1
Lepidoptera	8	8	0	0	0	0	0.0
Coleoptera	11	10	4	1	0	-1	-9.1
All taxa	109	99	32	12	2	-10	-9.2

4.3.3 Objective 4.2: Impact of molecular taxonomic analysis on the structure of flower-visitor networks

Flower-visitor networks constructed using MOTU-based insect taxa differed in size and some aspects of structure compared to morphology-based networks. Insect taxon richness decreased on average by 8.2%, from a mean of 28.4 ±2.1 for networks based on morphotaxa to 26.1 ±2.0 for networks based on molecular taxa (mean ±SE; Wilcoxon signed rank test W=0, P<0.01, n=12; Fig. 4.4; Table A4.8.1).

DNA sequence-based changes to specimen identifications and taxon richness translated into consistent minor changes in flower-visitor network structure. The number of unique links in the networks decreased on average by 5.4% from 45.6 ± 4.5 to 43.2 ± 4.3 links (W=0, P<0.01; Fig. 4.4). Similarly, interaction diversity, a measure of the effective number of links in the networks, decreased on average by 4.9% from 24.5 ± 2.1 to 23.4 ± 2.0 (W =0, P<0.01; Fig. 4.4). For insect taxa generality increased on average by 2.8% from 1.9 ± 0.1 to 2.0 ± 0.1 (W =66, P<0.01; Fig. 4.4), while for plant taxa vulnerability decreased on average by 4.9% from 6.4 ± 0.5 to 6.1 ± 0.5 (W =0, P<0.01; Fig. 4.4). Network connectance increased on average by 3.45% from 0.11 to 0.12 (W =74, P<0.001; Fig. 4.4). However, there were no changes in interaction evenness (W =40, P=0.96) or network specialisation (W =19, P=0.12; Fig. 4.4).

Although changes to taxonomy resulted in statistically significant changes to metrics (which were in some cases comparable to real ecological differences between flower-visitor communities – see Discussion), the visual difference between networks was minimal, even for the most strongly impacted network (Fig. 4.5). Hence, changes to visitor taxonomy and patterns of flower-visitor network structure had little impact on our ability to identify broad qualitative patterns that can be valuable for ecological

understanding, conservation or management. For example, visual examination of networks shows that most insect visits were to a small subset of floral species (Fig. 4.5 and A4.6), whilst almost all Hymenoptera (bees) visited a single floral species (*Centaurea cyanus*; Fig. A4.6).

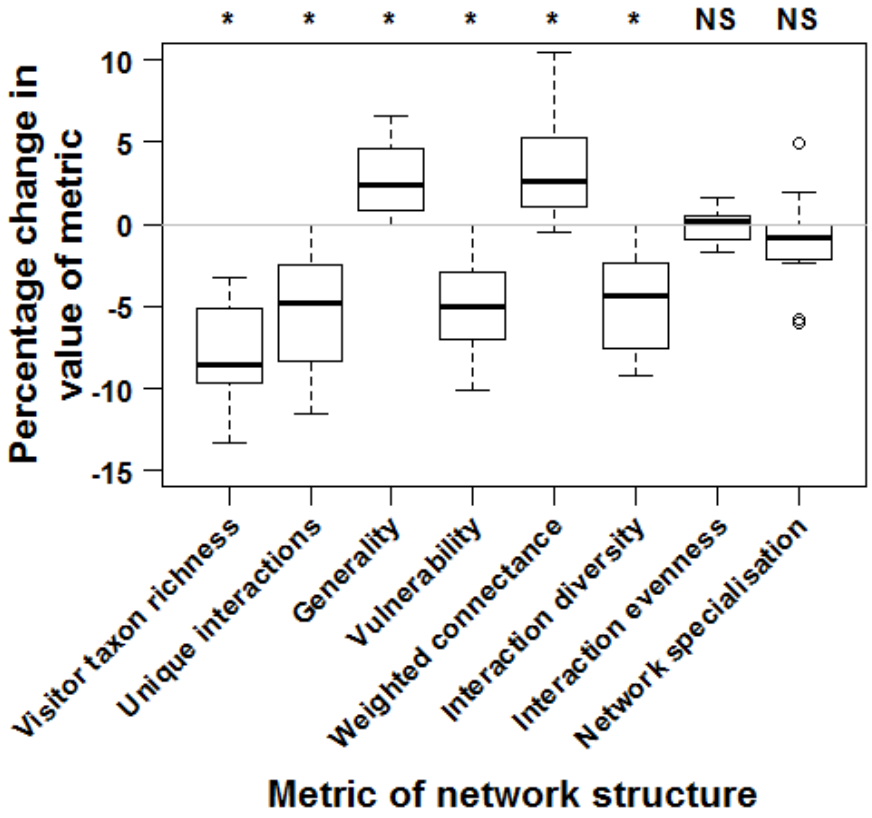
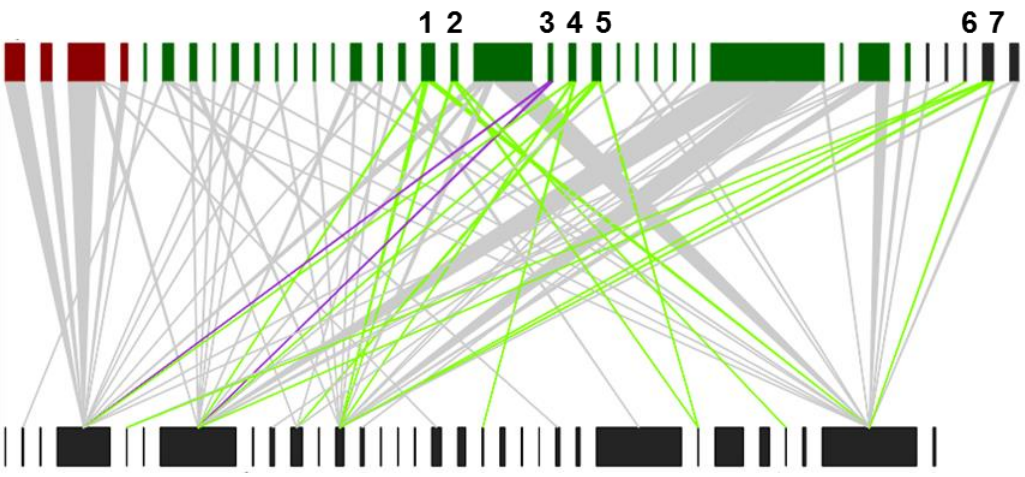


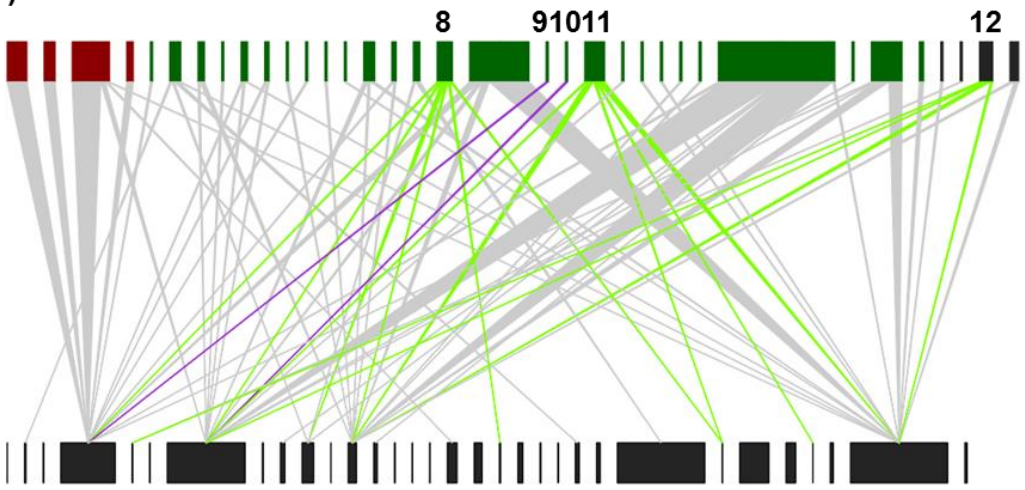
Figure 4.4: Percentage change in the structure of flower-visitor networks, when morphologically-defined visitor taxa are subject to supplementary molecular taxonomic analysis. Changes are differences in network properties for the same dataset before and after molecular taxonomic analysis. Network structure metrics are calculated for time-point specific flower-visitor networks for each of 4 replicate blocks surveyed at 3 time-points (n = 12). Wilcoxon signed rank tests were used to test the H_0 that the median change due to molecular information was zero. Significance levels indicate statistical evidence for differences using a Bonferroni adjusted alpha of 0.05/*k*, where *k* = 8 tests. Therefore: NS = p>(0.05/8); * = p<(0.01/8).

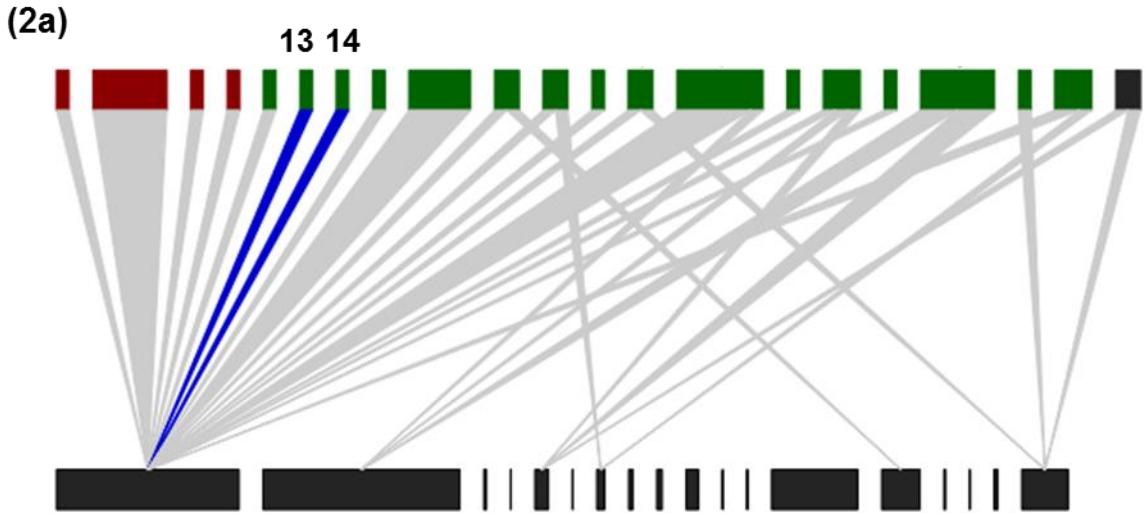
Figure 4.5 (below): Two exemplar pairs of flower-visitor networks with insect visitor taxa defined using either (a) morphology or (b) morphology supplemented with molecular taxonomic analysis. Networks 1 and 2 are exemplars of n=12 block by survey networks used for statistical analyses. Network 1 (Block A Round 2) had the highest absolute and relative change in visitor generality (0.16 or 6.6%), whilst network 2 (Block D Round 3) had the lowest absolute and relative change in visitor generality (0%). Upper bars represent insect flower-visitor taxa, whilst lower bars represent plant taxa. Widths are proportional to abundances. Links widths are proportional to interaction frequencies. Upper bar colours indicate insect orders: Hymenoptera (red), Diptera (green), and Coleoptera (black). Link colours highlight taxonomic changes due to molecular taxonomic analysis (see Table 4.2 for full definitions): no change (grey), lumping (green), combined splitting and lumping with no change in taxon richness (purple), with an a decrease (blue). Numbers indicate: 1 *Sphaerophoria scripta* (♂); 2 *Sphaerophoria* sp. (♀); 3 *Syrphus ribesii*; 4 *Syrphus vitripennis* (♀); 5 *Syrphus vitripennis* / *rectus* (♂); 6 *Oedemera lurida*; 7 *Oedemera nobilis* / *virescens*; 8 Syrphidae morphotype 05; 9 Syrphidae morphotype 06; 10 Syrphidae morphotype 11; 11 Syrphidae morphotype 02; 12 Coleoptera morphotype 04; 13 *Eupeodes latifasciatus*; 14 *Syrphus rectus* (♂); 15 Syrphidae morphotype 16. See A4.6 for fully-labelled exemplar networks.

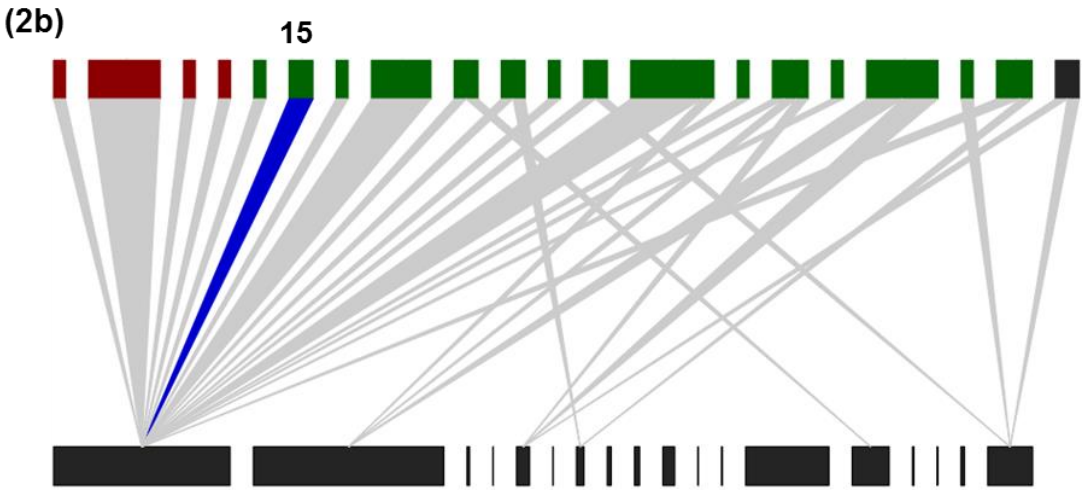
(1a)



(1b)




(2a)
13 14


(2b)
15

4.4 Discussion

Accurate identification of individuals to species is fundamental to the reliable construction of flower-visitor networks. To my knowledge, this study presents the first flower-visitor networks constructed using molecular taxonomic analysis to identify flower-visitors. Although most morphotaxa were not changed, use of molecular information resulted in substantial lumping of visitor taxa, decreasing taxon richness by ~9% from 109 to 99 taxa. These changes increased the average generalisation of visitor taxa, but this lead to only minor changes in the values of other quantitative metrics of network structure. In the following discussion, I consider the limitations of this study, and assess the broader implications of the increasing use of DNA sequence-based taxon identification for studies of flower-visitors networks.

4.4.1 Sequence-based identification of insects by delineation of sequences into MOTUs

MOTUs are clusters of sequences in which pairwise sequence divergence values are below a given threshold (Floyd *et al.* 2002; Blaxter *et al.* 2005; Floyd *et al.* 2009). MOTUs will be congruent with Linnaean species identified using morphological characters if: (i) species are monophyletic; (ii) sequence variation within species is lower than sequence variation between species (as is typically the case; Funk & Omland 2003; Hebert *et al.* 2003b; Hebert *et al.* 2004b); and (iii) a suitable threshold sequence divergence value can be identified (Hebert *et al.* 2003a; Acs *et al.* 2010). Hebert *et al.* (2003a) originally proposed that a pairwise distance of 2% (which enabled them to accurately distinguish 98% of 200 lepidopteran species) could provide a threshold suitable for a wider range of taxa. Although this approach has been criticised (Meyer & Paulay 2005; Wiemers & Fiedler 2007), studies continue to use an arbitrarily selected threshold of 2% (e.g. Alex Smith *et al.*

2013; Fernández-Flores *et al.* 2013). However, a more accurate taxon-specific threshold can often be identified by visualising the 'barcoding gap' from the variation within a sample of sequences (Fig. 4.1; Acs *et al.* 2010; Jones *et al.* 2011; Puillandre *et al.* 2012). The barcode gaps I selected using jMOTU for 5 taxonomic groups (Hymenoptera, Lepidoptera, Syrphid Diptera, Non-Syrphid Diptera and Coleoptera) had a maximum of 1.7% for non-syrphid Diptera (Table A4.4.2). My values are lower than in most previous studies, which have used a range of values including 1% (Hrcek *et al.* 2011), 1.6% (Smith *et al.* 2009), 2% (Smith *et al.* 2011; Strutzenberger *et al.* 2011; Alex Smith *et al.* 2013; Bribiesca-Contreras *et al.* 2013; Fernández-Flores *et al.* 2013; Stahlhut *et al.* 2013), 2.3% (Young *et al.* 2012), and 3% sequence divergence (Weigand *et al.* 2013). These values were either selected arbitrarily, justified by reference to previous studies, or estimated from the set of sampled sequences.

The threshold value estimated from a set of sequences is dependent on properties of the sample, especially the degree of intra- versus inter-specific sequence variation. The low sequence divergence thresholds identified in this study indicate low levels of sequence variation within taxa, whilst large barcoding gaps (e.g. plateaus ranging between 0.5-6.6% in Hymenoptera and 0-7.8% in Lepidoptera) indicate high levels of sequence variation between taxa (Figs. A4.4.1-A4.4.6). This pattern reflects that fact that a large proportion of morphotaxa comprised singleton specimens (39/103 morphotaxa), and hence contained no intra-specific sequence variation. Moreover, inter-specific variation tended to be high since almost half of morphotaxa came from families from which no other morphotaxa were sampled (14/30 families). Low levels of intra-specific variation may be exacerbated by the fact that flower-visitors were sampled from a single

location, where individuals may be closely related. Hence, the comparatively low thresholds likely reflect the fact that the objective of this study was to identify an ecological sample in order to construct MOTU-based flower-visitor networks, rather than to explore taxonomic boundaries within and between Linnaean species, which requires more comprehensive sampling. Thus, low threshold values may be a general feature for studies using MOTU-based analyses to identify ecological samples of diverse insect communities, due to limited sampling intensity across space, time and taxa.

4.4.2 Objective 4.1: The impact of molecular taxonomic analysis on the perceived number and relative abundance of insect taxa

DNA barcoding provides a useful approach for exploring taxonomic diversity in understudied groups, and for screening samples for misidentifications or for known or suspected morphologically cryptic species (Valentini *et al.* 2009; Joly *et al.* 2014; Creer *et al.* 2016). Whether MOTU identifications are concordant with Linnaean species depends on whether the assumptions of single-locus DNA barcoding hold across taxa (as described above). Numerous empirical studies have shown that these assumptions are often correct across a wide range of taxonomic groups (Hajibabaei *et al.* 2006; Smith *et al.* 2006; Dinca *et al.* 2011; Magnacca & Brown 2012; Schmidt *et al.* 2015). However, there are many counter examples in which assumptions are not met, for example in Hymenoptera (Nicholls *et al.* 2012), Diptera (Meier *et al.* 2006; Whitworth *et al.* 2007), and Lepidoptera (Wiemers & Fiedler 2007). In particular, many species are not monophyletic due to hybridisation and incomplete lineage sorting (Gompert *et al.* 2006; Wiemers & Fiedler 2007; Nicholls *et al.* 2012), resulting in paraphyletic and polyphyletic species which may comprise up to a quarter of animal species (Funk & Omland 2003). In such cases, single locus DNA barcoding provides inaccurate species

identifications, and independent corroborating evidence from nuclear markers is required to confirm species limits (Monaghan *et al.* 2005; Yang & Rannala 2010; David *et al.* 2012).

In this study, most MOTUs were congruent with morphotaxa. However, a large proportion of morphotaxa (29%) were changed by DNA barcoding through the loss or gain of individuals due to splitting or lumping. The most common type of change was full lumping of multiple morphotaxa into a single morphotaxon (Table 4.2), with a concomitant reduction in taxon richness (Table 4.3), although more complex combinations of splitting and lumping were common (Fig. 4.3). In some cases these changes improved the accuracy of taxonomic identifications, although in many cases this cannot be confirmed without further taxonomic or genetic investigation. Nonetheless, my approach allows examination of whether there are systematic biases between networks defined using morphology versus those incorporating molecular taxonomic information.

Important changes included the uniting of males and females identified to separate morphotaxa due to limitations in current keys into a single morphotaxon, pruning of misidentifications, and detection and reallocation of morphologically cryptic species. Uniting of males and females of the same species into a single morphotaxon was a common change in Diptera, since keys often do not fully resolve species for one or both sexes. For example, there are no known characters with which female *Sphaerophoria* can be resolved into species, yet 11 species have been described in Britain based on male genital characters (Stubbs & Falk 2002; Ball & Morris 2013). In this study, female *Sphaerophoria* were lumped with male *S. scripta* and *S. interrupta* (which can be distinguished by substantial differences in genital

morphology; Ball & Morris 2013). Hence, although the taxonomic accuracy was improved by uniting males and females of each species, the lumping of *S. scripta* and *S. interrupta* suggests a concurrent reduction in the accuracy of identifications. Clustering of sequences for these morphotaxa implies that either they have been erroneously described as separate species – due misdiagnosis of intraspecific genital variation (e.g. Jocqué 2002; Mutanen & Kaitala 2006; Packer *et al.* 2009) – or that they are independently evolving lineages that share mitochondria (due to introgression or incomplete lineage sorting; Funk & Omland 2003). Further analysis using nuclear genetic markers is required to test these hypotheses. Similar changes were observed for other dipterans, including male and female *Syrphus vitripennis* and *Syrphus rectus* (Syrphidae), and male and female morphotypes in family Anthomyiidae, referred to as *Botanophila* group D (males), *Delia* group C (males) and female anthomyiid morphotypes '5' and '6' (Fig. 4.3; Table A4.4.5).

For female *Syphus rectus*, taxonomic accuracy was improved by the pruning of a misidentification, since one of the two *S. rectus* females clustered into a MOTU with individuals in *Eupeodes latifasciatus* (Fig. 4.3). The remaining male and female *S. rectus* and *S. vitripennis* clustered into a single MOTU, implying that taxonomic accuracy improved by the uniting of male and female *S. vitripennis*. However, given that the taxonomic status of *S. rectus* in Europe is uncertain, with some authors suggesting it may be a "yellow-legged" form of *S. vitripennis* (Stubbs & Falk 2002), further taxonomic and genetic work is required to fully assess the merits of these changes.

For Diptera in family Anthomyiidae, changes partially represent an improvement in taxonomic accuracy, since a lack of keys to genus level for females precludes identification to the same morphotaxon as males. Hence, using molecular data, male and female morphotypes can be united into a single morphotaxon. However, further investigation is required to determine whether the clustering of anthomyiid individuals apparently from different genera (e.g. *Botanophila* and *Delia*) indicates misidentifications, limitations to keys, or complexity in their evolutionary histories, and hence sharing of haplotypes.

DNA barcoding also improved taxonomic accuracy through the detection of a morphologically cryptic species. For example, 12/15 *Bombus lucorum* were reallocated into a MOTU with all *Bombus terrestris* individuals. Studies often combine records for *Bombus terrestris* and *Bombus lucorum* (e.g. Dicks *et al.* 2002; Forup *et al.* 2008) because a lack of reliable morphological characters for distinguishing workers can lead to high rates of misidentification. Moreover, recent studies have shown that *B. lucorum* is one of 3 cryptic species, which cannot be distinguished on the basis of morphology (Murray *et al.* 2008; Carolan *et al.* 2012). Thus, DNA barcoding provides a valuable tool for targeted detection of a known cryptic species.

Overall, DNA barcoding resulted in numerous changes to insect identifications, which mainly affected Diptera. Although flies were the most diverse and abundant group in this dataset, this likely reflects the fact that flies are more generally diverse, difficult to identify and often lack well-developed keys. Although DNA barcoding often improved taxonomic accuracy, especially by uniting males and females, for many taxa, the merits of changes were inherently ambiguous. In many cases, corroborating

evidence from nuclear genetic markers or integrative taxonomic approaches will be required to resolve this ambiguity (e.g. Nicholls *et al.* 2012). Nonetheless, DNA barcoding provides a powerful tool to identify specimens to MOTUs when taxonomic limitation precludes species-level identifications (Packer *et al.* 2009). MOTUs can be used as surrogates for species in ecological studies requiring highly resolved taxonomic identifications (e.g. for estimating taxon richness or constructing interaction networks; Clare *et al.* 2013; Blaxter 2016). Given that researchers often use morphospecies/ morphotypes for these purposes, this represents a continuation of a long standing approach.

4.4.3 Objective 4.2: The impact of molecular information on flower-visitor network structure

Use of molecular information to identify flower-visitors resulted in consistent minor changes across replicate networks. Lumping of morphotaxa decreased taxon richness, reducing the number of unique links and interaction diversity (the effective number of links). Lumping also increased flower-visitor generality, reducing plant vulnerability and increasing overall network connectance. However, taxonomic changes had no effect on interaction evenness or network specialisation.

These changes were consistent with predicted changes for an idealised scenario in which use of molecular information results in the lumping of morphotaxa that visit a similar spectrum of floral partners, resulting in no overall change to the generalisation of individual visitor taxa (Table 4.4 – scenario III). The observed increase in generality was the only change to a metric that was not predicted under this simplified scenario. This largely reflects the simplicity of the scenario and its assumptions. The observed increase in generality suggests that lumped taxa did not fully overlap in their

floral partner spectrum. Given that lumped taxa tend to be closely related and hence visit similar floral partners, this may reflect an effect of low sampling intensity, with individuals in lumped morphotaxa randomly recorded on different floral species, rather than true differences in floral preferences.

Changes to network metrics appeared relatively small in absolute terms, but in some instances they were of a similar magnitude to reported changes in ecological studies of flower-visitor networks (Table 4.5). For example, changes to taxon definitions increased flower-visitor generality by 0.06 (2.8%), equivalent to almost half of the increase in generality of 0.12 (5.5%) in a restored vs. an unrestored heathland in Mauritius (Table 4.5; Kaiser-Bunbury *et al.* 2009). However, this was much less than the increase of 0.46 (28.6%) recorded for grasslands of organic vs. conventional dairy farms in Ireland (Power & Stout 2011). Similarly, changes to taxon definitions decreased plant vulnerability by -0.29 (-4.9%), which was a change of greater magnitude than the increase of 0.11 (3.8%) observed for organic vs. conventional farms, but was much less than the increase of 0.68 (15.3%) recorded for a restored heathland (Table 4.5). Thus, alternative techniques for taxon identification can result in estimates of network structure that differ by an amount comparable to real-world changes in flower-visitor communities due to habitat management. However, the change may be in the opposite direction. This reflects the fact that use of molecular data predominantly lumped visitor morphotaxa, whilst floral species identities remained constant, resulting in decreased visitor richness, unique links and plant vulnerability. In contrast, in ecological studies comparing habitats with contrasting management regimes, the richness and abundance of both flowers and visitors may increase (Kaiser-Bunbury *et al.* 2009) or floral

composition may change, resulting in concomitant shifts in visitor composition and hence network structure (Power & Stout 2011). Although changes to visitor taxonomy likely improved taxonomic accuracy and altered flower-visitor network structure, as yet there is no evidence that use of molecular information would change perceptions of ecological functions or alternative habitat management regimes, compared to traditional morphological taxonomy. We might expect impacts of DNA barcoding to be more pronounced in communities where identification to species is inherently more difficult, as in highly diverse and less well-described tropical faunas (e.g. Hebert *et al.* 2004a; Smith *et al.* 2006). However, the presence, direction and magnitude of change at a network level will depend on the frequency of different types of changes across the network.

More broadly, DNA barcoding has been used in other types of network, primarily insect host-parasitoid systems. In most, the predominant signal has been of increasing taxon richness by splitting morphologically cryptic taxa. For example, Kaartinen *et al.* (2010) analysed a host-parasitoid/inquiline network from pedunculate oak (*Quercus robur*) and found most changes resulting from DNA barcoding to involve splitting of morphotaxa, decreasing generality, vulnerability, and connectance (Table 4.5). Molecular taxonomic analyses also led to substantial reassortment of individuals among morphotaxa in the inquiline genus *Synergus* (Hymenoptera: Cynipidae) – a group known to be very difficult to identify morphologically, and to contain cryptic taxa (Acs *et al.* 2010). Similarly, Smith *et al.* (2011) found that DNA barcode analysis of a host-parasitoid network centred on pine feeding sawflies increased parasitoid taxon richness by ~41% by splitting of generalist morphotaxa into more specialist MOTUs, yielding a decrease in network connectance consistent with predictions presented here

for the splitting of a morphotaxon into multiple more specialist MOTUs (Table 4.4).

My study focussed on flower-visitor networks constructed for a geographically localised assemblage of visitors, from a comparatively well-described and species-poor fauna, which were subjected to substantial taxonomic effort to obtain morphotaxon identifications. Comparison with work on host-parasitoid networks, many of which are highly species rich, suggests that molecular taxonomic analysis of more diverse and less well-studied flower-visitor communities are more likely to yield a dominant signature of increased species richness, due to predominant splitting of morphotaxa. The impacts of such changes on the structure of a flower-visitor network will depend on the type of change (e.g. splitting vs. lumping), whether the affected taxa differ in the spectrum of floral partners that they visit (i.e. the relative level of specialisation/generalisation of MOTUs vs. morphotaxa), and the relative abundances of the affected taxa.

Table 4.4: A simplified version of Table 4.1 comparing predicted changes with observed changes in metrics of flower-visitor network structure when visitor taxa are resolved using molecular rather than morphological characters. Predictions are based on four simple possible impacts of molecular information, which consist of either the splitting of a morphotaxon, or lumping of several morphotaxa, resulting in either no change or an increase in the specialisation, or generalisation, of visitor taxa. Brackets '( )' indicate a change that is possible but less probable than the alternative under the same scenario.

	Predicted impact of molecular taxonomic analysis on metric values assuming:				**Observed impact of molecular taxonomic analysis on metric values**
	A morphotaxon is split		**Morphotaxa are lumped**		
Network property	**(i) No change in specialisation / generalisation of visitor taxa**	**(ii) A generalist taxon is split into more specialist taxa**	**(iii) No change in generalisation / specialisation of visitor taxa**	**(iv) Specialist taxa are lumped into a more generalist taxon**	
Insect visitor taxon richness (N)	+	+	−	−	−
Number of unique interactions	+	o	−	o	−
Generality (G_{qw})	o	−	o	+	+
Vulnerability (V_{qw})	+	o	−	o	−
Connectance (C_{qw})	o	o (−)	o	(+) o	o
Interaction diversity (e^{H_2})	+	o	−	o	−
Interaction evenness (IE_S)	o −	o	+ o	o	o
Network specialisation (H_2')	o	o	o	o	o

Table 4.5: Observed changes in network parameters/metrics due to molecular taxonomic analysis compared with previous studies of changes in ecological communities. Kaiser-Bunbury *et al.* (2009) took replicated samples from a restored vs. control Mauritanian heathland, whilst Power and Stout (2011) compared grasslands on 10 paired organic vs. conventional dairy farms. Data show mean estimates and changes for each parameter/metric. Morpho. vs. MOTU = morphological vs. morpho-molecular networks respectively. Control vs. Restored = unrestored vs. restored heathlands. Convent. Vs. Organic = conventional vs. organics dairy farms.

	This study				**Kaiser-Bunbury *et al.* 2009**				**Power and Stout (2011)**			
	Morpho.	MOTU	Change	%	Control	Restored	Change	%	Convent.	Organic	Change	%
Visitor richness	28.4	26.1	-2.3	-8.2	100	135	35	35.0	7.4	7.8	0.4	5.4
Unique interactions	45.6	43.2	-2.4	-5.4	534	744	210	39.3	10.3	14.3	4.0	38.8
Generality	1.94	2.00	0.06	2.8	2.20	2.32	0.12	5.5	1.61	2.07	0.46	28.6
Vulnerability	6.35	6.06	-0.29	-4.9	4.44	5.12	0.68	15.3	2.92	3.03	0.11	3.8
Connectance	0.114	0.117	0.004	3.5	0.082	0.075	-0.007	-8.5	0.19	0.19	0.00	0.0
Interaction diversity	24.5	23.4	-1.1	-4.9	-	-	-	-	-	-	-	-
Interaction evenness	0.57	0.57	0.00	0.0	0.70	0.70	0.00	0.0	-	-	-	-
Specialisation	0.54	0.53	-0.01	-1.1	-	-	-	-	-	-	-	-

Table 4.5: Observed changes in network parameters/metrics due to molecular taxonomic analysis compared with previous studies. Kaartinen *et al.* (2010) analysed networks from pedunculated oaks of leaf-mining and gall-inducing hosts and their parasitoids/inquilines, examining how DNA sequence-based identification of parasitoids and inquilines affected network metrics. Alex Smith *et al.* (2011) analysed the host-parasitoid network of the spruce budworm (*Choristoneura fumiferana*), comparing networks with morpho vs molecular parasitoid identifications. Data show mean estimates and changes for each parameter/metric. Morpho vs. MOTU = morphological vs. morpho-molecular networks respectively.

	This study				**Kaartinen *et al.* 2010**				**Alex Smith *et al.* 2011**			
	Morpho	MOTU	Change	%	Morpho	MOTU	Change	%	Morpho	MOTU	Change	%
Visitor richness	28.4	26.1	-2.3	-8.2	-	-	-	-	110	156	46.0	41.8
Unique interactions	45.6	43.2	-2.4	-5.4	-	-	-	-	336	449	113	33.6
Generality	1.94	2.00	0.06	2.8	2.32	2.16	-0.16	-6.9	-	-	-	-
Vulnerability	6.35	6.06	-0.29	-4.9	4.33	4.23	-0.10	-2.3	-	-	-	-
Connectance	0.114	0.117	0.004	3.5	0.13	0.11	-0.02	-15.4	0.03	0.02	-0.01	-33.3
Linkage density	-	-	-	-	3.32	3.19	-0.13	-3.9	-	-	-	-
Interaction diversity	24.5	23.4	-1.1	-4.9	-	-	-	-	-	-	-	-
Interaction evenness	0.57	0.57	0.00	0.0	0.5	0.49	-0.01	-2.0	-	-	-	-
Specialisation	0.54	0.53	-0.01	-1.1	-	-	-	-	-	-	-	-

4.5 Conclusions

Use of molecular information for specimen identification allows more accurate construction of flower-visitor networks, although it may introduce inaccuracies in groups for which the assumptions of single-locus barcoding are incorrect. For this well-studied fauna, MOTU-based flower-visitor networks were systematically biased toward fewer taxa and links, with more generalist visitors and specialist plants. However, overall, metrics for MOTU-based networks differed little from those of morphological networks (especially if improvements to taxonomic accuracy are discounted). Further studies are required to confirm this pattern, and explore impacts in less-well described communities, before more reliable generalisations can be made. Nevertheless, as molecular taxonomic methods becomes cheaper and more widely used they may provide a tractable alternative to morphological identification in capturing the main structural features of flower-visitor networks, especially for diverse and understudied areas.

Chapter 5: Floral resources and flower-visitation by insects in urban flower meadows.

5.1 Introduction

Flower-visiting insect communities are under pressure from multiple threats (Potts *et al.* 2010; Vanbergen *et al.* 2013; Goulson *et al.* 2015; Potts *et al.* 2016). Prime among these is the loss and degradation of flower-rich habitats (Carvell *et al.* 2006; Gerard *et al.* 2010; Senapathi *et al.* 2015), resulting in reduced availability of nesting, over-wintering and foraging resources (Goulson *et al.* 2015; Baude *et al.* 2016). This has led to widespread concerns that declines in flower-visitors may result in reduced pollination of wild plants and agricultural crops (Allen-Wardell *et al.* 1998; Klein *et al.* 2007; Potts *et al.* 2010). An important approach to halting and reversing flower-visiting insect declines is to increase the availability of foraging resources by creating, improving and linking flower-rich habitats (Dicks *et al.* 2010; Dicks *et al.* 2015; Gill *et al.* 2016).

Sowing of flower seed mixes is an effective and practical approach to creating diverse, flower-rich habitats on a large scale. In agricultural areas, flower seed mixes have been extensively tested as a means of increasing flower-rich habitats around field margins (e.g. Carvell *et al.* 2007; Haaland *et al.* 2011; Pywell *et al.* 2011; Blaauw & Isaacs 2014), which can increase inter-annual survival of bumblebee lineages in these landscapes (Carvell *et al.* 2017). This suggests that large-scale, flower-rich plantings may be effective in enhancing species persistence and population sizes more generally.

In urban areas, flower seed mixes are increasingly used as a cost-effective method of improving the aesthetic amenity value of urban parks and green spaces (Hitchmough & Dunnett 2004; Scott 2008; Bretzel *et al.*

2016). The resulting 'meadows' provide numerous colourful flowers, yet have lower ongoing labour and maintenance costs compared to short-mown amenity grasslands or traditional horticultural flowerbeds (Hitchmough & Dunnett 2004; Heatherington & Sargeant 2005). Given that funding for park maintenance is in decline (Dunnett *et al.* 2002; Barber 2007; Lambert 2014), flower seed mixes can help parks managers to meet their responsibilities to manage for both human amenity and biodiversity. However, a challenge for parks managers is making evidence-based decisions on which flower seed mixes best deliver benefits for both people and pollinators. For pollinators, this represents two challenges: (1) identifying which species, flower shapes, or combinations of species/flower shapes, provide the floral rewards required by flower-visiting insects; and (2) identifying whether patterns of insect visitation are determined by these resources.

Given human aesthetic preferences and social constraints (resulting from public complaints), parks managers often plant annual flower seed mixes designed primarily for human visual amenity. These urban or 'pictorial' meadows are designed using careful plant selection to provide a reliable display of large colourful flowers, flowering at high densities and over an extended flowering season (Dunnett & Hitchmough 2004; Dunnett 2008; Hitchmough 2017), rather than to explicitly support flower-visiting insects. Within these general specifications, pictorial seed mixes can vary substantially in species richness and composition, with mixes often designed around additional architectural traits such as height or colour schemes (Heatherington & Sargeant 2005; Hitchmough 2017). Hence, the choice of which flower seed mix is used for an urban planting (and which species it contains) will affect the amount of floral resources provided for flower-visiting insects.

Flower-visiting insect communities comprise a diversity of species with varying resource requirements (Proctor *et al.* 1996; Willmer 2011). Most flower-visiting insects obtain nutritional resources from flowers in the form of nectar and/or pollen (Proctor *et al.* 1996; Willmer 2011). Nectar is comprised mainly of water and sugars (Nicolson & Thornburg 2007), and is a primary source of energy for many groups, including bees and hoverflies (Nicolson 2011; Rotheray & Gilbert 2011). Pollen contains protein, carbohydrates, lipids, vitamins, and minerals (Roulston & Cane 2000), and is the primary source of protein from which bees and hoverflies provision their larvae and eggs, respectively (Nicolson 2011; Rotheray & Gilbert 2011). The characteristics of these resources (Potts *et al.* 2003; Potts *et al.* 2004; Ackermann & Weigend 2006), as well as their accessibility (Harder 1985; Branquart & Hemptinne 2000), are key determinants of whether flower-visitors can efficiently meet their nutritional needs (Ranta & Lundberg 1980b; Harder 1986; Kim *et al.* 2011; Balfour *et al.* 2013). For example, some flowers (such as poppies, *Papaver* spp.) produce only pollen, while others (such as dandelions, *Taraxacum* agg.) provide both pollen and nectar (Hicks *et al.* 2016). For species providing nectar rewards, floral corolla length determines the physical accessibility of nectar to flower-visitors with different tongue-lengths, with short-tongued visitors typically excluded from rewards in flowers with long corollas (Ranta & Lundberg 1980b; Gilbert 1981; Harder 1985; Branquart & Hemptinne 2000). Moreover, floral corolla length affects nectar sugar concentration and viscosity, with plants with deep corollas tending to produce more dilute nectars (Plowright 1987; Ackermann & Weigend 2006), which long-tongued pollinators require for efficient uptake (Harder 1986; Kim *et al.* 2011). Hence, a diversity of flower shapes and floral resource traits are required to support diverse communities of flower-visiting

insects (Potts *et al.* 2004; Bluthgen & Klein 2011; Venjakob *et al.* 2016). This diversity should also be available throughout the growing season to support visitors requiring resources at specific times of year or over an extended period, such as most solitary (Oertli *et al.* 2005) or eusocial bees, respectively (Westphal *et al.* 2009; Rundlof *et al.* 2014; Schellhorn *et al.* 2015).

Many of the features that enhance human visual amenity in pictorial meadows – such as the production of a high density of flowers over an extended flowering season – are also desirable in terms of providing resources for flower-visiting insects. However, few studies have examined the floral resources provided by annual pictorial meadows (e.g. Blackmore & Goulson 2014; Hicks *et al.* 2016), and none of these has examined the effects of seed mix choice or species composition on floral resource provision and insect visitation. Pictorial meadows seed mixes are often comprised of annual Asteraceae and Papaveraceae, which (per flower or flower head) typically provide low amounts of viscous or even crystalline nectar (Hicks *et al.* 2016). Hence, although they may provide pollen and nectar sources suitable for some flower visitors (such as hoverflies and short-tongued solitary bees), they likely provide little or no nectar for larger, long-tongued insects, such as bumblebees.

5.1.1 Objectives

In this study I examine the impacts of seed mix type and species composition on the floral resources and flower-visiting insect assemblages of different planted meadows. Using a field experiment, I compared floral resources and flower-visiting insect assemblages between meadows grown from 3 commercially-available annual flower seed mixes that differed in species and flower-shape richness (see Methods). These were the Short

Annual, Marmalade Annual and Cornfield Annual mixes sold by Pictorial Meadows Ltd. (see Methods). Mixes were chosen as contrasting exemplars from an array of commercial seed mixes, and were selected to contain annual species often used in pictorial mixes, including California poppy (*Escholozia californica*), Corn poppy (*Papaver rhoeas*) and Cornflower (*Centaurea cyanus*).

To examine whether floral resource provision could be improved whilst maintaining aesthetic designs, I also devised a nectar enrichment treatment for each seed mix intended to increase nectar sugar provision (see Methods, and Chapter 3).

I examined the floral resources and flower-visiting insect assemblages of planted meadows with three overall objectives.

Objective 5.1. ***To quantify and compare rewards per floral unit for each flowering species and the floral rewards provided per unit area by each meadow treatment.***

I identify high and low-rewarding species for pollen and nectar, asking whether the species selected to enrich seed mixes do in fact provide high nectar rewards. I then quantify reward contributions per unit area of meadow, for both intended (seed mix) and unintended (contaminant and weed) species in different seed mixes (see Chapter 3). To determine whether resource provisioning by commercially available seed mixes can be enhanced by seed mix manipulation, I also test whether meadows of enriched mixes produce more floral nectar sugar than standard formulations.

Objective 5.2. To examine patterns and floral resource correlates of insect visitation to planted meadows.

This objective can be broken down into three related questions:

(a) Did total seasonal richness or abundance of visitors differ between meadows of different mix types?

(b) How do patterns of visitation vary among intended (treatment) and unintended (contaminant and weed) floral species?

(c) Which floral resource measure best predicts bumblebee and hoverfly abundance in meadows?

Given low levels of replication for each seed mix, I compared seasonal richness and abundance of flower-visitors between planted meadows, and examine patterns of visitation among intended and unintended floral species. Since both of these categories are inevitably present in all planted meadows, understanding the contribution of each to visitation is important in assessing the biodiversity value of meadows as a whole. To assess whether patterns of visitation are predicted by floral resources, I examine correlations between visitor abundance and floral resources for bumblebees and hoverflies. Both of these groups are widely recognised as important, but somewhat ecologically different, components of pollinator communities. Both require pollen for maturation of eggs (female hoverflies; Rotheray & Gilbert 2011) or provisioning of larvae (bees; Roulston & Cane 2002; Müller *et al.* 2006; Brodschneider & Crailsheim 2010), and both require nectar for flight fuel (Nicolson 2011; van Rijn & Wäckers 2016), although bumblebees also incorporate nectar into larval food (Burkle & Irwin 2009). However, bumblebees have higher energy requirements due to their large body size and central-place foraging life history (Proctor *et al.* 1996; Willmer 2011). Hence, I predicted that bumblebee visitation would be correlated with nectar

rather than pollen resources, whilst female hoverfly visitation would be correlated with pollen rather than nectar resources.

Objective 5.3. ***To identify which plant species are most visited by bees and hoverflies.***

Bees and hoverflies are important pollinators that can be common and diverse in urban areas (Baldock *et al.* 2015; Hall *et al.* 2017), and which have overlapping nectar and pollen resource requirements with most other common flower-visiting insects. An improved understanding of which floral species are attractive to these groups can help to inform both improved seed mix choice and design. I first examine which floral species are most visited by different groups of bees and genera of hoverflies, and then examine whether any floral species are visited more than expected based on their floral abundance.

5.2 Materials and methods

5.2.1 Field experiment

Data were collected from a field experiment corresponding to the experimental design and seed mix treatments described for year 2013 in Chapter 2 General Methods. In brief, 6 flower seed mixes were sown in each of 4 replicate blocks (A, B, D, and E), with each seed mix randomly allocated to one of six 25 m^2 plots per block. Seed mixes were selected to comprise 2 formulations of each of 3 seed mix types: a Cornfield annual mix (comprised of 4 species) and two contrasting exemplars of pictorial seed mixes with either low or high species and flower-shape richness. These were the Marmalade mix (comprised of 6 species) and the Short mix (comprised of 13 species).

To explore whether floral nectar resource provision can be improved whilst maintaining aesthetic designs, sown seed mixes included 'standard' and 'nectar enriched' formulations of each seed mix type. Commercially available seed mixes provided 'standard' seed mix formulations, whilst 'enriched' formulations were created by Pictorial Meadows Ltd. using standard aesthetic designs as a baseline from which to increase the % seed weight of specific species that I predicted would provide comparatively large quantities of nectar sugar per flower or inflorescence based on available literature (Crawford 2000; Hooper & Taylor 2006; IBRA 2008; RHS 2011; The Xerces Society 2011; Kirk & Howes 2012). In the Short and Marmalade mixes these species were: *Centaurea cyanus*, *Convolvulus tricolor*, *Iberis umbellata* (Short mix) and *Coreopsis tinctoria* and *Rudbeckia hirta* (Marmalade mix). The Cornfield mix had only four species; therefore (to increase nectar sugar and to match the richness of the Marmalade mix) I added annual *Echium vulgare* and *Glebionis segetum*. The aim was to increase nectar sugar provision (and hence visitor abundance) per unit area of meadow, whilst maintaining the aesthetic character of the meadows. To ensure efficient use of seed, these changes to mix compositions required reducing the % seed weight of other species within mixes (see Chapter 2).

Blocks were separated by 80 metres or more and were prepared and sown in pairs on 29 April and 3 May 2013.

5.2.2 Meadow floral composition and flower-visiting insect surveys

Meadows were surveyed for their floral composition and flower-visitor assemblages at three time-points: in late July (30 July-3 August), late August (27 August-2 Sept.), and late September (20-27 Sept.; hereafter, survey rounds 1, 2 and 3, respectively).

Flower-visitors were surveyed by walking a 5 m-long by 2 m-wide transect through the centre of each 25 m^2 plot, catching by hand net all Hymenoptera, Diptera, Lepidoptera and Coleoptera seen contacting the reproductive parts of a flower. For each observed interaction, the flower species visited was recorded and the insect caught and killed to enable identification to species and to prevent resampling. To increase sample sizes this was repeated twice on the same day, with at least 30 min between the first and second transect walks within a given plot. Sampling effort was standardised by visually scanning each floral unit within meadows for insect visitors. A floral unit was defined as a flower or group of flowers that a medium-sized flower-visitor, such as a honeybee, can walk within, but from which it must fly rather than walk to reach an equivalent floral unit (Dicks *et al.* 2002). This general definition, as well as the specific botanical definitions used for each species (see Table 2.1), matched those used in other similar studies of flower-visitor interactions in Britain (Baude *et al.* 2016; Hicks *et al.* 2016). All flower-visitor surveys were conducted between 10.00 and 18:00 hrs during warm, dry weather, with temperature in the shade greater than 15 ºC and wind speed lower than a moderate breeze (4 on the Beaufort scale). Flower visitors are likely to be active under these conditions, but because variation temperature and wind speed can influence flower visitor activity

both were recorded for each survey and were incorporated into analyses of pollinator abundance (see section 5.2.6 Data Analysis, Objective 5.2c, below).

Surveys of meadow floral composition were performed within 48 hrs of the corresponding flower-visitor survey for a given plot and survey round. Floral composition was quantified by counting floral units of all flowering species in five 1 m^2 quadrats per replicate meadow. Quadrats were located contiguously on alternating sides of the flower-visitor sampling transect, with random right/left placement of the first quadrat and alternating left/right placement of subsequent quadrats.

Each floral species within each field plot was classified into one of three categories: 'treatment', 'contaminants' or 'weeds' (hereafter: 'floral categories'). Weeds consisted of naturally-occurring ruderal or arable species. Treatment and contaminant floral categories contained species present in at least one sown seed mix. However, for a given meadow, whether a species was classified as treatment or contaminant depended on whether it was intended (treatment) or unintended (contaminant) with respect to the initial seed mix. This enabled examination of the impact of different floral categories on floral resources and insect visitation. Given that there was no practical way to distinguish in the field between wild *Tripleurospermum inodorum* (a weed; Asteraceae) and the sown cultivar present in Cornfield seed mixes, all *T. inodorum* were classified as either intended treatment plants (in meadows of Cornfield mixes) or unintended contaminants (in meadows of Marmalade and Short mixes).

5.2.3 Insect identification

Insects were identified using morphology, supplemented for some taxa by DNA barcode information (see Chapter 4). Firstly, all insects (n=1570) were identified to either Linnaean species using taxon-specific keys (92% of specimens), or to morphotype (8% of specimens). Secondly, to screen flower-visitor assemblages for misidentifications and morphologically cryptic species, a subset of specimens were selected for DNA sequence-based identification (DNA barcoding; 56% of n=1570). Resource and time limitations precluded generation of DNA barcode sequences for all specimens, and subsampling was designed to target those taxa in which morphological identification was most challenging, either due to lack of taxonomic resources, or due to known existence of cryptic species that cannot reliably be separated using morphological characters. DNA barcode identifications were then extrapolated from the sequenced subset to the full specimen set, which was straightforward since all conflicts between morphotaxon and MOTU designations occurred in groups for which all individuals were sequenced. Full details of morphology- and molecular-based specimen identifications can be found in Chapter 4 sections 4.2.3 and 4.2.4.

5.2.4 Floral resource quantification

Floral resources were quantified per species in terms of the average daily nectar sugar mass and pollen volume provided per floral unit. This approach provided per-species estimates that are comparable between species with diverse floral morphologies, tractable to collect for a large number of species, and which match recent community level studies of floral resource provision (Müller *et al.* 2006; Baude *et al.* 2016; Hicks *et al.* 2016).

Combined with quantitative data on meadow floral composition, per floral unit resource estimates allow comparison of community level floral resource provision between different meadows. For each species, I either generated my own data for plants flowering in Sheffield, or used data from a previous study using the same methodology to quantify the floral rewards provided by meadows in Edinburgh (Hicks *et al.* 2016; see Table A5.1). My approach has several limitations, including collection of data over three years (2012-2014) and two locations (Edinburgh or Sheffield, UK; see Discussion). Nevertheless, this methodology is considered sufficient to quantify the approximate magnitude of floral rewards provided per floral unit (Baude *et al.* 2016; Hicks *et al.* 2016).

Nectar quantification per-floral unit

For each species, nectar sugar mass per floral unit per day (μg/day) was quantified as (the mean nectar sugar mass provided per flower in 24 hours (μg)) x (the mean number of flowers per floral unit). Nectar samples were collected between 10:00-18:00 hrs on days with no rain from flowers bagged for 24 hours to exclude insect visits. For species providing large quantities of nectar, nectar was sampled directly from flowers using 1.0 μl microcapillaries (VWR International, UK). For species providing either small quantities or highly viscous nectar, 1-5 μl of distilled water was added to nectaries, and left for 1 minute, before the resulting nectar solution was collected using 1.0 μl microcapillaries. For these species nectaries were rinsed twice to maximise the amount of nectar sugar sampled. Given the difficulty of extracting all nectar sugar from a flower, these protocols provide a lower bound estimate for the actual amount of nectar sugar produced by flowers. All species were sampled by rising with distilled water, except *Lamium album*

and *Linaria maroccana*. Floral unit definitions for each species are show in Table 2.2 General Methods.

For each nectar sample, sugar concentration was measured in degrees Brix (g sucrose/100 g solution), using a handheld refractometer modified for low volumes (Bellingham and Stanley Ltd.). The sugar mass in each nectar sample (μg; weight) was quantified using the equation: $s = 10dvC$, in which v is the volume of the sample (μl; volume), and d is the density of a sucrose solution (w/v) at concentration C (g sucrose/100 g solution; w/w; Bolten *et al.* 1979; Prŷs-Jones & Corbet 2011). Sample volume was calculated from the length of the liquid column inside constant bore microcapillaries. The density of sugar was estimated as $d = 0.0037921C + 0.0000178C^2 + 0.9988603$ (Prŷs-Jones & Corbet 2011), using measurements of sugar concentration corrected for variation in room temperature using a standardised table of correction values (Bellingham and Stanley Ltd.).

To estimate mean nectar sugar mass per flower, nectar samples were collected from 10-20 flowers from at least 5 plants. The mean number of open flowers per floral unit was estimated by counting the number of mature but un-wilted male and female flowers in a sample of 10-20 floral units per species. The product of these two values provided a point estimate of mean resources per floral unit. Where possible, I collected two independent spatially replicated estimates per species of nectar sugar provision per floral unit, which were averaged to provide a single point estimate of resource provision per floral unit. Estimated nectar rewards per floral unit/species are shown in Appendix 5 Table A5.1.

Pollen quantification per-floral unit

Pollen samples were collected from flowers allowed to open in the lab from mature buds gathered in the field. For each species, pollen volume per floral unit per day (μl/day) was quantified as the 'total volume of pollen produced per floral unit (μl)' divided by 'total floral unit longevity in days'. Pollen volume per floral unit was estimated as (the mean number of pollen grains per anther or floret) x (the mean volume of a pollen grain (μl)) x (the mean number of anthers or florets per floral unit).

The mean number of pollen grains per anther or floret was estimated for a known number of anthers or (for Asteraceae) disc florets collected into 1.5 ml tubes containing 70% ethanol. The number of anthers or florets collected per tube varied between species (from 10 to 150 anthers or 5 to 70 florets) to ensure sufficient pollen was available for quantification. For each species and replicate (see below), I estimated pollen grain numbers per anther and pollen grain volume for between 1-4 independent tubes, each containing anthers or florets from at least 5 different plants.

Pollen was extracted from anthers and florets through a process of vortexing, sonicating (using a Dawe sonicleaner), and filtering (using a handheld pipette), which was repeated three times, before samples were dried and re-suspended in a known volume of 70% ethanol. The number of pollen grains per sampling tube was estimated as the product of (resuspension volume (μl)) and (pollen grain concentration/μl). Pollen grain concentration was estimated for each of three 10 μl subsamples on a haemocytometer slide using counts of five 0.1 μl grid squares. For each species, the number of pollen grains per anther or floret was then calculated as the number of pollen grains per sampling tube divided by the number of

anthers or florets initially collected. Concurrently, pollen grain volume was estimated for at least 40 pollen grains per replicate per species using the formula for a 3D ellipsoid: Volume = $(4/3)*\pi*(A/2)*(B/2)^2$, in which A is the major axis and B the minor axis of a pollen grain.

Variation in pollen availability among individuals in a species was incorporated by repeating (where possible) the procedure above for two spatial replicates per species. However, since scaling up to a meadow level required a single estimate of daily pollen volume per floral unit per species, replicate values for each floral trait were averaged and then used to provide a single point estimate of resource provision per floral unit. For each species, estimated pollen rewards per floral unit are shown in Appendix 5 Table A5.1.

The number of anthers or florets per floral unit of each species was estimated from counts performed in the field from 10-20 floral units per species, depending on availability.

Floral unit longevity was estimated using a method based on monitoring the numbers of newly-opened and newly-closed floral units in a fixed area, or from a randomly selected and marked sample (Hicks *et al.* 2016). Assuming that the population of floral units for a given species is roughly stable, the floral longevity in days can be estimated as $(2a + (b-c))/((b+c)/d)$, where 'a' is the total number of open floral units on the first observation, 'b' is the total number of newly-opened floral units on the second observation, 'c' is the total number of newly-closed floral units on the second observation, and 'd' is the number of days between first and second observations (Hicks *et al.* 2016). To meet the assumption that the floral population was roughly stable, species were sampled as close as possible to

their peak flowering period, while their floral units were highly abundant. Sampling was performed during periods of warm dry weather, and where possible on at least 50 floral units from 5 or more plants. Marked areas or floral units were surveyed 24 hours after the first observation. For species with long-lasting flowers (such as Asteraceae), marked areas or floral units were monitored every 24 hours for multiple days until a subset of floral units had newly-closed and others had opened.

Floral resource quantification per unit area of meadow

For each surveyed meadow, daily floral resources per unit area of meadow (measured as nectar sugar mass or pollen volume) were quantified as the sum across plant species of the product for each plant species of: (daily floral rewards per floral unit) x (the number of floral units per species in 5 m^2 of meadow).

5.2.5 Data analysis

(i) Objective 5.1: *Quantification and comparison of floral rewards per-floral unit and per-unit area of meadow.*

To compare floral reward provision between species recorded in meadows, species were ranked by their daily estimated nectar (μg/day) or pollen (μl/day) production.

To test for differences in total nectar sugar mass or pollen volume provided by meadows of each seed mix treatment, I used Gamma GLMMs with a log-link function, implemented in R package 'lme4' (Bates *et al.* 2015). Fixed effects consisted of mix type, formulation and round, with an interaction between mix type and round. Block was fitted as a random effect. Models were checked for heteroskedasticity. Log-likelihood ratio tests were used to test main effects and interactions (Table A5.4). For interactions

between mix type and round, conditional pairwise contrasts were used to test for differences in estimated marginal means between each level of each factor, within each level of the corresponding interacting factor. *P*-values were adjusted to account for multiple tests using the 'mvt' method in R package 'lsmeans' (Lenth 2016). This method adjusts the critical value used to calculate confidence intervals and *p*-values, using a multivariate *t* distribution for *k* pairwise contrasts (Lenth 2016).

(ii) Objective 5.2: *Examination of patterns and floral resource correlates of insect visitation to planted meadows.*

(a) *Did total seasonal richness or abundance of visitors differ between meadows of different mix types?*

(b) *Were visitors mainly on intended (treatment) or unintended (contaminant and weed) floral species?*

To investigate patterns and drivers of insect visitation, I examined the total seasonal richness and abundance of three categories of visitors (all insects, bumblebees and hoverflies) to each meadow treatment at three floral scales: (i) the full meadow community; (ii) treatment, contaminant and weed floral categories within each mix type; and (iii) individual floral species within each mix type.

To investigate patterns of visitation at a meadow community scale, I quantified total seasonal abundance, richness and diversity of visitors to each meadow treatment for the three insect categories. Given that estimates of species richness are affected by sample sizes, I also generated sample size corrected estimates using the Chao1 estimator, a non-parametric estimator of asymptotic species richness for abundance data (Chao 1984). Moreover, given that species richness does not account for variation in species abundances, I also calculated two diversity indices: exponential Shannon's

entropy ('Shannon's diversity'), and inverse Simpson's concentration ('Simpson's diversity'), which incorporate variation in species abundances and describe the 'effective number of species' observed in meadows of each mix type over the season (Chao & Jost 2012). Shannon's diversity weights species in proportion to their relative abundance, providing a measure of the number of 'common' species in the community (Chao & Jost 2012). In contrast, Simpson's diversity penalises the contribution of rare species, providing a measure of the number of 'highly abundant' species in the community (Chao *et al.* 2014). Estimates of Chao1 species richness, Shannon's diversity and Simpson's diversity were calculated using EstimateS v.9.1 (Colwell 2013). Given the low replication inherent to this experiment, models testing for effects of mix type, formulation and round on visitor abundance and richness failed to converge. Hence, I rank and compare mean estimates of visitor abundance, richness and diversity between mix types.

To assess the impact of each floral category (treatment species, contaminants and weeds) on patterns of visitation, I calculated the total seasonal richness and abundance of each visitor category (all insects, bumblebees and hoverflies) for each floral category within each mix type. Visitor richness (Chao1) and diversity (Shannon and Simpson's diversities) were not estimated for individual floral categories (or for individual floral species) due to small samples sizes at these finer scales.

(c) *Are bumblebee or hoverfly visits to meadows better predicted by floral units, nectar sugar mass or pollen volume?*

To investigate which floral resources best predict bumblebee or hoverfly visits to meadows, I compared, for each insect group, measures of model fit among models containing either floral units, nectar sugar or pollen

volume as predictors. For each insect group, candidate models had identical fixed and random effect structures, except that models contained fixed effects of either: (i) floral units per meadow (5 m2); (ii) nectar sugar mass per meadow (mg/5 m2/day); or (iii) pollen volume per meadow (ml/5 m2/day).

For models of bumblebee abundance, I used Poisson GLMMs with a log-link function. Fixed effects included survey round, temperature and wind speed (at the start of each transect walk), and either: (i) floral units; (ii) nectar sugar mass; or (iii) pollen volume per meadow. Block was fitted as a random effect. Models were tested for heteroskedasticity and overdispersion. Poisson models for hoverfly abundance were overdispersed; hence, for models of hoverfly abundance, I constructed negative binomial GLMMs using function 'glmer.nb' in R package 'lme4' (Bates et al. 2015). Fixed effects included wind speed and survey round, and either: i) floral units; (ii) nectar sugar mass; or (iii) pollen volume per meadow. Temperature had no effect on hoverfly abundance whether fit in combination with wind speed or separately, and was removed from models to reduce model complexity and ensure model convergence.

Candidate models were compared for two measures of goodness-of-fit: Akaike's information criterion (AIC) and an R^2 analogue derived for GLMMs (R^2_{GLMM}, hereafter R^2). AIC provides a relative measure of model fit that enables identification of the 'best' model among a set of candidate models (Johnson & Omland 2004). It does not provide information about absolute model fit or the proportion of variance explained by the model, but enables selection of the most parsimonious model from a set of candidate models (Johnson & Omland 2004). AIC can be calculated as: AIC = -2(log-likelihood) + 2p, where p is the number of parameters estimated in the model

(Johnson & Omland 2004). Here, AIC was used to rank candidate models, with the model with the lowest AIC deemed to provide a better fit to the data. Given that each model contains an identical number of parameters (p), the model with the lowest AIC was the model with the lowest -2(log-likelihood). Hence, for each insect group, the best fitting model was the model that maximised the log-likelihood of the model given the data.

In contrast, R^2 provides a measure of the proportion of variance explained by a model, which can function as an absolute measure of goodness-of-fit (Nakagawa & Schielzeth 2013). There are several ways to define measures of R^2, which has hindered their development and use in studies using LMMs and GLMMs (Nakagawa & Schielzeth 2013). I used the function 'sem.model.fits' in R package 'piecewiseSEM' (Lefcheck 2016). This returns marginal and conditional R^2 values for GLMMs (Nakagawa & Schielzeth 2013), with marginal R^2 values based on fixed effects only and conditional R^2 values based on fixed and random effects. For each insect group, the model with the highest R^2 value explained the most variance in visitor abundance in meadows and was considered to be the best fitting model.

For both bumblebees and hoverflies two alternative sets of analyses were performed. For bumblebees, models of floral abundance, nectar sugar mass or pollen volume were constructed and compared for data from either (i) all floral species; or (ii) sown species only. Models using data for sown species only were constructed because no bumblebees were recorded visiting any weed floral units during surveys, even though floral units of weed species comprised 22% of all recorded floral units. Given that most weed floral units were produced by species with small flowers, such as *Polygonum aviculare* or *Persicaria maculosa*, data from weeds may have contributed a

substantial amount of noise to the dataset. However, results of models using data for sown species were fully consistent with results from those using data for all floral species. Results are reported for models of sown species only, with models for all floral species in Appendix A5.

In contrast to bumblebees, hoverflies were recorded visiting weed floral units; therefore, models for hoverflies used data from all floral species. However, models of floral abundance, nectar sugar mass or pollen volume were constructed and compared for data from either: (i) all hoverflies; or (ii) female hoverflies only. Given that reproduction imposes greater physiological requirements for protein from pollen on female hoverflies compared to males, I predicted that pollen volume per meadow would be a better predictor of female hoverfly visits than for hoverflies. However, results were consistent between models using data for female hoverflies or data for all hoverflies. Results for models of female hoverflies are reported in the main text, with models for all hoverflies in the presented in Appendix A5.

(iii) Objective 5.3: *Which species are most visited by bees and hoverflies?*

Given substantial floral contamination in meadows of Marmalade and Short seed mixes (Chapter 3), and strong effects of contaminants on patterns of insect visitation to these meadows (this chapter), comparison of flower-visitors between meadow treatments could have led to misleading conclusions on the visitors attracted to different seed mix types. Instead, I quantified visits by bee species and hoverfly genera to specific plant species.

To assess whether any floral species were visited more than expected based on their floral abundance, I compared for each mix type the proportion

of floral units provided over all blocks and survey rounds by each plant species, with the proportion of visits they received from: (a) all insects; (b) bumblebees; or (c) hoverflies. This approach does not directly test for preferential visitation, or control for spatial or temporal asynchrony between flower and visitor species, and their relative abundances, but does provide a crude indicator of which species may be preferentially visited by different groups of flower-visitor.

5.3 Results

5.3.1 Objective 5.1: Quantifying and comparing floral rewards per-floral unit and per-unit area of meadow.

Daily nectar and pollen rewards per-floral unit were each quantified for 40 species, with both resources quantified for 37 species (Table A5.1). Floral reward data for most species were collected directly from experimental meadows in Sheffield (nectar: 22 species; pollen: 25 species). Additional floral nectar and pollen rewards data, predominantly for weeds, were available from a study performed in Edinburgh using identical methods (nectar:18 species; pollen: 15 species; Hicks *et al.* 2016). In both cases, although the 40 species were different, they collectively comprised over 98% of floral units recorded in each replicate field plot in each survey round (except for a single plot in round 1 where floral reward data was available for species comprising 92% of the floral units present).

(a) *Which species had the highest nectar and pollen rewards per floral unit?*

Nectar rewards per floral unit varied substantially among species. Asteraceae species, whose floral units are capitulate inflorescences, comprised eight of the 10 top-ranked species for nectar sugar mass (μg

sugar/floral unit/day, Fig. 5.1): *Cirsium arvense* (2608.9; ranked 1st); *Rudbeckia hirta* (1843.3; 2nd); *Hypochaeris radicata* (1843.2; 3rd); *Centaurea cyanus* (822.5; 4th); *Coreopsis tinctoria* (629.6; 6th); *Sonchus asper* (593.8; 8th), *Sonchus oleraceus* (568.8; 9th) and *Glebionis segetum* (564.1; 10th). Apart from Asteraceae, the top nectar producers were two weed species: *Medicago lupulina* (1202.9; 4th overall; Fabaceae), for which floral units were also inflorescences, and *Lamium album* (651.6; 6th overall; Lamiaceae). The lowest ranking sown species were the poppies *Papaver rhoeas* and *Eschscholzia californica*, neither of which produced any detectable nectar. Most weeds produced small floral units providing comparatively little nectar compared to most sown species (Fig. 5.1).

From a seed mix perspective, Asteraceae contributed the top-ranked nectar species. The top 2 species in the Marmalade mix were *Rudbeckia hirta* and *Coreopsis tinctoria* (ranked 2nd and 7th), in the Short mix were *Centaurea cyanus* and *Coreopsis tinctoria* (5th and 7th), and in the Cornfield mix were *Centaurea cyanus* and *Glebionis segetum* (5th and 10th).

The top-ranked weed species, *Cirsium arvense* and *Hypochaeris radicata* (ranked 1st and 3rd overall; Fig. 5.1), produced more nectar sugar per floral unit than almost all sown species. However, most weeds produced small floral units providing comparatively little nectar compared to most sown species (Fig. 5.1).

Pollen volume per floral unit per day also varied substantially among species (Fig. 5.2). The two top-ranked species were both sown species of poppy (Papaveraceae; all values for pollen provision are in µl/floral unit/day): *Papaver rhoeas* (10.6) produced over 4 times as much as the second-ranked species, *Eschscholzia californica* (2.5). The next three ranked species

were all sown species of Asteraceae: *Glebionis segetum* (2.0; 3rd); *Coreopsis tinctoria* (1.5; 4th); and *Rudbeckia hirta* (1.2; 5th). The sown species providing the lowest amounts of pollen per floral unit were either species with small single-flower floral units, such *Linaria maroccana* (0.091; 25th) and *Gypsophila elegans* (0.089; 26th), or species with floral units comprising an inflorescence that had on average few flowers per floral unit, such as *Silene armeria* (0.04/day; 28th) and *Iberis umbellata* (0.03; 30th).

Most weed species produced small floral units and provided less pollen per floral unit than sown species (Fig. 5.2). The 5 top-ranked weed species were all Asteraceae: *Hypochaeris radicata* (0.365; 13th); *Scorzoneroides autumnalis* (0.2738; 15th); *Achillea millefolium* (0.247; 16th); *Matricaria discoidea* (0.1337; 18th); and *Sonchus asper* (0.12; 19th).

There was no apparent relationship across species between the amount of pollen and the amount of nectar provided per floral unit (Fig. 5.3). While some species such as *Papaver rhoeas* and *Eschscholzia californica* provided lots of pollen but almost no nectar rewards, others (including the weeds *Cirsium arvense and Hypochaeris radicata*) provided lots of nectar sugar per floral unit but comparatively little pollen. Species that provided relatively high amounts of both pollen and nectar per floral unit were all in family Asteraceae (*Glebionis segetum*, *Rudbeckia hirta*, *Coreopsis tinctoria* and *Centaurea cyanus* (Fig 5.3).

(b) *Did the species selected for enrichment rank highly for nectar rewards within their respective seed mix types?*

The species chosen for enrichment tended to rank highly for nectar rewards within their respective seed mix types (Fig. 5.1). For the Marmalade mix, the enriched species (*Rudbeckia hirta* and *Coreopsis tinctoria*) were the

top-ranked species in the mix for daily nectar sugar per floral unit. For the Short mix, one enriched species (*Centaurea cyanus*) was the top-ranked species in the mix, although another (*Iberis umbellata*) was ranked second bottom, whilst for the third (*Convolvulus tricolor*) no nectar reward data could be gathered since it produced too few floral units. For the Cornfield mix, the two enriched species (*Glebionis segetum* and *Echium vulgare* 'Blue Bedder') ranked second and third for nectar rewards within the mix.

However, enrichment also required concurrent reduction in the proportional contributions of other species within each mix to ensure that valuable seed was not wasted and that plant performance was not reduced by competition. For the Marmalade mix, the reduced species (*Glebionis segetum*) ranked third in the mix (below the enriched species). For the Short mix, the reduced species (*Coreopsis tinctoria* and *Gypsophila elegans*) ranked second and fifth, respectively (below *C. cyanus* but above *I. umbellata*). For the Cornfield mix, the reduced species (*C. cyanus* and *Agrostemma githago*) ranked top and fourth respectively (above and below the enriched species). Hence, for each seed mix type, the enriched species were either the top or were above average nectar producers, indicating that *a priori* predictions were sufficient to identify high ranking species, although the effectiveness of enrichment at a meadow scale is also contingent on relative changes in floral abundance between species that were enriched, not changed or reduced (see Discussion).

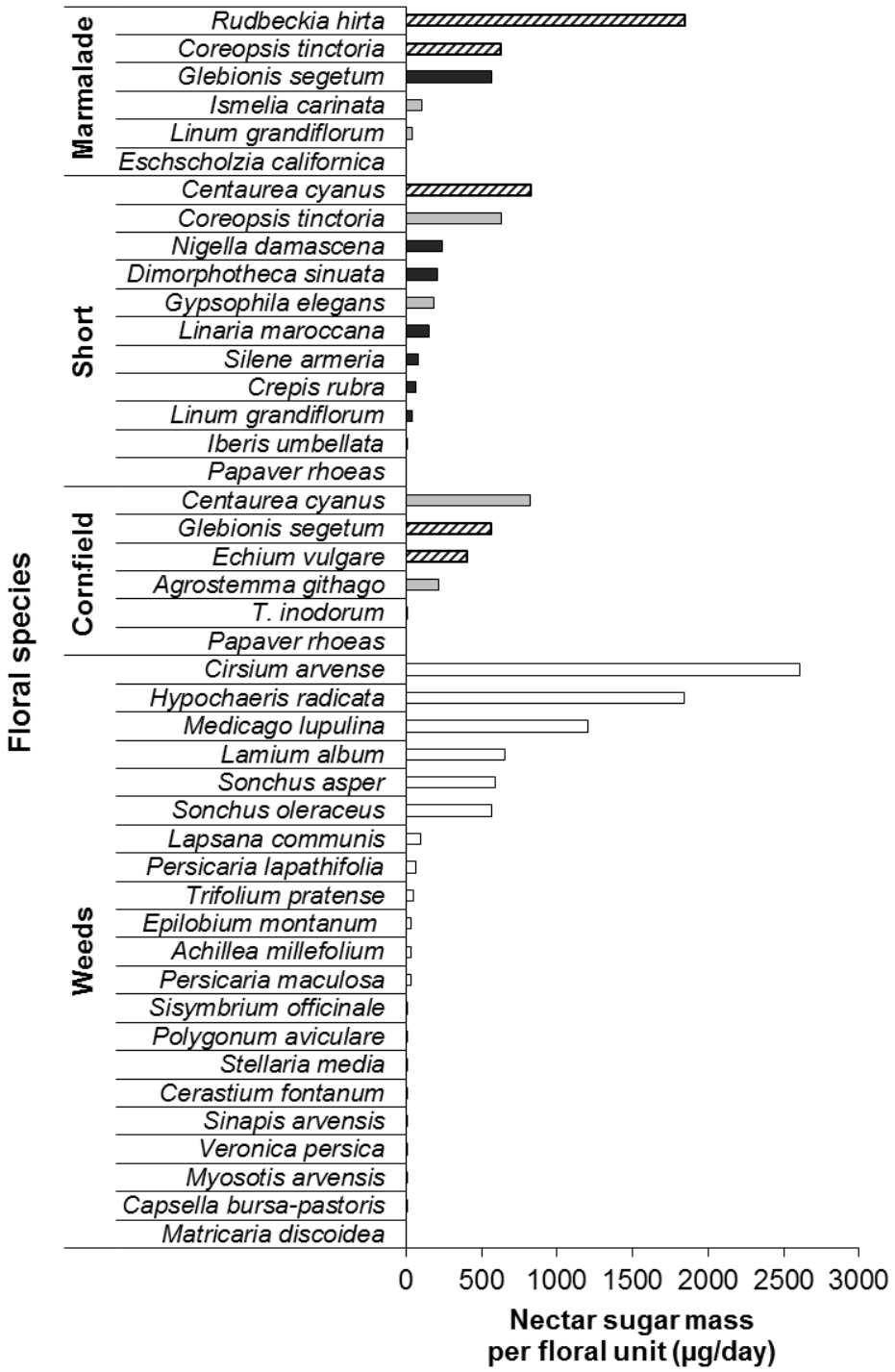


Figure 5.1: Mean nectar sugar mass per 24h per floral unit for species in meadows of Marmalade, Short or Cornfield seed mixes. Sown species are ranked within their respective seed mix types, with weeds ranked below. For sown species, colours indicate floral amendment category, with species either increased (hatched), decreased (grey) or not changed (black) as a % of seed mix seed weight. Values are point estimates.

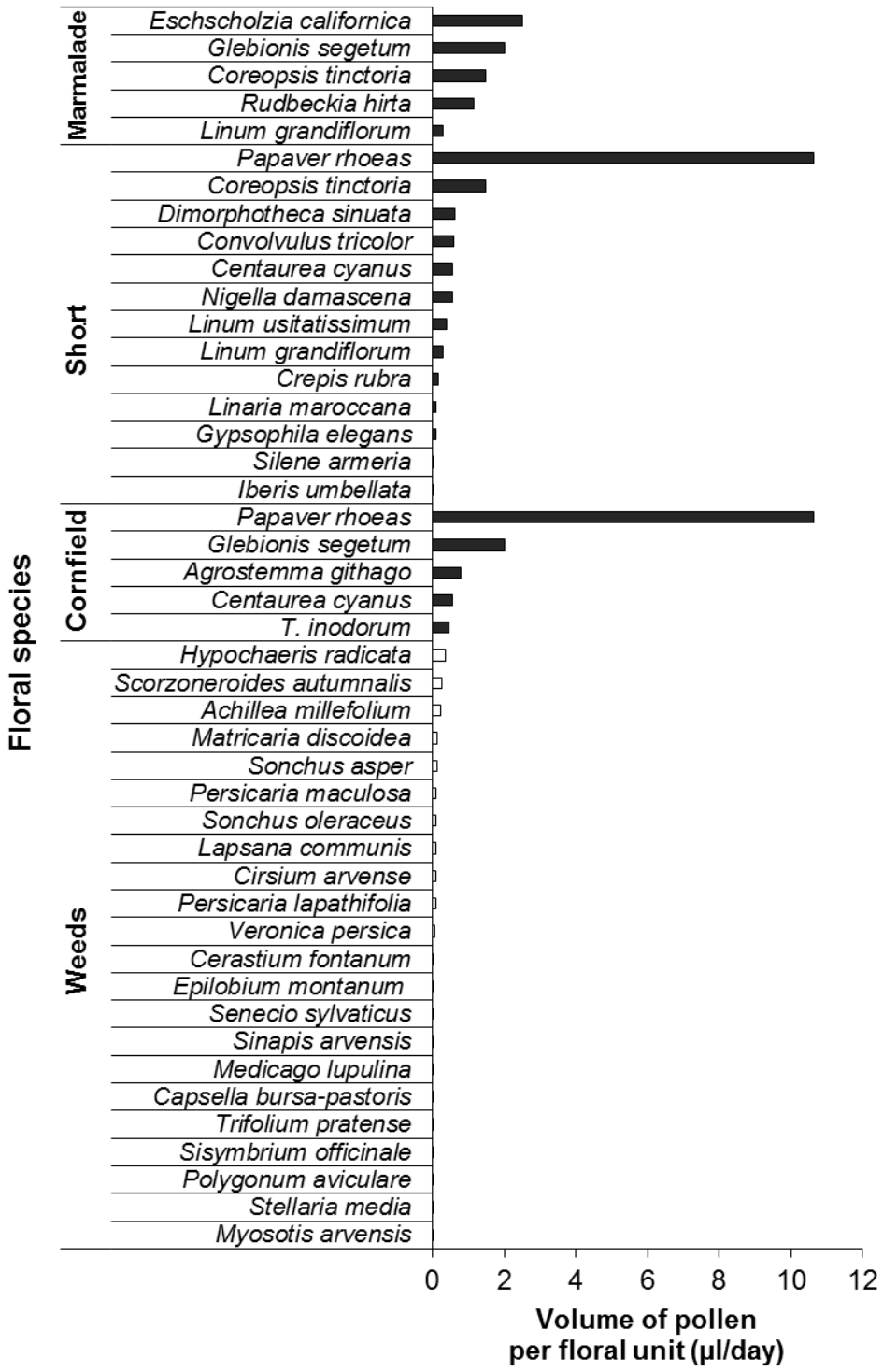


Figure 5.2: Mean pollen volume per 24h per floral unit for species flowering in meadows of Marmalade, Short and Cornfield seed mixes. Sown species are ranked by estimated mean pollen volume, with weeds ranked below. Sown species are shown in black, weeds are shown in white. Values are point estimates.

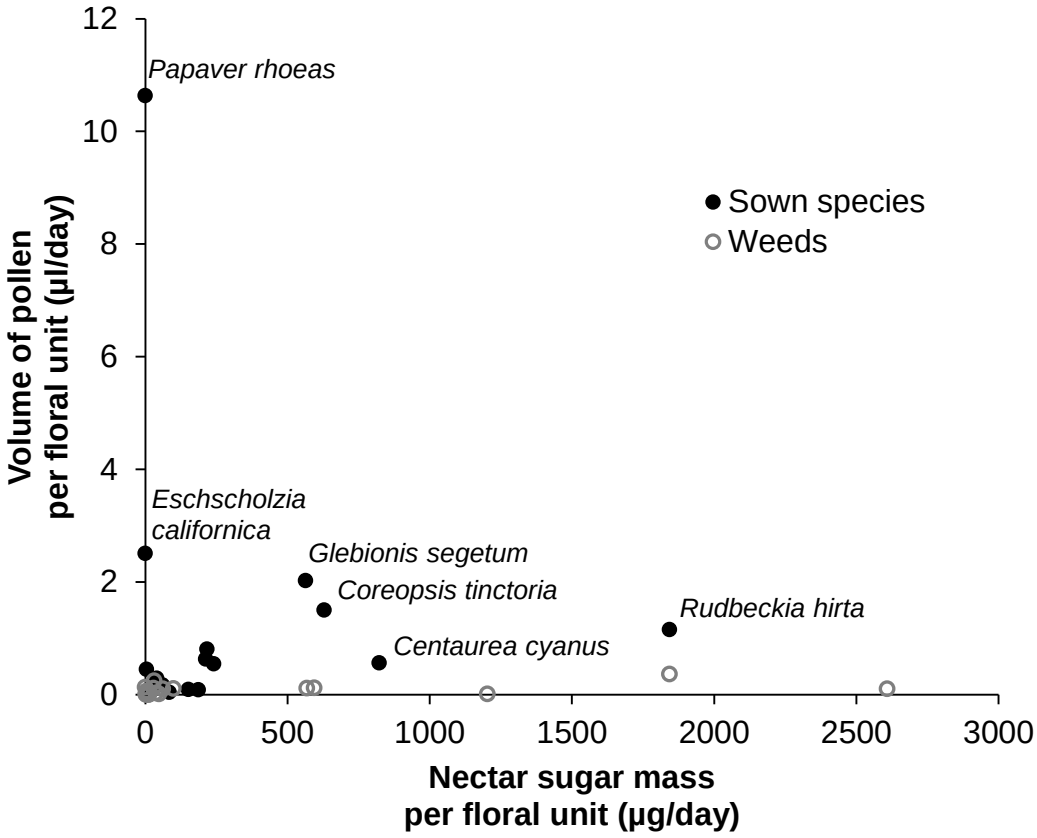


Figure 5.3: The relationship between pollen volume per 24h per floral unit and nectar sugar mass per 24h per floral unit for species flowering in meadows of Marmalade, Short and Cornfield seed mixes. Values are point estimates.

(c) Did floral nectar and pollen reward provision differ between meadows of different mix types, and did the enrichment of seed mixes enhance nectar rewards relative to standard formulations?

There was no consistent difference over the season (i.e. survey rounds) in the nectar sugar mass provided by different seed mix treatments. However, there was a significant interaction between mix type and survey round (LRT: χ^2=10.92, df=4, p=0.027; Tables. 5.1 and A5.2.1). In rounds 1 and 2, nectar sugar provision per meadow did not differ among mix types, but in round 3 Marmalade mix meadows provided more than double the nectar sugar mass of Cornfield mix meadows, with Short mix meadows providing

an intermediate amount (Fig. 5.4a). It is noteworthy that unintended/ contaminant Centaurea cyanus was the largest single contributor of nectar rewards in Marmalade mix meadows in round 1, although as survey rounds progressed the contribution of C. cyanus declined, while the contribution of late-flowering species Rudbeckia hirta and Coreopsis tinctoria increased, making Marmalade mix meadows the most rewarding for nectar sugar in round 3 (Fig. A5.3). In the absence of C. cyanus, Marmalade mix meadows would likely have provided comparatively little nectar sugar in round 1 (Fig. A5.3).

Similarly, there was also no consistent difference over the season in the pollen volume provided by different seed mix treatments. Again, however, there was a significant interaction between mix type and round (LRT: χ^2=12.3, df=4, p=0.015; Tables 5.2 and A5.2.1). In round 1, Cornfield mix meadows provided more than double the pollen volume of Short mix meadows, with Marmalade mix meadows providing an intermediate amount (Fig. 5.5a). In rounds 2 and 3, meadows of all three seed mixes produced similar amounts of pollen, although the absolute volume of pollen produced in each mix was higher in round 2 than in round 3 (Fig. 5.5a). It is noteworthy that unintended/ contaminant *Tripleurospermum inodorum* was the largest single contributor of pollen rewards in Marmalade mix meadows in round 1, but by round 2 this contribution had declined substantially, whilst *C. cyanus* contributed relatively little pollen throughout surveys (Fig. A5.3). In the absence of *T. inodorum* and *C. cyanus*, Marmalade mix meadows would likely have provided less pollen round 1 than Cornfield mix meadows (Fig. A5.3). Thus, in the absence of contaminants, Cornfield mix meadows may have provided more nectar than Marmalade and more pollen than both Marmalade and Short mix meadows early the season (round 1), but less

nectar and pollen than both Marmalade and Short mix meadows later in the season (round 3), as late-flowering non-native species began to bloom (Fig. A5.3).

There was also no consistent pattern across mix types to variation in nectar and pollen resources between survey rounds. Marmalade mix meadows showed no differences between rounds in either nectar sugar mass (Fig. 5.4b) or pollen volume (Fig. 5.5b). However, patterns for this seed mix type are difficult to interpret due to high contributions from contaminant floral species (Tables 5.1 and 5.2), although it seems likely that in the absence of contaminants both nectar and pollen resource provision in round 1 would be lower than in rounds 2 and 3 (Fig. A5.3). In Short mix meadows, nectar sugar mass in round 3 was half that in round 2, with round 1 roughly intermediate (Fig. 5.4b), but there was no difference between rounds in pollen volume (Fig. 5.5b). In Cornfield mix meadows, both nectar sugar mass (Fig. 5.4b) and pollen volume (Fig. 5.5b) were higher in rounds 1 and 2 than in round 3. Overall, weeds contributed low quantities of nectar sugar and pollen per meadow per round in each of the three seed mix types (Tables 5.1 & 5.2).

Table 5.1: Results from a GLMM testing for an effect of mix type, enrichment and round on meadow floral nectar sugar mass (see Table A5.2.1 for full model). Significant results from log-likelihood ratio tests are in bold. Estimates of nectar sugar mass per meadow type for each round (mean±SE) are calculated from raw data and are averaged across standard/enriched formulations and blocks (n=4). Estimates of nectar sugar mass per meadow per round (mean±SE) are calculated from raw data and averaged across formulations, mix types and blocks (n=4).

	Mean nectar sugar mass per meadow (mg/5 m^2/day)			**Effect of seed mix type and round**	
	Seed mix type				
Round	**Marmalade**	**Short**	**Cornfield**	***χ^2***	***p-value***
Round 1	245.1 ± 51.6	248.2 ± 31.9	236.7 ± 28.7	Interaction mix type & round: χ^2=10.9;*p*=**0.027** (see Tab. A5.2.1 & Fig. 5.4)	
Round 2	329.3 ± 44.9	402.3 ±124.5	211.1 ± 34.1		
Round 3	278.7 ± 17.9	187.6 ± 33.8	105.3 ± 10.8		
	Mean nectar sugar mass per meadow per round (mg/5 m^2/day/round)			**Effect of seed mix type and round**	
	Seed mix type			***χ^2***	***p-value***
Floral category	**Marmalade**	**Short**	**Cornfield**		
Total	284.4 ± 20.6	279.4 ± 50.5	184.4 ± 20.3	-	-
Treatment	169.3 ± 32.2	263.3 ± 46.7	169.6 ± 18.1		
Contaminant	103.7 ± 30.8	5.8 ± 2.9	5.1 ± 2.6	-	-
Weed	11.3 ± 3.8	10.3 ± 4.7	9.7 ± 4.5		

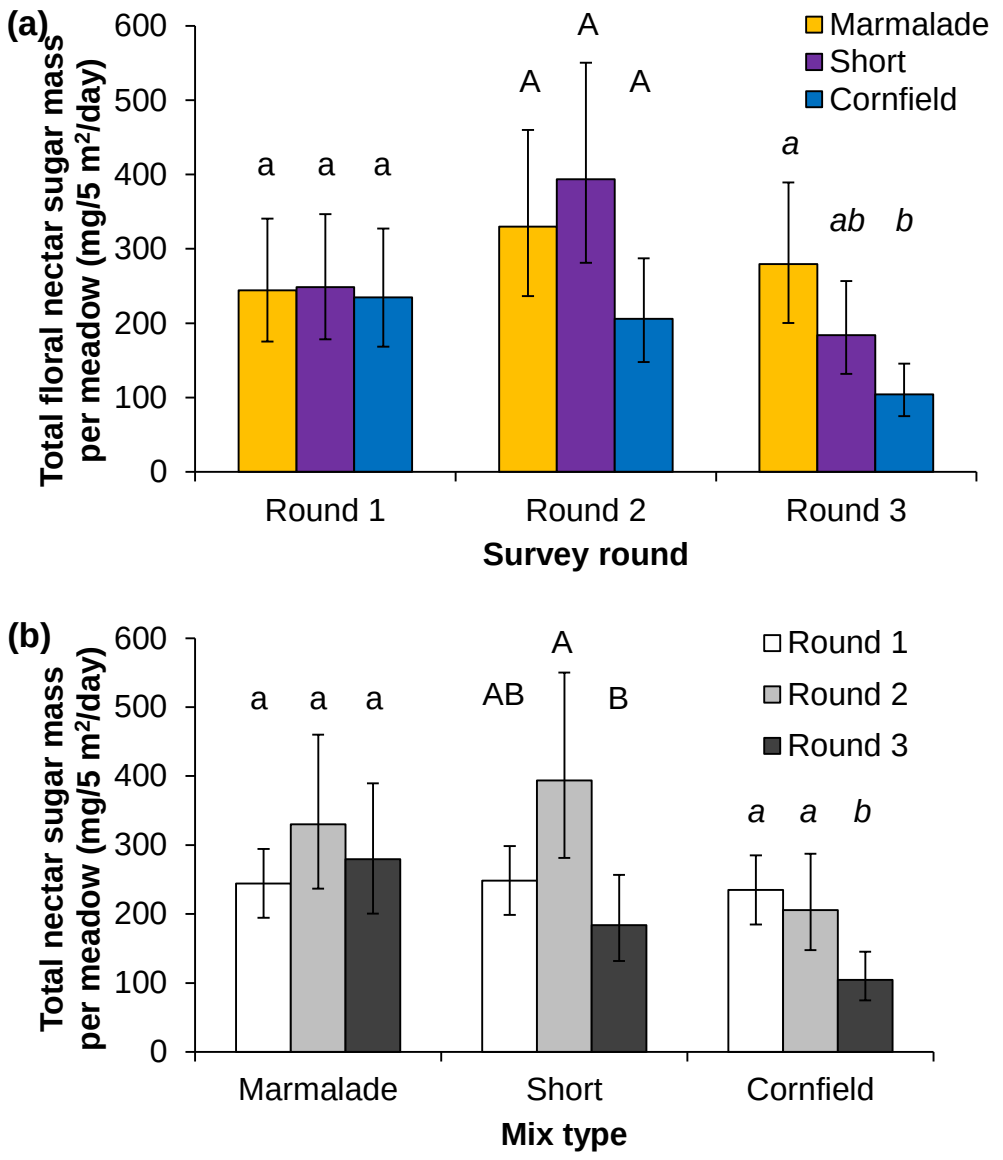


Figure 5.4: Mean (±95% CI) nectar sugar mass per meadow, showing tests for: (a) differences within each survey round between meadows of different seed mix types; and (b) differences within meadows of each seed mix type between different survey rounds. Values are predicted marginal means from a GLMM averaged over levels of seed mix formulation and block (Tables 5.1, A5.2.1, and A5.2.2). Significant contrasts are indicated by different letters, with contrasts only valid within x-axis categories (indicated by letter font and case).

Table 5.2: Results from a GLMM testing for effects of mix type, enrichment and round on the volume of pollen provided per meadow (see Table A5.2.1 for full model). Significant results from log-likelihood ratio tests are in bold. Estimates of pollen volume per meadow type in each round (mean±SE) are calculated from raw data and averaged across enriched/standard formulations and blocks (n=4). Estimates of pollen volume per meadow per round (mean±SE) are calculated from raw data and averaged across formulations, mix types and blocks (n=4).

	Mean pollen volume per meadow (ml/5 m^2/day)			**Effect of seed mix type and round**	
	Seed mix type				
Round	**Marmalade**	**Short**	**Cornfield**	***χ^2***	***p-value***
Round 1	0.72 ±0.18	0.36 ± 0.11	0.80 ± 0.11	Interaction mix type & round: χ^2=12.30; *p*=**0.015** (see Tab. A5.2.1 & Fig. 5.5)	
Round 2	0.73 ±0.16	0.68 ± 0.23	0.94 ± 0.35		
Round 3	0.42 ±0.11	0.32 ± 0.07	0.21 ± 0.03		
	Mean pollen volume per meadow per round (ml/5 m^2/day/round)			**Effect of seed mix type and round**	
	Seed mix type			***χ^2***	***p-value***
Floral category	**Marmalade**	**Short**	**Cornfield**		
Total	0.626 ±0.046	0.452 ±0.111	0.651 ±0.142	-	-
Treatment	0.423 ±0.109	0.404 ±0.101	0.628 ±0.144		
Contaminant	0.189 ±0.085	0.04 ±0.014	0.018 ±0.005	-	-
Weed	0.014 ±0.007	0.008 ±0.003	0.005 ±0.001		

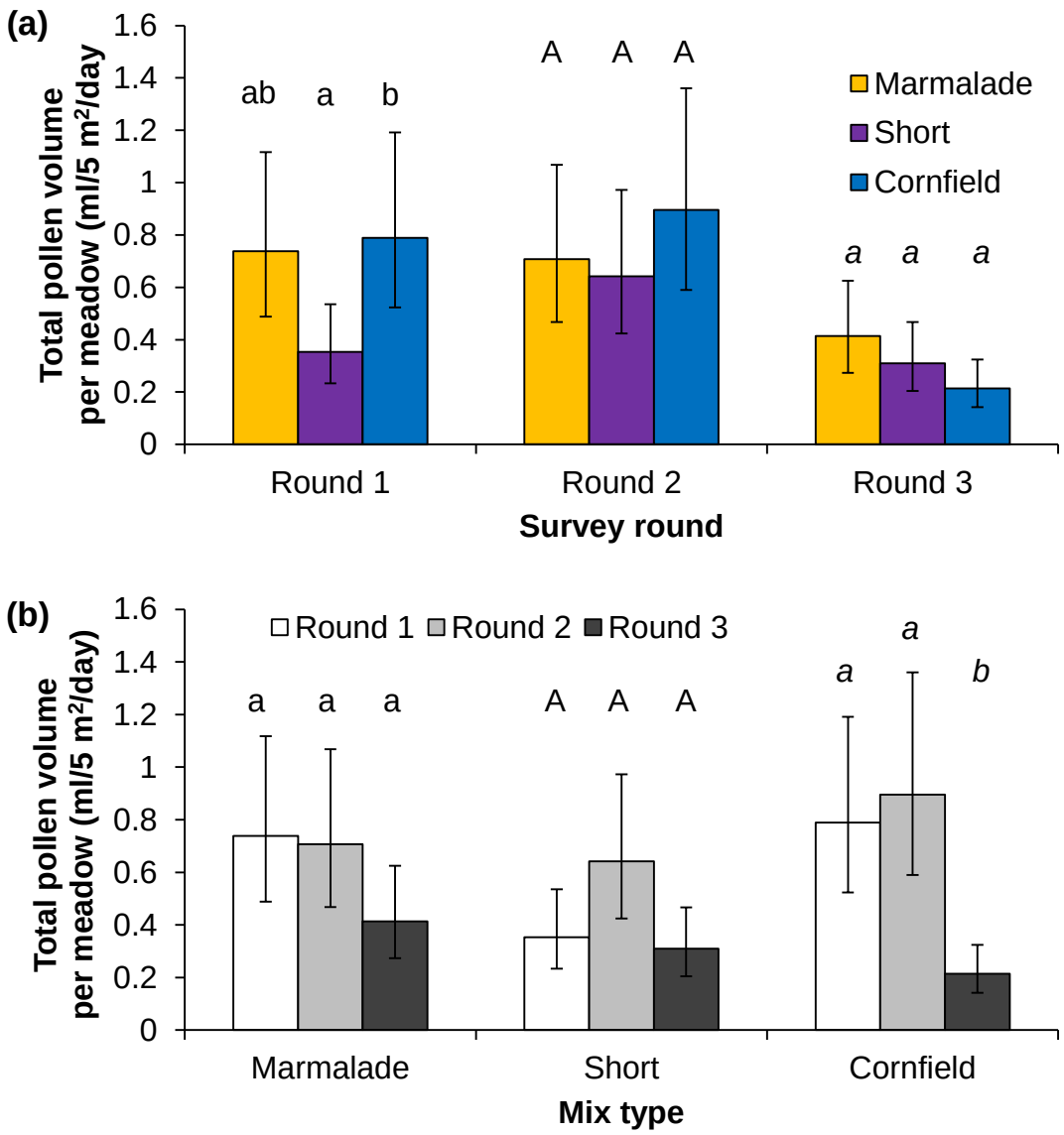


Figure 5.5: Mean (±95% CI) pollen volume per meadow, showing tests for: (a) differences within each survey round between meadows of different seed mix types; and (b) differences within meadows of each mix type between different survey rounds. Values are predicted marginal means from a GLMM averaged over levels of seed mix formulation and block (Tables 5.2, A5.2.1, and A5.2.2). Significant contrasts are indicated by different letters, with contrasts only valid within x-axis categories (indicated by letter font and case).

5.3.2 Objective 5.2: Examining patterns and drivers of insect visitation in meadows of different seed mix treatments.

(a) *Did total seasonal richness or abundance of visitors differ between meadows of different seed mix types?*

Formal statistical tests of differences in visitor abundance and richness could not be carried out due to low replication (see Methods). Nevertheless, total seasonal abundance of visitors ranked Marmalade > Cornfield > Short (Table 5.3). In contrast, total seasonal richness and diversity of insect visitors (whether direct counts, Chao1 richness estimates or diversity indices) were similar in meadows of all three mix types (Table 5.3). Both bumblebees and hoverflies showed no apparent differences in total seasonal abundance, richness or diversity between meadows of different mix types (Table 5.3).

Table 5.3 (on subsequent page): The mean (±SE) total abundance, richness, and diversity of insect visits to meadows of Marmalade, Short and Cornfield seed mixes. Results are shown for all insects, bumblebees, and hoverflies. For each group, values of abundance and richness are presented: (i) for all visitors regardless of the species they visited ('total' meadow values); and (ii) separately for visitors to different floral categories (treatment, contaminants, or weeds). Values represent estimates for 20 m^2 of meadow, calculated as averages over standard/enriched treatments and blocks (n = 4). Abbreviations: Abund. = abundance; Emp. = empirical richness; Chao1 = Chao1 estimated richness; Shan. = exponential Shannon diversity; Simp. = inverse Simpson's diversity.

Taxa	Mix	Floral category	Mean total-seasonal visitor abundance, species richness and diversity ± SE				
			Abun.	Emp.	Chao1	Shan.	Simp.
All insects	Marmalade	Total	80.3±4.5	22.9±1.5	38.3±2.8	11.1±1.4	6.5±1.1
		Treatment	48.0±12.9	14.1±2.8	-	-	-
		Contaminant	32.0±12.8	12.0±4.4	-	-	-
		Weed	0.3±0.1	0.3±0.1	-	-	-
	Short	Total	46.4±7.4	18.0±2.2	32.8±6.7	12.6±1.5	9.6±1.4
		Treatment	31.3±5.6	13.5±1.2	-	-	-
		Contaminant	13.2±4.5	6.0±1.8	-	-	-
		Weed	2.0±0.9	1.6±0.8	-	-	-
	Cornfield	Total	69.6±14.3	22.9±1.3	38.7±4.8	14.8±0.4	10.0±0.8
		Treatment	66.3±14.9	21.8±1.7	-	-	-
		Contaminant	2.3±0.4	1.8±0.4	-	-	-
		Weed	1.1±0.4	1.0±0.3	-	-	-
Bumblebees	Marmalade	Total	8.3±1.9	2.5±0.6	2.6±0.7	2.3±0.5	2.1±0.4
		Treatment	0.6±0.1	0.6±0.1	-	-	-
		Contaminant	7.6±2.0	2.3±0.6	-	-	-
		Weed	0.0±0.0	0.0±0.0	-	-	-
	Short	Total	11.1±0.9	3.4±0.2	3.7±0.3	2.9±0.2	2.6±0.2
		Treatment	11.0±0.9	3.4±0.2	-	-	-
		Contaminant	0.1±0.1	0.1±0.1	-	-	-
		Weed	0.0±0.0	0.0±0.0	-	-	-
	Cornfield	Total	8.9±0.8	3.0±0.0	2.9±0.1	2.6±0.1	2.4±0.2
		Treatment	8.9±0.8	3.0±0.0	-	-	-
		Contaminant	0.0±0.0	0.0±0.0	-	-	-
		Weed	0.0±0.0	0.0±0.0	-	-	-
Hoverflies	Marmalade	Total	16.3±2.3	8.1±0.6	11.8±1.8	6.8±0.4	5.9±0.3
		Treatment	9.8±4.2	5.0±1.7	-	-	-
		Contaminant	6.5±2.5	4.1±1.5	-	-	-
		Weed	0.0±0.0	0.0±0.0	-	-	-
	Short	Total	11.8±2.9	6.8±1.2	9.9±1.6	5.8±0.9	5.1±0.9
		Treatment	8.3±1.6	5.4±0.9	-	-	-
		Contaminant	2.3±1.2	1.9±0.9	-	-	-
		Weed	1.3±0.5	1.0±0.4	-	-	-
	Cornfield	Total	17.3±4.6	7.6±0.6	11.8±2.3	6.3±0.5	5.5±0.6
		Treatment	15.8±4.5	7.1±0.8	-	-	-
		Contaminant	0.9±0.1	0.8±0.1	-	-	-
		Weed	0.6±0.3	0.6±0.3	-	-	-

(b) *How do patterns of visitation vary among intended (treatment) and unintended (contaminant and weed) floral species?*

Most insect visits were to flowers of treatment species in all meadow types (Table 5.3). Most visits by bumblebees were to treatment species in Short and Cornfield mix meadows (~99% and 100%, respectively), while hoverflies mainly visited treatment species in Cornfield mix meadows (~91%). However, substantial percentages of insect visits in some meadow types were to unintended species. For example, over a third of all visits to Marmalade mix meadows and a quarter of all visits to Short mix meadows were to unintended contaminant species (Table 5.3). Contaminant species received a significant proportion of visits by bumblebees (~ 92%) and hoverflies (> 40%) in Marmalade mix meadows, and ~ 20% of hoverfly visits in Short mix meadows (Table 5.3). Few insects, and no bumblebees, were recorded visiting weeds, despite high weed floral abundance (Table 5.3).

(c) Which floral resource measure best predicts bumblebee or hoverfly abundance?

Bumblebee abundance was positively correlated with the number of floral units of sown (treatment and contaminant) species (LRT: χ^2=4.55, df=1, p=0.0328; Table 5.3) and the total mass of nectar sugar provided by these species (χ^2=24.28, df=1, p=<0.001; Table 5.3), but not with the total volume of pollen they provided (χ^2=2.12, df=1, p=0.15). The model containing floral nectar sugar had the highest log-likelihood given the data (i.e. the lowest AIC value; Table 5.3). However, the proportion of variance explained (marginal and conditional R^2) did not differ qualitatively among models for each floral resource measure (Table 5.3). Results were consistent when data from weed species were included (see Table A5.4.1).

Table 5.3: GLMMs testing for an effect of meadow-level floral abundance, nectar sugar mass or pollen volume on bumblebee abundance in meadows. Models contained fixed effects of temperature, wind speed and round, with block as a random effect. Results are shown for models with predictors (floral units, nectar and pollen) comprising sown species only (treatment and contaminants). Significant results from log-likelihood ratio tests are highlighted in bold. Models with the lowest AIC or R^2 are highlighted in bold. R^2 values are marginal R^2 or conditional R^2 calculated using the 'sem.model.fits' function in R package 'piecewiseSEM'. Parameter estimates ±SE are slopes from model summaries which indicate the direction of the relationship and should not be compared for effect sizes.

Response: Bumblebee abundance								
Model	**Fixed effects**	**Effect of predictor**				**R^2**		**Parameter Estimate ±SE**
		df	**AIC**	**χ^2**	**p-value**	**Marg.**	**Con.**	
Floral model	Full model AIC		305.9			0.602	**0.668**	
	Temperature	1	303.9	0.03	0.86			
	Windspeed	3	307.2	7.21	0.0654			
	Floral units	1	308.5	4.55	**0.0328**			0.073±0.033
	Round	2	318.2	16.25	**<0.001**			
Nectar model	Full model AIC		**286.2**			**0.665**	0.665	
	Temperature	1	284.5	0.32	0.57			
	Windspeed	3	285.7	5.47	0.1402			
	Nectar	1	308.5	24.28	**<0.001**			0.157±0.028
	Round	2	301.5	19.32	**<0.001**			
Pollen model	Full model AIC		308.3			0.602	0.663	
	Temperature	1	306.4	0.07	0.79			
	Windspeed	3	310.2	7.83	**0.0498**			
	Pollen	1	308.5	2.12	0.15			0.049±0.036
	Round	2	319.5	15.1	**<0.001**			

Conversely, female hoverfly abundance was positively correlated to the total volume of pollen (χ^2= 9.56, df= 1, p=0.002) and the total mass of nectar sugar provided per meadow per day (χ^2= 5.36, df=1, p= 0.02), but not with the total number of floral units in meadows (χ^2=1.61, df=1, p= 0.21; Table 5.4). The model containing pollen volume had the highest log-likelihood given the data (i.e. the lowest AIC value; Table 5.4). Again, the proportion of variance explained (marginal and conditional R^2) did not differ qualitatively among models for each floral resource measure (Table 5.4). Results were consistent for models containing data for all hoverflies individuals (see Table A5.4.2).

Table 5.4: GLMMs testing for an effect of meadow-level floral abundance, nectar sugar mass or pollen volume on female hoverfly abundance in meadows. Models contained fixed effects of wind speed and round, with block as random effect. Temperature had no significant effect, whether fit singly or in combination with wind speed, and was removed to ensure model convergence. Significant results from log-likelihood ratio tests are highlighted in bold. Models with the lowest AIC and R^2 are highlighted in bold. R^2 values are marginal R^2 or conditional R^2 calculated using the 'sem.model.fits' function in R package 'piecewiseSEM'. Parameter estimates ±SE are slopes from model summaries which indicate the direction of the relationship and should not be compared for effect sizes.

Response: Female hoverfly abundance								
Model	**Fixed effects**	**Effect of predictor**				**R^2**		**Effect size Estimate ±SE**
		df	**AIC**	**χ^2**	**p-value**	**Marg.**	**Con.**	
Floral model	Full model AIC		303.3			0.768	0.768	
	Windspeed	3	304.3	6.96	0.07			
	Floral units	1	302.9	1.61	0.21			0.063±0.051
	Round	2	351.7	52.40	**<0.001**			
Nectar model	Full model AIC		300.3			0.758	0.769	
	Windspeed	3	300.1	5.74	0.13			
	Nectar	1	303.7	5.36	**0.02**			0.092±0.041
	Round	2	353.1	56.78	**<0.001**			
Pollen model	Full model AIC		**296.4**			**0.778**	**0.778**	
	Windspeed	3	297.8	7.39	0.06			
	Pollen	1	304.0	9.56	**0.002**			**0.133**±0.043
	Round	2	358.6	66.18	**<0.001**			

5.3.3 Objective 5.3: Examining patterns of bee and hoverfly visitation to individual flowering species.

(a) *Which species are most visited by bees and hoverflies?*

For bumblebees, 91% of 226 individuals were sampled from *Centaurea cyanus* (Fig. 5.6a). Three species (*Bombus lapidarius*, *Bombus pascuorum,* and *Bombus terrestris*) collectively comprised ~96% of all recorded bumblebee visits. Almost all visits by three rarer bumblebee species (*Bombus pratorum, Bombus lucorum, B. hypnorum* and *B. sylvestris*) were to *C. cyanus* (Fig. 5.6b). Only *Bombus hortorum*, for which only two individuals were recorded, did not visit *C. cyanus*, being only found on either *Linaria maroccana* or *Agrostemma githago* (Fig. 5.6b). No bumblebees were recorded visiting weeds.

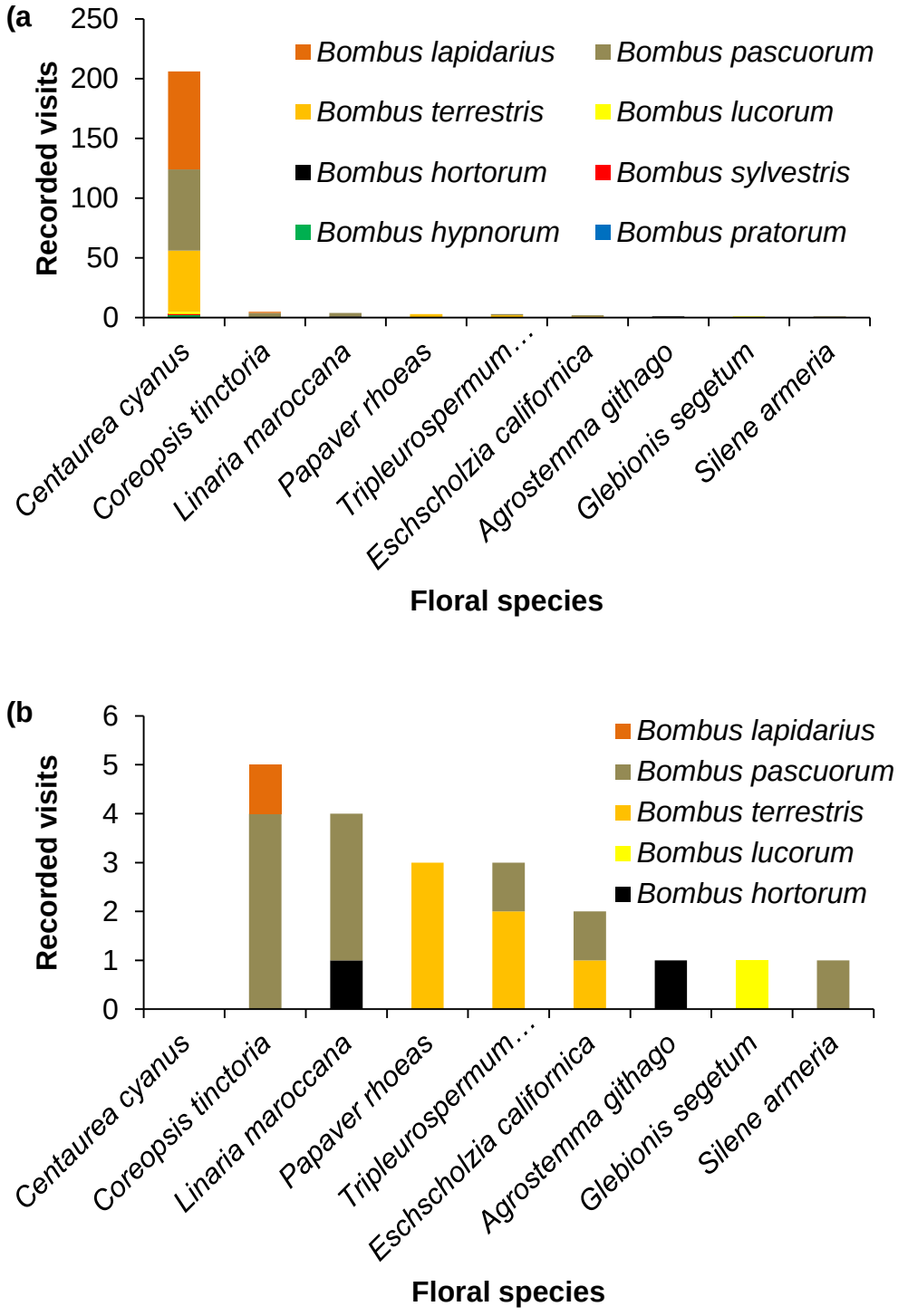


Figure 5.6: Bumblebee visits to different floral species across all meadow types. Panel (a) includes visits to *Centaurea cyanus*; Panel (b) excludes visits to *Centaurea cyanus*. *T. inodorum* = *Tripleurospermum inodorum*. *E. californica* = *Eschscholzia californica*. Note: bumblebee species listed in panel 'a' but not 'b' were visiting *C. cyanus*.

For honeybees, 88% of 88 individuals were sampled from *Centaurea cyanus* (Fig. 5.7), with a further 10% of individuals recorded visiting *Glebionis segetum*. No honeybees were recorded visiting weeds.

For solitary bees, only 29 individuals were recorded during surveys. *Colletes daviesanus*, the most common solitary bee (21 individuals), was sampled almost exclusively from *Tripleurospermum inodorum* (20 individuals; Fig. 5.8). *Tripleurospermum inodorum* and *Glebionis segetum* were visited by *Lasioglossum smeathmanellum* and *Andrena minutula*, whilst *Andrena bicolor* was sampled from *Coreopsis tinctoria* and the weed specis *Persicaria lapathifolia* (Fig. 5.8).

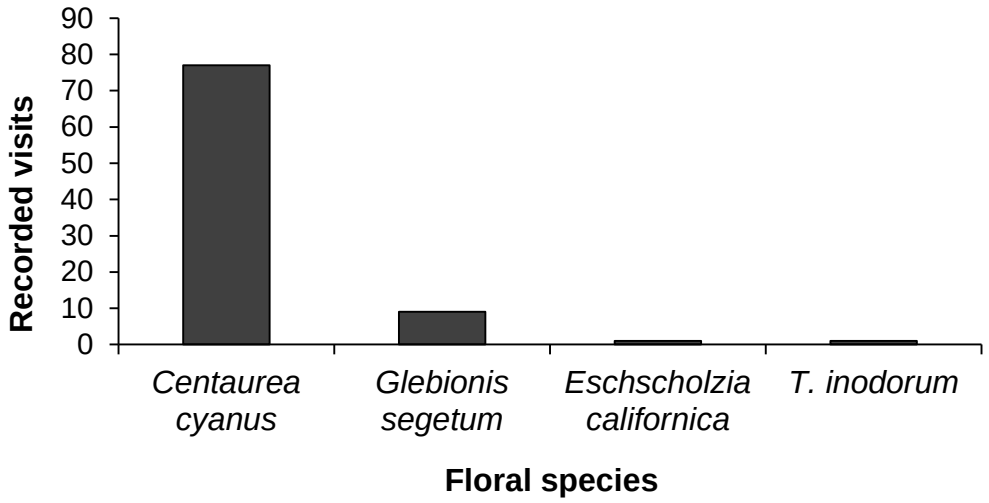


Figure 5.7: Honeybee visits to different floral species across all meadow types. *T. inodorum = Tripleurospermum inodorum*.

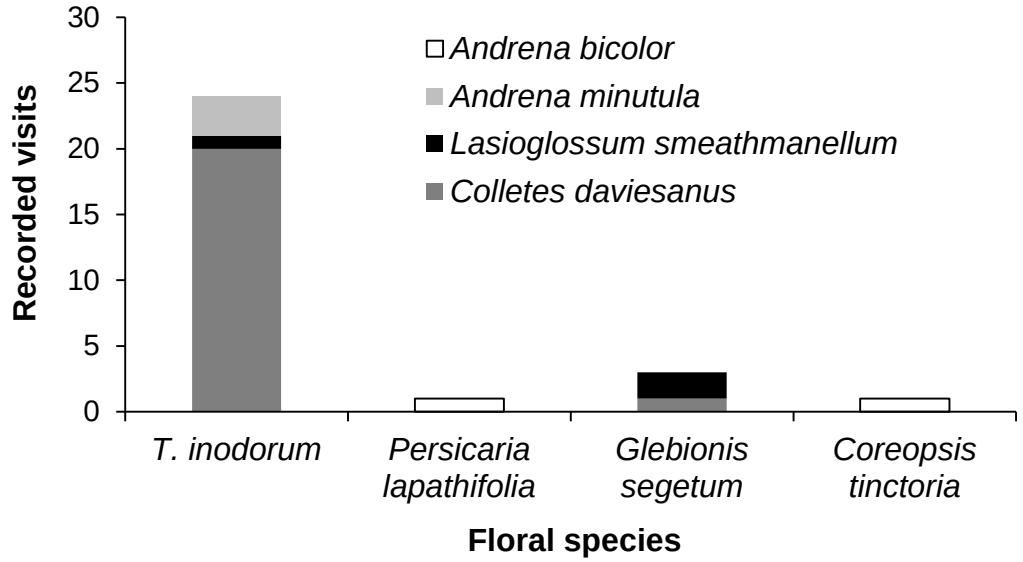


Figure 5.8: Solitary bee visits to different floral species across all meadow types. *T. inodorum* = *Tripleurospermum inodorum*.

For hoverflies, 362 individuals of 26 species in 15 genera were recorded during surveys. Of these, 90% of individuals came from 7 common genera, *Eupeodes*, *Eristalis*, *Helophilus*, *Platycheirus*, *Sphaerophoria*, *Syritta* and *Syrphus*. 89% of hoverflies were sampled from just five species: *C. cyanus*, *T. inodorum*, *G. segetum*, *Coreopsis tinctoria* or *Eschscholzia californica* (Fig. 9). Only 15 individual hoverflies were sampled from weeds: these were mainly on *Scorzoneroides autumnalis* (6 individuals) and *Polygonum aviculare* agg. (3 individuals), but also *Capsella bursa-pastoris*, *Lactuca serriola*, *Lapsana communis*, *Scorzoneroides autumnalis*, *Sisymbrium officinale* and *Sonchus asper*.

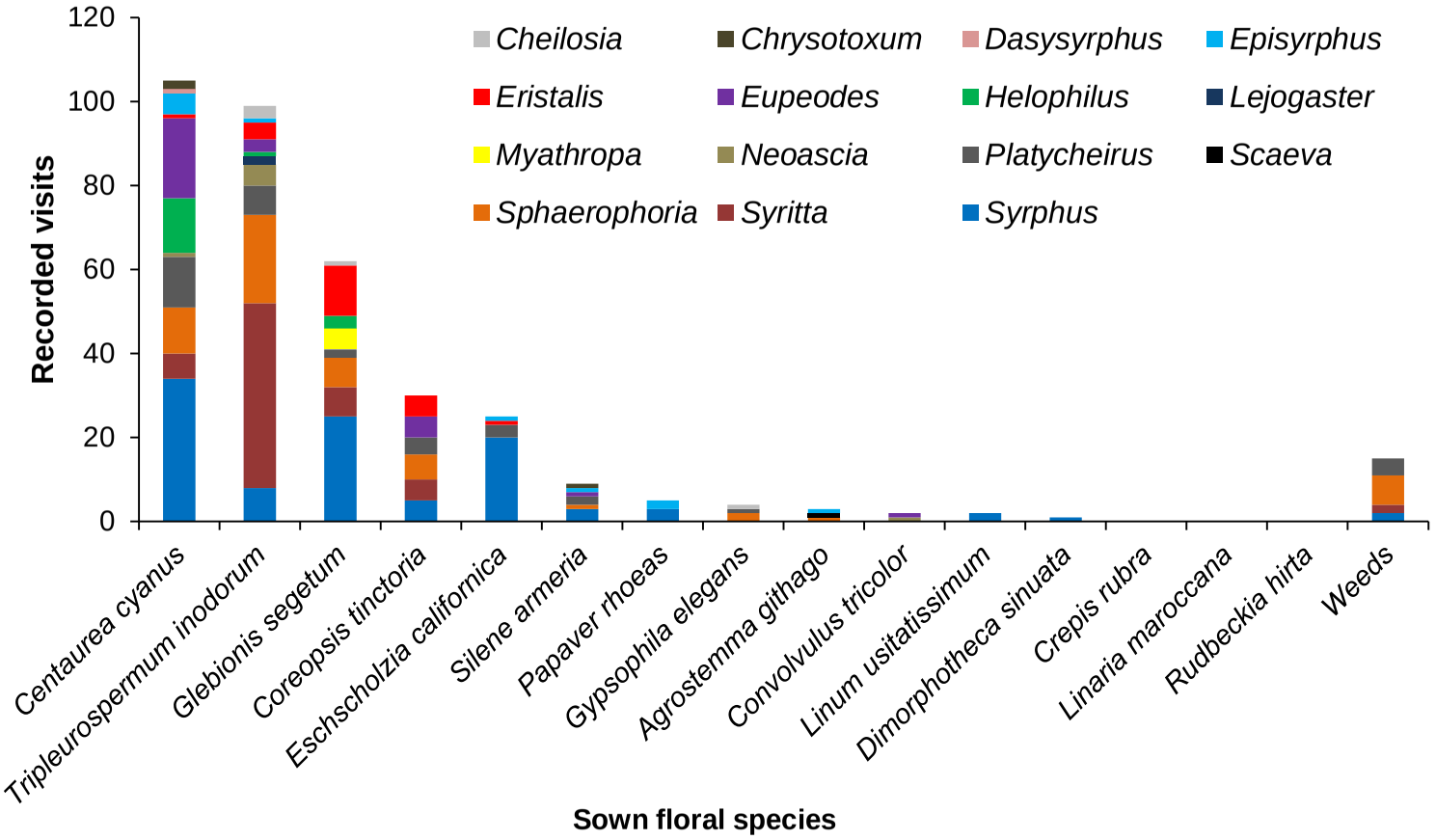


Figure 5.9: Hoverfly visits to floral species across all meadow types, for each sown species and for weeds collectively.

(b) *Were any species visited more than expected given their floral abundances?*

Across mix types, two flower species consistently appeared to receive more insect visits than expected given their proportional contribution of floral units to the meadow (Fig. 5.10). These species were *Centaurea cyanus* and *Glebionis segetum*. This was particularly true for *C. cyanus*, which was highly visited by bumblebees regardless of the proportion of floral units it contributed to meadows (Fig. 5.11). Patterns were less pronounced for hoverflies, although both *C. cyanus* and *G. segetum* appeared to attract disproportionately more individuals than their proportional contribution to floral units across meadows (Fig. 5.12). Although insects frequently visited *T. inodorum*, the number of individuals visiting this species was proportional to its contribution to floral units in meadows (Fig 5.10). It is noteworthy that the pattern described above (section 5.3.3 objective 5.2b), showing that visitation to contaminants was highest in meadows of Marmalade mixes, largely resulted from the fact that all three of these highly visited species were treatment species in meadows of Cornfield mixes (with the exception of *G. segetum* in standard Cornfield mixes), but only one was considered a treatment species in each of Marmalade (*G. segetum*) and Short mixes (*C. cyanus*).

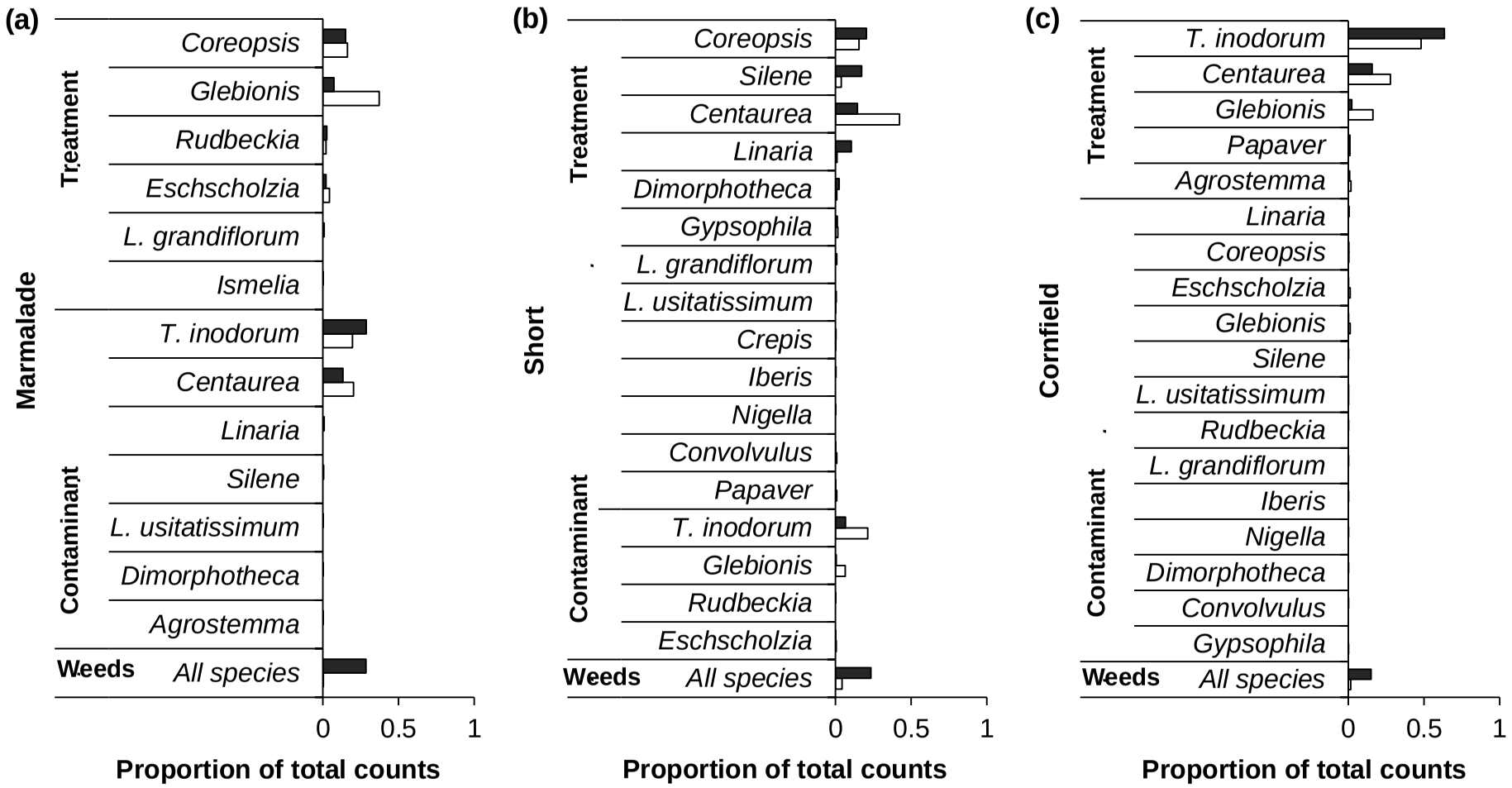


Figure 5.10: The proportional contribution of individual sown species (and weeds collectively) to total seasonal counts of floral units (black bars) and flower-visiting insects (white bars) to meadows of Marmalade, Short and Cornfield annual mixes.

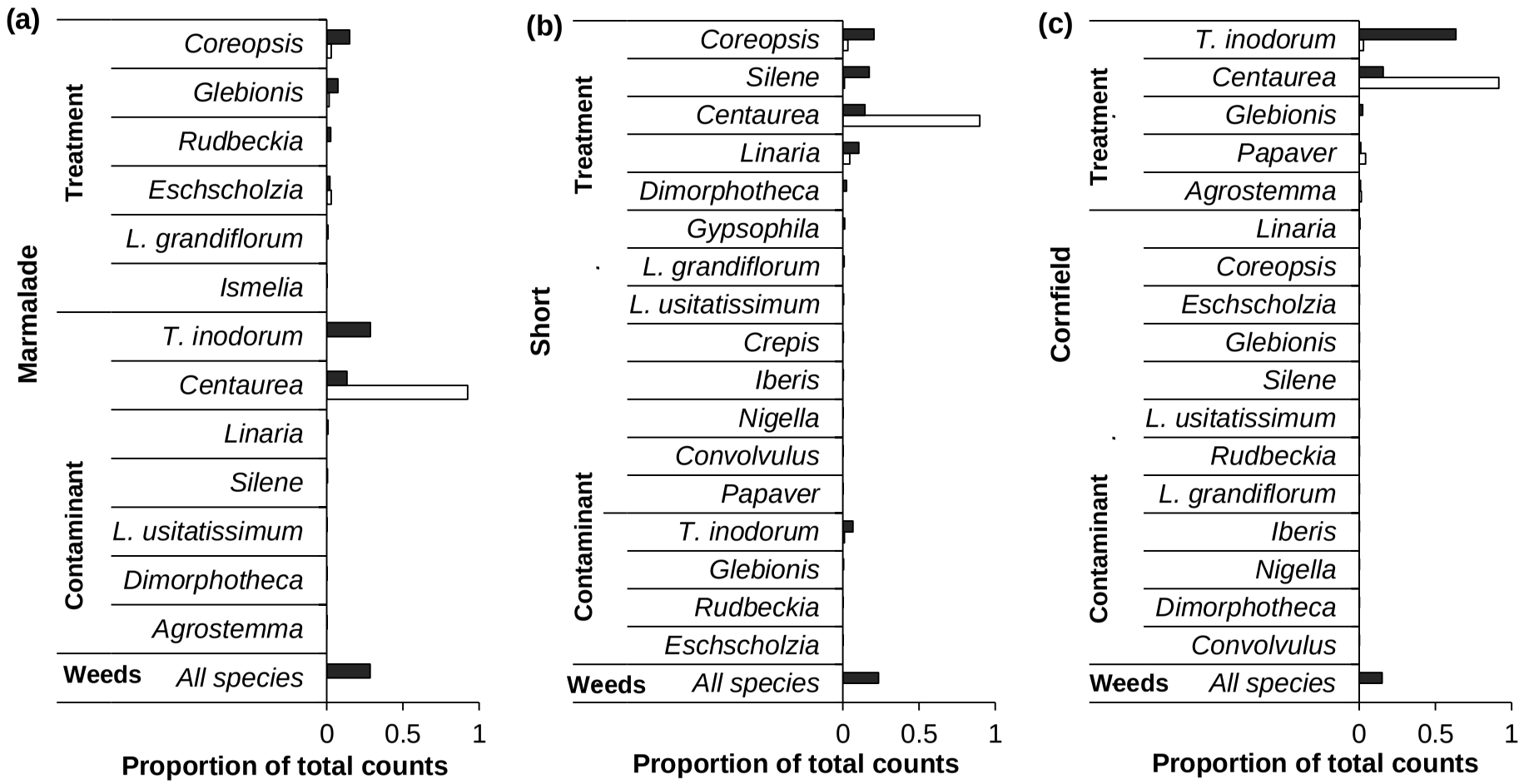


Figure 5.11: The proportional contribution of individual sown species (and weeds collectively) to total seasonal counts of floral units (black bars) and bumblebee visits (white bars) in meadows of Marmalade, Short and Cornfield annual mixes

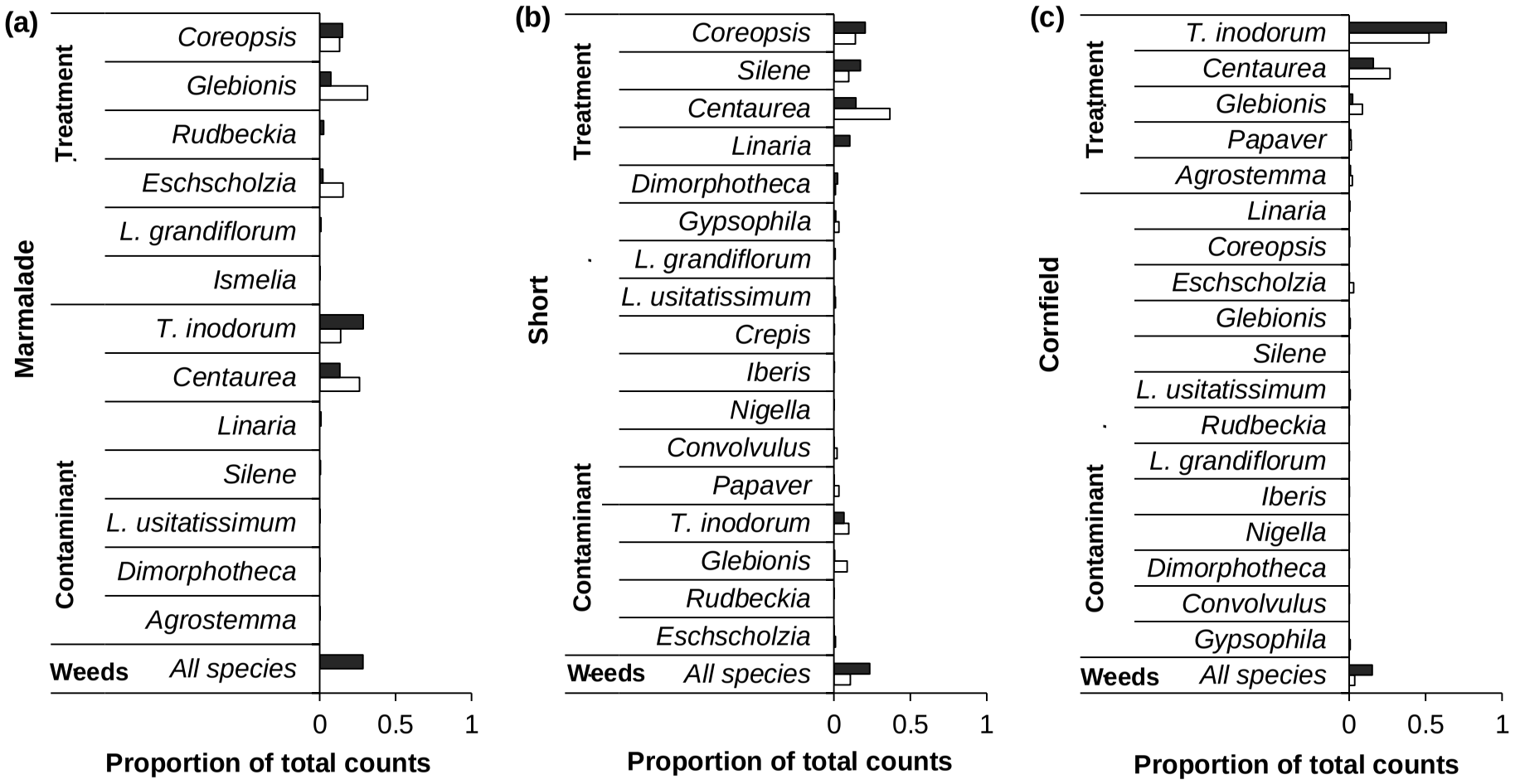


Figure 5.12: The proportional contribution of individual sown species (and weeds collectively) to total seasonal counts of floral units (black bars) and hoverfly visits (white bars) in Marmalade, Short and Cornfield annual mixes.

5.4 Discussion

My main aims in this study were to examine the effects of seed mix choice and species composition on floral reward provision and insect visitation to meadows of annual pictorial flower seed mixes. I also aimed to test whether nectar rewards can be increased in meadows by simply increasing the proportional contribution of specific floral species within seed mixes. In the following discussion, I first examine which factors affect the value of planted meadows as foraging habitat for flower-visiting insects. I then discuss methodological issues with the quantification of floral nectar and pollen rewards. Finally, I discuss variation in floral rewards and patterns of visitation between floral species and meadows, and consider the implications and limitations of this study for improving the design and use of flower seed mixes.

5.4.1 Factors affecting the value of planted meadows as foraging habitat for flower-visiting insect assemblages.

The value of a planted meadow as foraging habitat for flower-visiting insect assemblages is largely dependent on which floral species it contains, their floral abundance through space and time, and the floral shapes and floral resources per floral unit of these species (Dicks *et al.* 2015; Hicks *et al.* 2016). The first two of these depend on: (a) the species composition of the seed mix sowed; (b) the species composition of the soil seed bank; and (c) the many factors that influence the germination, establishment, growth, and flowering of each seed mix or seed bank species (Aldrich 2002; Glover 2014; Long *et al.* 2015). These factors include ground preparations, seed quality, sowing season, weather, soil characteristics, seasonal phenology, and ecological interactions between individual plants and species (Aldrich 2002; Glover 2014; Long *et al.* 2015) . Environmental factors, such as soil fertility and moisture, also influence flower size and the resources individual plants can invest in floral nectar and pollen rewards (e.g. Leiss & Klinkhamer 2005;

Farkas *et al.* 2012). The floral resources provided by planted meadows are therefore contingent on multiple factors, only some of which can be controlled through design and management. Prime among these - for landscape managers - are which seed mix is chosen for an urban planting scheme and - for seed mix designers - which species are included in mixes and in what proportions.

Selection of plant species is partially constrained by the design objectives for pictorial meadows, which may require plants individually to possess certain traits (e.g. high germination for a given environment), and collectively to complement (e.g. growth form, colour) or contrast with each other (e.g. flowering phenology). Nevertheless, plant selection is likely the prime route by which seed mix designers can influence floral resource provision for flower-visiting insects. For example, informed plant selection may (at least in principle) allow manipulation of the available flower shapes and floral resources, along with their relative densities (through relative seed proportions) and seasonal distribution. In practice, data on floral resources, as well as the relative germination rates and competitive abilities are currently limited (Dunnett & Hitchmough 2004; Köppler & Hitchmough 2015). Hence, to date, seed mix choice and design have largely been guided by simple general principles.

To support diverse flower-visitor communities, seed mixes should include an array of species. These should be selected both individually and collectively to provide a diversity of nectar and pollen sources with different flower shapes and floral resource traits, with variation in flowering season across species such that each type of resource and flower shape are available throughout the season. Further, a mix should incorporate functional redundancy in case of failure of any single species to flower. Finally, seed mixes should include any cornucopia species which meet horticultural

criteria and provide unusually abundant resources or are visited by an usually wide range of species (Corbet 2006). Even these basic traits are, however, not known for many of the species appropriate for urban meadows (Dunnett & Hitchmough 2004; Köppler & Hitchmough 2015).

5.4.2 Objective 5.1: Quantification of floral rewards and the effectiveness of nectar enrichment treatment.

Limitations in the quantification of floral rewards

Quantification of floral reward provision at a community level required estimation of floral reward provision per floral unit and number of floral units per species per unit area of meadow for each species. The sum for all species of the product (for each species) of these two values yields an estimate of the total floral rewards provided per unit area of meadow. There are advantages and limitations to this approach.

Firstly, this approach enables quantification of floral reward provision per species in units which are tractable to measure and comparable between species. For example, quantification of pollen volume requires less pollen (and hence sampling effort) than quantification of pollen mass, which would be intractable to quantify for species providing small amounts of pollen. Similarly, quantification of daily nectar rewards controls for diurnal variation in nectar sugar standing crop (Corbet 2003), which would be intractable to characterise at both a species and community level. Estimation of daily nectar rewards requires bagging flowers for 24 hours to prevent removal by insects (Kearns & Inouye 1993; Dafni *et al.* 2005). This approach provides a useful standardised measure of nectar sugar provision for cross-species comparisons, although it may underestimate daily nectar rewards for species which can reabsorb excess sugar or secrete additional sugar in response to depletion by insects (Corbet 2003).

Secondly, quantification of floral rewards per floral unit typically requires data on multiple floral traits, which are each measured with sampling error that is compounded as rewards are scaled up to a floral unit level. For example, quantification of nectar sugar mass per floral unit requires estimation of nectar sugar mass per flower and the number of open flowers per floral unit (see Methods). The main challenge in floral reward quantification (and an important source of sampling error) lies in the extraction of small quantities of nectar or pollen from small flowers or florets (Kearns & Inouye 1993; Dafni *et al.* 2005). Pollen can be extracted relatively easily from samples of anthers or florets by sonication and filtering, which can be repeated until all pollen grains have been released from vegetative tissues. Complete extraction of nectar sugar from small flowers or florets is more difficult, especially since there is no way to be sure that the water added during rinsing is mixed with the nectar sugar inside a flower. Moreover, even when effective mixing has occurred, the water added to a flower is often not fully recovered due to adhesion to plant tissues. Estimates of floral nectar rewards are therefore likely to be underestimates.

Thirdly, although these measures can be used to quantify nectar and pollen resources and they enable comparison between plant species and meadows, they do not incorporate important factors which affect the accessibility and value of rewards to different groups of flower-visitors, such flower shape/corolla depth (Ranta & Lundberg 1980a; Harder 1985; Gilbert 1981; Branquart & Hemptinne 2000), or nectar concentration/volume (as discussed below; Harder 1986; Potts *et al.* 2004; Kim *et al.* 2011).

Despite these limitations, most species whose floral nectar or pollen rewards were quantified in both Edinburgh and Sheffield yielded similar reward estimates – to an order of magnitude, and differences between replicate estimates of floral rewards for individual species were generally

small relative to differences between species (Table A5.1). Of the 9 species for which nectar rewards were quantified in both locations, 5 species had similar estimates of nectar sugar mass. These included Asteraceae species with composite floral units comprising multiple small florets, such as *Centaurea cyanus* (Sheffield: 822.5 μg/floral unit/day; Edinburgh: 895.8) and *Glebionis segetum* (Sheff: 564.1; Ed: 931.2), as well as species with single-flowered floral units, such as *Linum grandiflorum* (Sheff: 40.4, Ed: 50.2). Similarly, of the 12 species for which pollen volume was quantified in both locations, 7 had similar estimates. This included species with large single-flowered floral units providing large volumes of pollen – such as *Eschscholzia californica* (Sheff: 2.51; Ed: 2.41), as well species with small single flowers or composite floral units, such *Veronica persica* (Sheff: 0.06; Ed: 0.03) and *C. cyanus* respectively (Sheff: 0.56; Ed: 0.55).

Nevertheless, there were discrepancies between estimates made in Sheffield and in Edinburgh (Table A5.1). For example, estimated pollen volume per floral unit for *Papaver rhoeas* was twice as high in Sheffield as in Edinburgh (Sheff: 10.64 μl/day; Ed: 5.96). Similarly, estimated nectar sugar per floral unit for *Myosotis arvensis* in Sheffield was one seventh of that in Edinburgh (Sheff: 3.26 μg/day; Ed: 21.83), while estimated nectar sugar for *T. inodorum* in Edinburgh was 3 orders of magnitude higher than the value for Sheffield (Sheff: 4.8; Ed: 1415.8). Given that floral traits are measured with sampling error and are likely subject to natural variation associated with environmental conditions and plant genotypes (especially for different cultivars), the origins of these discrepancies are not clear. For *T. inodorum*, differences between estimates may be due environmental differences between cities, genetic or genetic-by- environment differences between the provenances represented in each city, or sampling error – especially given that floral units of *T. inodorum* are comprised of tiny florets from which it is difficult to rinse all the nectar.

Although the per-floral unit floral reward estimates generated in this study are approximate, and were collected in two cities over several years, they are sufficient to identify species providing high *versus* low floral rewards. Further replication of these daily estimates, as well as studies examining floral reward variation among cultivars in space and time, are required to fully assess floral nectar and pollen provision for different species (for an example in nectar bearing trees, see Somme *et al.* 2016).

Quantification of floral rewards per unit area of meadow is sensitive to sampling effort, given that species vary naturally in their distribution and aggregation within a meadow (Hicks *et al.* 2016). The sampled area in my study (5 m^2 per replicate meadow) may not have been enough to detect between-treatment variation in the floral abundance of an individual species, which is likely highly stochastic on the scale of a few metres (see Chapter 3). A previous study on planted urban meadows found little difference between mean meadow floral reward estimates for surveys of 7 m^2 and 20 m^2 in 300 m^2 meadows (Hicks *et al.* 2016). Hence, although a higher sampling intensity is desirable, 5 m^2 per meadow is a high proportion (50%) of the area surveyed for flower-visitors and is likely adequate to compare mean estimates of floral rewards between treatments.

Floral reward provision by individual species

For most plant species, few quantitative data are available on floral rewards provided for flower-visitors. However, over many decades horticulturalists, gardeners and beekeepers have accumulated many anecdotal records on the value of different plant species for bees (especially honeybees). This information is often presented in books or in the form of lists of recommended species (e.g. Crawford 2000; Hooper & Taylor 2006; IBRA 2008; RHS 2011; The Xerces Society 2011; Kirk & Howes 2012), which typically simply list recommended species but may provide qualitative

indicators for comparing the relative values of different species (e.g. Kirk and Howes 2012). The UK's Royal Horticultural Society has even developed a trademarked labelling system, so that plants featuring in its 'Perfect for Pollinators' recommended species lists can be more effectively marketed to horticulturalists and gardeners. Although undoubtedly of educational value, recent studies have shown that these lists often include poor recommendations, miss good plants and provide little detail on relative values of different plants or on the sources of information used (Garbuzov & Ratnieks 2014). Moreover, empirical evaluation has shown that, although plant species/varieties with a recommendation typically receive more insect visits than those without, variation in visitation is high, with some recommended plants poorly visited and some non-recommended plants highly visited (Shackleton & Ratnieks 2016; Garbuzov *et al.* 2017). Hence, there is a need for more comparative data on the relative values of different plant species for flower-visitors.

I found floral nectar and pollen reward provision per floral unit to vary hugely among species. This was partly due to variation in the structure and size of floral units among species. For example, species with floral units defined as an inflorescence (or capitulum) rather than as a single 'botanical' flower - such as species in family Asteraceae - tended to provide high quantities of floral rewards per floral unit per day. Moreover, species with large singleton flowers tended to provide more floral rewards than species with small singleton flowers (Figs. 5.1 and 5.2). The data presented here, in concordance with previous studies (Hicks *et al.* 2016), show that *Centaurea cyanus* (2nd for nectar) and *Papaver rhoeas* (1st for pollen) provide amongst the most floral rewards of any species used in annual flower meadow seed mixes. Although *Papaver rhoeas*, along with *Eschscholzia californica* (also family Papaveraceae), provided the most pollen rewards by volume, these species provided almost no nectar sugar (Fig. 5.1). Few species provided large

quantities of both nectar and pollen rewards, but the four highest combined producers were all in family Asteraceae: *Glebionis segetum*, *Rudbeckia hirta*, *Coreopsis tinctoria* and *Centaurea cyanus* (Fig. 5.3). Although weeds can provide large amounts of nectar per floral unit - for example, weed species comprised 5 of the top 10 nectar producers (including the top-ranked species, *Cirsium arvense*) - most weeds species had small flowers and provided low amounts of nectar and pollen compared to sown species (Fig. 5.3). Nevertheless, this shows weeds can be valuable for flower-visitors.

Does enrichment of seed mixes with highly rewarding species increase meadow-level rewards?

Floral resource provision at a meadow scale is a function of meadow floral composition (Chapter 3), the per-floral unit rewards provided by individual species (above), the densities of floral units of each species, and their individual and relative flowering phenologies. Each of these is a parameter that can be manipulated by seed mix designers through judicious selection and mixing of different species/varieties. This study incorporated a nectar enrichment treatment, which was predicated on increasing the floral abundance of species whose rewards were higher than the average for the mix. One aspect of enrichment is that it also requires reduction in the contribution of other species (to avoid reduced flowering success through competition), so enrichment with the wrong species can reduce floral reward at a meadow level. The enrichment treatments I used were based on information available at the time, and can be interpreted in light of floral reward data I generated for all of the species concerned.

My floral resource data show that generally I enriched for the right species (i.e. those with the highest nectar rewards per floral unit), given the premise of my enrichment treatment. In the Marmalade mix I enriched with the top 2 species (*R. hirta* and *G. segetum*), in the Short mix I enriched with the top species (*C. cyanus*) but also the 2nd bottom species (*Iberis umbellata*), and in

the Cornfield mix I enriched with the 2nd and 3rd ranked species (*G. segetum* and *Echium vulgare*). However, my treatment also reduced the abundance by seed of the top-ranked species in the Cornfield (*C. cyanus*) and the 2nd top species in the Short mix (*C. tinctoria*). Given quantitative reward data, of the type I have now generated, I would have altered my enrichment treatments to increase the proportion of *C. tinctoria* in the Short mix and *C. cyanus* in the Cornfield mix.

While I enriched for highly rewarding species, I found no evidence that this increased nectar sugar at the meadow level (Table A5.2.1). There are a number of possible reasons for this result.

Firstly, my sampled meadows may have been too small to detect a small but otherwise consistent effect of enrichment (a false negative, or Type 2 error). This could be addressed by working with larger meadow replicates, and/or larger numbers of sample quadrats (as explored by Hicks *et al.* 2016).

Second, manipulation of the proportional seed weights of species in seed mixes may not increase the product of resources per floral unit and floral abundance, due to combined effects of seed germination success (which is likely highly stochastic) and plant performance given the meadow location and potential competition with contaminant species.

Thirdly, reward contributions by unintended contaminants, which included highly rewarding species such as *C. cyanus*, may have swamped any effects of enriched species, and hence compromised the internal validity of treatments (Campbell & Stanley 1963; Brewer & Crano 2014; Chapter 3). Given that contamination reduced differences in meadow floral composition between seed mix types, whilst also often increasing variation within seed mix types (see Chapter 3), contamination both homogenised estimates of

floral reward provision between mix types and reduced the power to detect an effects of enrichment within mix types.

Fourthly, and in addition to the effects of contamination, this experiment was not designed using a formal power analysis and likely does not have the replication necessary for the detection of an effect of enrichment. This experiment had only four replicate blocks, which were surveyed at only 3 time-points, yielding a dataset with only 72 data points. Given the experiment and sampling design, models were specified to account for variation associated with seed mix type, mix formulation, block and survey round. Despite fitting block as a random effect to save degrees of freedom, all models were likely overfitted for the dataset. Hence, regardless of treatment validity issues, model parameter estimates and their standard errors – as well as model predictions - may not be representative of general population level patterns.

Thus, although there was no evidence for an effect of seed mix enrichment on the floral abundance of enriched species, or on total nectar sugar mass provision, this experiment does not provide a comprehensive test of this intervention. It is likely that enrichment of seed mixes for a relatively competitive species would result in a detectable increase its floral abundance in a large meadow planting, covering several hundred square metres. Nevertheless, focussing on flower shapes, floral reward traits and flowering phenologies of individual floral species, using judiciously selected combinations to ensure floral rewards are available from a range of sources over a long season, is likely to be a more powerful approach to improving floral resource provision in meadows.

Floral resource provision at a meadow scale

As with previous studies, floral resources in all meadows rose and fell through the season (Figs. 5.4b and 5.5b; Hicks *et al.* 2016) and varied

substantially between replicates meadows of the same seed mix (Figs. 5.4a and 5.5a), with most floral rewards at any given time provided by a small subset of species (Fig. A5.3; Hicks *et al.* 2016). Although no amenity grassland controls were incorporated into this study (since blocks were located in either long-term pasture or horticultural trial beds), all meadows provided hundreds of floral units per m^2, equivalent to at least an order of magnitude more floral units than amenity grassland (Blackmore & Goulson 2014), even under relaxed mowing regimes (Garbuzov *et al.* 2015). Nectar and pollen reward provision per unit area were also two-orders of magnitude higher for all mix types than mean estimates for amenity grasslands surveyed across Britain using the same methodology (Hicks *et al.* 2016).

My estimates of floral nectar rewards for all mix types were an order of magnitude higher per unit area than previous estimates for annual meadows (Table 5.5; Hicks *et al.* 2016). Previous work found perennial meadows to produce much higher sugar rewards than annual meadows, and my annual meadow data are closer to these perennial values (with Marmalade and Short mix meadows providing respectively 84% and 83% of the average sugar mass provided by a perennial seed mix in Hicks *et al.* 2016). My estimates of pollen rewards per-unit area/round were of the same order of magnitude as previous estimates for both annual and perennial meadows (Table 5.5; Hicks *et al.* 2016). As highlighted at the start of this Discussion, many factors are likely to influence the resources produced by a given seed mix, and further studies are required to tease apart the reasons for variation in floral rewards between meadows and replicates. However, it is clear that planted meadows produce substantially more nectar and pollen than amenity grassland.

Table 5.5: Comparison of the estimated floral resources provided per m^2 by planted meadows and amenity grassland from this and several recent studies. Hicks *et al.* (2016) quantified floral resources for 4 treatments comprising an annual mix sown for one or two years, a perennial mix and an amenity grassland control. Each treatment was surveyed in the second year of the experiment, with 'year one' annuals only sown in the second year. Blackmore *et al.* (2014) surveyed planted meadows grown from a mix comprising annuals and perennials, with surveys in the same year for meadows and controls in their first (year one) or second year (year two). Garbuzov *et al.* (2015) surveyed amenity grasslands subject to different intensities of management. All values are means per m^2 per survey. Hicks *et al.* did not publish floral density data.

Seed mix type / ground cover / management type	Data origin	Floral units (m^2)	Nectar rewards ($mg/m^2/day$)	Pollen rewards ($ml/m^2/day$)
Marmalade annual	This study	184.5	56.88	0.1252
Short annual	This study	183.5	55.88	0.0904
Cornfield annual	This study	230.1	36.88	0.1302
Annuals (year one)	Hicks *et al.* 2016	-	10.82	0.0327
Annuals (year two)	Hicks *et al.* 2016	-	10.59	0.0287
Perennials (year two)	Hicks *et al.* 2016	-	67.55	0.0549
Amenity grass (year two)	Hicks *et al.* 2016	-	0.49	0.0005
Sown mix (year one)	Blackmore *et al.* 2014	70	-	-
Sown mix (year two)	Blackmore *et al.* 2014	95	-	-
Control (year one)	Blackmore *et al.* 2014	1	-	-
Control (year two)	Blackmore *et al.* 2014	12	-	-
Regular mowing	Garbuzov *et al.* 2015	5.5	-	-
Mowing until July	Garbuzov *et al.* 2015	8.8	-	-
Mowing until June	Garbuzov *et al.* 2015	9.7	-	-
No mowing	Garbuzov *et al.* 2015	16.9	-	-

The extent to which reward values can ever be attributed to a specific mix depends on the contribution of non-mix (contaminant and weed) species, and these will be a variable feature of resulting meadows wherever a given seed mix is sown. In my meadows, contaminants varied in impact between mixes. For example, contaminants contributed on average 36% of nectar rewards and 34% of pollen volume to Marmalade meadows, but only 2% and 9% respectively to Short mix meadows (Table 5.1). Though some weeds produced high nectar rewards per floral unit (Fig. 5.1), these were generally too rare to substantially influence meadow level floral rewards. In contrast, those weeds contributing many floral units tended to have small singleton flowers providing low levels of reward per floral unit, and so

contributed low absolute quantities of nectar or pollen at the meadow level (Fig. A5.3).

5.4.3 Objective 5.2 and 5.3: on patterns and drivers of insect visitation to meadows and individual floral species

Patterns and drivers of visitation to meadows

Just as the floral composition of the meadow that results from sowing a given seed mix cannot be fully predicted from the seed mix alone (Chapter 3), the composition of a flower visitor assemblage in a meadow cannot be predicted from a seed mix. However, in principle, it would be possible to define an associated flower-visitor assemblage probabilistically by sampling over many replicate plots incorporating environmental variation and natural variation in unintended contaminants /weeds (which will both affect meadow floral composition). In this study, there were high levels of inter-annual floral contamination between seed mix treatments due to repetition of the same experiment twice over two years, but without specific fixed plots per treatment (Chapter 3). As a result patterns of visitation at a community scale are biased due to low treatment validity (Campbell & Stanley 1963; Brewer & Crano 2014), with meadow floral compositions often unrepresentative of underlying seed mix treatments (Chapter 3). Although most flower visits in each mix type were to treatment species, a large proportion of insect visits, especially by bumblebees and hoverflies, were to contaminants in Marmalade and Short mix meadows. Hence, visitor abundance, richness and diversity cannot be reliably compared between mix types. Even in the absence of contamination, the experimental design used for this study would not have provided the power required to statistically compare meadow level patterns of visitation (see Methods). Nevertheless, it was possible to examine whether floral resource levels predicted insect visitation rates and to identify plants that are visited by particular visitor taxa.

Which species are most visited in planted meadows?

Even to a casual observer, some species in my planted meadows were visited at high frequency, particularly Cornflower (*C. cyanus*), Scentless mayweed (*Tripleurospermum inodorum*) and Field marigold (*Glebionis segetum*). Almost all bumblebee visits were to *C. cyanus* (whether present as a mix or contaminant species). *C. cyanus* was one of four species to provide high levels of both nectar and pollen (along with *Rudbeckia hirta*, *Coreopsis tinctoria* and *G. segetum*), but was much more highly visited, especially by bumblebees and honeybees. In the absence of contaminants from this species, very few bumblebees would have been recorded in meadows of Marmalade mixes, suggesting that the Marmalade mix provides little floral rewards suitable for large long-tongued insects compared to Short and Cornfield mixes. As a counter example, *Dimorphotheca sinuata* (a South African daisy) was abundant in meadows and provided pollen and nectar rewards, but was rarely visited by insects. Field observations suggest that pollen was rarely fully released from florets in this species, perhaps because pollen release is controlled by temperature or humidity cues that are not available in urban Sheffield. These examples show that floral rewards alone do not necessarily predict visitation frequency given the pool of available visitors, implying that other floral traits, such as reward accessibility (e.g. corolla shape; Ranta & Lundberg 1980a; Harder 1985) or reward characteristics (e.g. nectar concentration/volume; Harder 1986; Kim *et al.* 2011) influence visitor preferences. This suggests that 'bottom-up' design, based on known pollinator preferences, can be incorporated into the design of meadow seed mixes.

Although weeds can contribute large numbers of floral units to planted meadows, they were visited rarely relative to their abundance in my surveys. However, given that small flower-visiting insects (such as small dipteran visitors) may be under-sampled (due to the higher likelihood of

observing larger insects during surveys, the value of weeds to flower-visitors is likely underestimated.

Do meadow resource levels predict insect visitation rates?

An important question in meadow seed mix design is whether one can increase the value of meadows for pollinators (as indicated by visitation rates) by increasing the nectar sugar and/or pollen volume provided by constituent plant species. The three measures of resources I used (floral unit counts, total nectar sugar mass, total pollen volume) encompass different trophic resource types (i.e. nectar sugar *versus* pollen protein) and investments of investigator time (floral unit counts, *versus* additional quantification and scaling of these counts by resource values per floral unit). These considerations were encompassed in my approach and analyses.

I found that visitation rates for bumblebees were positively correlated with floral units and nectar sugar, whilst female hoverflies were positively correlated with nectar sugar and pollen volume. However, for both of these taxa, there was no qualitative difference in the proportion of variation explained by either model (as measured by R^2 values). For bumblebees, this pattern likely resulted from the fact that they mainly visited a single species (*Centaurea cyanus* – as detailed below) which was both highly abundant and a high nectar but not pollen producer. For female hoverflies, this pattern likely resulted from the fact that most hoverflies visited a subset of species in family Asteraceae, which each provided high quantities of both nectar and pollen. Although these patterns are concordant with *a priori* predictions that bumblebees (as large-bodied central place foragers) will be more strongly dependent on nectar sugar rewards than hoverflies, this approach cannot demonstrate that rewards cause patterns of visitation, which requires a study in which visitation surveys are performed on sets of floral species with consistent flower shapes but variable floral rewards (e.g. Fowler *et al.* 2016).

5.5 Conclusions

This study contributes to a small but growing literature on the amount of nectar and pollen rewards provided by individual species and meadows grown from flower seed mixes. Furthermore, this is the first study to compare the floral rewards and flower-visitor assemblages of meadows grown from different annual pictorial flower seed mixes. Results show that floral species vary substantially in the amount of pollen and nectar they provide, and in their attractiveness to flower-visitors. Hence, evidence-based seed mix design should use a 'bottom up' strategy, incorporating knowledge of floral species traits (such as flowering phenology), and the floral preferences of different groups of pollinators, into plant species selection processes. This study also found that most bumblebees and honeybees visited *Centaurea cyanus*, which was only present in Marmalade mixes as a contaminant. Thus, the choice of which seed mix to plant can have important consequences for specific groups of flower-visitors, and this should be considered along with aesthetic considerations during seed mix choice.

Chapter 6: General Discussion

6.1 Overview

Urban parks and green spaces are often planted with pictorial meadows flower seed mixes to enhance their aesthetic amenity value and the resources they provide for flowering-visiting insects (Hitchmough 2011; Bretzel *et al*. 2016; Gill et al. 2016). However, there is little quantitative data available to guide the design or choice of meadow seed mixes that provide high quantities of pollen and nectar for a diversity of insect species (but see Baude *et al*. 2016; Hicks *et al*. 2016). Hence, the design and use of pictorial mixes has tended to focus on their aesthetic benefits, with little consideration given to variation in the floral resources and flower-visitor assemblages of different plant species/cultivars.

The aim of my thesis was therefore to improve our understanding of the floral resources provided by pictorial meadows for flower-visiting insects, by: (i) quantifying the floral resources provided by meadows of three commercially-available, exemplar pictorial meadows mixes; (ii) testing whether floral resource provision can be improved, whilst maintaining aesthetic designs, by enriching seed mixes for species producing high quantities of nectar; and (iii) quantifying patterns of insect visitation to meadows of these standard commercial and nectar-enriched seed mixes. I also (iv) used DNA barcoding to corroborate/correct morphology-based insect identifications, testing whether use of molecular information changes our perceptions of flower-visitor richness and the structure of flower-visitor interaction networks. In this Discussion, I briefly summarise my key findings and consider their implications for seed mix design and use, whilst highlighting valuable avenues for future research.

6.2 Key findings

6.2.1 Chapter 3

In Chapter 3, I examined the flowering performance of sown seed mixes. I found that seed mixes sown grew into flower-rich meadows, but that floral composition was highly variable, with meadows of different seed mix types often more similar in floral composition than replicates of the same mix type (Chapter 3). This was largely due to the widespread presence of numerous floral units of unintended contaminant species. Contamination mainly affected meadows of Marmalade and Short mixes, since the two most abundant contaminants *T. inodorum* and *C. cyanus* were unintended components of Marmalade (both species) and Short mix meadows (*T. inodorum* only). These species likely germinated from the soil seed bank either from seed that survived from 2012 (Chapter 2) or -for *T. inodorum* - from a naturally-occurring weed seed bank. Finally, there was no evidence for an effect of enrichment on the floral abundance of enriched species, although this experiment likely provides an unreliable test of this intervention (as discussed below).

6.2.2 Chapter 4

In Chapter 4, I examined the effect of DNA barcoding on our perceptions of flower-visitor assemblage composition and flower-visitor network structure. I found that most morphotaxa from a well-described insect fauna were not changed by molecular taxonomic analysis. However, splitting and/or lumping processes affected almost one third of morphotaxa, which collectively comprised most individuals, although the distribution of individuals across these morphotaxa was highly skewed. The predominant change was lumping of morphotaxa, which appeared mainly to: unite males and females from sexually dimorphic species; reallocate specimens between

morphologically cryptic species; and clarify misidentifications; although further taxonomic and molecular analysis is required to fully assess the accuracy of such changes. These DNA barcode-based changes to visitor taxonomy resulted in consistent minor changes in network size and structure across replicate networks. Lumping of morphotaxa decreased taxon richness, reducing the number of unique links and interaction diversity (the effective number of links). Lumping also increased flower-visitor generality, reducing plant vulnerability and increasing overall network connectance. However, taxonomic changes had no effect on interaction evenness or network specialisation. Thus, for this well-studied fauna, DNA barcode-based flower-visitor networks were systematically biased toward fewer taxa and links, with more generalist visitors and specialist plants.

6.2.3 Chapter 5

In Chapter 5, I quantified per-floral unit pollen and nectar rewards for each plant species, and examined the impacts of seed mix type and composition on floral resources and flower-visitor assemblages.

At a species level, both nectar and pollen rewards per floral unit varied substantially among species. For nectar, the top 4 ranked sown species were all species Asteraceae, which also ranked highly for pollen rewards. Weeds provided little pollen but the top weed species for nectar were among the top nectar producers in meadows. Much of the variation in rewards appeared to be related to floral unit size, with species with small floral units providing comparatively little nectar or pollen compared to those with large floral units.

At a meadow level, floral nectar and pollen rewards often varied across mix types and survey rounds (Figs. 5.4 & 5.5). Most of the floral resources available from the meadow are provided by a small subset of species. The high contributions of *C. cyanus* and *T. inodorum* to nectar and pollen rewards respectively in meadows of Marmalade mixes make direct comparisons between seed mix types difficult. However, excluding contaminants, Cornfield mix meadows appear to provide more nectar than Marmalade and more pollen than Marmalade and Short mix meadows early the season (round 1), but less nectar and pollen than Marmalade and Short mix meadows later in the season, as late-flowering non-native species begin to bloom. The enrichment treatment did not increase floral nectar sugar mass in meadows of enriched versus standard seed mixes (Table A5.2.1).

For flower-visitor assemblages, there were not qualitative differences in visitor abundance, diversity or richness between mix types, although meadow scale patterns were confounded by contaminants, which were frequently visited. Bumblebee abundance was positively correlated with the floral abundance of sown (treatment and contaminant) species and total nectar sugar mass, but not with total pollen volume (Table 5.3). In contrast, female hoverfly abundance was positively correlated with total pollen volume and total nectar sugar mass, but not with floral abundance (Table 5.4). However, the proportion of variance explained (marginal and conditional R^2 values) did not differ qualitatively between models for either bumblebees or hoverflies (Tables 5.3 & 5.4).

For individual plant species, patterns of visitation were highly skewed, with 4 abundant species (*Tripleurospermum inodorum*, *Centaurea cyanus*, *Glebionis segetum* and *Coreopsis tinctoria*) receiving most flower-visits.

Most bumblebees and honeybees visited *C. cyanus*, whilst most solitary bees visited *T. inodorum*. Most hoverfly visits were to just 5 species: *C. cyanus*, *T. inodorum*, *G. segetum*, *C. tinctoria* and *Eschscholzia californica* (Fig. 5.9). However, *C. cyanus* and *G. segetum* were the only species that appeared to be visited preferentially given their abundance in meadows.

6.3 Growing meadows from seed

Previous studies have shown that sowing flower seed mixes can be an effective method of increasing floral abundance and richness in urban parks and greenspaces (e.g. Hitchmough & Woudstra 1999; Hitchmough 2000; Hitchmough *et al.* 2003; Blackmore & Goulson 2014; Hicks *et al.* 2016). Pictorial seed mixes are often favoured because they are designed using careful plant selection to provide a reliable floral display (Hitchmough 2004a, 2004b, 2008, 2011) – although as I found during this research even these will fail under extreme conditions, such as the high levels of rainfall that occurred during the first iteration of my experiment in summer 2012 (Chapter 2). When these seed mixes are sown the floral composition of the resulting meadow is determined by multiple interacting processes, including (a) seed mix composition, (b) soil seed bank composition, and (c) the many factors that influence germination, growth and flowering of each seed mix/bank species (see Chapter 3 and Aldrich 2002; Glover 2014; Long *et al.* 2015).

For seed mix users, the most direct ways to influence the character of meadows is through the choice of which seed mix to sow (discussed below) and through appropriate ground preparations prior to sowing. These typically include the initial removal of vegetation/turf grass, followed by ground rotovation and – once seeds from the seed bank have germinated – one or two herbicide applications (Prentis & Norton 1992; Highways Agency

1993; Aldrich 2002). On the day of sowing, the ground should also be raked to a fine tilth to enhance seed-soil contact and air and water infiltration (Prentis & Norton 1992; Highways Agency 1993; Aldrich 2002). Although these measures are effective and cost-effective (compared to alternatives approaches such as mulching), weeds and unintended contaminant species, such as garden escapes, are likely to occur regardless of these preparations and whenever flower seed mixes are sown (Haigh 1980; Baker 1989; Thompson *et al.* 2005; Albrecht *et al.* 2011).

In my experiment, all of these preparations were undertaken, yet weeds and contaminants were widespread and abundant across replicate meadows (Chapter 3). Contaminants in 2013 likely originated from seed sown in 2012, which germinated from the seed bank. From the point of view of experimentation, this had the important consequence of undermining the internal validity of seed mix treatments, reducing my ability to directly attribute community scale patterns to the seed mix treatments (Campbell & Stanley 1963; Brewer & Crano 2014). One approach to avoiding similar issues in future studies would be to apply sterile soil mulch to plots to suppress germination of weeds and contaminants (Aldrich *et al.* 2002). Although an effective mulch (of an appropriate depth) would improve the internal validity of treatments, the materials, transport and labour required for mulching are expensive, therefore the external validity of treatments (the extent to which results can be generalised) will be substantially reduced. Hence, a better approach for future studies would be to mark out permanent plots so that the same seed mix can be applied to the same plot in experiments lasting multiple years (see below). Although some weeds and inter-annual contaminants would likely survive ground preparations, this approach would prevent cross-contamination between treatments, thereby

enhancing the internal validity of treatments whilst maintaining high external validity.

From a horticultural point of view, suppression of contaminants and weeds is desirable to reduce competition and enhance establishment of sown species. Recent studies have shown that people prefer colourful urban plantings with high flower cover (Hoyle *et al.* 2017) and some structural diversity (i.e. meadows that are tall or medium in height; Southon *et al.* 2017). Hence, the extent to which contaminants and weeds disrupt the intended aesthetic character of a meadow will depend on their density and aesthetic character (colour, size) in relation to sown species. Given that, from a flower-visitors perspective, it is the floral resources rather than origins of contaminants or weeds that are important, these species should be considered valuable providers of floral rewards in planted meadows (Hicks *et al.* 2016).

6.4 Limitations and possible improvements to experimentation

In this thesis, I designed an experiment to compare traits of replicate meadows grown from seed mix treatments. There are several limitations to my approach, which stem from resource constraints and horticultural and statistical naivety at the outset of this project, but from which lessons may be garnered to improve future studies of planted meadows.

Firstly, I did not include controls in this experiment. This was motivated by the observation that blocks were not located in amenity grassland, but in an array of different habitats, including horticultural trial beds, waste ground and former pasture. I therefore judged that controls (regardless of whether they were defined as pre- or post-ground

preparations) were not of intrinsic interest. However, I neglected to consider that controls enable quantification of the contribution of soil seed bank species to meadows, which would have enabled more accurate assessment of the extent of contamination by *T. inodorum* cultivars versus germination of the wild type as a weed (Chapter 3). Future studies can increase the external validity of treatments by planting meadows in amenity grasslands and should incorporate controls to fully discern environmental and treatment effects.

Secondly, I attempted to enrich seed mixes for particular species (with the actual amendments designed by Pictorial Meadows Ltd.) without an expectation of the effect size that particular changes in seed mixes would have on floral abundance in meadows. The ability to detect an effect of enrichment will depend on multiple factors, including the degree of enrichment (% change in seed weight), the average effect size of this change on floral abundance, the scale of plantings/sampling intensity, the degree of variability in germination/growth/flowering associated with environmental variation and the number of replicates per treatment (discussed below). In principle, a calibration could be performed to quantify effect sizes for different degrees of change in percentage seed weight. However, in practice, given that my enrichment treatment involved manipulating multiple species, this would require testing a multidimensional array of mixes, testing different combinations of change for different species in microcosms to account for interactions between species (Köppler & Hitchmough 2015). These experiments would be complex, time-consuming and subject to effects of scale and sampling intensity. Hence, enrichment of existing seed mixes, whilst a valid horticultural intervention, is difficult to reliably test for multiple species in multiple mixes. In the event, my experiment showed no

evidence for an effect of enrichment, but this was an unreliable test of the intervention (see Chapters 3 and 5). Future studies of flower meadows should avoid the complexity of manipulating commercial seed mixes, focusing instead on robustly comparing different types of seed mixes. Given that few people have explicitly compared the floral rewards and flower-visitors of different urban/pictorial meadow seed mixes, a simple experiment comparing meadows of different seed mix types would yield more robust and high impact science.

Thirdly, and related to the point above, I planted seed mixes using a randomised complex block design, without performing a formal power analysis, but rather allowed practical limitations of land and labour to guide experiment and sampling design. As consequence of land, labour and weather-related constraints, this experiment had only 4 replicates per treatment (with 2 others destroyed due to horticultural issues; Chapter 2), which were each surveyed 3 times, yielding a dataset with only 72 data points. Given the experiment and sampling design, models were specified to account for variation associated with seed mix type, mix formulation, block and survey round. Despite fitting block as a random effect to save degrees of freedom, all models were likely overfitted for the dataset. Hence, regardless of treatment validity issues, model parameter estimates and their standard errors – as well as model predictions - may not be representative of general population level patterns. Moreover, this number and spacing of surveys was not enough to accurately characterise seasonal trends in flower-visitors or floral resources. Future studies would benefit from the early consultation of an experienced statistician familiar agricultural experiments and a formal power analysis. This study, and other recent studies (Blackmore & Goulson 2014; Hicks *et al.* 2016), provide a useful starting point for future power

analyses by providing approximate estimates of treatment effect sizes and variability, which can inform estimation of the spatial and temporal replication required to robustly test hypotheses.

Fourthly, and as described above, this study was not explicitly planned as a multi-year experiment assessing inter-annual variation in flower seed mix treatments. However, inter-annual variation in meadow composition is likely to be substantial. For example, regardless of contamination or the potential for accumulation of weeds over successive years, there was a marked contrast in the floral composition of the subset of field plots that flowered in 2012 and those that flowered in 2013. In particular, floral units of *Linaria maroccana* were evenly and densely distributed throughout meadows of Short mixes in 2012, whilst in 2013 they were comparatively rare and distributed patchily (personal observation). Future studies would benefit from marking blocks and field plots so that seed mix treatments can be applied to the same plots over multiple years. In this study, blocks were rotovated on mass, plots/blocks were not located in precisely the same place, and the sowing layout was randomised in both the first and second years. This resulted in extensive floral species contamination, which substantially affected the inferences that can be drawn from this study (as discussed above).

6.5 Quantification of floral resources / the attractiveness of flower species to visitors

The absolute and relative values of different floral species for a given visitor taxon are difficult to quantity, and these will vary for different floral species across visitor taxa. For example, even for two floral species with similar floral shapes (accessibilities) providing rewards with similar characteristics (e.g. nectar volume/concentration combinations), their absolute and relative values to visitors in a meadow may depend on their densities (which affects the balance between use and gain of resources (Ishii *et al.* 2008; Dauber *et al.* 2010) and flowering phenologies (since a species flowering when other resources are scarce will be more valuable to insect visitors). Nevertheless, understanding the relative value of different floral species and mixes for different visitor taxa is an important part of improving floral resource provision for flower-visitors through evidence-based meadow seed mix design and choice.

In this thesis, I explored the relative value of different floral species and seed mixes for flower-visiting insects in terms of their daily floral nectar and pollen rewards. For this I quantified the floral rewards provided by each floral species in terms of the daily floral nectar sugar and pollen volume they produced per floral unit, using methods increasingly used for community level floral resource quantification (Baude *et al.* 2016; Hicks *et al.* 2016). This approach is advantageous since it enables quantification of rewards per floral unit per species in units that are tractable to measure and comparable between species with diverse floral morphologies. For example, quantification of pollen volume is more tractable than pollen mass, since it requires less pollen (and hence fewer samples) and can be quantified for species providing very little pollen. Similarly, quantification of daily nectar

rewards controls for high diurnal variation in nectar sugar standing crop (Corbet 2003), providing a standardised measure of nectar provision which can be compared across species. Moreover, these measures can be combined with estimates of floral unit density per species in habitats to quantify daily floral reward provision at a meadow (Hicks *et al.* 2016) or landscape scale (Baude *et al.* 2016).

Using this approach, I found that pollen and nectar rewards per floral unit varied substantially among species, with relatively few species providing both high nectar and pollen rewards (Chapter 5). At a meadow scale, floral rewards varied across mix types and survey rounds, but patterns were confounded by the large contribution from contaminants. As with previous studies, there was considerable spatial variation in resources between replicate meadows, and most resources were provided by a subset of species at any given time of during the season (Hicks *et al.* 2016).

This approach also has a number of important limitations. Firstly, whilst nectar sugar is a directly metabolised nutritional reward sought after by flower-visitors, there is no direct link between pollen volume and pollen nutritional value. Although there is a strong correlation across species between pollen volume and pollen mass (Roulston *et al.* 2000), the proportional protein content and amino acid profiles of pollen varies between species (Roulston *et al.* 2000; Roulston & Cane 2002) Pollen volume therefore provides a tractable but crude measure for comparing pollen resources between species and mixes.

Secondly, differences in patterns of visitation between floral species are often due to differences in characteristics other than daily nectar sugar mass. For example, patterns of visitation are often determined by a

combination of flower shape (corolla depth) and nectar concentration and volume, since both flower shape (Ranta & Lundberg 1980a; Harder 1985; Gilbert 1981; Branquart & Hemptinne 2000) and nectar volume and concentration can affect reward accessibility and foraging efficiency (Harder 1986; Kim *et al.* 2011). This likely explains why most bumblebees and honeybees visited *Centaurea cyanus*, even though its per-floral unit floral nectar sugar mass rewards were unexceptional compared to other species of Asteraceae (Fig. 5.3). In contrast, pollen is usually more widely accessible than nectar, since anthers are usually located towards the mouth of a flower (Corbet 2006; Willmer 2011).

Thus, although the measures of floral nectar and pollen rewards used in this thesis can be compared between species and scaled to a community level, they do not account for important traits that affect the value of plants as forage resources for different groups of flower visitors. Although the methods can be used to quantify and compare floral nectar sugar and pollen rewards between meadows and treatments, these values are not necessarily meaningful when considering flower-visitor ecology or seed mix design. For example, a meadow comprised of a single floral species providing lots of nectar may provide more sugar in absolute terms than a diverse meadow containing more nectar volume/concentration combinations, although the latter is likely to support a higher functional diversity of flower-visiting insects (e.g. Potts *et al.* 2003; Potts *et al.* 2004).

For future studies, a more useful approach to exploring floral resource provision at a meadow scale might be to quantify daily floral nectar and pollen rewards using the methods described above (or by quantifying pollen protein content for pollen rewards), but with floral species (and hence

meadow scale rewards) divided into functional types based on an appropriate criterion, such as flower shape (Corbet 2006; Willmer 2011) or nectar holder depth (Stang *et al.* 2006). This would enable more realistic quantification of the floral resources available to different groups of visitors. However, this assumes that appropriate functional types can be defined, and still requires time-consuming quantification of floral nectar and pollen rewards using techniques that would be difficult for horticultural practitioners to implement, given the equipment required.

Given the limitations described above a more useful approach for improving seed mix design may be to focus on individual species and better quantify their floral traits (such as flower shape, nectar concentration/volume and flowering phenology) and flower-visitor assemblages. This information could then be used to judiciously select combinations of species to ensure floral rewards with different characteristics are available throughout the season. The challenge is to ensure that estimates are comparable between species. Future studies could examine whether it is possible to design an insect visitation assay that horticultural practitioners could use to test individual seed mix species (or new candidate species/cultivars) for their flower-visitor assemblages. For example, Fijen and Kleijn (2017) examined the relationship between observation duration and the accuracy of estimates of visitation rates in leek-seed production fields, by collecting a large dataset over six day-long observations and calculating the 'minimum observation period' required to obtain an accurate estimate of the full visitation rate, under a variety of conditions (e.g. different weather, times of day, different days, different fields, etc.). They concluded that reliable estimates of visitation rates required observations on multiple days, but recommended that accurate estimates of visitation rates could be obtained more consistently

by standardising sampling effort to a standard number of visitor observations (which can then be used to calculate visitors/time; Fijen & Kleijn 2017). In a horticultural context, a similar approach might explore how to develop a standardised protocol that could compare visitation rates and visitor assemblage compositions (for broad functional types) between candidate floral species. Given that horticulturalists and the designers of urban/pictorial meadows are often trialling new species and cultivars, whilst many horticulturalists acknowledge that future horticultural practice will require greater understanding of individual plant species (Köppler & Hitchmough 2015) and ecosystem service contributions (Cameron & Blanusa 2016), a simple assay of visitation rates may be valuable for seed mix designers. Although these rates would not translate directly to mixes, since meadows would rarely contain monocultural species patches, this approach could provide useful information on which plants are visited by broad functional groups such as bumblebees, honeybees, solitary bees, or flies.

6.6 Methods for the identification of flower-visiting insects and their network consequences.

Reliable and informative flower-visitor networks are underpinned by appropriate sampling regimes and accurate species identifications. However, whilst previous studies have addressed the network consequences of alternative sampling methods (Gibson *et al.* 2011) and intensities (Hegland *et al.* 2010), as well as natural temporal variation in flower-visitor communities (see Burkle & Alarcon 2011 and references therein), the effects of errors or biases caused by the taxonomic impediment on perceptions of flower-visitor networks are heretofore unexamined. In this thesis, I assessed the effect of using molecular markers to revise morphology-based taxon identifications on the structure of flower-visitor networks (Chapter 4). I found that MOTU-

based networks were systematically biased towards fewer taxa and links, with more generalist visitors and more specialist plants, and higher overall connectance (Chapter 4). Taxonomic changes will have improved the accuracy of specimen identifications and estimates of network metrics if the sequenced species are monophyletic at the barcode locus and intra-specific variation is lower than inter-specific variation. However, flower-visitor assemblages comprise a diversity of taxa, which vary in their recent evolutionary histories and the degree to which they have been studied taxonomically, therefore for many groups further taxonomic and genetic investigation is required to determine whether changes represent an increase or decrease in taxonomic accuracy. Nevertheless, many taxonomic changes, such as lumping of separate sexually dimorphic male and female morphotaxa or reallocated misidentifications of *Bombus terrestris*/*B. lucorum*, are likely to represent improvements to taxonomic accuracy.

Network metrics in some cases differed between morphological and MOTU-based networks by a magnitude lower than but comparable to differences recorded between habitats in two ecological studies. Thus, there were significant biases between networks constructed using morphology versus those constructed using molecular information. It is not clear which set of networks is most accurate, but it is likely that each contains errors and biases which affect different taxa depending on the method of taxon identification. Hence, discounting the proportion of changes to visitor taxonomy that represent improvements in taxonomic accuracy, we may regard estimates of network structure based on MOTU-identifications to be about as accurate as those based on morphological identifications – suggesting that past studies based on morphology and future studies based on DNA barcoding may be comparable. However, this study focussed on

flower-visitor communities of a comparatively well-described and low diversity fauna. We might expect impacts of DNA barcoding to be more pronounced in highly diverse and less well-described tropical faunas, where species level identifications are inherently more difficult. In contrast to patterns reported here, more diverse and less well-studied fauna may yield a dominant splitting signature (e.g. Hebert *et al.* 2004; Smith *et al.* 2006). However, the direction and magnitude will depend on the type of change (splitting vs. lumping), whether affected taxa differ in the spectrum of floral partners they visit, and their relative abundances. Given these contingencies the current paucity of studies precludes generalisation of the impact of DNA barcoding on estimates of flower-visitor network structure. Further studies in both tropical and temperate faunas are required to fully assess the impact of taxon identification methods on our perceptions of species richness and flower-visitor network structure.

Although sequencing costs have declined over recent decades, the cost of Sanger sequencing has stabilised at around US$ 5.00 per animal specimen (for PCR, purification and sequencing; Cameron *et al.* 2006) and US$ 3.00–7.50 per plant specimen (De Mattia *et al.* 2012). These costs are between 1.7 and 3.4 times the unit cost of traditional morphological identification, but costs may rise to as much as 10 times morphological taxonomy if sequencing fails and significant resequencing is required (Stein *et al.* 2014). Although affordable for small samples, these costs are likely to prevent use of DNA barcoding as a default identification method in ecological studies until or unless costs decline further. Nevertheless, as DNA barcoding can provide higher taxonomic resolution with faster sample processing times, whilst shifting the taxonomic burden from specialist taxonomic skills to more generalist laboratory skills, its use in routine ecological studies in likely to

increase in future. For resource efficiency, future studies may adopt a mixed identification strategy in which all specimens are initially identified using morphological characters, with only those groups known or suspected to comprise sexually dimorphic males and females or to contain morphologically cryptic species subsequently identified using DNA barcoding.

Given the differences in network metrics presented in this study, it is important to consider how increasing use of DNA barcoding will impact cross-comparisons of network studies. Use of either morphological or morpho-molecular identifications (or even solely MOTUs) may not change inferences when comparing networks within individual studies or systems (e.g. between different types of habitat management), although it is important that treatments and methods of identification are not confounded during study design. However, the use of different methods may have important implications when comparing across studies or systems, and may bias comparisons if the magnitude and direction of effects due to identification methodology are not taken into account.

6.7 Concluding remarks

The research outlined in this thesis has contributed to our current state of knowledge in a number of ways. Firstly, few studies have quantified the floral resources provided by annual meadows. Hence, this study contributes to a growing literature and growing resource of quantitative data on floral resources of species used in seed mixes or that grow naturally in Britain. Secondly, this thesis presents the first study to examine the impact of seed mix choice and hence composition (in terms of annual seed mix formulations, rather than annual versus perennial mixes) on the floral

resources provided for flower-visiting insect assemblages. Mixes designed on colour schemes (the Marmalade mix) may provide almost no nectar rewards for bumblebees and honeybees. Thirdly, this thesis presents the first study – to my knowledge – to compare the structure of flower-visitor networks constructed using molecular information, rather than solely traditional morphological taxonomy. Overall, this thesis shows that most annual meadows will improve floral resources over amenity grassland in urban areas, but some mixes do not provide nectar sources for species requiring large quantities of rewards, such as bumblebees. Hence, further research is required to identify suitable species and alternative management practices to enhance floral resources for the full suite of flower-visitors in urban areas.

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Appendices

Appendix A2

A2.1 Full species list with floral unit definitions

Table A2.1: A list of the species recorded in meadows of blocks A, B, D and E, along with their horticultural categories and floral unit definitions. Horticultural categories were defined based on whether species were listed as part of seed mixes or not. Floral unit definitions were defined for convenience to enable quantification of the contribution of each species to 'flowers', floral rewards and insect visitation in meadows. For species with 'single' floral units, a single botanical flower (i.e. perianth plus reproductive organs) was defined as a single floral unit. This included species with large single flowers, such as *Papaver rhoeas*. For species of Asteraceae, 'composite' disk-shaped inflorescences were defined single floral units. For Fabaceae and most Polygonaceae, single inflorescences were defined as single floral units. For Iberis umbellata and *Silene armeria*, floral units were defined as a cluster of flowers, with the average number of flowers per cluster counted and then averaged over all recorded clusters.

Species	Family	Horticultural category	Floral unit definition
Agrostemma githago	Caryophyllaceae	seed mixes	single
Centaurea cyanus	Asteraceae	seed mixes	composite
Convolvulus tricolor	Convolvulaceae	seed mixes	composite
Coreopsis tinctoria	Asteraceae	seed mixes	composite
Crepis rubra	Asteraceae	seed mixes	composite
Dimorphotheca sinuata	Asteraceae	seed mixes	composite
Echium vulgare	Boraginaceae	seed mixes	single
Eschscholzia californica	Papaveraceae	seed mixes	single
Glebionis segetum	Asteraceae	seed mixes	composite
Gypsophila elegans	Caryophyllaceae	seed mixes	single
Iberis umbellata	Brassicaceae	seed mixes	umbel
Ismelia carinata	Asteraceae	seed mixes	composite
Linaria maroccana	Plantaginaceae	seed mixes	single
Linum grandiflorum	Linaceae	seed mixes	single
Linum usitatissimum	Linaceae	seed mixes	single
Nigella damascena	Asteraceae	seed mixes	composite
Papaver rhoeas	Papaveraceae	seed mixes	single
Rudbeckia hirta	Asteraceae	seed mixes	composite
Silene armeria	Caryophyllaceae	seed mixes	cluster
Tripleurospermum inodorum	Asteraceae	seed mixes	composite
Achillea millefolium	Asteraceae	weeds	composite
Brassica rapa	Brassicaceae	weeds	single
Capsella bursa-pastoris	Brassicaceae	weeds	single
Cerastium fontanum	Caryophyllaceae	weeds	single
Cirsium arvense	Asteraceae	weeds	composite

Epilobium hirsutum	Onagraceae	weeds	single
Epilobium montanum	Onagraceae	weeds	single
Fallopia convolvulus	Polygonaceae	weeds	single
Galium aparine	Rubiaceae	weeds	single
Gilia achilleifolia	Polemoniaceae	weeds	single
Hypochaeris radicata	Asteraceae	weeds	composite
Lactuca serriola	Asteraceae	weeds	composite
Lamium album	Lamiaceae	weeds	single
Lapsana communis	Asteraceae	weeds	composite
Lepidium sativum	Brassicaceae	weeds	single
Matricaria discoidea	Asteraceae	weeds	composite
Medicago lupulina	Fabaceae	weeds	inflorescence
Myosotis arvensis	Boraginaceae	weeds	single
Persicaria lapathifolia	Polygonaceae	weeds	inflorescence
Persicaria maculosa	Polygonaceae	weeds	inflorescence
Polygonum aviculare	Polygonaceae	weeds	single
Scorzoneroides autumnalis	Asteraceae	weeds	composite
Senecio sylvaticus	Asteraceae	weeds	composite
Sinapis arvensis	Brassicaceae	weeds	single
Sisymbrium officinale	Brassicaceae	weeds	single
Sonchus asper	Asteraceae	weeds	composite
Sonchus oleraceus	Asteraceae	weeds	composite
Spergula arvensis	Caryophyllaceae	weeds	single
Stellaria media	Caryophyllaceae	weeds	single
Trifolium pratense	Fabaceae	weeds	inflorescence
Veronica persica	Plantaginaceae	weeds	single

A2.2 The extent of contamination by Glebionis segetum and Echium vulgare ‘Blue Bedder’ across replicate plots

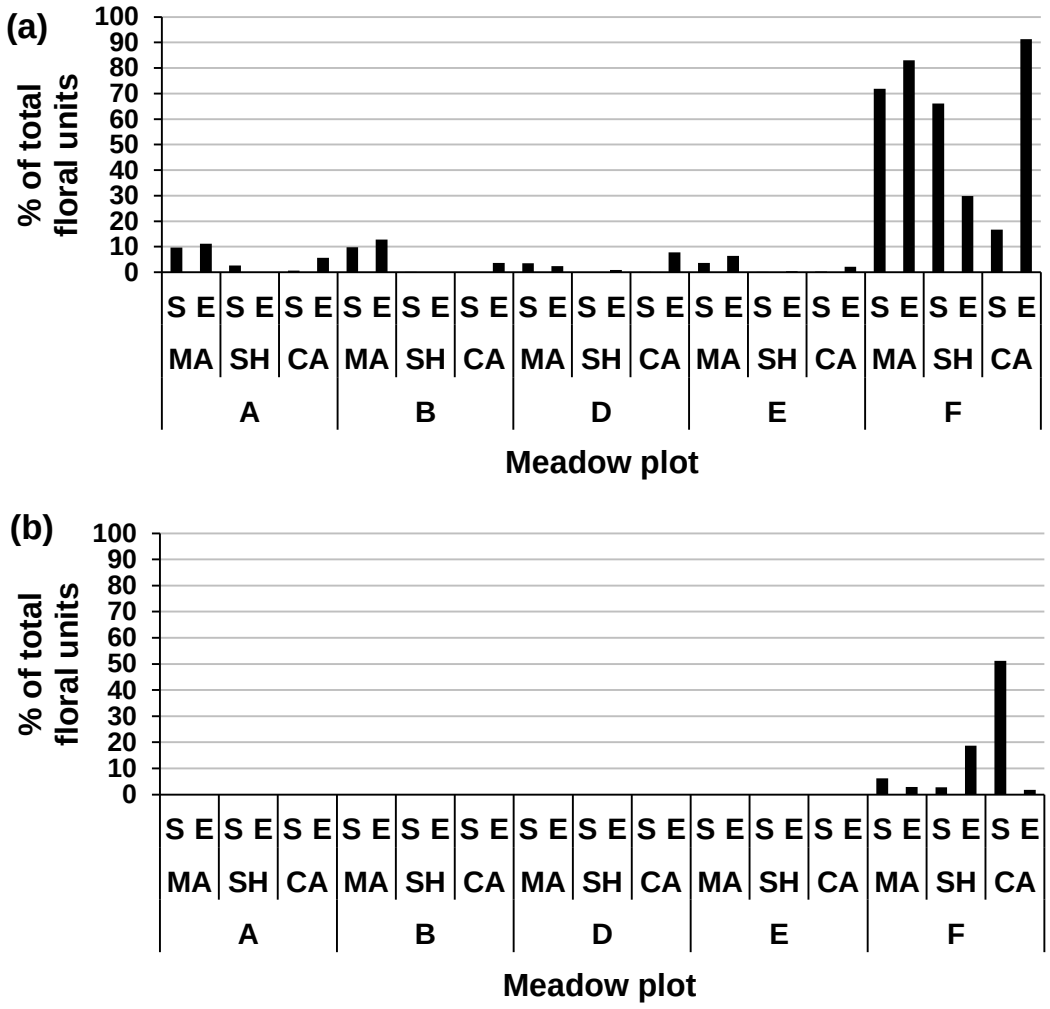
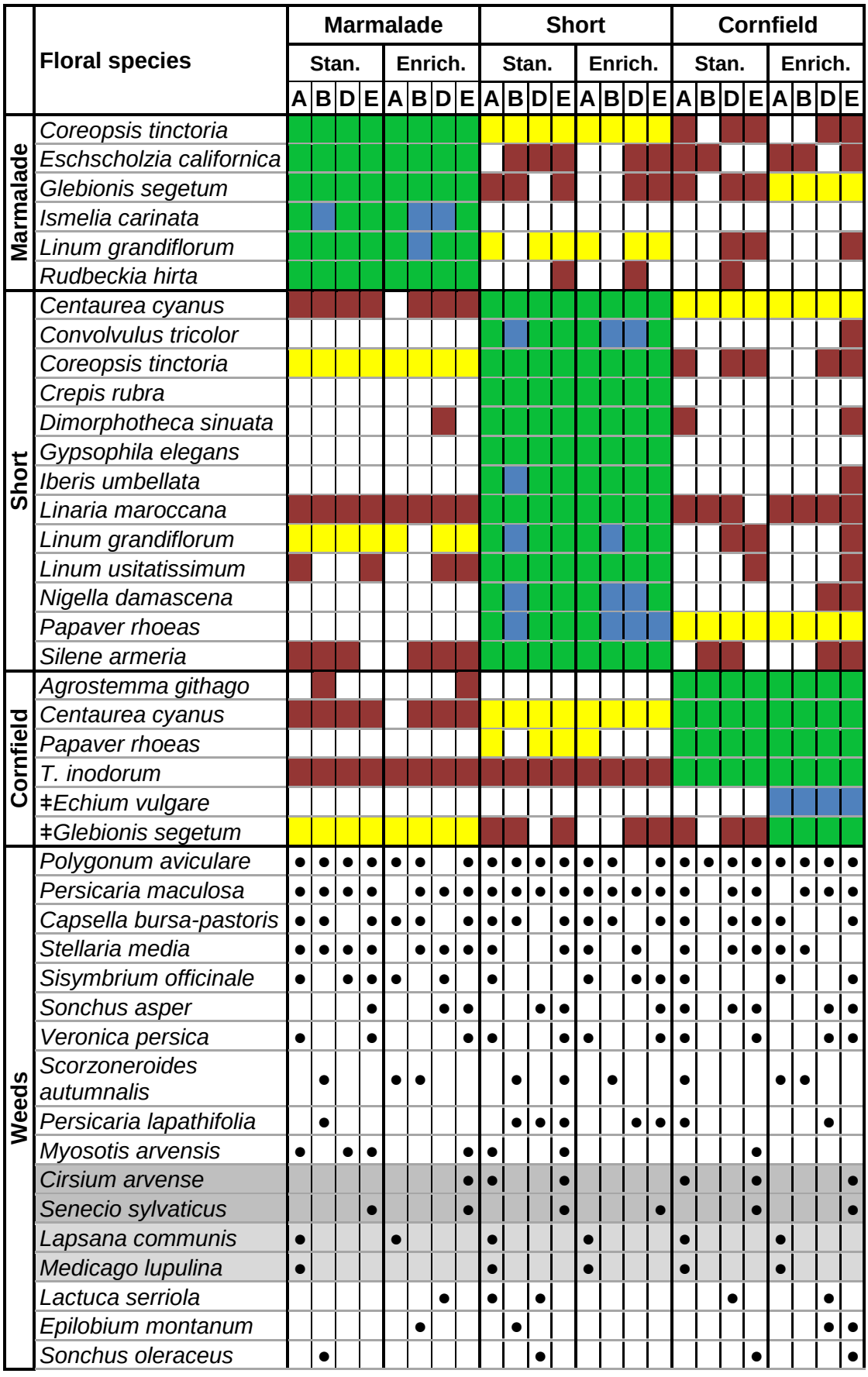


Figure A2.1: The proportion of floral units recorded during 3 floral surveys in each meadow plot that were produced by (a) *Glebionis segetum* or (b) *Echium vulgare* ‘Blue Bedder’. Letters A, B, D, E and F indicate the corresponding replicate block. ‘MA’, ‘SH’ and ‘CA’ refer to Marmalade, Short and Cornfield annual seed mix types, respectively. Letters ‘S’ and ‘E’ indicate standard and enriched versions of each seed mix type. In block ‘F’, most floral units were produced by *Glebionis segetum* and *Echium vulgare* ‘Blue Bedder’, both of which germinated from the seed bank and grew extensively throughout the block. Meadows in Block ‘F’ therefore differed markedly in floral composition from meadows in other replicate blocks and were not representative of the sown seed mix treatments.

Appendix A3

A3.1 Flowering performance of seed mix treatments

Table A3.1.1: A list for each of 24 replicate meadows showing species recorded flowering during 3 survey rounds. Columns represent individual field plots, located in one of four replicate blocks (A, B, D or E) and sown with either a standard or enriched version of one of three seed mix types. Rows list all the flowering species recorded across all field plots, as well as the full composition of each seed mix type, including species present in multiple seed mix types. **Green cells = treatment floral units**; Green cells indicate a intended treatment species recorded flowering in a plot in which it was sown (column entry). **Red cells = contaminant floral units**; Red cells indicate the presence of unintended species not was listed as part of the respective seed mix treatment. For species in multiple seed mixes, there was no way to distinguish, within a given field plot, whether plants originated from the seed mix treatment or from contaminant seeds in the seed bank or from adjacent field plots. For these species, plants/floral units were assumed to originate from seed mixes rather than from contaminant seeds. To enable easy comparison of species composition between seed mix treatments, **yellow squares** are used to fill cells next to duplicate list entries for these species. Filled circles ('●') indicate the presence of floral units of weed species. Weed species are ordered by the number of replicate meadows in which they occur, while **grey shading** highlights species providing clear qualitative exemplars of the spatial structuring of weed floral composition within blocks**. White cells** indicate that no floral units of that species were recorded in the respective meadow during surveys, while **blue cells** indicate the absence of species expected to be present. See table 3.2 for quantitative summary. **T.inodorum* represents *Tripleurospermum inodorum*. ǂ Note: *Echium vulgare* 'Blue Bedder' and *Glebionis segetum* were only present in the enriched version of the Cornfield Annual seed mixes.


Marmalade
Short
Cornfield
Floral species
Marmalade
Short
Cornfield
Stan.
Enrich.
Stan.
Enrich.
Stan.
Enrich.
A B D E A B D E A B D E A B D E A B D E A B D E
Marmalade
Coreopsis tinctoria
Eschscholzia californica
Glebionis segetum
Ismelia carinata
Linum grandiflorum
Rudbeckia hirta
Short
Centaurea cyanus
Convolvulus tricolor
Coreopsis tinctoria
Crepis rubra
Dimorphotheca sinuata
Gypsophila elegans
Iberis umbellata
Linaria maroccana
Linum grandiflorum
Linum usitatissimum
Nigella damascena
Papaver rhoeas
Silene armeria
Cornfield
Agrostemma githago
Centaurea cyanus
Papaver rhoeas
T. inodorum
‡Echium vulgare
‡Glebionis segetum
Weeds
Polygonum aviculare
Persicaria maculosa
Capsella bursa-pastoris
Stellaria media
Sisymbrium officinale
Sonchus asper
Veronica persica
Scorzoneroides autumnalis
Persicaria lapathifolia
Myosotis arvensis
Cirsium arvense
Senecio sylvaticus
Lapsana communis
Medicago lupulina
Lactuca serriola
Epilobium montanum
Sonchus oleraceus

	Hypochaeris radicata		●				●												●				●		
	Spergula arvensis		●								●								●						
	Achillea millefolium						●							●	●										
	Galium aparine					●				●												●			
	Matricaria discoidea					●		●						●											
	Sinapis arvensis					●				●															
	Trifolium pratense		●																						●
	Brassica rapa					●																			
	Cerastium fontanum						●																		
	Lamium album						●																		
	Lepidium sativum											●													
	Gilia achilleifolia													●											
	Fallopia convolvulus																						●		
	Epilobium hirsutum																								●

Table A3.1.2: Presence/absence lists of the species recorded flowering in four replicate blocks of meadows. Rows list all floral species recorded across replicate blocks during the season, as well as the full composition of the seed mixes used in this study. Columns represent individual replicate blocks (A, B, D or E) and show a consensus list of species recorded flowering within a given block, either for the whole season or for each of the three survey rounds, in late July, late August or late September. ***Squares*** ('■') indicate a species recorded flowering within a given block during at least one survey round. ***Circles*** ('●') indicate a species recorded flowering in given block in a particular survey round. Blank cells indicate that no floral units of the respective species were recorded in a given block during the respective time-period. ‡ Note: *Echium vulgare 'Blue Bedder'* and *Glebionis segetum* were only present in the enriched version of the Cornfield Annual seed mixes.

Mix	Floral species	Block				Survey round											
						July				August				Sept.			
		A	B	C	D	A	B	D	E	A	B	D	E	A	B	D	E
Marmalade	*Coreopsis tinctoria*	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Eschscholzia californica	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Glebionis segetum	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Ismelia carinata	■		■	■	●			●	●		●	●	●			
	Linum grandiflorum	■	■	■	■	●		●	●	●	●	●	●	●		●	●
	Rudbeckia hirta	■	■	■	■						●	●	●	●	●	●	●
Short	*Centaurea cyanus*	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Convolvulus tricolor	■		■	■	●		●	●	●		●	●				●
	Coreopsis tinctoria	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Crepis rubra	■	■	■	■	●	●	●	●	●	●	●	●	●			●
	Dimorphotheca sinuata	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●

	Gypsophila elegans	■	■	■	■	●	●	●	●	●	●	●	●	●		●
	Iberis umbellata	■	■	■	■	●		●	●	●		●	●	●	●	●	●
	Linaria maroccana	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Linum grandiflorum	■	■	■	■	●		●	●	●	●	●	●	●		●	●
	Linum usitatissimum	■	■	■	■		●	●	●	●		●	●	●	●	●	●
	Nigella damascena	■		■	■	●		●	●	●							
	Papaver rhoeas	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Silene armeria	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
Cornfield	*Agrostemma githago*	■	■	■	■	●	●	●	●	●	●		●	●			
	Centaurea cyanus	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Papaver rhoeas	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Tripleurospermum inodorum	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	‡*Echium vulgare*																
	‡*Glebionis segetum*	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
Weeds	*Persicaria lapathifolia*	■	■	■	■	●	●	●	●								
	Persicaria maculosa	■	■	■	■	●	●	●	●	●	●			●	●		
	Capsella bursa-pastoris	■	■	■	■	●	●	●	●	●	●		●				●
	Polygonum aviculare agg.	■	■	■	■	●	●		●	●	●	●	●	●	●		●
	Stellaria media	■	■	■	■	●	●	●	●	●	●	●	●	●	●	●	●
	Epilobium montanum		■	■	■			●			●	●	●				
	Sonchus oleraceus		■	■	■						●					●	●
	Myosotis arvensis	■		■	■				●	●			●	●		●	●
	Sisymbrium officinale	■		■	■	●		●	●	●		●	●	●			●
	Sonchus asper	■		■	■			●	●	●		●	●			●	●
	Veronica persica	■		■	■			●	●	●			●	●			●
	Scorzoneroides autumnalis	■	■		■	●	●			●	●		●	●	●		
	Achillea millefolium	■	■				●			●							
	Cirsium arvense	■			■				●	●			●	●			●
	Matricaria discoidea		■		■		●						●				●
	Sinapis arvensis			■	■			●	●								
	Lactuca serriola	■		■				●				●		●		●	
	Trifolium pratense	■		■		●		●									
	Fallopia convolvulus	■								●							
	Gilia achilleifolia	■												●			
	Lapsana communis	■				●				●				●			
	Medicago lupulina	■				●				●				●			
	Cerastium fontanum		■												●		
	Hypochaeris radicata		■				●				●				●		
	Lamium album		■				●										
	Spergula arvensis		■				●								●		
	Epilobium hirsutum			■				●									
	Brassica rapa				■								●				
	Galium aparine				■				●				●				
	Lepidium sativum				■				●								
	Senecio sylvaticus				■				●				●				

A3.2 Floral abundance distributions for flowering species recorded in this study

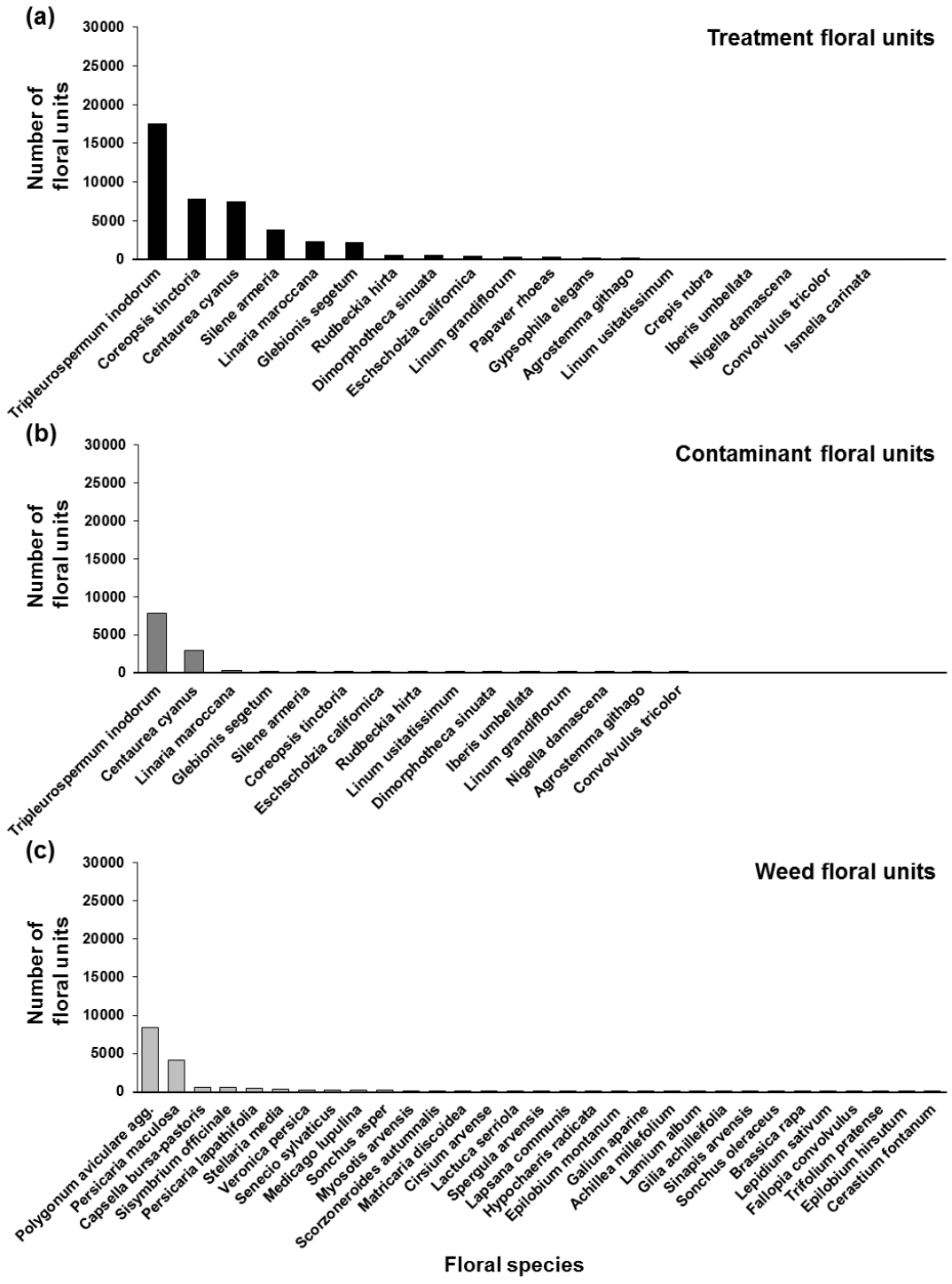


Figure A3.2.1: Floral abundance distributions for the floral species recorded in all replicate meadows over the season, quantified separately for (a) intended treatment species, (b) unintended contaminants, (c) weeds.

A3.3 Comparison of empirical and estimated floral species richness for meadows of each seed mix treatment

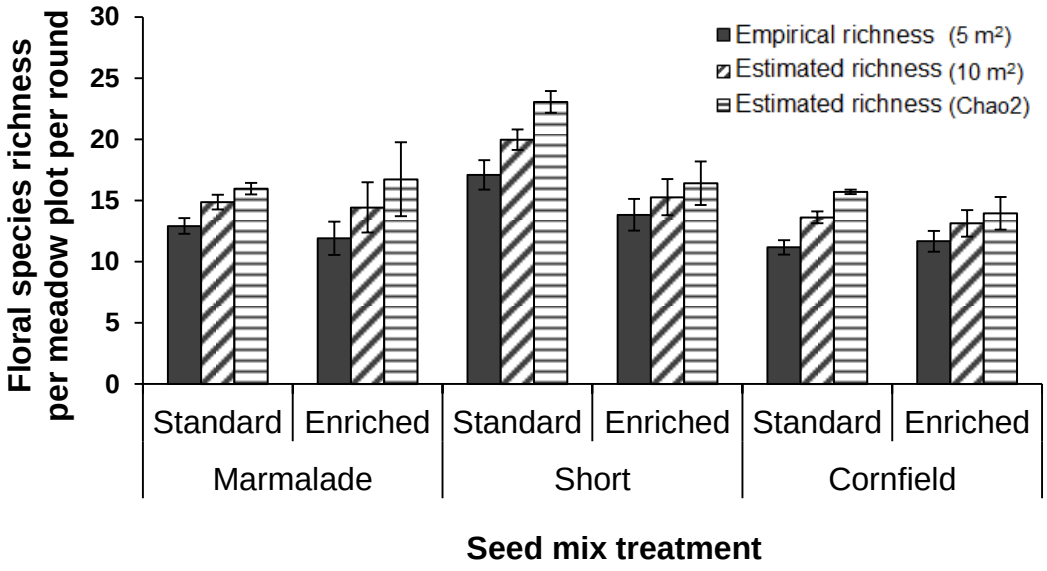
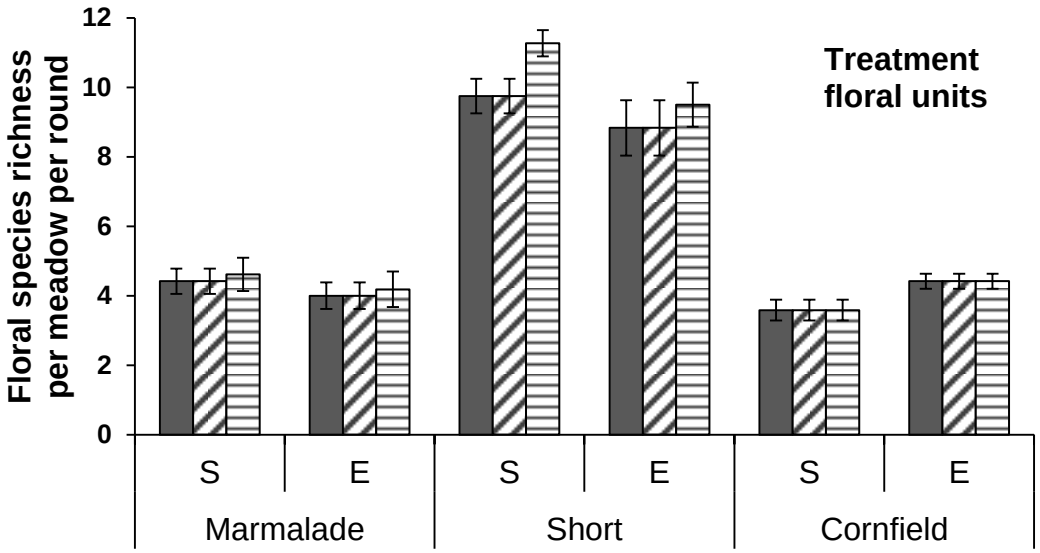
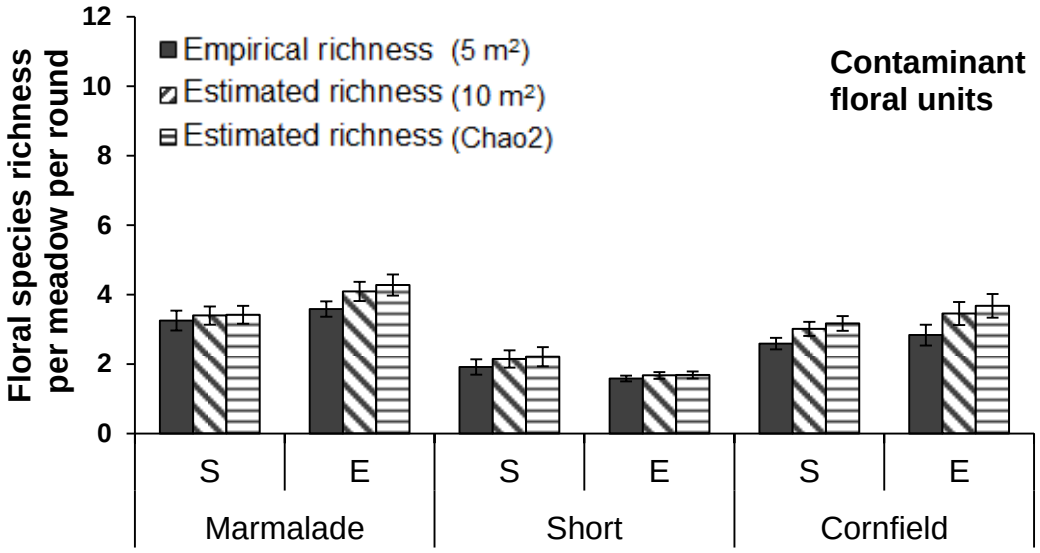


Figure A3.3.1: Total floral species richness per meadow per round in meadows of different seed mix treatments, for three alternative methods of species richness estimation: (i) empirical richness in 5 m^2 per meadow per round (5 m^2/round); (ii) extrapolated richness in 10 m^2 per meadow per round (10 m^2/round); and (iii) Chao2 estimated asymptotic ('true') species richness per meadow per round.

Table A3.3.1: Mean estimates ±SE of sample coverage, empirical species richness (5 m^2 per meadow), extrapolated species richness (10 m^2 per meadow), Chao2 species richness, and empirical Shannon and Simpson's diversities for meadows of different seed mix treatments within each of 3 survey rounds.

Round	Mix type	Enrich. status	All flowering species							
			Sample coverage ± SE	Empirical richness ± SE	Extrapolated Richness (10 m^2)		'Chao2' richness		Empirical Shannon ± SE	Empirical Simpson ± SE
					Mean±SE	change	Mean±SE	change		
Round 1	Marmalade	Standard	**0.95** ± 0.02	13.00 ± 1.08	14.5 ± 1.55	1.55	15.3 ± 1.95	2.35	5.29 ± 1.21	4.26 ± 1.09
		Enriched	**0.90** ± 0.01	13.75 ± 2.01	16.5 ± 2.45	2.83	19.0 ± 2.89	5.25	4.29 ± 0.79	3.12 ± 0.55
	Short	Standard	**0.92** ± 0.02	18.75 ± 0.85	21.3 ± 0.68	2.65	22.9 ± 1.46	4.20	6.94 ± 0.35	5.06 ± 0.41
		Enriched	**0.94** ± 0.01	16.00 ± 1.77	17.8 ± 1.45	1.88	19.3 ± 1.02	3.33	6.44 ± 0.88	4.50 ± 0.69
	Cornfield	Standard	**0.91** ± 0.02	11.75 ± 1.31	13.9 ± 1.61	2.18	15.8 ± 2.53	4.13	3.68 ± 0.90	2.77 ± 0.68
		Enriched	**0.96** ± 0.01	12.25 ± 1.31	13.3 ± 1.75	1.14	13.7 ± 1.96	1.52	5.2 ± 0.92	3.97 ± 0.81
Round 2	Marmalade	Standard	**0.92** ± 0.01	14.00 ± 1.87	16.0 ± 2.00	2.03	16.8 ± 2.13	2.88	6.14 ± 1.40	4.76 ± 1.09
		Enriched	**0.85** ± 0.03	12.75 ± 0.85	16.3 ± 1.22	3.64	20.4 ± 2.22	7.70	4.50 ± 0.78	3.40 ± 0.59
	Short	Standard	**0.94** ± 0.02	17.75 ± 3.11	20.0 ± 3.36	2.27	21.5 ± 3.41	3.83	6.87 ± 1.61	5.01 ± 1.16
		Enriched	**0.97** ± 0.01	14.00 ± 2.48	15.1 ± 3.25	1.14	16.7 ± 4.68	2.70	6.86 ± 0.86	5.61 ± 0.81
	Cornfield	Standard	**0.89** ± 0.01	11.75 ± 2.59	14.2 ± 3.34	2.54	15.9 ± 3.94	4.17	3.13 ± 0.61	2.44 ± 0.44
		Enriched	**0.91** ± 0.02	12.75 ± 2.56	14.8 ± 2.90	2.13	16.3 ± 2.89	3.62	3.81 ± 1.40	2.68 ± 0.93
Round 3	Marmalade	Standard	**0.90** ± 0.02	11.75 ± 1.43	14.0 ± 1.82	2.26	15.6 ± 2.36	3.90	6.89 ± 1.49	5.88 ± 1.43
		Enriched	**0.95** ± 0.01	9.25 ± 1.43	10.3 ± 1.81	1.07	10.7 ± 2.04	1.50	4.37 ± 0.95	3.37 ± 0.69
	Short	Standard	**0.89** ± 0.03	14.75 ± 2.75	18.4 ± 3.75	3.73	24.6 ± 6.97	9.88	4.67 ± 1.32	3.29 ± 0.96
		Enriched	**0.93** ± 0.01	11.50 ± 2.36	12.8 ± 2.82	1.30	13.1 ± 2.96	1.68	4.93 ± 0.72	3.84 ± 0.54
	Cornfield	Standard	**0.86** ± 0.01	10.00 ± 1.77	12.6 ± 2.44	2.66	15.3 ± 3.61	5.35	3.48 ± 0.61	2.54 ± 0.52
		Enriched	**0.93** ± 0.03	10.00 ± 1.00	11.1 ± 1.21	1.12	11.7 ± 1.43	1.73	5.27 ± 0.60	4.22 ± 0.39


Floral species richness per meadow per round
12
10
8
6
4
2
0
Treatment floral units
S
E
S
E
S
E
Marmalade
Short
Cornfield


Floral species richness per meadow per round
12
10
8
6
4
2
0
Empirical richness (5 m²)
Estimated richness (10 m²)
Estimated richness (Chao2)
Contaminant floral units
S
E
S
E
S
E
Marmalade
Short
Cornfield

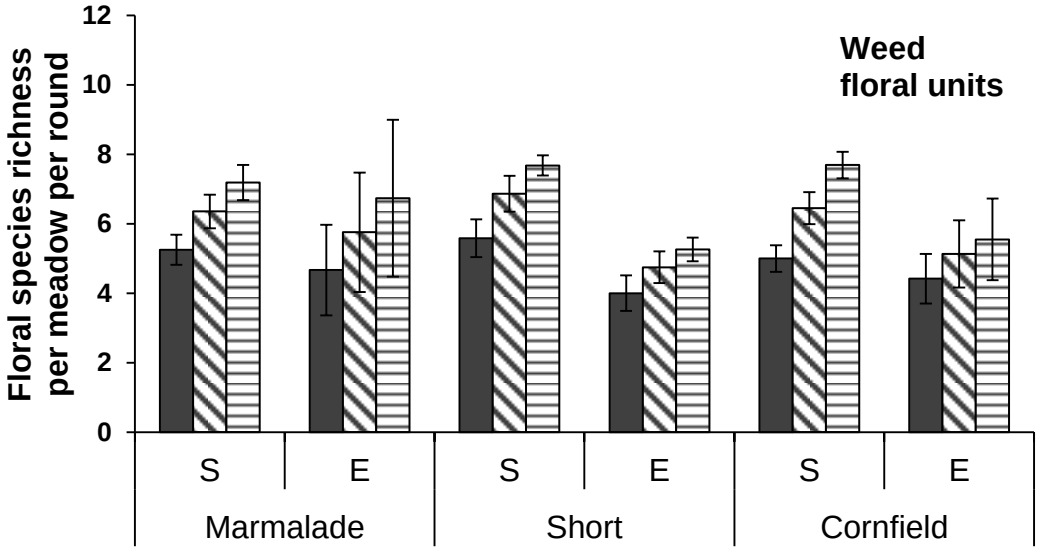

Floral species richness per meadow per round
12
10
8
6
4
2
0
Weed floral units
S
E
S
E
S
E
Marmalade
Short
Cornfield

Figure A3.3.2: Estimated floral species richness per meadow per round for each of three floral categories (treatment, contaminant and weed) in meadows of different seed mix treatments. Floral species richness was estimated using three alternative methods: (i) empirical richness in 5 m^2 per meadow per round (5 m^2/round); (ii) extrapolated richness in 10 m^2 per meadow per round (10 m^2/round); and (iii) Chao2 estimated asymptotic ('true') species richness per meadow per round.

Table 3.3.2: Mean estimates ±SE of sample coverage, empirical species richness (5 m^2 per meadow), and Chao2 species richness of treatment contaminant and weed floral categories in meadows of different seed mix treatments within each of 3 survey rounds.

Round	Mix type	Enrich. status	Treatment			Contaminants			Weeds		
			Sample coverage ± SE	Emp. richness ± SE	Chao2 richness ± SE	Sample coverage ± SE	Emp. richness ± SE	Chao2 richness ± SE	Sample coverage ± SE	Emp. richness ± SE	Chao2 richness ± SE
Round 1	Marmalade	Standard	**0.99**±0.02	3.75±0.25	3.85±0.30	**0.94**±0.04	3.25±0.48	3.45±0.61	**0.83**±0.09	6.00±1.22	8.20±1.80
		Enriched	**0.97**±0.02	3.50±0.29	3.60±0.36	**0.67**±0.23	3.25±0.85	3.85±1.34	**0.84**±0.07	7.00±1.15	10.50±3.05
	Short	Standard	**0.95**±0.01	10.25±0.85	11.20±0.57	**0.82**±0.08	2.25±0.25	2.65±0.65	**0.83**±0.06	6.25±0.62	7.90±0.44
		Enriched	**0.96**±0.02	9.50±1.04	10.30±1.24	**0.71**±0.24	1.50±0.29	1.50±0.29	**0.88**±0.06	5.00±0.70	5.85±1.01
	Cornfield	Standard	**1.00**±0.00	4.00±0.00	4.00±0.00	**0.92**±0.05	2.25±0.75	2.75±1.11	**0.68**±0.06	5.50±1.32	8.43±2.01
		Enriched	**1.00**±0.00	4.75±0.25	4.75±0.25	**0.67**±0.23	2.25±0.48	3.00±0.92	**0.92**±0.03	5.25±0.75	6.25±1.53
Round 2	Marmalade	Standard	**0.96**±0.03	5.00±0.00	5.50±0.38	**0.93**±0.01	3.75±0.48	3.85±0.57	**0.66**±0.22	5.25±1.43	6.65±1.78
		Enriched	**0.94**±0.02	4.75±0.25	5.20±0.29	**0.66**±0.22	3.50±0.50	4.10±0.47	**0.61**±0.20	4.50±0.50	7.00±1.28
	Short	Standard	**0.97**±0.02	10.25±1.55	11.95±2.59	**0.75**±0.25	1.50±0.29	1.70±0.44	**0.68**±0.23	6.00±1.73	8.03±2.28
		Enriched	**0.99**±0.00	9.75±1.38	9.95±1.42	**0.67**±0.24	1.50±0.50	1.70±0.70	**0.90**±0.08	3.67±1.04	5.26±2.40
	Cornfield	Standard	**0.97**±0.02	3.75±0.25	3.75±0.25	**0.60**±0.20	2.75±0.75	3.35±1.10	**0.79**±0.08	5.25±1.79	7.48±3.34
		Enriched	**0.99**±0.02	4.50±0.29	4.50±0.29	**0.66**±0.24	3.25±1.65	4.08±2.46	**0.81**±0.05	5.00±0.91	7.15±1.64
Round 3	Marmalade	Standard	**0.96**±0.02	4.50±0.50	4.50±0.50	**0.87**±0.06	2.75±0.48	2.95±0.55	**0.78**±0.11	4.50±0.86	6.70±2.56
		Enriched	**1.00**±0.00	3.75±0.95	3.75±0.95	**0.82**±0.06	4.00±0.87	4.87±1.36	**0.67**±0.22	2.50±0.64	2.70±0.75
	Short	Standard	**0.92**±0.03	8.75±0.85	10.65±1.58	**0.89**±0.10	2.00±0.50	2.27±0.71	**0.58**±0.19	4.50±1.65	7.10±3.44
		Enriched	**0.96**±0.01	7.25±1.80	8.25±2.10	**0.67**±0.23	1.75±0.48	1.85±0.51	**0.89**±0.07	3.33±0.76	4.66±1.73
	Cornfield	Standard	**0.96**±0.02	3.00±0.41	3.00±0.41	**0.74**±0.07	2.75±0.25	3.40±0.55	**0.83**±0.07	4.25±1.65	7.15±3.51
		Enriched	**0.99**±0.02	4.00±0.00	4.00±0.00	**0.60**±0.20	3.00±0.71	3.95±1.16	**0.91**±0.07	3.00±0.40	3.25±0.48

A3.4 Model summaries for species richness and diversity, including comparison of alternative model structures

Models for 'total' meadow floral species richness and diversity

Table A3.4.1: Results of 'fixed round' and 'random round' models of floral species richness and floral diversity. Empirical species richness was modelled using a log-link Poisson GLMM. Extrapolated richness and Chao2 richness were modelled using log-link Gamma. Shannon and Simpson's diversities were modelled using LMMs. All fixed round models contained fixed effects of 'mix type', 'mix formulation' and 'round', with 'block' as a random effect. Random round models were identical except that round was fit as a random effect. The GLMM for empirical species richness also contained 'total floral abundance' as an additional covariate to control for variation in sampling effort. Results show single term deletion log-likelihood ratio tests. The random round model for Chao2 estimated richness did not convergence therefore results are not shown.

		Model structure							
		Models with 'round' as fixed				Models with 'round' as random			
Response	**Predictors**	df	AIC	X^2	p-value	df	AIC	X^2	p-value
Total floral species richness	Full model AIC		388.06				381.54		
	log(Floral abundance+1)	1	380.04	1.20	0.27	1	383.80	4.26	**0.03892**
	Mix type	2	392.62	15.78	**0.00037**	2	393.08	15.54	**0.00042**
	Formulation	1	380.89	2.06	0.15	1	381.51	1.97	0.16
	Round	2	379.54	2.70	0.26	-	-	-	-
Extra-polated richness	Full model AIC		405.97				408.42		
	Mix type	2	416.11	14.13	**0.00085**	2	418.38	13.95	**0.00093**
	Formulation	1	407.97	3.99	**0.0456**	1	410.29	3.86	**0.04929**
	Round	2	415.45	13.48	**0.00118**	-	-	-	-
Chao2 richness	Full model AIC		450.04			-	-	-	-
	Mix type	2	456.16	10.11	**0.00634**	-	-	-	-
	Formulation	1	452.19	4.14	**0.042**	-	-	-	-
	Round	2	453.79	7.75	**0.0208**	-	-	-	-
Total Shannon diversity	Full model AIC		304.85				303.47		
	Mix type	2	316.17	15.32	**0.00047**	2	314.67	15.19	**0.0005**
	Formulation	1	303.01	0.15	0.69	1	301.62	0.15	0.69
	Round	2	301.47	0.62	0.73	-	-	-	-
Total Simpsons diversity	Full model AIC		279.82				277.92		
	Mix type	2	287.69	11.86	**0.00265**	2	285.78	11.85	**0.00267**
	Formulation	1	278.01	0.18	0.67	1	276.11	0.18	0.67
	Round	2	275.92	0.10	0.95	-	-	-	-

Table 3.4.2: Model summaries for 'fixed round' and 'random round' Poisson GLMMs of empirical floral species richness. Models contain fixed effects of 'mix type', 'mix formulation' and 'total floral abundance' to account for variation in sampling effort. Round is fit as either a fixed or random effect in 'fixed round' or 'random round models, respectively, while 'block' is fit as a random effect in both models. There were no qualitative differences in coefficient estimates between fixed versus random round models.

	Response: Total floral species richness							
	Model structure							
	Fixed round				Random round			
Predictors	Estimate ± SE	*z*	*P*	Model fit	Estimate ± SE	*z*	*P*	Model fit
intercept	2.043±0.479	4.274	**<0.001**	AIC	1.507±0.345	4.366	**<0.001**	AIC
log floral abundance	0.072±0.065	1.1	0.27	380.8	0.143±0.049	2.953	**0.0032**	381.5
mix type: marmalade	0.089±0.084	1.067	0.29	logLik	0.094±0.084	1.116	0.26	logLik
mix type: short	0.303±0.079	3.811	**<0.001**	-182.4	0.302±0.079	3.797	**<0.001**	-183.8
formulation: enriched	-0.094±0.065	-1.436	0.15		-0.092±0.065	-1.405	0.16	
round: round 2	-0.017±0.078	-0.224	0.82		-	-	-	
round: round 3	-0.165±0.106	-1.557	0.12		-	-	-	
Random effects	Variance	SD			Variance	SD		
block	0.0251	0.1586			0.0271	0.1645		
round	-	-			0.0000	0.0000		

Table A3.4.3: Pairwise contrasts of empirical floral species richness between meadows of difference seed mix types. To control for inflated type 1 errors due to multiple testing, estimated marginal means (lsmeans) were calculated for each seed mix type and pairwise contrasts performed using Tukey's HSD test to maintain a family-wise type-1 error rate of 0.05. This post-hoc analysis was performed for the 'Fixed round' model (above). LS means (± SE) are averaged over levels of enrichment and round. Asymptotic 95% confidence intervals are calculated using R package 'lsmeans'. P-values are adjusted using Tukey's HSD method for a family of 3 simultaneous contrasts. Model scale estimates are a log scale, while response scale estimates are back-transformed to the data scale.

Post-hoc Tukey contrasts for 'Fixed round' model of empirical species richness								
		Model scale estimates				**Response scale estimates**		
Estimated mean	**Mix type**	**LS mean ± SE**	**df**	**Lower 95% CI**	**Upper 95% CI**	**Mean ± SE**	**Lower 95% CI**	**Upper 95% CI**
	Marmalade	2.5 ± 0.1	NA	2.3	2.7	12.5 ± 1.25	10.2	15.2
	Short	2.7 ± 0.1	NA	2.6	2.9	15.4 ± 1.48	12.8	18.6
	Cornfield	2.4 ± 0.1	NA	2.2	2.6	11.4 ± 1.15	9.3	13.9
Pairwise contrasts	**Contrast**	**Estimated difference ±SE**	**df**	***z*-ratio**	***P*-value**	**Estimated ratio of means ± SE**	**Estimated % difference ± SE**	
	Marmalade-Cornfield	-0.089 ± 0.084	NA	-1.06685	0.54	0.915 ± 0.077	91.5 ± 7.7	
	Short -Cornfield	-0.303 ± 0.079	NA	-3.81148	**0.00041**	0.738 ± 0.059	73.8 ± 5.9	
	Short - Marmalade	-0.214 ± 0.078	NA	-2.74886	**0.01648**	0.807 ± 0.063	80.7 ± 6.3	

Table A3.4.4: Model summaries for 'fixed round' and 'random round' Gamma GLMMs of extrapolated floral species richness. Models contain fixed effects of 'mix type' and 'mix formulation'. Round is fit as either a fixed or random effect in 'fixed round' or 'random round models', respectively, while 'block' is fit as a random effect in both models. There were no qualitative differences in coefficient estimates between fixed versus random round models.

	Response: Extra-polated richness (10 m^2 per meadow)							
	Model structure							
	Fixed round				Random round			
Predictors	Estimate ± SE	*t*	*P*	Model fit	Estimate ± SE	*t*	*P*	Model fit
intercept	2.716 ± 0.136	20.041	**<0.001**	AIC	2.619 ± 0.188	13.958	**<0.001**	AIC
-	-	-	-	406.0	-	-	-	408.4
mix type: marmalade	0.1001± 0.068	1.467	0.14	logLik	0.101 ± 0.069	1.464	0.14	logLik
mix type: short	0.266 ± 0.068	3.896	**<0.001**	-195	0.266 ± 0.069	3.872	**0.0001**	-197.2
formulation: enriched	-0.113± 0.056	-2.027	**0.0427**	Dev.	-0.112± 0.056	-1.992	**0.0464**	Dev.
round: round 2	-0.029± 0.068	-0.429	0.67	390.0	**-**	**-**	**-**	394.4
round: round 3	-0.244± 0.068	-3.571	**<0.001**	df: 64	**-**	**-**	**-**	df: 65
Random	Variance	SD			Variance	SD		
block	0.0121	0.1099			0.0134	0.1158		
round	**-**	**-**			0.0067	0.0817		
residual	0.0583	0.2414			0.0612	0.2473		

Table A3.4.5: Model summary for a Gamma GLMM of Chao2 species richness. Model contains fixed effects of 'mix type' and 'mix formulation' and 'round', while 'block' is fit as a random effect in both models. A 'random round' model with round fit as a random effect failed to converge as is not presented.

	Response: Chao2 richness			
	Model structure: Fixed round			
Predictors	Estimate ± SE	*z*	*P*	Model fit
intercept	2.815 ± 0.149	18.794	**<0.001**	AIC
-	-	-	-	450
mix type: marmalade	0.1102 ± 0.084	1.307	**0.191**	logLik
mix type: short	0.275 ± 0.084	3.263	**0.0011**	-217
formulation: enriched	-0.143 ± 0.069	-2.066	**0.0388**	Dev.
round: round 2	-0.01 ± 0.085	-0.123	0.90	434
round: round 3	-0.218 ± 0.085	-2.566	**0.0103**	df: 64
Random	Variance	SD		
block	0.0165	0.1284		
round	**-**	**-**		
residual	0.0932	0.3052		

Table A3.4.6: Model summaries for LMMs of Shannon diversity. Models contain fixed effects of 'mix type' and 'mix formulation'. Round is fit as either a fixed or random effect in 'fixed round' or 'random round models', respectively, while 'block' is fit as a random effect in both models. There were no qualitative differences in coefficient estimates between fixed versus random round models. R package 'lmerTest' was used to estimate approximated Satterthwaite degrees of freedom for model coefficients confidence intervals (Kuznetsova, Brockhoff and Christensen, 2016).

	Response: Shannon diversity									
	Model structure									
	Fixed round					Random round				
Predictors	Estimate ± SE	df	*t*	*P*	Model fit	Estimate ± SE	df	*t*	*P*	Model fit
intercept	4.329 ± 0.804	6.68	5.383	**0.0012**	REML	4.178 ± 0.747	5	5.595	**0.0025**	REML
mix type: marmalade	1.153 ± 0.509	63	2.264	**0.0270**	criterion	1.153 ± 0.503	65	2.29	**0.0253**	criterion
mix type: short	2.025 ± 0.509	63	3.978	**<0.001**	286.7	2.025 ± 0.503	65	4.022	**<0.001**	288
formulation: enriched	-0.158± 0.416	63	-0.381	0.701		-0.158 ± 0.411	65	-0.385	0.701	
round: round 2	-0.085± 0.509	63	-0.167	0.87		-		-	-	
round: round 3	-0.369± 0.509	63	-0.725	0.47		-		-	-	
Random	Variance	SD				Variance	SD			
block	1.551	1.245				1.554	1.247			
round	-	-				0.000	0.000			
residual	3.109	1.763				3.041	1.744			

Table A3.4.7: Pairwise contrasts of Shannon diversity between meadows of difference seed mix types. Post-hoc analysis was performed for the 'Fixed round' model. Estimated marginal means (LS means ± SE) are averaged over levels of enrichment and round. Asymptotic 95% confidence intervals are calculated using R package 'lsmeans'. P-values are adjusted using Tukey's HSD method for a family of 3 simultaneous contrasts.

Post-hoc Tukey contrasts for 'Fixed round' model of Shannon diversity					
Estimated mean	**Mix type**	**Estimates (model & response)**			
		LS mean ± SE	**df**	**Lower 95% CI**	**Upper 95% CI**
	Marmalade	5.25 ± 0.72	4.31	3.31	7.19
	Short	6.12 ± 0.72	4.31	4.18	8.06
	Cornfield	4.10 ± 0.72	4.31	2.16	6.04
Pairwise contrasts	**Contrast**	**Estimated difference in means ± SE**	**df**	***z*-ratio**	***P*-value**
	Marmalade-Cornfield	-1.15 ± 0.51	63	-2.26	(0.0684)
	Short -Cornfield	-2.03 ± 0.51	63	-3.98	**0.0005**
	Short - Marmalade	-0.87 ± 0.51	63	-1.71	0.21

Table A3.4.8: Model summaries for LMMs of Simpson's diversity. Models contain fixed effects of 'mix type' and 'mix formulation'. Round is fit as either a fixed or random effect in 'fixed round' or 'random round models', respectively, while 'block' is fit as a random effect in both models. There were no qualitative differences in coefficient estimates between fixed versus random round models. R package 'lmerTest' was used to estimate approximated Satterthwaite degrees of freedom for model coefficients confidence intervals (Kuznetsova, Brockhoff and Christensen, 2016).

	Response: Simpson's diversity									
	Model structure									
	Fixed round					Random round				
Predictors	Estimate ± SE	df	*t*	*P*	Model fit	Estimate ± SE	df	*t*	*P*	Model fit
intercept	3.197 ± 0.638	7.64	5.012	**0.0012**	REML	3.181 ± 0.585	5.48	5.434	**0.00216**	REML
mix type: marmalade	1.027 ± 0.430	63	2.387	**0.02**	criterion	1.027 ± 0.424	65	2.423	**0.0182**	criterion
mix type: short	1.449 ± 0.430	63	3.37	**0.0013**	263.9	1.449 ± 0.424	65	3.421	**0.00108**	264
formulation: enriched	-0.146± 0.351	63	-0.414	0.68		-0.146 ± 0.346	65	-0.421	0.68	
round: round 2	0.039 ± 0.430	63	0.091	0.93		-		-	-	
round: round 3	-0.089± 0.430	63	-0.207	0.84		-		-	-	
Random	Variance	SD				Variance	SD			
block	0.8882	0.9424				0.8918	0.9443			
round	-	-				0.000	0.000			
residual	2.2201	1.49				2.1549	1.468			

Table A3.4.9: Pairwise contrasts of Simpson's diversity between meadows of difference seed mix types. Post-hoc analysis was performed for the 'Fixed round' model. Estimated marginal means (LS means ± SE) are averaged over levels of enrichment and round. Asymptotic 95% confidence intervals are calculated using R package 'lsmeans'. P-values are adjusted using Tukey's HSD method for a family of 3 simultaneous contrasts.

Post-hoc Tukey contrasts for 'Fixed round' model of Simpson's diversity					
Estimated mean	**Mix type**	**Estimates (model & response)**			
		LS mean ± SE	**df**	**Lower 95% CI**	**Upper 95% CI**
	Marmalade	3.11 ± 0.56	4.63	1.63	4.59
	Short	4.14 ± 0.56	4.63	2.66	5.61
	Cornfield	4.56 ± 0.56	4.63	3.08	6.03
Pairwise contrasts	**Contrast**	**Estimated difference in means ± SE**	**df**	***z*-ratio**	***P*-value**
	Marmalade-Cornfield	-1.03 ± 0.43	63	-2.39	(0.0516)
	Short - Cornfield	-1.45 ± 0.43	63	-3.37	**0.0036**
	Short - Marmalade	-0.42 ± 0.43	63	-0.98	0.59

Models for richness and diversity of floral categories

Table A3.4.10: Results of Poisson GLMM (using a log-link function) for floral species richness of different floral categories in meadows of different seed mix treatments. Fixed effects were mix type, formulation, floral category and round, with an interaction between floral category and mix. Block was fit as a random effect.

Predictors (fixed effects)	**Response:** Floral species richness							
	Model structure							
	Models with 'round' as fixed				Models with 'round' as random			
	df	AIC	χ^2	p-value	df	AIC	χ^2	p-value
Full model AIC		811.2				809.3		
log(Floral abundance+1)	1	850.1	40.81	**<0.001**	1	853.8	46.36	**<0.001**
Mix type	NA	NA	NA	NA	NA	NA	NA	NA
Floral category	NA	NA	NA	NA	NA	NA	NA	NA
Mix type * Floral category	4	859.9	56.60	**<0.001**	4	858.0	56.56	**<0.001**
Formulation	1	811.1	1.81	0.1784	1	809.3	1.80	0.1796
Round	2	807.5	0.21	0.8988	-	-	-	-

Table A3.4.11: Summary of Poisson GLMMs.

Predictors	**Response:** Empirical species richness (in $5m^2$)							
	Model structure							
	Fixed round				Random round			
	Estimate ± SE	*z*	*P*	Model fit	Estimate ± SE	*z*	*P*	Model fit
intercept	0.568±0.185	3.077	**0.0021**	AIC	0.533±0.168	3.171	**0.0015**	AIC
log floral abundance	0.180±0.035	5.164	**<0.001**	800.5	0.188±0.032	5.834	**<0.001**	798.9
mix type: marmalade	-0.265±0.194	-1.346	0.18	logLik	-0.281±0.192	-1.467	0.14	logLik
mix type: short	-0.623±0.204	-3.049	**0.0023**	-385.3	-0.628±0.204	-3.079	**0.0021**	-385.5
class: treatment	-0.296±0.208	-1.424	0.16		-0.324±0.202	-1.603	0.11	
class: weed	0.149±0.174	0.854	0.39		0.132±0.172	0.768	0.44	
formulation: enriched	-0.079±0.065	-1.209	0.23		-0.079±0.065	-1.208	0.23	
round: round 2	0.006±0.077	0.074	0.94		-	-		
round: round 3	-0.046±0.0878	-0.519	0.60		-	-		
mix type-floral category: marmalade-	0.508±0.259	1.963	**0.0496**		0.535±0.255	2.094	**0.0363**	

treatment							
mix type- floral category: short-treatment	1.488±0.239	6.233	**<0.001**		1.493±0.239	6.259	**<0.001**
mix type- floral category: marmalade-weed	0.293±0.232	1.263	0.21		0.311±0.230	1.353	0.18
mix type- floral category: Short - weed	0.625±0.244	2.565	**0.0103**		0.629±0.244	2.586	**0.0097**
Random	Variance	SD			Variance	SD	
ID	0	0			0	0	
block	0.01807	0.1344			0.01812	0.1346	
round	-	-			0	0	

Table A3.4.12 (on subsequent pages): Pairwise contrasts of floral species richness between different levels of seed mix type and between different floral categories (treatment, contaminant and weed). There was a significant interaction was between mix type and floral category (Table A3.4.2); hence, conditional pairwise contrasts were performed comparing levels of each factor within each level of the corresponding interacting factor. Conditional pairwise contrasts test differences in estimated marginal means of floral species richness between: (a) seed mix types, for each floral category: treatment, contaminant or weed; and (b) floral categories (treatment, contaminant and weed) within seed mix types. P-values were adjusted to account for 18 simultaneous tests using the 'mvt' method in R package 'lsmeans'. This method provides a one-step adjustment (similar to classical Bonferroni) that adjusts the critical value used to calculate confidence intervals and p-values, using a multivariate t distribution for the maximum of k estimates. This Post-hoc analysis was performed on a model for empirical floral species richness with sampling round fitted as a fixed effect ('Fixed round' model; Table A3.4.4). Results are averaged over levels of enrichment and round.

(a) Difference in species richness between seed mix types for treatment, contaminant or weed floral categories									
			Model scale estimates				Response scale estimates		
	Level of floral category	Level of seed mix type	LS mean ± SE	df	Lower 95% CI	Upper 95% CI	Mean ± SE	Lower 95% CI	Upper 95% CI
Estimated means	Treatment	Marmalade	1.52 ± 0.12	NA	1.28	1.76	4.59 ± 0.56	3.61	5.84
		Short	2.11 ± 0.09	NA	1.92	2.31	8.28 ± 0.81	6.83	10.04
		Cornfield	1.24 ± 0.13	NA	0.99	1.49	3.47 ± 0.44	2.71	4.43
	Contaminant	Marmalade	1.29 ± 0.13	NA	1.03	1.55	3.64 ± 0.49	2.79	4.73
		Short	0.97 ± 0.19	NA	0.61	1.34	2.65 ± 0.49	1.84	3.82
		Cornfield	1.64 ± 0.18	NA	1.29	1.99	5.16 ± 0.91	3.65	7.29
	Weed	Marmalade	1.76 ± 0.12	NA	1.53	1.99	5.79 ± 0.68	4.59	7.29
		Short	1.69 ± 0.12	NA	1.46	1.94	5.47 ± 0.67	4.30	6.96
		Cornfield	1.73 ± 0.12	NA	1.49	1.97	5.66 ± 0.69	4.46	7.17
			Model scale estimates				Response scale estimates		
Pair-wise contrasts	Level of floral category	Contrast	Estimated difference in means ± SE	df	*z*-ratio	*P*-value	Estimated ratio of means ± SE		Estimated % difference between means ± SE
	Treatment	Cornfield - Marmalade	-0.28 ± 0.15	NA	-1.907	0.49	0.755 ± 0.111		75.5 ± 11.1
		Cornfield - Short	-0.87 ± 0.12	NA	-7.125	**<0.001**	0.419 ± 0.051		41.9 ± 5.1
		Marmalade - Short	-0.59 ± 0.12	NA	-4.753	**<0.001**	0.555 ± 0.069		55.5 ± 6.9
	Contaminant	Cornfield - Marmalade	0.35 ± 0.19	NA	1.821	0.55	1.419 ± 0.272		141.9 ± 27.3
		Cornfield - Short	0.67 ± 0.20	NA	3.276	**0.0165**	1.949 ± 0.397		194.9 ± 39.7
		Marmalade - Short	0.32 ± 0.20	NA	1.564	0.74	1.374 ± 0.279		137.4 ± 27.9
	Weed	Cornfield - Marmalade	-0.02 ± 0.13	NA	-0.181	1	0.977 ± 0.128		97.7 ± 12.8
		Cornfield - Short	0.03 ± 0.14	NA	0.246	1	1.034 ± 0.139		103.4 ± 13.9
		Marmalade - Short	0.06 ± 0.13	NA	0.428	0.99	1.059 ± 0.141		105.9 ± 14.1

(b) Differences in species richness within seed mix types for treatment, contaminant and weed floral categories

	Level of seed mix type	Level of Floral category	Model scale estimates				Response scale estimates		
			LS mean ± SE	df	Lower 95% CI	Upper 95% CI	Mean ± SE	Lower 95% CI	Upper 95% CI
Estimated means	Marmalade	Treatment	1.52 ± 0.12	NA	1.28	1.76	4.59 ± 0.56	3.61	5.84
		Contaminant	1.29 ± 0.13	NA	1.03	1.55	3.64 ± 0.49	2.79	4.73
		Weed	1.76 ± 0.12	NA	1.53	1.99	5.79 ± 0.68	4.59	7.29
	Short	Treatment	2.11 ± 0.09	NA	1.92	2.31	8.28 ± 0.81	6.83	10.04
		Contaminant	0.97 ± 0.19	NA	0.61	1.34	2.65 ± 0.49	1.84	3.82
		Weed	1.69 ± 0.12	NA	1.46	1.94	5.47 ± 0.67	4.30	6.96
	Cornfield	Treatment	1.24 ± 0.13	NA	0.99	1.49	3.47 ± 0.44	2.71	4.43
		Contaminant	1.64 ± 0.18	NA	1.29	1.99	5.16 ± 0.91	3.65	7.29
		Weed	1.73 ± 0.12	NA	1.49	1.97	5.66 ± 0.69	4.46	7.17
	Level of seed mix type	Contrast	Model scale estimates				Response scale estimates		
			Estimated difference in means ±SE	df	*z*-ratio	*P*-value	Estimated ratio of means ± SE		Estimated % difference between means ± SE
Pair-wise contrasts	Marmalade	Contaminant - Treatment	-0.23 ± 0.15	NA	-1.538	0.75	0.79 ± 0.12		79.3 ± 11.9
		Contaminant - Weed	-0.47 ± 0.15	NA	-3.171	**0.023**	0.63 ± 0.09		62.8 ± 9.2
		Treatment - Weed	-0.23 ± 0.14	NA	-1.712	0.63	0.79 ± 0.11		79.3 ± 10.8
	Short	Contaminant - Treatment	-1.14 ± 0.19	NA	-5.901	**<0.001**	0.32 ± 0.06		31.9 ± 6.2
		Contaminant - Weed	-0.73 ± 0.19	NA	-3.817	**0.0023**	0.48 ± 0.09		48.4 ±9.2
		Treatment - Weed	0.41 ± 0.13	NA	3.288	**0.0152**	1.51 ± 0.19		151.3 ± 19.1
	Cornfield	Contaminant - Treatment	0.39 ± 0.21	NA	1.943	**0.465**	1.49 ± 0.31		148.9 ± 30.5
		Contaminant - Weed	-0.09 ± 0.17	NA	-0.529	0.99	0.91 ± 0.16		91.3 ± 15.8
		Treatment - Weed	-0.49 ± 0.15	NA	-3.294	**0.0152**	0.61 ±0.09		61.3 ± 9.1

A3.5 Model summaries for species richness and diversity, including comparison of alternative model structures

Model for 'total' meadow floral abundance

Table A3.5.1: Results of Poisson GLMM (using a log-link function) for total floral abundance in meadows of different seed mix treatments. Fixed effects were mix type, formulation and round, with an interaction between mix type and round. Block was fit as a random effect. A random effect of observation ID (for n=72 observations) was fitted to control overdispersion. Results show single term deletion log-likelihood ratio tests.

Predictors (fixed effects)	**Response:** Total floral abundance							
	Model structure							
	Models with 'round' as fixed				Models with 'round' as random			
	df	AIC	χ^2	p-value	df	AIC	χ^2	p-value
Full model AIC		1085.3				1093.6		
Mix type	2	1080.9	0.36	0.83	2	1089.9	0.35	0.84
Formulation	1	1083.5	0.20	0.65	1	1091.8	0.18	0.67
Round	2	1126.0	45.5	**<0.001**	-	-	-	-
Mix type*Round	4	1084.5	7.25	0.12	-	-	-	-

Table A3.5.2: Summary of Poisson GLMMs (using a log-link functions) for total floral abundance (response)

Predictors	**Model structure**							
	Fixed round				Random round			
	Estimate ± SE	*z*	*P*	Model fit	Estimate ± SE	*z*	*P*	Model fit
intercept	7.13±0.17	41.69	**<0.001**	AIC	6.71±0.32	20.997	**<0.001**	AIC
mix type: marmalade	-0.09±0.15	-0.58	0.56	1084.5	-0.09±0.15	-0.568	0.57	1093.6
mix type: short	-0.02±0.15	-0.12	0.90	logLik	-0.02±0.15	-0.123	0.90	logLik
formulation: enriched	-0.05±0.12	-0.44	0.66	-534.3	-0.05±0.12	-0.429	0.67	-539.8
round: round 2	-0.17±0.15	-1.17	0.24		-	-	-	
round: round 3	-1.09±0.15	-7.48	**<0.001**		-	-	-	
Random effects	Variance	SD			Variance	SD		
ID	0.25538	0.5054			0.2633	0.5132		
block	0.03147	0.1774			0.0432	0.2078		
round	**-**	**-**			0.2297	0.4793		

Table A3.5.3: Pairwise contrasts of total floral abundance between survey rounds. Estimated marginal means (LS means ± SE) are averaged over levels of mix type and enrichment. Asymptotic 95% confidence intervals are calculated using R package 'lsmeans'. P-values are adjusted using Tukey's HSD method for a family of 3 simultaneous contrasts. Model scale estimates are a log scale, while response scale estimates are back-transformed to the data scale.

Post-hoc Tukey contrasts for 'Fixed round' model of total floral abundance								
		Model scale estimates				**Response scale estimates**		
Estimated mean	**Survey round**	**LS mean ± SE**	**df**	**Lower 95% CI**	**Upper 95% CI**	**Mean ± SE**	**Lower 95% CI**	**Upper 95% CI**
	Round 1	7.07 ± 0.14	NA	6.80	7.34	1176.7 ± 160.3	901.0	1536.7
	Round 2	6.89 ± 0.14	NA	6.63	7.17	992.1 ± 135.2	759.6	1295.7
	Round 3	5.98 ± 0.14	NA	5.71	6.24	393.8 ± 53.8	301.4	514.6
Pairwise contrasts	**Contrast**	**Estimated difference ±SE**	**df**	***z*-ratio**	***P*-value**	**Estimated ratio of means ± SE**	**Estimated % difference ± SE**	
	Round 1 - Round 2	0.17 ± 0.15	NA	1.17	0.47	1.19 ± 0.17	118.6 ± 17.3	
	Round 1 - Round 3	1.09 ± 0.15	NA	7.48	**<0.001**	2.99 ± 0.44	298.8 ± 43.8	
	Round 2 - Round 3	0.92 ± 0.15	NA	6.31	**<0.001**	2.52 ± 0.37	251.9 ± 36.9	

Model for floral abundance of floral categories

Table 3.5.4: Results of a Poisson GLMM (using a log-link function) for the floral abundance of different floral categories in meadows of different seed mix treatments. Fixed effects were mix type, formulation, floral category and round, with an interaction between floral category and mix type. Block was fit as a random effect. A random round model did not converge and is therefore not presented.

Predictors (fixed effects)	**Response:** Floral abundance			
	Model structure			
	Models with 'round' as fixed			
	df	AIC	X^2	p-value
Full model AIC		2725.8		
Mix type	NA	NA	NA	NA
Floral category	NA	NA	NA	NA
Mix type * Floral category	4	2804.9	87.188	**<0.001**
Formulation	1	2723.9	0.106	0.75
Round	2	2764.8	42.996	**<0.001**

Table 3.5.5: Summary of Poisson GLMMs (using a log-link functions).

	Response: Floral abundance of floral categories			
	Model structure			
	Fixed round			
Predictors	Estimate ± SE	*z*	*P*	Model fit
Intercept	7.19 ± 0.23	30.834	**<0.001**	AIC
mix type: marmalade	-1.00 ± 0.28	-3.566	0.0003	2725.8
mix type: short	-0.24 ± 0.28	-0.845	0.39	logLik
class:contaminant	-3.82 ± 0.29	-13.384	**<0.001**	-1348.9
class: weed	-1.63 ± 0.28	-5.855	**<0.001**	dev
formulation: enriched	-0.04 ± 0.13	-0.325	0.75	2697.8
round: round 2	-0.30 ± 0.17	-1.796	0.073	df.resid
round: round 3	-1.13 ± 0.17	-6.561	**<0.001**	202
mix type-floral category: marmalade-contaminant	3.82 ± 0.41	9.306	**<0.001**	
mix type- floral category: short- contaminant	1.30 ± 0.41	3.21	0.0013	
mix type- floral category: marmalade-weed	1.37 ± 0.39	3.467	0.0005	
mix type- floral category: Short - weed	0.43 ± 0.39	1.101	0.27	
Random	Variance	SD		
Observation ID	**-**	**-**		
block	0.0000	0.0000		
round	**-**	**-**		

Table A3.5.6 (on subsequent pages): Pairwise contrasts of floral abundance of different floral categories. Conditional pairwise contrasts were performed comparing levels of each factor within each level of the corresponding interacting factor. Pairwise differences in estimated marginal means of floral abundance were calculated: (a) between seed mix types, for each floral category: treatment, contaminant or weed; and (b) within seed mix types, between floral categories (treatment, contaminant and weed). *P*-values were adjusted to account for 18 simultaneous tests using the 'mvt' method in R package 'lsmeans'. Results are averaged over levels of enrichment and round.

(a) Difference in floral abundance between seed mix types for treatment, contaminant or weed floral categories									
			Model scale estimates				Response scale estimates		
Estimated means	Level of floral category	Level of seed mix type	LS mean ± SE	df	Lower 95% CI	Upper 95% CI	Mean ± SE	Lower 95% CI	Upper 95% CI
	Treatment	Marmalade	5.69 ± 0.19	NA	5.30	6.09	297.21 ± 59.38	200.91	439.67
		Short	6.46 ± 0.19	NA	6.08	6.85	641.36 ± 126.16	436.17	943.07
		Cornfield	6.69 ± 0.19	NA	6.31	7.09	812.03 ± 160.82	550.80	1197.14
	Contaminant	Marmalade	5.70 ± 0.20	NA	5.31	6.09	299.30 ± 60.07	201.97	443.55
		Short	3.95 ± 0.20	NA	3.56	4.34	51.92 ± 10.45	34.99	77.03
		Cornfield	2.88 ± 0.21	NA	2.48	3.29	17.88 ± 3.67	11.96	26.72484
	Weed	Marmalade	5.44 ± 0.19	NA	5.05	5.82	229.67 ± 45.07	156.33	337.39
		Short	5.27 ± 0.19	NA	4.88	5.65	193.52 ± 37.99	131.72	284.32
		Cornfield	5.07 ± 0.19	NA	4.68	5.45	158.86 ± 31.19	108.11	233.43
			Model scale estimates				Response scale estimates		
Pair-wise contrasts	Level of floral category	Contrast	Estimated difference in means ± SE	df	*z*-ratio	*P*-value	Estimated ratio of means ± SE		Estimated % difference between means ± SE
	Treatment	Cornfield - Marmalade	1.01 ± 0.28	NA	3.566	**0.006**	2.73 ± 0.77		273.22 ± 77.01
		Cornfield - Short	0.24 ± 0.28	NA	0.845	0.99	1.27 ± 0.35		126.61 ± 35.36
		Marmalade - Short	-0.77 ± 0.28	NA	-2.769	0.0785	0.46 ± 0.13		46.34 ± 12.87
	Contaminant	Cornfield - Marmalade	-2.82 ± 0.29	NA	-9.68	**<0.0001**	0.06 ± 0.02		5.97 ± 1.74
		Cornfield - Short	-1.07 ± 0.29	NA	-3.666	**0.0041**	0.34 ± 0.10		34.43 ± 10.01
		Marmalade - Short	1.75 ± 0.28	NA	6.294	**<0.0001**	5.77 ± 1.60		576.46 ± 160.43
	Weed	Cornfield - Marmalade	-0.37 ± 0.28	NA	-1.327	0.88	0.69 ± 0.19		69.17 ± 19.21
		Cornfield - Short	-0.19 ± 0.28	NA	-0.711	0.99	0.82 ± 0.23		82.09 ± 22.79
		Marmalade - Short	0.17 ± 0.28	NA	0.617	0.99	1.19 ± 0.33		118.68 ± 32.92

(b) Differences in floral abundance within seed mix types for treatment, contaminant and weed floral categories

	Level of seed mix type	Level of Floral category	Model scale estimates				Response scale estimates		
			LS mean ± SE	df	Lower 95% CI	Upper 95% CI	Mean ± SE	Lower 95% CI	Upper 95% CI
Estimated means	Marmalade	Treatment	5.69 ± 0.19	NA	5.30	6.09	297.21 ± 59.38	200.91	439.67
		Contaminant	5.7 ± 0.20	NA	5.30	6.09	299.30 ± 60.07	201.97	443.55
		Weed	5.44 ± 0.19	NA	5.05	5.82	229.67 ± 45.07	156.33	337.39
	Short	Treatment	6.46 ± 0.19	NA	6.08	6.85	641.36 ± 126.16	436.17	943.07
		Contaminant	3.95 ± 0.20	NA	3.56	4.34	51.92 ± 10.45	34.99	77.03
		Weed	5.27 ± 0.19	NA	4.88	5.65	193.52 ± 37.99	131.72	284.32
	Cornfield	Treatment	6.69 ± 0.19	NA	6.31	7.09	812.03 ± 160.82	550.80	1197.14
		Contaminant	2.88 ± 0.21	NA	2.48	3.29	17.88 ± 3.67	11.96	26.73
		Weed	5.07 ± 0.19	NA	4.68	5.45	158.86 ± 31.19	108.11	233.43

	Level of seed mix type	Contrast	Model scale estimates				Response scale estimates	
			Estimated difference in means ±SE	df	*z*-ratio	*P*-value	Estimated ratio of means ± SE	Estimated % difference between means ± SE
Pair-wise contrasts	Marmalade	Treatment - Contaminant	-0.01 ± 0.29	NA	-0.024	1.00	0.99 ± 0.29	99.30 ± 28.66
		Treatment - Weed	0.26 ± 0.28	NA	0.918	0.99	1.29 ± 0.36	129.41 ± 36.36
		Contaminant - Weed	0.27 ± 0.28	NA	0.948	0.98	1.30 ± 0.36	130.32 ± 36.41
	Short	Treatment - Contaminant	2.51 ± 0.28	NA	8.854	**<0.0001**	12.35 ± 3.51	1235.26 ± 350.70
		Treatment - Weed	1.19 ± 0.28	NA	4.308	**0.0003**	3.31 ± 0.92	331.41 ± 92.17
		Contaminant - Weed	-1.32 ± 0.28	NA	-4.686	**0.0001**	0.27 ± 0.08	26.83 ± 7.53
	Cornfield	Treatment - Contaminant	3.82 ± 0.29	NA	13.383	**<0.0001**	45.42 ± 12.95	4542.43 ± 1295.26
		Treatment - Weed	1.63 ± 0.28	NA	5.854	**<0.0001**	5.11 ± 1.43	511.17 ± 142.48
		Contaminant - Weed	-2.19 ± 0.28	NA	-7.7	**<0.0001**	0.11 ± 0.03	11.25 ± 3.19

Appendix A4

A4.1 Morphological identification of insects

A4.1.1 Protocols used to identify different taxonomic groups

Insect specimens were identified morphologically either to Linnaean species or to morphotypes. Taxon-appropriate keys were used to identify informative characters for sorting specimens into morphotypes, using external morphological characters (excluding internal genitalia). A small number of specimens with damage to anatomy bearing important diagnostic characters, such as missing or broken legs, were allocated to morphotaxa (Linnaean species and morphotypes) based on morphological similarity in the totality of available morphological characters.

Table A4.1: Summary of the protocols and taxonomic literature used to morphologically identify different taxonomic groups. Protocol numbers and literature refer to identification within the associated taxonomic level. The two protocols were: (1) full identification to species using taxon-specific keys; or (2) identification to morphotype using taxon-appropriate keys to highlight informative characters. All Lepidoptera were identified by Dr. Keith Bland of the National Museum of Scotland. Families marked with an asterisk were identified to morphotype using family-level keys since they were represented by single specimens.

Order	Family	Protocol 1	Protocol 2	Source
Hymenoptera	Families	x	-	Goulet and Huber, 2013
	Andrenidae	x	x	BWARS
	Apidae	x	-	Benton, 2006; Baldock, 2008; Prŷs-Jones and Corbet, 2011
	Colletidae	x	-	BWARS
	Crabronidae	-	x	*
	Halictidae	x	-	BWARS
	Tenthredinidae	-	x	*
	Vespidae	-	x	*
Diptera	Families	x	-	Unwin, 1981; Oosterbroek, 2006; Ball, 2008
	Anthomyiidae	x	x	Ackland, 2012
	Calliphoridae	x	x	van Emden, 1954; Jewiss-Gaines *et al.*, 2012; Falk, 2016
	Bibionidae	-	x	*
	Lonchopteridae	-	x	*
	Milichiidae	-	x	Brake, 2000
	Muscidae	x	x	D'Assis Fonseca, 1968
	Psilidae	-	x	*
	Sarcophagidae	x	x	van Emden, 1954; Hackston, 2015; Falk, unpublished
	Scathophagidae	x	-	Ball, 2014
	Sepsidae	x	-	Pont & Meier, 2002
	Syrphidae	x	x	Stubbs and Falk, 2002; Ball and Morris, 2013
	Tachinidae	x	x	van Emden, 1954; Belshaw, 1993
Lepidoptera	Families	x	-	N/A
	Choreutidae	x	-	N/A
	Hesperiidae	x	-	N/A
	Lycaenidae	x	-	N/A
	Nymphalidae	x	-	N/A
	Pieridae	x	-	N/A
	Zygaenidae	x	-	N/A
Coleoptera	Families	x	-	Unwin, 1984
	Apionidae	-	x	Gurney, 2016
	Aphodiidae	-	x	*
	Cantharidae	x	-	Eversham, 2006
	Chrysomelidae	-	x	*
	Kateridae	x	-	Kirk-Spriggs, 1996
	Nitidulidae	x	-	Kirk-Spriggs 1996; Hackston, 2009
	Curculionidae	-	x	Gurney, 2016
	Oedemeridae	x	-	Hackston 2014
	Phalacridae	x	-	Telfer, 2013

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A4.2 Selection of specimens for DNA barcoding

Table A4.2: Summary of insect flower-visitor sample sizes, the proportions selected for sequencing and the proportions successfully sequenced.

	Number of individuals in sample	Number of individuals selected for sequencing	% individuals selected for sequencing	Number of individuals successfully PCR amplified	% of individuals selected for sequencing successfully amplified	Number of individuals successfully sequenced	% of PCR amplicons successfully sequenced	**% of total insect sample successfully sequenced**
Hymenoptera	347	117	33.7	116	99.1	116	100.0	33.4
Diptera	1003	552	55.0	546	98.9	542	99.3	54.0
Lepidoptera	46	40	87.0	40	100.0	40	100.0	87.0
Coleoptera	174	172	98.9	172	100.0	172	100.0	98.9
Total	1570	881	56.1	874	99.2	870	99.5	55.4

A4.3 Selection of DNA loci and the primers used for sequencing

Table A4.3.1: Primer combinations and sequences used to target, amplify and sequence fragments of the cytochrome c oxidase 1 gene (CO1).

Primer set	Target	Region	Primer sequence 5' => 3'	Target length	References
LepF1 LepR1	CO1	5' CO1 (Folmer region)	ATTCAACCAATCATAAAGATATTGG TAAACTTCTGGATGTCCAAAAAATCA	658 bp	Hebert *el al*., 2004 Hebert *el al*., 2004
LCO1490 HCO2198	CO1	5' CO1 (Folmer region)	GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA	658 bp	Folmer *et al*., 1994 Folmer *et al*., 1994
MLepF1 HCO2198 LepR1	CO1	5' CO1 (Folmer region)	GCTTTCCCACGAATAAATAATA - -	407 bp	Hajibabaei *et al*., 2006 - -
LepF1 LCO1490 MLepR2	CO1	5' CO1 (Folmer region)	- - GTTCAWCCWGTWCCWGCYCCATTTTC	307 bp	- - Hebert *et al*., 2013
SJerry_F SPatR	CO1	3' CO1	CAACATYTATTYTGATTYTTTGG GCACTAWTCTGCCATATTAGA	900 bp	Timmermans *et al*., 2010 Timmermans *et al*., 2010

Hymenoptera, Diptera and Lepidoptera were sequenced for the standard animal barcode region of CO1, known as the Folmer region. In contrast, Coleoptera were sequenced for two non-overlapping, contiguous regions of CO1.

Coleoptera were initially screened for a 900 bp 3' region of CO1, using a primer pair (SJerryF/SPatR) developed using Coleoptera DNA sequences (Timmermans *et al*. 2010). This region is known to amplify more easily than the Folmer region in some groups of beetles including flower-visiting pollen beetles (Nitidulidae and Meligethinae; Ouvrard *et al.* 2016). However, amplification of this region was not successful for all specimens, with the spread of amplification failures across taxa suggesting that failures may have been due to a combination of fragmented DNA (from low quality DNA extractions) and a relatively long target fragment.

Samples that failed to amplify using SJerryF/SPatR were then progressively screened for the full length Folmer region, or for two shorter sequences within the Folmer region (Table A4.3.1). Given that there is no overlap between the Folmer region and the region amplified by SjerryF/SpatR, specimens sequenced for these regions could not be directly clustered together into MOTUs using pairwise sequence divergence and were analysed separately. In order to cross-validate MOTUs defined using separate loci, for each morphotaxon, a subset of the specimens that were successfully sequenced for the region amplified by SjerryF/SpatR were sequenced for the Folmer region.

Specimens from 6 morphotaxa were sequenced for both regions of CO1, enabling sequences from the same individuals to be included in both sequence clustering analyses and the resulting MOTUs cross-validated.

However, for 5 morphotaxa, sequences were obtained for only one region of CO1 (Table A4.3.2). This precluded full pairwise comparison of sequence divergence among all Coleopteran morphotaxa. The resulting MOTUs for these taxa are therefore partially defined using morphological characters and are explicitly morpho-molecular taxa. However, given that these morphotaxa come from morphologically distinct families or genera, the morphotaxa and morpho-molecular taxa defined for Coleoptera are robust and delineated without bias.

Table A4.3.2: Summary of the numbers of individuals in each beetle morphotaxon along with the number sequenced for each of two contiguous regions of CO1.

Morphotaxon	Total number of individuals	Number of individuals sequenced	Number of individuals sequenced for 5' CO1 (Folmer Region)	Number of individuals sequenced for 3' CO1
Aphodiidae morphotype1	1	1	0	1
Chrysomelidae morphotype1	1	1	0	1
Mecinus pascuorum	1	1	0	1
Omphalapion morphotype1	1	1	1	0
Rhagonycha fulva	3	2	0	2
Meligethes aeneus	3	3	3	2
Meligethes viridescens	2	2	2	1
Oedemera lurida	4	4	4	1
Oedemera nobilis/virescens	8	8	8	7
Olibrus aeneus	148	147	72	81
Olibrus pygmaeus	2	2	2	1
Total	174	172	92	98

A4.4 Summary of jMOTU and ABGD analyses

Sequences were clustered into molecular operational taxonomic units using two approaches: jMOTU and ABGD. The aim of these approaches is to avoid the arbitrary selection of a sequence divergence clustering threshold by examining a series of candidate thresholds for a given dataset and highlighting the presence of a 'barcoding gap', enabling data driven selection of a sequence clustering threshold.

For analysis, sequences were separated into six groups based on relatedness and CO1 region: Hymenoptera, syrphid Diptera, non-syrphid Diptera, Lepidoptera, Coleoptera (5' Folmer region) and Coleoptera (3' CO1).

For syrphid Diptera and non-syrphid Diptera, short sequences were recovered for some specimens due to poor quality sequencing data. For a small number of species pairs these sequences did not overlap.

In jMOTU, all sequences of syrphid Diptera or non-syrphid Diptera were clustered into MOTUs in a single analysis for each group. However, for ABGD, the simultaneous clustering of all sequences in each of these two groups was precluded by short sequences, which for some sequence pairs did not overlap and prevented calculation of a pairwise distance matrix. To cluster all sequences into MOTU and enable cross-validation of MOTU designations, sequences for each of these two taxonomic groups were analysed in ABGD in two batches, alternately excluding short sequences covering either the 5' end or the 3' end of the Folmer region. The MOTUs defined for these analyses were then compared to generate consensus MOTU designations, cross-validating the MOTU designations of sequences excluded from one or other analysis.

For the four other groups of sequences analysed in jMOTU and ABGD, all sequences overlapped sufficiently for a pairwise distance matrix to be calculated for ABGD.

Figures A4.4.1: Hymenoptera

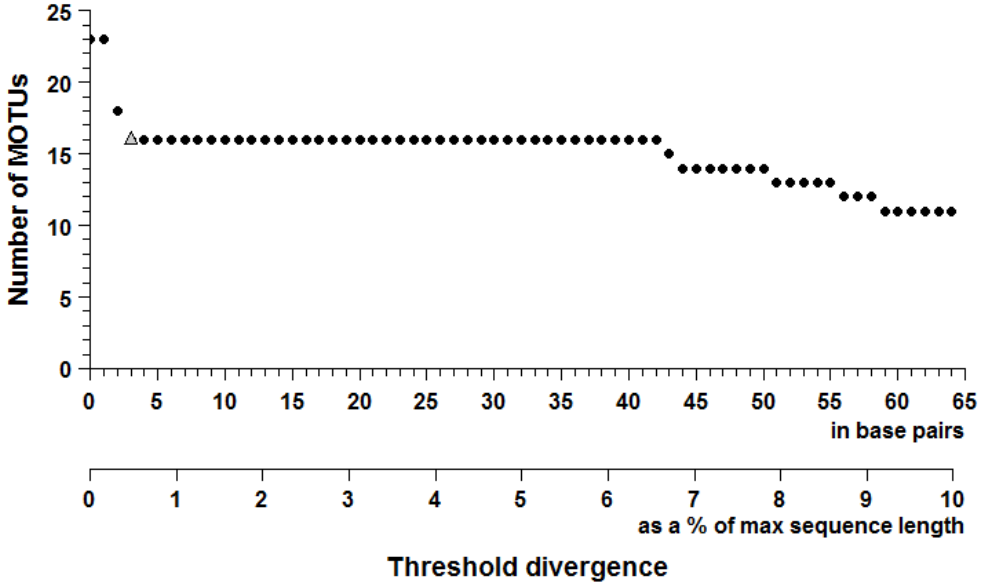


Figure A4.4.2: Diptera: Syrphidae

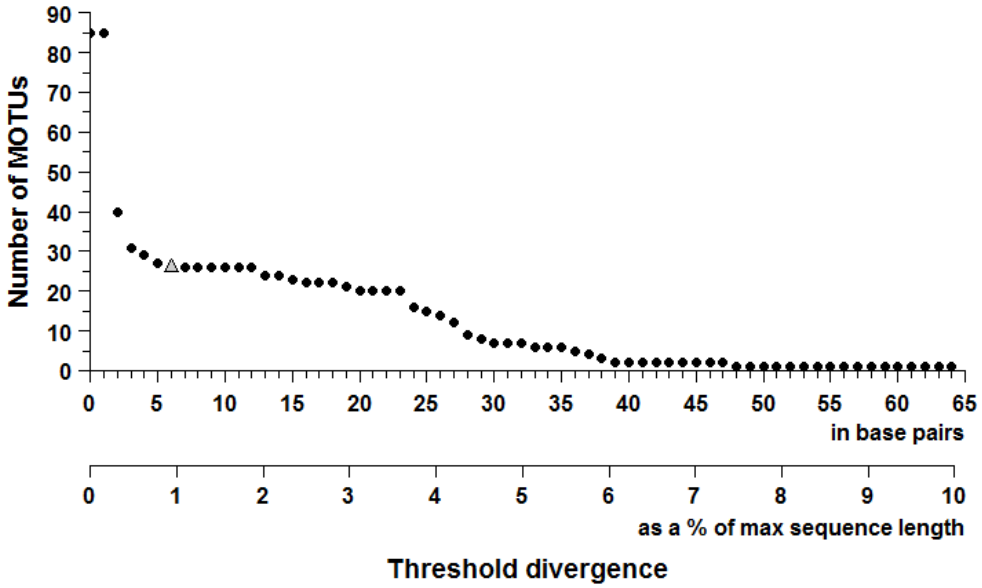


Figure A4.4.3a: Non-syrphid Diptera

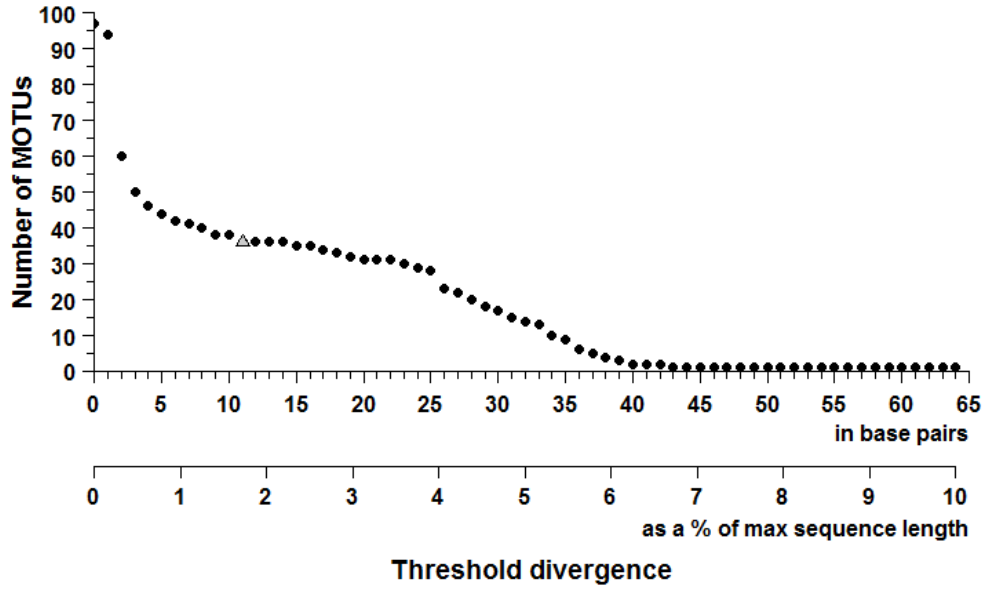


Figure A4.4.4: Lepidoptera

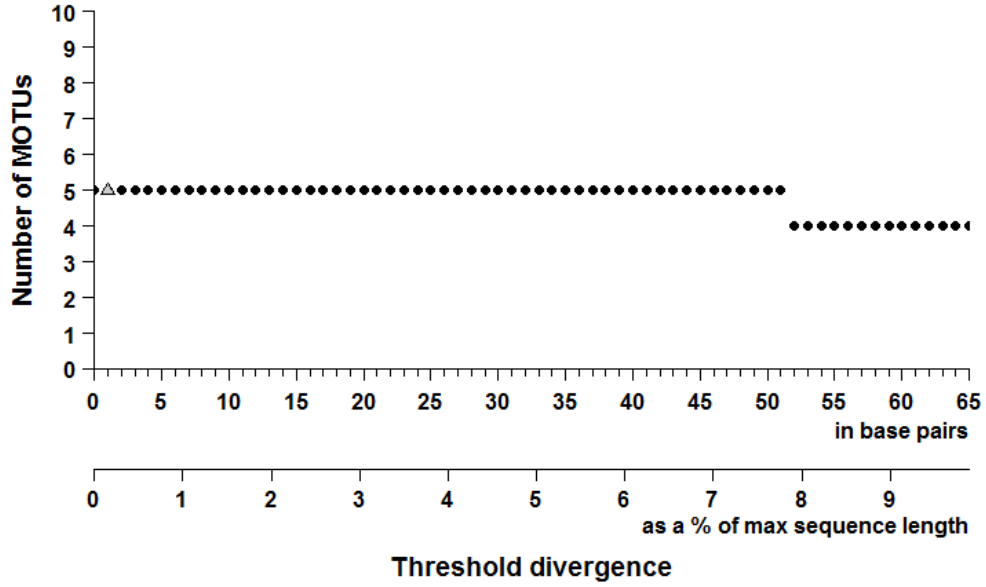


Figure A4.4.5a: Coleoptera - 5' CO1 (Folmer region)

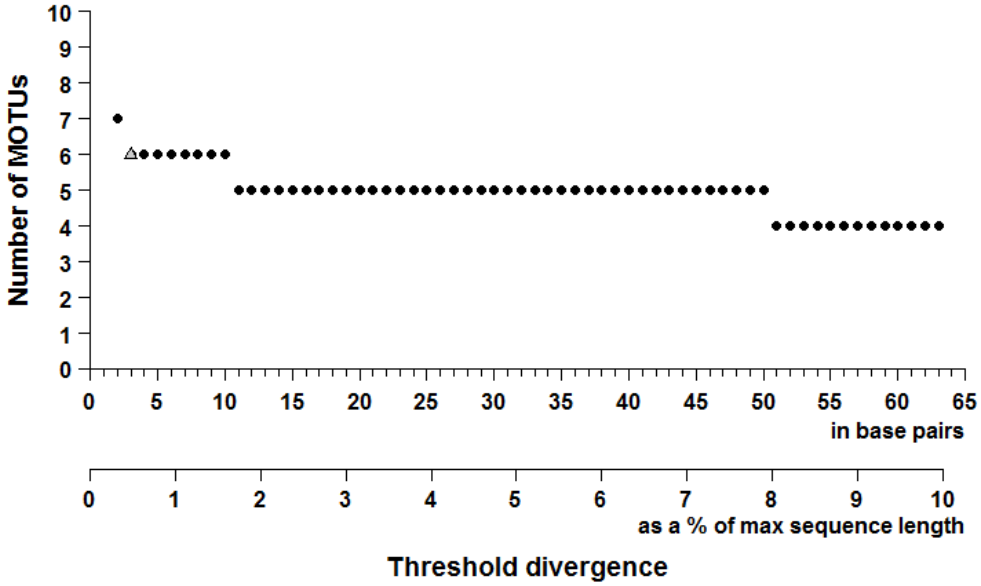
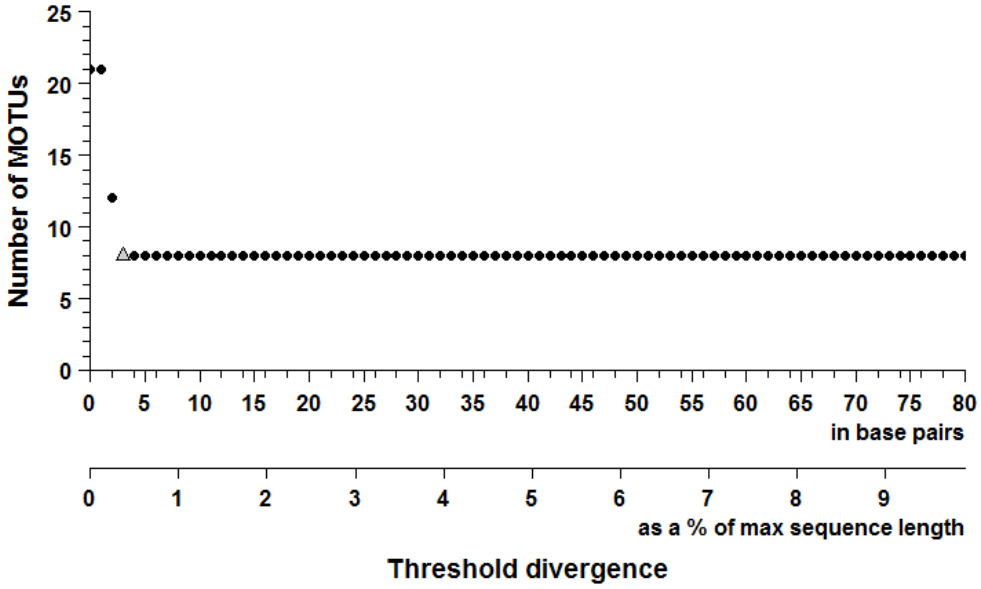


Figure A4.4.6a: Coleoptera - 3' CO1



Figures A4.4.1-A4.4.6: The number of MOTUs defined for at alternative candidate pairwise sequence divergence clustering thresholds in jMOTU. For jMOTU analyses, the threshold was identified as the lower limit of the first major plateau encountered as thresholds increased from 0 bp (indicated by a grey triangle '△'). Threshold divergence as a % of maximum sequence length was calculated relative to the maximum sequence length for each taxonomic group or CO1 region. Figures for ABGD are not shown since patterns were consistent with jMOTU analyses.

A4.4.2 Comparison of MOTUs defined by alternative sequence clustering programmes

Table A4.4.2 provides a comparison of the MOTUs defined by alternative sequence clustering programmes. For jMOTU analyses, main plateaus were identified from figures A4.4.1-A4.4.6a with sequence clustering thresholds defined as the lower limit in base pairs of the main plateau. Sequence diversity below this threshold is likely to be intraspecific diversity and sequence diversity above the main plateau is likely to be interspecific diversity. Clustering thresholds as a % of maximum sequence length are calculated relative to the maximum sequence length for each taxonomic group or CO1 region. For ABGD analyses, ABGD figures are not shown but MOTUs are shown in Table A4.4.2.

For Coleoptera, consensus MOTUs were generated by cross-validating MOTUs defined separately for the Folmer region and the 3' region of CO1, using individuals that were sequenced for both regions. The consensus number of MOTUs was lower than the sum of MOTUs for both regions because the some MOTUs were present in both samples.

Table A4.4.2: Summary of the sequence divergence thresholds used to cluster sequences of different orders and CO1 regions into MOTUs, and the number of MOTUs defined, in jMOTU and ABGD.

	Divergence thresholds and MOTUs defined in jMOTU						**Divergence thresholds and MOTUs defined in ABGD**					
	Plateau in bp	Plateau as a % of max seq. length	Threshold in bp	Threshold as a % of max seq. length	Number of MOTUs	Total number of MOTUs	Plateau in % prior intraspecific divergence		% prior intraspecific divergence		Number of MOTUs	Total number of MOTUs
Hymenoptera	3 - 42 bp	0.5 - 6.6	3 bp	0.5	16	16	0.1 – 6.2		0.1		16	16
Lepidoptera	0 - 51 bp	0 - 7.8	0 bp	0	5	5	0.1 - 10		0.1		5	5
Coleoptera 5’ CO1	3 - 10 bp	0.5 -1.6	3 bp	0.5	6	10	0.1 - 10		0.1		5	9
Coleoptera 3’ CO1	3 - 94 bp	0.4 - 11.6	3 bp	0.4	8		0.1 - 10		0.1		8	
							5’ Folmer	3’ Folmer	5’ Folmer	3’ Folmer	5’ Folmer	3’ Folmer
Syrphid Diptera	6 - 12 bp	0.9 - 1.9	6 bp	0.9	26	62	0.4 - 2	0.36 - 2	0.36	0.36	24	25	62
Non-Syrphid Diptera	11 - 14 bp	1.7 - 2.2	11 bp	1.7	36		0.8 - 5.3	0.5 - 4.5	0.79	0.49	33	37

A4.4.3 Comparison of MOTUs defined in ABGD for analyses excluding short sequences covering either the 5’ end or the 3’ end of the Folmer region

In jMOTU, all sequences of syrphid Diptera or non-syrphid Diptera were clustered into MOTUs in a single analysis for each group. However, for ABGD, the simultaneous clustering of all sequences in each of these two groups was precluded by a few short sequences, which did not overlap and prevented calculation of a pairwise distance matrix. To cluster all sequences into MOTU and enable cross-validation of MOTU designations, sequences for each of these two taxonomic groups were analysed in two batches, alternately excluding short sequences covering either the 5’ end or the 3’ end of the Folmer region. The MOTUs defined for these analyses were then compared to generate consensus MOTU designations, cross-validating the MOTU designations of sequences excluded from one or other analysis.

The majority of MOTU designations were identical, given that all long or full length sequences were included in both sets of analyses. The only differences between analyses were in two non-syrphid Dipteran clades. For these two clades, MOTU designations were discordant between analyses excluding either short 3’ or short 5’ Folmer sequences (Table A4.4.3). However, MOTU designations for the dataset excluding short 5’ Folmer sequences were fully concordant with MOTUs defined in jMOTU. This pattern is consistent with previous research demonstrating that the 3’ end of the Folmer region is more variable and therefore has greater power to resolve taxa. For full comparison of MOTU definitions between ABGD and jMOTU approaches, we therefore selected MOTUs defined using a subset of sequences that excluded short sequences at the 5’ end of the Folmer region.

Table A4.4.3: Clades for which discordant results were returned for ABDG analyses that excluded short, non-overlapping sequences covering either the 5' or 3' end of the Folmer region. Apart from the sequence for specimen B_0634, for which a short sequence at the 3' end of the Folmer region was recovered, all sequences recovered for specimens in these clades were long or full length sequences. Colours aid comparison of differences in specimen MOTU designations. All MOTUs are defined at threshold of main plateau.

Clade	Specimen code	Morphological identification	jMOTU analysis	ABGD analyses	
				MOTUs for a subset of seqs. excluding short 3' Folmer region sequences (5'end analysis)	MOTUs for a subset of seqs. excluding short 5' Folmer region sequences (3' end analysis)
1	A_0553	*Sarcophaga subvicina*	MOTU0012	5end_MOTU0012	3end_MOTU0022
	B_0275	*Sarcphaga melanura*	MOTU0011	5end_MOTU0011	3end_MOTU0011
	B_0516	*Sarcophaga carnaria/variegata*	MOTU0012	5end_MOTU0012	3end_MOTU0022
	D_0433	*Sarcophaga carnaria/variegata*	MOTU0023	5end_MOTU0012	3end_MOTU0024
	D_0453	*Sarcophaga carnaria/variegata*	MOTU0023	5end_MOTU0012	3end_MOTU0024
	D_0487	*Sarcphaga melanura*	MOTU0011	5end_MOTU0011	3end_MOTU0011
	D_0532	*Sarcophaga carnaria/variegata*	MOTU0030	5end_MOTU0012	3end_MOTU0023
	D_0541	*Sarcophaga carnaria/variegata*	MOTU0030	5end_MOTU0012	3end_MOTU0023
2	A_0269	*Helina setiventris*	MOTU0032	5end_ MOTU0013	3end_MOTU0014
	A_0424	*Helina reversio*	MOTU0006	5end_ MOTU0013	3end_MOTU0013
	A_0542	*Helina reversio*	MOTU0004	5end_ MOTU0013	3end_MOTU0015
	B_0634	*Helina reversio*	MOTU0006	NA	3end_MOTU0013
	D_0534	*Helina reversio*	MOTU0006	5end_ MOTU0013	3end_MOTU0013
	D_0585	*Helina reversio*	MOTU0006	5end_ MOTU0013	3end_MOTU0013
	E_0226	*Helina reversio*	MOTU0006	5end_ MOTU0013	3end_MOTU0013
	E_0314	*Helina reversio*	MOTU0006	5end_ MOTU0013	3end_MOTU0013
	E_0604	*Helina reversio*	MOTU0006	5end_ MOTU0013	3end_MOTU0013

A4.4.4 Comparison of differences in the MOTUs defined by alternative sequence clustering approaches

Molecular taxa defined in jMOTU and ABGD were almost fully concordant. Sequences were recovered from 103 out of the 109 morphologically-defined taxa. From these, 93 MOTUs were delineated by jMOTU. For ABGD, 92 MOTUs were delineated with 88 corresponding exactly to equivalent MOTUs defined by jMOTU. For 3 clades, MOTU definitions were discordant between jMOTU and ABGD (Table A4.4.4). For syrphid Dipteran and Coleopteran clades, the MOTUs defined by jMOTU were more congruent with morphological identifications. However, for a non-syrphid Dipteran clade, MOTUs defined by ABGD appeared more consistent with morphotaxon designations.

Overall, MOTU designations differed little between jMOTU and ABGD, while, for those which did differ, jMOTU designations were on average more consistent with morphological identifications. Given that the sequence clustering approach of jMOTU is also simpler and easier to implement, we selected jMOTU molecular taxon definitions for full comparison with morphologically-defined taxa.

Table A4.4.4: Clades for which discordant results were returned for jMOTU versus ABGD analyses.

Clade	Group	Morphological identification	jMOTU analysis	ABGD analyses
			MOTUs defined at threshold of main plateau	**MOTUs defined at threshold of main plateau**
1	Syrphid Diptera	*Chrysotoxum bicinctum*	syrDip_08	sDip_08&26
		Chrysotoxum festivum	syrDip_26	
2	Non-syrphid Diptera	Botanophila group D (♂)	nsDip_14	nsDip_14a
		Anthomyiidae morphotype 5 (♀)		
		Delia group C (♂)		nsDip_14b
		Anthomyiidae morphotype 6 (♀)		
3	Coleoptera	*Oedemera lurida*	Col_06	Col_04&06
		Oedemera nobilis / virescens	Col_04	

A4.4.5 Full results of jMOTU analyses

Table A4.4.5: Summary of changes in morphotaxon definitions due to molecular taxonomic analysis. Morphotaxa affected by changes are highlighted in yellow. Colours indicate the five potential types of change: (i) **Green:** full lumping of individuals from multiple morphotaxa into a single morpho-molecular taxon; (ii) **Orange:** full splitting of individuals in a single morphotaxon into multiple morpho-molecular taxa; (iii) **Purple:** combined splitting and lumping of individuals from multiple morphotaxa with no net change in taxon richness; (iv) **Dark Blue**: combined splitting and lumping of individuals from multiple morphotaxa with a net decrease in taxon richness; (v) **Light Blue:** combined splitting and lumping of individuals from multiple morphotaxa with a net increase in taxon richness.

Taxa defined by morphology				Taxa defined using jMOTU		
	Morphotaxon	Individuals	Individuals sequenced	**MOTUs**	Individuals per MOTU	Changes relative to morphotaxa
Hymenoptera	*Ametastegia morphotype 1*	1	1	Hym_16	1	
	Andrena morphotype 1	2	2	Hym_03	2	
	Andrena morphotype 2 (♂)	1	1	Hym_12	3	*Andrena* lumped
	Andrena morphotype 3 (♀)	2	2			
	Apis mellifera	88	0	NA	NA	
	Bombus hortorum	2	2	Hym_02	2	
	Bombus hypnorum	1	1	Hym_07	1	
	Bombus lapidarius	83	10	Hym_06	10	
	Bombus lucorum	15	15	Hym_09	3	Minus 12 '*B. lucorum*'
	Bombus pascuorum	78	8	Hym_05	8	
	Bombus pratorum	1	1	Hym_15	1	
	Bombus sylvestris	1	1	Hym_08	1	
	Bombus terrestris	45	45	Hym_10	57	Plus 12 '*B. lucorum*'
	Colletes daviesanus	21	21	Hym_11	21	
	Crossocerus sp.	1	1	Hym_04	1	
	Lasioglossum smeathmanellum	3	3	Hym_14	3	
	Tenthredo arcuata	1	1	Hym_01	1	
	Vespula vulgaris	1	1	Hym_13	1	
Lepidoptera	*Aglais urticae*	1	0	NA	NA	
	Anthophila fabriciana	34	34	Lep_01	34	
	Lycaena phlaeas	1	1	Lep_04	1	
	Maniola jurtina	3	0	NA	NA	
	Ochlodes venata	1	1	Lep_03	1	
	Pieris brassicae	2	0	NA	NA	
	Thymelicus sylvestris	3	3	Lep_02	3	
	Zygaena filipendulae	1	1	Lep_05	1	

	Morphotaxon	Individuals	Individuals sequenced	MOTUs	Individuals per MOTU	Changes relative to morphotaxa
Coleoptera	*Mecinus pascuorum*	1	1	Col_10	1	
	Meligethes aeneus	3	3	Col_05	3	
	Meligethes viridescens	2	2	Col_01	2	
	Aphodiidae morphotype 1	1	1	Col_09	1	
	Chrysomelidae morphotype 1	1	1	Col_08	1	
	Oedemera lurida	4	4	Col_06	3	Minus one *O. lurida*
	Oedemera nobilis / virescens	8	8	Col_04	9	Plus one *O. lurida*
	Olibrus aeneus	148	147	Col_02	149	All *Olibrus* spp. lumped
	Olibrus pygmaeus	2	2			
	Omphalapion sp.	1	1	Col_03	1	
	Rhagonycha fulva	3	2	Col_07	2	
Syrphid Diptera	*Cheilosia vernalis*	5	5	syrDip_14	5	
	Chrysotoxum bicinctum	2	2	syrDip_08	2	
	Chrysotoxum festivum	1	1	syrDip_26	1	
	Dasysyrphus albostriatus	1	1	syrDip_25	1	
	Episyrphus balteatus	11	2	syrDip_18	2	
	Eristalis arbustorum	10	2	syrDip_17	2	
	Eristalis pertinax	6	6	syrDip_15	6	
	Eristalis tenax	7	2	syrDip_01	2	
	Eupeodes corollae	1	1	syrDip_21	1	
	Eupeodes luniger	20	20	syrDip_10	20	
	Helophilus pendulus	16	2	syrDip_09	2	
	Helophilus trivittatus	1	1	syrDip_22	1	
	Lejogaster metallina	2	2	syrDip_07	2	
	Myathropa florea	5	5	syrDip_04	5	
	Neoascia podagrica	7	7	syrDip_19	7	
	Platycheirus albimanus	20	20	syrDip_03	20	
	Platycheirus granditarsus	1	1	syrDip_12	1	
	Platycheirus manicatus	7	7	syrDip_20	7	
	Platycheirus scutatus s.l.	7	7	syrDip_23	7	
	Scaeva pyrastri	1	1	syrDip_24	1	
	Sphaerophoria interrupta (♂)	5	5	syrDip_05	56	All *Sphaerophoria* lumped
	Sphaerophoria scripta (♂)	28	28			
	Sphaerophoria sp. (♀)	23	23			
	Syritta pipiens	64	63	syrDip_13	63	
	Syrphus ribesii	37	37	syrDip_11	35	Minus two '*S. ribesii*'
	Syrphus torvus	11	11	syrDip_06	13	Plus two '*S. ribesii*'
	Eupeodes latifasciatus	7	7	syrDip_16	8	Plus one '*Syrphus rectus*'
	Syrphus rectus (♀)	2	2	NA	0	Minus both individuals
	Syrphus vitripennis (♀)	48	8	syrDip_02	15	All *S. vitripennis / rectus* lumped, plus one *Syrphus rectus*
	Syrphus vitripennis / rectus (♂)	6	6			

	Morphotaxon	Individuals	Individuals sequenced	MOTUs	Individuals per MOTU	Changes relative to morphotaxa
Non-syrphid Diptera	*Bellardia sp.*	1	1	nsDip_34	1	
	Botanophila group A (♂)	1	1	nsDip_31	1	
	Botanophila group D (♂)	1	1	nsDip_14	12	All *Botanophila* group D, Anthomyiidae morphotype 5, *Delia* group C & Anthomyiidae morphotype 6 lumped
	Anthomyiidae morphotype 5 (♀)	5	5			
	Delia group C (♂)	3	3			
	Anthomyiidae morphotype 6 (♀)	4	3			
	Coenosia sp.	1	1	nsDip_16	1	
	Eriothrix rufomaculata	29	5	nsDip_35	5	
	Exorista mimula/rustica	2	2	nsDip_25	2	
	Gymnocheta viridis	1	1	nsDip_18	1	
	Helina reversio	8	8	nsDip_04	1	Plus one *H. reversio*
				nsDip_06	7	Minus one *H. reversio*
	Helina setiventris	1	1	nsDip_30	1	
	Lucilia sp. (morphotype 1)	1	1	nsDip_33	1	
	Lucilia richardsi	2	2	nsDip_09	53	All *Lucilia richardsi* and *L. sericata* lumped
	Lucilia sericata	402	51			
	Lydella stabulans	1	1	nsDip_20	1	
	Anthomyiidae morphotype 1 (♀)	2	2	nsDip_17	2	
	Bibionidae morphotype 1	1	0	NA	NA	
	Lonchopteridae morphotype 1	1	1	nsDip_10	1	
	Psilidae morphotype 1	1	1	nsDip_07	1	
	Paradelia group A	1	0	NA	NA	
	Paregle sp. (♂)	5	4	nsDip_08	9	All *Paregle* and Anthomyiidae morphotype 3 lumped
	Anthomyiidae morphotype 3 (♀)	7	5			
	Pegoplata group A (♂)	14	14	nsDip_15	1	Plus one *Pegoplata* group A
	Anthomyiidae morphotype 2 (♀)	14	13	nsDip_01	26	*Pegoplata* group A & Anthomyiidae morphotype 2 lumped, minus one *Pegoplata* group A
	Phryxe heraclei	1	1	nsDip_32	1	
	Pollenia augustigena	1	1	nsDip_29	1	
	Pollenia rudis	4	4	nsDip_13	4	
	Sarcophaga carnaria / variegata	5	5	nsDip_22	2	Plus two *S. carnaria / variegata*
				nsDip_28	2	Plus two *S. carnaria / variegata*
	Sarcophaga subvicina	1	1	nsDip_12	2	One *S. carnaria / variegata* & all *S. subvicina* lumped
	Sarcophaga crassimargo	1	1	nsDip_36	1	
	Sarcophaga dissimilis	1	1	nsDip_03	1	
	Sarcophaga melanura	2	2	nsDip_11	2	
	Sarcophaga nigriventris	54	52	nsDip_02	52	
	Scathophaga stercoraria	6	6	nsDip_24	6	
	Sepsis cynipsea	4	4	nsDip_21	4	
	Sepsis fulgens	21	21	nsDip_05	23	All *Sepsis fulgens* & *S. orthocnemis* lumped
	Sepsis orthocnemis	2	2			
	Siphona geniculate	26	26	nsDip_19	26	
	Stomorhina lunata	1	1	nsDip_26	1	
	Muscinae morphotype 1	1	1	nsDip_27	1	
	Phaoniinae morphotype 1	1	1	nsDip_23	1	

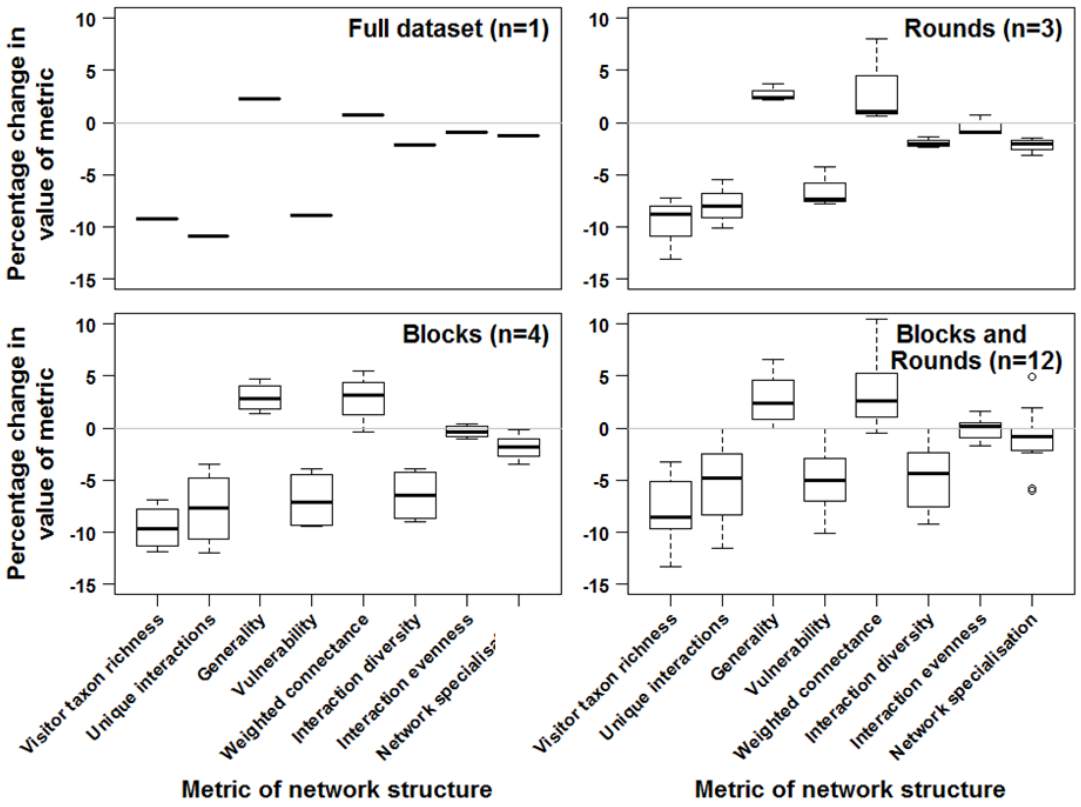
A4.5 Change in network structure due to molecular taxonomic analysis

Table A4.5.1: Change in flower-visitor network parameters when visitor taxa are defined by either morphology or morphology supplemented with molecular information. Network parameter and metric values are calculated for (a) seasonal networks for each of four replicate experimental blocks (n = 4) and (b) time-point specific networks for each of four replicate blocks surveyed at 3 time-points (n = 12). Values are means (± SE). *P*-values are for Wilcoxon signed rank tests, implemented using a normal approximation and continuity correction. Significance levels indicate significance at a Bonferonni adjusted alpha of 0.05/*k*, where *k* = 8 tests: ns > (0.05/8); * < (0.01/8).

	(a) Seasonal networks of experiment blocks (n=4)							
Metric	**Value for morpho-webs**	**SE**	**Value for morpho-molecular webs**	**SE**	**Mean change**	**SE**	**% Change**	**SE**
Floral species richness	34.8	1.8	34.8	1.8	0.0	0.0	0.0	0.0
No. of floral species visited	15.0	2.1	15.0	2.1	0.0	0.0	0.0	0.0
Observed interactions	392.5	23.4	392.5	23.4	0.0	0.0	0.0	0.0
Visitor taxon richness	57.8	0.6	52.3	0.9	-5.5	0.7	-9.5	1.1
Unique interactions	109.3	4.4	101.0	5.6	-8.3	1.9	-7.7	1.9
Generality ($\mathrm{G_{qw}}$)	2.35	0.13	2.42	0.14	0.07	0.02	2.95	0.71
Vulnerability ($\mathrm{V_{qw}}$)	9.55	0.42	8.89	0.37	-0.66	0.15	-6.88	1.42
Weighted connectance	0.08	0.01	0.08	0.01	0.00	0.00	2.87	1.20
Interaction diversity (e^{H_2})	43.49	3.40	40.80	3.69	-2.69	0.40	-6.44	1.28
Interaction evenness (IE_S)	0.56	0.01	0.56	0.01	0.00	0.00	-0.31	0.33
Specialisation (H_2')	0.49	0.04	0.48	0.04	-0.01	0.00	-1.83	0.67

Metric	(b) Time-point specific networks of experiment blocks (n=12)										
	Value for morpho-webs	**SE**	**Value for morpho-molecular webs**	**SE**	**Mean change**	**SE**	**% Change**	**SE**	**Wilcoxon signed rank test statistic (*W*)**	***P*-value**	
Floral species richness	26.2	1.4	26.2	1.4	0.0	0.0	0.0	0.0	na	na	
No. of floral species visited	9.2	0.7	9.2	0.7	0.0	0.0	0.0	0.0	na	na	
Observed interactions	130.8	15.2	130.8	15.2	0.0	0.0	0.0	0.0	na	na	
Visitor taxon richness	28.4	2.1	26.1	2.0	-2.3	0.4	-8.2	1.2	0	**0.00249**	*
Unique interactions	45.6	4.5	43.2	4.3	-2.4	0.5	-5.4	1.1	0	**0.00386**	*
Generality ($\mathrm{G_{qw}}$)	1.94	0.12	2.00	0.13	0.06	0.02	2.82	0.65	66	**0.00386**	*
Vulnerability ($\mathrm{V_{qw}}$)	6.35	0.48	6.06	0.48	-0.29	0.05	-4.86	0.86	0	**0.00386**	*
Weighted connectance	0.11	0.01	0.12	0.01	0.00	0.00	3.45	0.96	74	0.0068	ns
Interaction diversity (e^{H_2})	24.52	2.06	23.39	2.04	-1.13	0.23	-4.87	0.86	0	**0.00386**	*
Interaction evenness (IE_S)	0.57	0.01	0.57	0.01	0.00	0.00	-0.05	0.27	40	0.9687	ns
Specialisation (H_2')	0.54	0.03	0.53	0.03	-0.01	0.00	-1.07	0.86	19	0.1261	ns

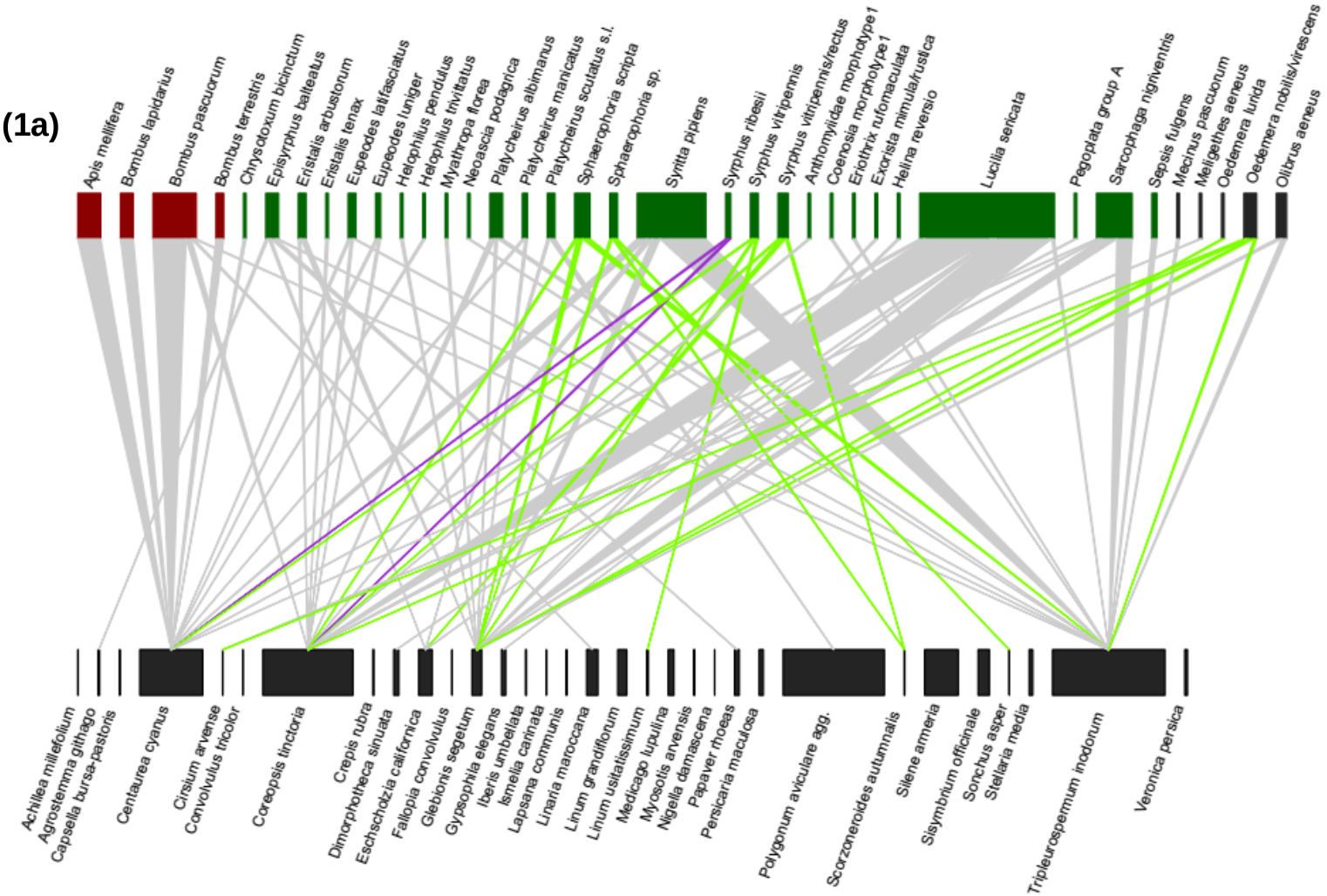
Figure A4.5.1: Percentage change in metrics of network structure for datasets with visitor taxa defined by either morphological or molecular information. Changes are differences in network metrics for the same dataset before and after molecular taxonomic analysis. Network structure metrics are calculated for: (a) a single network containing all data for the field site over the season (n=1); (b) networks for each of 3 survey time-points (n = 3); (c) seasonal networks for each of four replicate experimental blocks (n = 4); (d) time-point specific networks for each of 4 replicate blocks surveyed at 3 time-points (n = 12).



A4.6 Fully-labelled exemplar networks

Figure A4.6 (below): Two exemplar pairs of flower-visitor networks with insect visitor taxa defined using either (a) morphology or (b) morphology supplemented with molecular taxonomic analysis. Networks 1 and 2 are exemplars of n=12 block by survey networks used for statistical analyses. Network 1 (Block A Round 2) had the highest absolute and relative change in visitor generality (0.16 or 6.6%), whilst network 2 (Block D Round 3) had the lowest absolute and relative change in visitor generality (0%).

(1a)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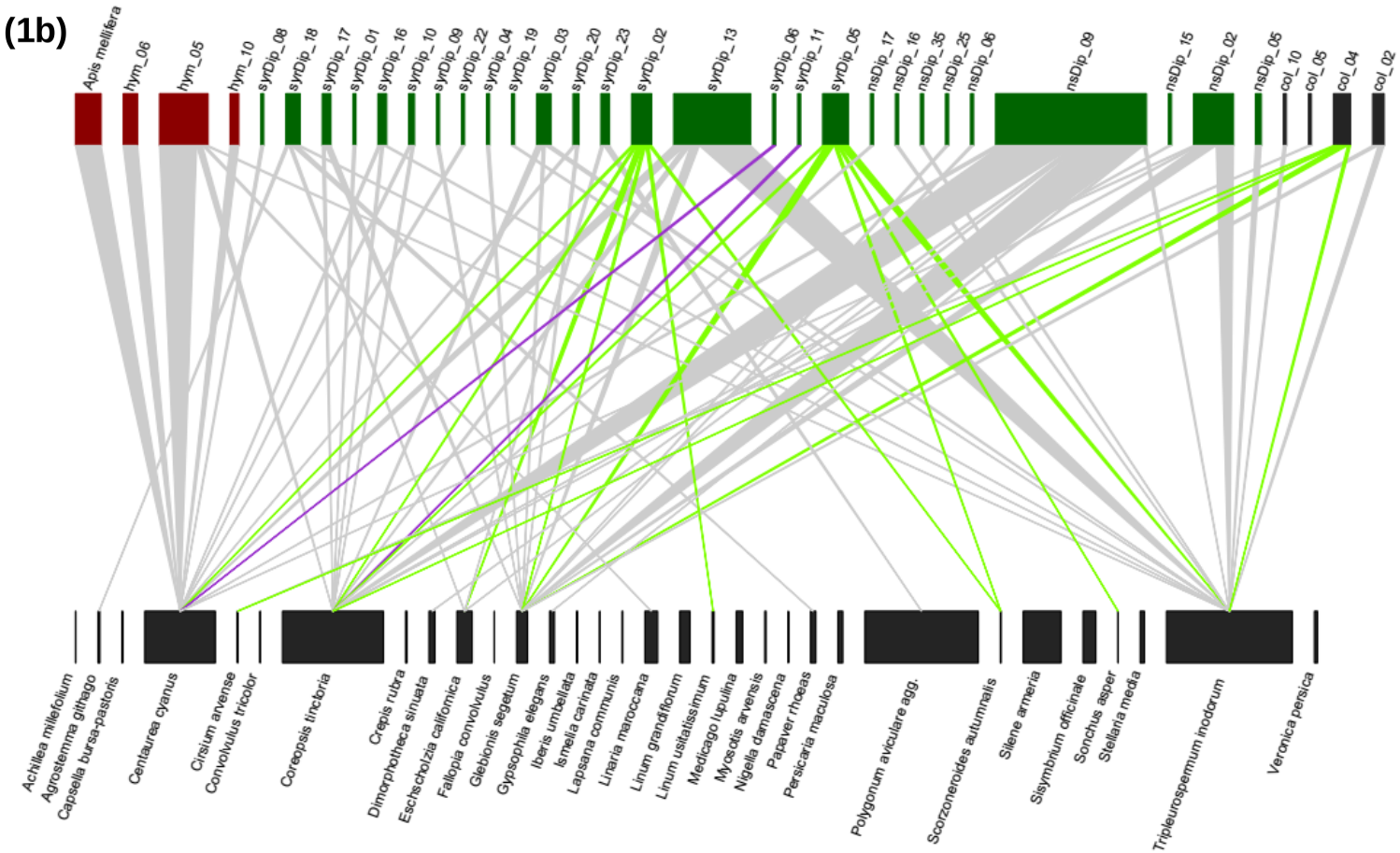
Eristalis tenax
Eupeodes latifasciatus
Eupeodes luniger
Helophilus pendulus
Helophilus trivittatus
Myathropa florea
Neoascia podagrica
Platycheirus albimanus
Platycheirus manicatus
Platycheirus scutatus s.l.
Sphaerophoria scripta
Sphaerophoria sp.
Syritta pipiens
Syrphus ribesii
Syrphus vitripennis
Syrphus vitripennis/rectus
Anthomyiidae morphotype 1
Coenosia morphotype 1
Eriothrix rufomaculata
Exorista mimula/rustica
Helina reversio
Lucilia sericata
Pegoplata group A
Sarcophaga nigriventris
Sepsis fulgens
Mecinus pascuorum
Meligethes aeneus
Oedemera lurida
Oedemera nobilis/virescens
Olibrus aeneus
Achillea millefolium
Agrostemma githago
Capsella bursa-pastoris
Centaurea cyanus
Cirsium arvense
Convolvulus tricolor
Coreopsis tinctoria
Crepis rubra
Dimorphotheca sinuata
Eschscholzia californica
Fallopia convolvulus
Glebionis segetum
Gypsophila elegans
Iberis umbellata
Ismelia carinata
Lapsana communis
Linaria marocana
Linum grandiflorum
Linum usitatissimum
Medicago lupulina
Myosotis arvensis
Nigella damascena
Papaver rhoeas
Persicaria maculosa
Polygonum aviculare agg.
Scorzoneroides autumnalis
Silene armeria
Sisymbrium officinale
Sonchus asper
Stellaria media
Tripleurospermum inodorum
Veronica persica

(1b)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syrDip_05
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nsDip_16
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col_02
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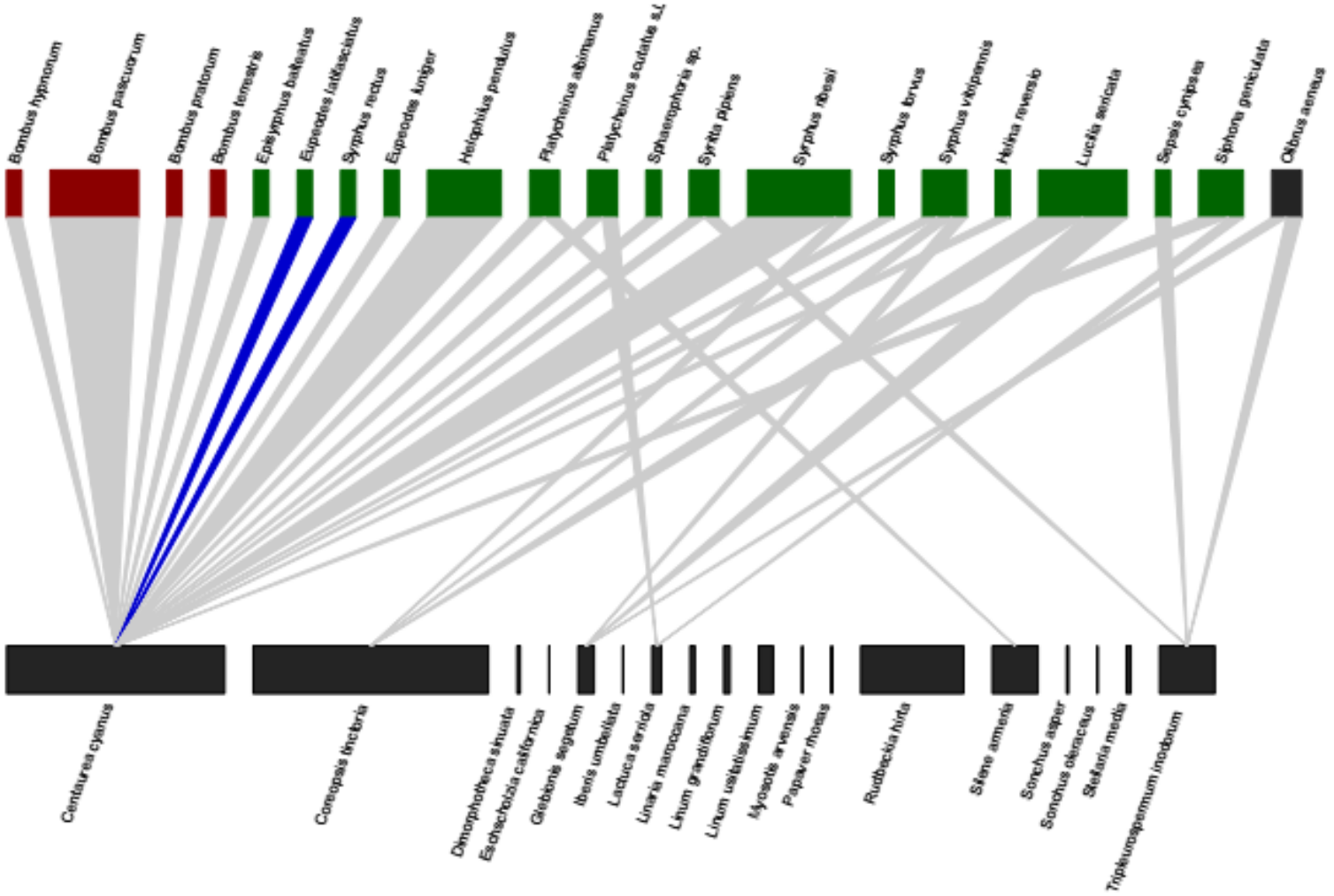
Agrostemma githago
Capsella bursa-pastoris
Centaurea cyanus
Cirsium arvense
Convolvulus tricolor
Coreopsis tinctoria
Crepis rubra
Dimorphotheca sinuata
Eschscholzia californica
Fallopia convolvulus
Glebionis segetum
Gypsophila elegans
Iberis umbellata
Ismelia carinata
Lapsana communis
Linaria maroccana
Linum grandiflorum
Linum usitatissimum
Medicago lupulina
Myosotis arvensis
Nigella damascena
Papaver rhoeas
Persicaria maculosa
Polygonum aviculare agg.
Scorzoneroides autumnalis
Silene armeria
Sisymbrium officinale
Sonchus asper
Stellaria media
Tripleurospermum inodorum
Veronica persica

(2a)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Syrphus rectus
Eupeodes luniger
Helophilus pendulus
Platycheirus albimanus
Platycheirus scutatus s.l.
Sphaerophoria sp.
Syritta pipiens
Syrphus ribesii
Syrphus torvus
Syrphus vitripennis
Helina reversio
Lucilia sericata
Sepsis cynipsea
Siphona geniculata
Oldenus aeneus
Centaurea cyanus
Coreopsis tinctoria
Dimorphotheca sinuata
Eschscholzia californica
Glebionis segetum
Iberis umbellata
Lactuca serriola
Linaria maroccana
Linum grandiflorum
Linum usitatissimum
Myosotis arvensis
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Silene armeria
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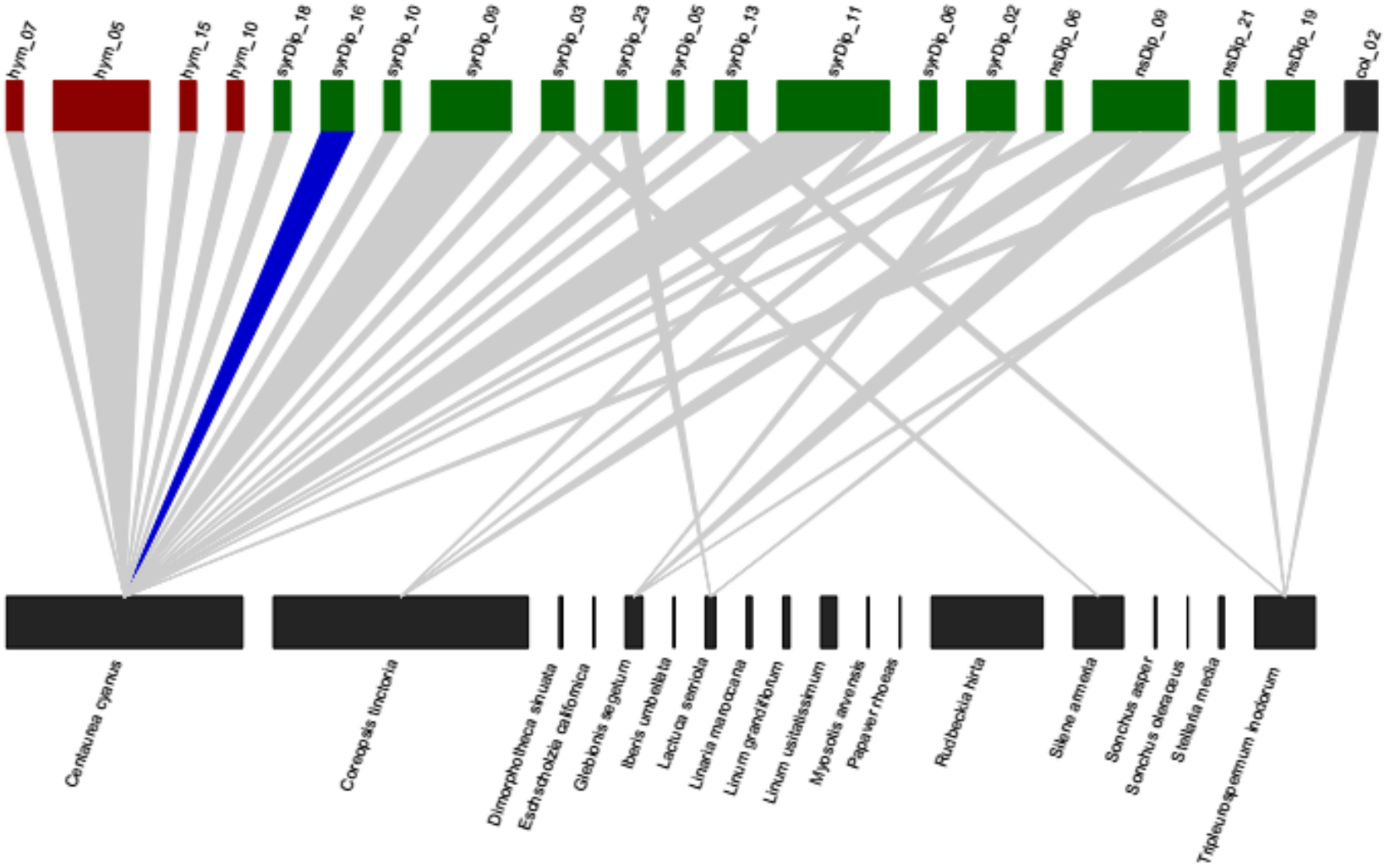
Sonchus oleraceus
Stellaria media
Tripleurospermum inodorum

(2b)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Eschscholzia californica
Gilia tricolor
Iberis umbellata
Lactuca serriola
Linaria maroccana
Linum grandiflorum
Linum usitatissimum
Myosotis arvensis
Papaver rhoeas
Rudbeckia hirta
Silene armeria
Sonchus asper
Sonchus oleraceus
Stellaria media
Tripleurospermum inodorum

Appendix A5

A5.1 Table of pollen and nectar values

Table A5.1: A list of the 50 floral species (plus *Echium vulgare* 'Blue Bedder') recorded in meadows, along with their status (sown vs. weed species) and estimated floral rewards. Floral reward data for sown species were mainly collected in Sheffield. For species not sampled in Sheffield, per-floral unit estimates of floral reward provision were available for a similar dataset collected in Edinburgh using identical methods (Hicks *et al*. 2016).

Species	Status	Nectar sugar mass per floral unit (µg/floral unit/day)		Pollen volume per floral unit (µl/floral unit/day)	
		Sheffield	Edinburgh	Sheffield	Edinburgh
Agrostemma githago	Sown	217.66	-	0.807	-
Centaurea cyanus	Sown	822.53	895.83	0.562	0.547
Convolvulus tricolor	Sown	-	-	0.586	-
Coreopsis tinctoria	Sown	629.58	133.47	1.499	0.455
Crepis rubra	Sown	61.26	-	0.171	-
Dimorphotheca sinuata	Sown	212.54	-	0.629	-
Echium vulgare 'B.B.'	Sown	401.66	-	-	-
Eschscholzia californica	Sown	0.00	10.32	2.505	2.407
Glebionis segetum	Sown	564.09	931.21	2.026	0.609
Gypsophila elegans	Sown	186.60	369.23	0.089	0.072
Iberis umbellata	Sown	2.21	-	0.029	-
Ismelia carinata	Sown	101.30	-	-	-
Linaria maroccana	Sown	151.98	-	0.091	-
Linum grandiflorum	Sown	40.40	50.21	0.289	0.707
Linum usitatissimum	Sown	-	-	0.399	-
Nigella damascena	Sown	-	240.3	-	0.549
Papaver rhoeas	Sown	-	0.57	10.639	5.958
Rudbeckia hirta	Sown	1843.31	-	1.157	-
Silene armeria	Sown	83.83	-	0.038	-
T. inodorum	Sown	4.75	1415.79	0.449	0.348
Achillea millefolium	Weed	-	31.11	-	0.247
Brassica rapa	Weed	-	-	-	-
Capsella bursa-pastoris	Weed	2.26	9.052	0.013	0.001
Cerastium fontanum	Weed	-	11.59	-	0.035
Cirsium arvense	Weed	-	2608.94	-	0.104
Epilobium hirsutum	Weed	-	-	-	-
Epilobium montanum	Weed	-	36.57	-	0.026
Fallopia convolvulus	Weed	-	-	-	-
Galium aparine	Weed	-	-	-	-
Gilia achilleifolia	Weed	-	-	-	-

Hypochaeris radicata	Weed	-	1843.24	-	0.365
Lactuca serriola	Weed	-	-	-	-
Lamium album	Weed	651.64	-	-	-
Lapsana communis	Weed	-	99.35	-	0.107
Lepidium sativum	Weed	-	-	-	-
Matricaria discoidea	Weed	-	0.00	-	0.134
Medicago lupulina	Weed	1202.88	-	0.014	-
Myosotis arvensis	Weed	3.26	21.83	-	0.0001
Persicaria lapathifolia	Weed	61.56	-	0.1025	-
Persicaria maculosa	Weed	-	29.59	0.11	0.002
Polygonum aviculare	Weed	12.18	-	0.0049	0.003
Scorzoneroides autumnalis	Weed	-	-	-	0.274
Senecio sylvaticus	Weed	-	-	0.0184	-
Sinapis arvensis	Weed	-	5.69	-	0.015
Sisymbrium officinale	Weed	12.89	-	0.0115	-
Sonchus asper	Weed	-	593.83	-	0.120
Sonchus oleraceus	Weed	-	568.84	-	0.112
Spergula arvensis	Weed	-	-	-	-
Stellaria media	Weed	-	11.92	-	0.001
Trifolium pratense	Weed	-	48.37	-	0.012
Veronica persica	Weed	-	4.69	-	0.063

A5.2 Models of nectar and pollen

Table A5.2.1: Results of Gamma GLMMs (using a log-link function) for total nectar sugar mass and total pollen volume in meadows of different seed mix treatments. Fixed effects were mix type, formulation and round, with an interaction between mix type and round, and block as a random effect. Results show single-term deletion log-likelihood ratio tests.

Response	Predictors (fixed effects)	Effect of predictor			
		df	**AIC**	**χ^2**	**p-value**
Floral nectar sugar mass per meadow	Full model AIC		1885.6		
	Mix type	-	-	-	-
	Formulation	1	1884.2	0.542	0.46
	Round	-	-	-	-
	Mix type*Round	4	1888.5	10.92	**0.027**
Floral pollen volume per meadow	Full model AIC		1021.9		
	Mix type	-	-	-	-
	Formulation	1	1020	0.0261	0.87
	Round	-	-	-	-
	Mix type*Round	4	1026.2	12.304	**0.015**

Table: A5.2.2 (on subsequent pages): Pairwise contrasts of total floral nectar sugar mass between different levels of seed mix type and survey rounds. There was a significant interaction was between mix type and survey round (Table A5.2.1); hence, conditional pairwise contrasts were performed comparing levels of each factor within each level of the corresponding interacting factor. Conditional pairwise contrasts test differences in estimated marginal means of total floral nectar sugar mass: (a) between seed mix types, within each survey round; and (b) between survey rounds for each seed mix type. Models performed on data in µg/5 m^2/day, but response scale estimates are in mg/5 m^2/day. P-values were adjusted to account for 18 simultaneous tests using the 'mvt' method in R package 'lsmeans'.

(a) Difference in total floral nectar sugar mass between seed mix types within rounds

	Survey round	Level of seed mix type	Model scale estimates				Response scale estimates (mg/5 m^2/day)		
			LS mean ± SE	df	Lower 95% CI	Upper 95% CI	Mean ± SE	Lower 95% CI	Upper 95% CI
Estimated means	Round 1	Marmalade	12.41 ± 0.17	NA	12.07	12.74	244.4 ± 41.5	175.2	340.8
		Short	12.42 ± 0.17	NA	12.09	12.76	248.5 ± 42.2	178.1	346.6
		Cornfield	12.37 ± 0.17	NA	12.03	12.69	234.8 ± 39.8	168.5	327.4
	Round 2	Marmalade	12.71 ± 0.17	NA	12.37	13.04	329.9 ± 55.9	236.6	459.9
		Short	12.88 ± 0.17	NA	12.55	13.22	393.5 ± 67.3	281.3	550.3
		Cornfield	12.23 ± 0.17	NA	11.90	12.57	205.8 ± 34.9	147.6	287.1
	Round 3	Marmalade	12.54 ± 0.17	NA	12.21	12.87	279.4 ± 47.3	200.5	389.3
		Short	12.12 ± 0.17	NA	11.79	12.45	183.9 ± 31.2	131.8	256.5
		Cornfield	11.56 ± 0.17	NA	11.22	11.89	104.3 ± 17.7	74.9	145.4

	Survey round	Contrast	Model scale estimates				Response scale estimates	
			Estimated difference in means ± SE	df	*z*-ratio	*P*-value	Estimated ratio of means ± SE	Estimated % difference between means ± SE
Pair-wise contrasts	Round 1	Cornfield - Marmalade	-0.039 ± 0.221	NA	-0.179	1	0.961	96.1
		Cornfield - Short	-0.057 ± 0.222	NA	-0.255	1	0.945	94.5
		Marmalade - Short	-0.017 ± 0.221	NA	-0.076	1	0.983	98.3
	Round 2	Cornfield - Marmalade	-0.472 ± 0.222	NA	-2.126	0.35	0.624	62.4
		Cornfield - Short	-0.648 ± 0.222	NA	-2.919	**0.0515**	0.523	52.3
		Marmalade - Short	-0.176 ± 0.223	NA	-0.79	0.99	0.8381	83.81
	Round 3	Cornfield - Marmalade	-0.985 ± 0.221	NA	-4.45	**0.0001**	0.373	37.3
		Cornfield - Short	-0.567 ± 0.221	NA	-2.561	0.14	0.567	56.7
		Marmalade - Short	0.418 ± 0.222	NA	1.886	0.51	1.519	151.9

(b) Differences in floral nectar sugar mass between rounds within seed mix type

	Level of seed mix type	Level of Floral category	Model scale estimates				Response scale estimates (mg/5 m^2/day)		
			LS mean ± SE	df	Lower 95% CI	Upper 95% CI	Mean ± SE	Lower 95% CI	Upper 95% CI
Estimated means	Marmalade	Round 1	12.41 ± 0.17	NA	12.07	12.74	244.4 ± 41.5	175.2	340.8
		Round 2	12.71 ± 0.17	NA	12.37	13.04	329.9 ± 55.9	236.6	459.9
		Round 3	12.54 ± 0.17	NA	12.21	12.87	279.4 ± 47.3	200.5	389.3
	Short	Round 1	12.42 ± 0.17	NA	12.09	12.76	248.5 ± 42.2	178.1	346.6
		Round 2	12.88 ± 0.17	NA	12.55	13.22	393.5 ± 67.3	281.3	550.3
		Round 3	12.12 ± 0.17	NA	11.79	12.45	183.9 ± 31.2	131.8	256.5
	Cornfield	Round 1	12.37 ± 0.17	NA	12.03	12.69	234.8 ± 39.8	168.5	327.4
		Round 2	12.23 ± 0.17	NA	11.90	12.57	205.8 ± 34.9	147.6	287.1
		Round 3	11.56 ± 0.17	NA	11.22	11.89	104.3 ± 17.7	74.9	145.4

	Level of seed mix type	Contrast	Model scale estimates				Response scale estimates	
			Estimated difference in means ±SE	df	*z*-ratio	*P*-value	Estimated ratio of means ± SE	Estimated % difference between means ± SE
Pair-wise contrasts	Marmalade	Round 1 – Round 2	-0.300 ±0.222	NA	-1.353	0.86	0.741	74.1
		Round 1 – Round 3	-0.134 ± 0.222	NA	-0.604	0.99	0.875	87.5
		Round 2 – Round 3	0.166 ± 0.221	NA	0.752	0.99	1.181	118.1
	Short	Round 1 – Round 2	-0.459 ± 0.223	NA	-2.06	0.39	0.632	63.2
		Round 1 – Round 3	0.301 ± 0.222	NA	1.356	0.86	1.351	135.1
		Round 2 – Round 3	0.761 ± 0.221	NA	3.436	**0.0095**	2.139	213.9
	Cornfield	Round 1 – Round 2	0.811 ± 0.221	NA	3.67	**0.0041**	1.141	114.1
		Round 1 – Round 3	0.132 ± 0.221	NA	0.595	0.99	2.251	225.1
		Round 2 – Round 3	0.679 ± 0.221	NA	3.071	**0.0323**	1.973	197.3

Table A5.2.3 (on subsequent pages): Pairwise contrasts of total floral pollen volume between different levels of seed mix type and survey rounds. There was a significant interaction was between mix type and survey round (Table A5.2.1); hence, conditional pairwise contrasts were performed comparing levels of each factor within each level of the corresponding interacting factor. Conditional pairwise contrasts test differences in estimated marginal means of total floral pollen volume: (a) between seed mix types, within each survey round; and (b) between survey rounds for each seed mix type. Models performed on data in µl/5 m^2/day, but response scale estimates are in ml/5 m^2/day. P-values were adjusted to account for 18 simultaneous tests using the 'mvt' method in R package 'lsmeans'.

(a) Difference in total floral pollen volume between seed mix types within rounds

Estimated means	Survey round	Level of seed mix type	Model scale estimates				Response scale estimates (ml/5 m^2/day)		
			LS mean ± SE	df	Lower 95% CI	Upper 95% CI	Mean ± SE	Lower 95% CI	Upper 95% CI
	Round 1	Marmalade	6.605 ± 0.211	NA	6.1905	7.0185	0.738 ± 0.156	0.488	1.117
		Short	5.868 ± 0.211	NA	6.1483	6.9741	0.354 ± 0.075	0.234	0.535
		Cornfield	6.671 ± 0.210	NA	5.6114	6.4384	0.789 ± 0.166	0.523	1.192
	Round 2	Marmalade	6.561 ±0.211	NA	5.4542	6.2826	0.707 ± 0 .149	0.468	1.069
		Short	6.465 ± 0.212	NA	6.0494	6.8797	0.642 ± 0.136	0.424	0.972
		Cornfield	6.797 ± 0.213	NA	5.3208	6.1465	0.895 ± 0.191	0.589	1.359
	Round 3	Marmalade	6.025 ± 0.211	NA	6.2596	7.0832	0.414 ± 0.087	0.274	0.625
		Short	5.734 ± 0.211	NA	6.3796	7.2151	0.309 ± 0.065	0.205	0.467
		Cornfield	5.367 ± 0.210	NA	4.9557	5.7795	0.214 ± 0.045	0.142	0.324

Pair-wise contrasts	Survey round	Contrast	Model scale estimates				Response scale estimates	
			Estimated difference in means ± SE	df	*z*-ratio	*P*-value	Estimated ratio of means ± SE	Estimated % difference between means ± SE
	Round 1	Cornfield - Marmalade	0.067 ± 0.255	NA	0.263	1	1.069	106.9
		Cornfield - Short	0.803 ± 0.254	NA	3.157	**0.0246**	2.232	223.2
		Marmalade - Short	0.736 ± 0.254	NA	2.901	**0.0537**	2.088	208.8
	Round 2	Cornfield - Marmalade	0.236 ± 0.254	NA	0.93	0.98	1.266	126.6
		Cornfield - Short	0.333 ± 0.258	NA	1.289	0.89	1.395	139.5
		Marmalade - Short	0.097 ± 0.256	NA	0.378	1	1.102	110.2
	Round 3	Cornfield - Marmalade	-0.657 ± 0.254	NA	-2.589	0.13	0.518	51.8
		Cornfield - Short	-0.366 ± 0.255	NA	-1.438	0.82	0.693	69.3
		Marmalade - Short	0.291 ± 0.255	NA	1.142	0.94	1.338	133.8

(b) Differences in floral pollen volume between rounds within seed mix type

	Level of seed mix type	Level of Floral category	Model scale estimates				Response scale estimates (ml/5 m^2/day)		
			LS mean ± SE	df	Lower 95% CI	Upper 95% CI	Mean ± SE	Lower 95% CI	Upper 95% CI
Estimated means	Marmalade	Round 1	6.605 ± 0.211	NA	6.191	7.019	0.738 ± 0.156	0.488	1.117
		Round 2	6.561 ± 0.211	NA	6.148	6.974	0.707 ± 0.149	0.468	1.069
		Round 3	6.025 ± 0.211	NA	5.611	6.438	0.414 ± 0.087	0.274	0.625
	Short	Round 1	5.868 ± 0.211	NA	5.454	6.283	0.354 ± 0.075	0.234	0.535
		Round 2	6.465 ± 0.212	NA	6.049	6.879	0.642 ± 0.136	0.424	0.972
		Round 3	5.734 ± 0.211	NA	5.321	6.147	0.309 ± 0.065	0.205	0.467
	Cornfield	Round 1	6.671 ± 0.210	NA	6.259	7.083	0.789 ± 0.166	0.523	1.192
		Round 2	6.797 ± 0.213	NA	6.379	7.215	0.895 ± 0.191	0.589	1.359
		Round 3	5.368 ± 0.210	NA	4.956	5.779	0.214 ± 0.045	0.142	0.324

	Level of seed mix type	Contrast	Model scale estimates				Response scale estimates	
			Estimated difference in means ±SE	df	*z*-ratio	*P*-value	Estimated ratio of means ± SE	Estimated % difference between means ± SE
Pair-wise contrasts	Marmalade	Round 1 – Round 2	0.043 ± 0.257	NA	0.169	1	1.044	104.4
		Round 1 – Round 3	0.579 ± 0.257	NA	2.256	0.27	1.785	178.5
		Round 2 – Round 3	0.536 ± 0.254	NA	2.111	0.36	1.709	170.9
	Short	Round 1 – Round 2	-0.596 ± 0.256	NA	-2.33	0.23	0.551	55.1
		Round 1 – Round 3	0.135 ± 0.255	NA	0.529	0.99	1.144	114.4
		Round 2 – Round 3	0.731 ± 0.254	NA	2.877	**0.0587**	2.077	207.7
	Cornfield	Round 1 – Round 2	-0.126 ± 0.255	NA	-0.493	0.99	0.882	88.2
		Round 1 – Round 3	1.304 ± 0.254	NA	5.13	**<.0001**	3.683	368.3
		Round 2 – Round 3	1.429 ± 0.257	NA	5.574	**<.0001**	4.178	417.8

A5.3 Contributions of individuals species to floral resources

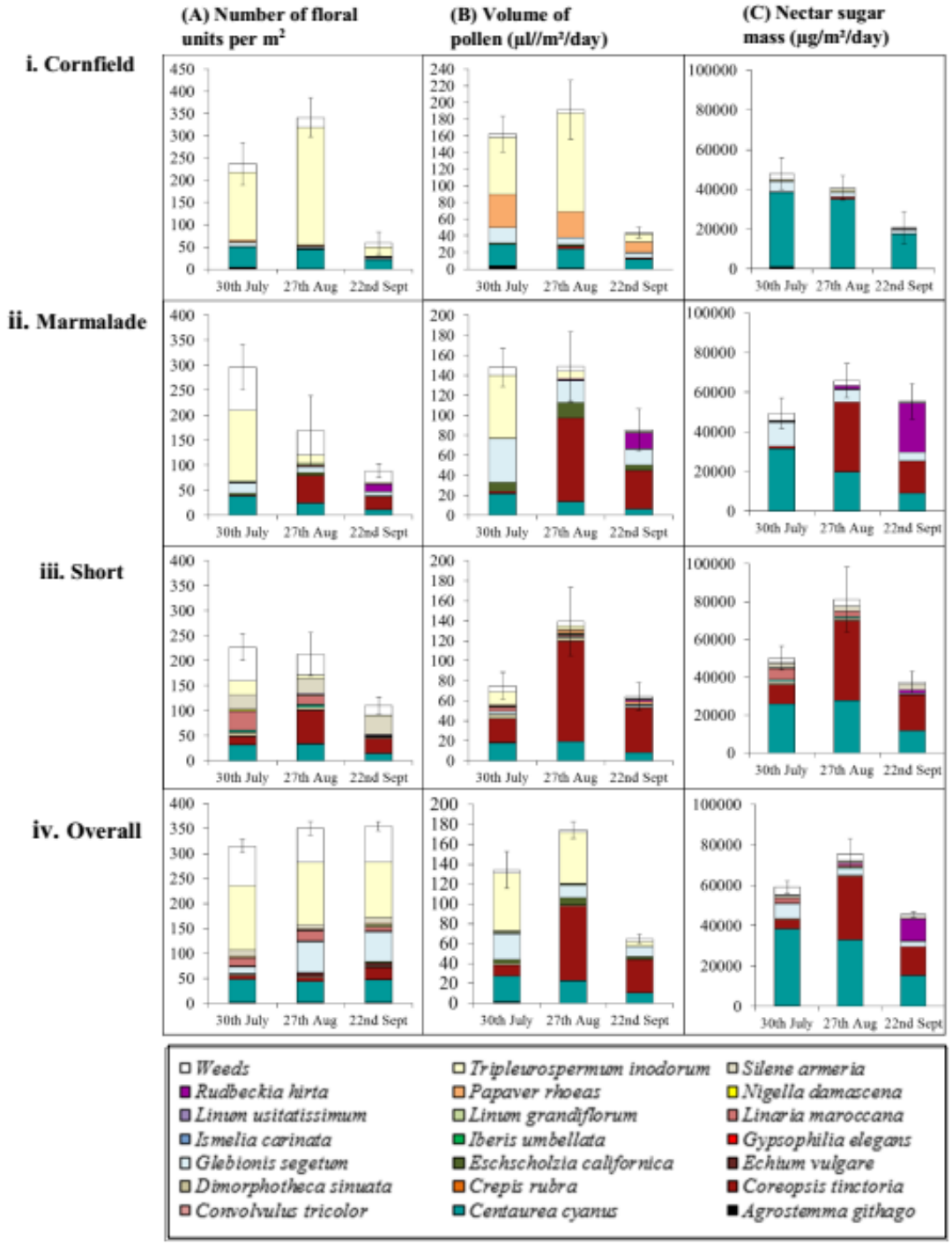

Figure A5.3 (from Plenderleith 2016 – see Declaration): The contribution of individual species to floral resources in planted meadows of different mix types over the season, for floral units, daily pollen volume and daily nectar sugar. Values are means (±SE) averaged across standard/enriched treatments and blocks (n=4). Bars show total floral units, pollen volume and nectar sugar mass per round (1, 2 and 3). Colours show mean species contributions, with weeds shown collectively.

A5.4 Models of flower visitor abundance

Table A5.4.1: Results of Poisson GLMMs testing the effect of either total floral unit abundance per meadow (5 m^2), total nectar sugar mass (mg/5 m^2/day) or total pollen volume (ml/5 m^2/day) on bumblebee abundance. Models contained consistent fixed effects of temperature, wind speed and round, with block as a random effect. Results are shown for models containing predictors (floral units, nectar and pollen) based on full floral communities, including weeds. Significant results from log-likelihood ratio tests of each fixed effect are highlighted in bold. Models with the lowest AIC and R^2 are highlighted in bold. R^2 values are calculated using the 'sem.model.fits' function in R package 'piecewiseSEM'. Marg. = marginal R^2; Con. = conditional R^2.

Response: Bumblebee abundance							
		Effect of predictor				***R*²**	
Model	**Fixed effects**	df	AIC	χ^2	p-value	Marg.	Con.
Floral model	Full model AIC		309.5			0.589	0.589
	Temperature	1	307.5	0.05	0.82		
	Windspeed	3	314.2	10.71	**0.0134**		
	Total floral units	1	308.5	1.03	0.31		
	Round	2	328.9	23.49	**<0.001**		
Nectar model	Full model AIC		**285.5**			**0.655**	**0.655**
	Temperature	1	283.9	0.41	0.52		
	Windspeed	3	284.1	4.67	0.19		
	Total nectar	1	308.5	25.04	**<0.001**		
	Round	2	300.4	18.94	**<0.001**		
Pollen model	Full model AIC		308.5			0.601	0.664
	Temperature	1	306.6	0.06	0.81		
	Windspeed	3	310.2	7.71	0.0524		
	Total pollen	1	308.5	1.96	0.16		
	Round	2	319.7	15.19	**<0.001**		

Table A5.4.2: Results of Negative Binomial GLMMs testing the effect of either total floral unit abundance per meadow (5 m^2), total nectar sugar mass (mg/5 m^2/day) or total pollen volume (ml/5 m^2/day) on hoverfly abundance. Models contained consistent fixed effects of wind speed and round, with block as a random effect. Temperature had no effect and was removed to aid convergence. Results are shown for models for the full hoverfly assemblage, including males. Significant results from log-likelihood ratio tests of each fixed effect are highlighted in bold. Models with the lowest AIC and R^2 are highlighted in bold. R^2 values are calculated using the 'sem.model.fits' function in R package 'piecewiseSEM'. Marg. = marginal R^2; Con. = conditional R^2.

Response: Total hoverfly abundance							
		Effect of predictor				***R*²**	
Model	**Fixed effects**	df	AIC	χ^2	p-value	Marg.	Con.
Floral model	Full model AIC		354.9			0.732	0.732
	Windspeed	3	360.2	11.22	**0.011**		
	Floral units (total)	1	355.1	2.14	0.14		
	Round	2	396.3	45.36	**<0.001**		
Nectar model	Full model AIC		355.9			0.728	0.728
	Windspeed	3	361.5	11.59	**0.0089**		
	Nectar (total)	1	355.1	1.23	0.27		
	Round	2	394.9	42.98	**<0.001**		
Pollen model	Full model AIC		**347.3**			**0.741**	**0.749**
	Windspeed	3	349.3	8.04	**0.0453**		
	Pollen (total)	1	355.9	10.68	**0.0011**		
	Round	2	399.4	56.14	**<0.001**		