

Pollinator community and function: in oilseed rape fields and in drought-stressed grassland.

Submitted by Benjamin B Phillips to the University of Exeter
as a thesis for the degree of
Master of Science by Research in Biological Sciences
In December 2016.

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ABSTRACT

This thesis explores (i) how pollinator community characteristics relate to pollination service, and (ii) how pollinator communities will be affected by climate change-induced drought.

Chapter 1 gives an overview of the evidence for a relationship between biodiversity and ecosystem services (BES), with particular reference to pollination services. The major threats to pollinators are discussed, including how these affect the characteristics of pollinator communities and ultimately, pollination services.

Chapter 2 explores how pollinator community characteristics affect the pollination service to winter-sown oilseed rape, an economically important crop. A modelling approach was used to estimate the pollination service that is provided by different flower visitors and to explore the mechanisms that are driving the pollination service in this system. Overall, the contribution of bees and non-bees to pollination service was estimated to be similar. Functional group richness had a positive effect on estimated pollen deposition, providing evidence for a BES relationship due to community structuring. However, the abundance of common species was the primary driver of pollination service.

Chapter 3 explores how pollinators are likely to be affected by drought events, which are predicted to increase in frequency and intensity due to climate change. In a field experiment in calcareous grassland, rooves were placed over plant communities to simulate a single period of drought. Subsequent effects on floral resources and flower visitor activity were measured. Flowers in the drought treatment were less likely to contain nectar and racemes had fewer flowers overall. At the community scale, there were substantially fewer flowers in the drought treatment, suggesting that drought events will cause periods of floral resource scarcity.

In both case studies, changes to pollinator communities, by loss of biodiversity or due to effects of drought, have the potential to reduce pollination of both crop and wild plants.

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AUTHOR'S DECLARATION

The work that is described in this thesis was undertaken as part of the Wessex Biodiversity and Ecosystem Service Sustainability (BESS) project, which aims to understand how biodiversity underpins the ecosystem functions and services that landscapes provide.

In chapter 2, the work that is described took place as part of BESS work package 4, which studies crop production, and was led by Dr Rosalind Shaw (RS) and Prof Juliet Osborne (JO). The 2014/2015 study fields were identified by RS, and community survey data (pan trap, quadrat, and transect data) were collected by RS and field team. Benjamin Phillips (BP) assisted with the identification of invertebrates in pan traps. Visitation effectiveness data were collected by RS in 2015 and by BP in 2016. BP was responsible for all laboratory analysis of visitation effectiveness. BP was responsible for laboratory analysis of pollen loads and trait measurements, with assistance from Aidan Williams (undergraduate honours project). BP created and programmed the model, and was responsible for subsequent data analysis, modelling, and writing up. Ideas were informed by discussion with RS and JO at each step.

In chapter 3, the work that is described took place as part of BESS work package 2, which looks at climate regulation, and was led by Dr Ellen Fry (EF) and Prof Richard Bardgett (RB). EF and RB established the field site, plots, and experimental treatments. BP was responsible for planning the pollinator experiment within the existing experimental setup, collecting the data (with assistance from RS and field team), analysing the data, and writing up. Ideas were informed by discussion with RS and JO at each step.

Drafts of this thesis were improved by comments from RS, JO, and Alice Lord.

ACKNOWLEDGEMENTS

Many individuals deserve to be acknowledged for their direct or indirect contribution to the work that is described in this thesis. I am very grateful to the numerous people who assisted with the data collection that made this project possible, including Toby Doyle, Victoria Mallott, Ciara McGinley, Aidan Williams, Matthew Holland, Emma Higgins, Joanna Savage, Richard Comont, and Carly Benefer. I am also very grateful to the members of the University of Exeter Bee Group for stimulating discussion, particularly about methodologies and the interpretation of the results. I would like to thank landowners and land managers for allowing access to field sites, and Ellen Fry and Richard Bardgett for allowing use of the drought experiment and for being extremely generous with their time and support. I would also like to thank my inspirational supervisors, Ros Shaw and Juliet Osborne, for the ideas that formed the basis of this thesis and for advice, discussion, and support at every step. I am particularly indebted to Ros Shaw, who spent numerous hours providing assistance and training in laboratory, field, and computational skills, and for allowing me to work on the oilseed rape experiment after years of hard work developing it. I would like to thank The Access & Achievement Foundation and the Haberdashers' Educational Foundation for providing personal financial assistance, which allowed me to undertake this project. Finally, I would like to thank my partner, Alice, my mother, Sharon, and my friends for their continued encouragement and support throughout this project. The work that is described in this thesis took place as part of the Wessex Biodiversity and Ecosystem Service Sustainability (BESS) project, which is part of the BESS programme, and supported by the Natural Environment Research Council.

Chapter 1:

General Introduction

Biodiversity, Ecosystem Functioning, & Ecosystem Services

The relationship between biodiversity and ecosystem functioning (BEF) is a major focus of ecological research. Theory suggests that biodiversity determines ecosystem functioning because the presence and abundance of species determine ecosystem processes and interactions. Although the general concept can be traced as far back as Darwin (Darwin, 1859), it has become particularly important in recent years due to the unprecedented rate of species loss. If the BEF relationship holds true, this will result in dramatic degradation of ecosystem functioning and stability (Cardinale et al., 2012; Naeem et al., 2012).

Theoretically, the BEF relationship is somewhat intuitive. With a general decrease in biodiversity, there is predicted to be a general decrease in ecosystem functioning (Hooper et al., 2012). As species differ in their ecological niche, including their use of particular resources, greater species diversity is associated with greater niche diversity. This is known as niche complementarity and it is expected to result in an increase in the range of resources that can be utilised by the community. Therefore, the addition of new species to a community is predicted to provide a synergistic benefit to functioning. Diversity may benefit ecosystem functioning in two ways: (i) Directly because the community performs a wider range of functions, and (ii) Indirectly because, as a consequence of this, a greater number of resources are available, and therefore a greater abundance of individuals can be supported.

Where the niches of two species overlap, and are therefore not complementary, there is niche redundancy. Although niche redundancy is not predicted to increase ecosystem functioning, it may provide resilience to environmental stress by acting as a buffer against species loss (Oliver et al., 2015). Therefore, it is functional diversity, in terms of the variety of niches, that is predicted to determine ecosystem functioning, rather than species diversity *per se* (Cadotte et al., 2011; Gagic et al., 2015; Tilman et al., 1997).

Over the past twenty years, BEF research has resulted in a vast literature. Biodiversity has been shown to relate to a wide variety of ecosystem functions in a wide range of systems, including plants (Cardinale et al., 2004; 2011), belowground communities (Bardgett & van der Putten, 2014), bacteria (Bell et al., 2005), and ocean ecosystems (Stachowicz et al., 2007; Worm et al.,

2006). Reviews of BEF studies have provided unequivocal support for the existence of BEF relationships (Balvanera et al., 2006; Cardinale et al., 2009; 2012; Hooper et al., 2005; Loreau, 2000; Loreau et al., 2001; Pasari et al., 2013; Tilman et al., 2012). However, there remains some uncertainty about how widespread and important this phenomenon is because some studies have found little or no effect (Wardle et al., 1997), and the relationship does not appear to apply to all ecosystem functions (for example, water filtration; Cardinale et al., 2012). Furthermore, methodological issues have been a major limitation and a historical source of criticism (Huston, 1997; Huston et al., 2000). Early experiments have had issues with separating causality from correlation, and this has affected the interpretation of their results (Cardinale et al., 2012). Field experiments have often made it difficult to attribute differences in ecosystem functioning to diversity, rather than to other confounding factors, whilst for laboratory and microcosm experiments, there have been concerns about biological relevance (Cardinale et al., 2012).

Although methodologies have been refined, some difficulties do remain. For example, it is often difficult to separate the relative contribution of diversity and abundance because these are confounded. Whilst greater abundance may result in greater observed diversity because more individuals are sampled per equivalent sampling effort, this can often be resolved by using methods such as rarefaction (Gotelli & Colwell, 2001). However, diversity may also be a driver of abundance because greater niche complementarity may result in a greater number of individuals being supported per unit area. Even when diversity can be isolated as having an effect, there are multiple possible mechanisms for a BEF relationship. The observed relationship may be due to the benefits of multiple species and niche complementarity, or as a mathematical phenomenon, with greater diversity resulting in a greater likelihood of functionally important species being present in the community. Evidence suggests that both of these mechanisms may be of comparable importance (Cardinale et al., 2011). Furthermore, functionally important species may be more or less likely to appear in diverse communities due to non-random community structuring. For example, functionally important species may be more likely to appear in diverse communities if they are dependent upon the presence of other species. Although much progress has been made, there is clearly further to go in order to disentangle this array of mechanisms.

When ecosystem processes provide benefits to human beings, they are termed ecosystem services. Ecosystem services are defined as “the aspects of ecosystems utilised (actively or passively) to produce human well-being” (Boyd & Banzhaf, 2007; Fisher et al., 2009; Millennium Ecosystem Assessment, 2005). The term incorporates a wide range of benefits to humanity. Whilst services such as water filtration and nutrient cycling provide obvious benefits, more elusive services such as exposure to nature have been shown to provide benefits that justify their inclusion, even though they are difficult to quantify (Keniger et al., 2013).

The agricultural industry utilises a range of ecosystem services (Power, 2010; Zhang et al., 2007). For example, naturally occurring predators and parasites reduce populations of crop pests, and decomposers recycle nutrients and remove dung which would otherwise support pests and increase risk of disease to cattle (Zhang et al., 2007). Pollinators provide an important ecosystem service by transferring pollen between crop flowers leading to marketable produce (Klein et al., 2007).

It is possible to produce an economic valuation of ecosystem services by considering the cost of them being degraded or lost altogether. By doing so, long-term costs can be incorporated into short-term economics, allowing sustainable practices to be identified. When this is done, it becomes apparent, even to non-conservationists, that it is important to protect ecosystems. Although the concept of ecosystem valuation is a relatively recent phenomenon, it is increasingly being used by policy makers in decision-making processes (Fisher et al., 2008; Millennium Ecosystem Assessment, 2005).

A branch of BEF research focuses solely on ecosystem functioning that is beneficial to human beings by looking at the relationship between biodiversity and ecosystem services (BES). Although this field is rapidly expanding, there is already evidence for such relationships (Balvanera et al., 2006; Cardinale et al., 2012; Mace et al., 2012). BES relationships have been found in a diverse range of study systems, including plants (Isbell et al., 2011; Quijas et al., 2010), bacterial communities (Bell et al., 2005), and ocean ecosystems (Worm et al., 2006). If biodiversity is demonstrated to be an important driver of ecosystem services, it provides a financial incentive against biodiversity-degrading activity. Furthermore, it encourages the promotion of biodiversity through strategic

management, in the form of ecological intensification (Bommarco et al., 2013; Pywell et al., 2015; Tittonell et al., 2014). Although biodiversity has often been shown to have an effect on ecosystem services, other factors such as the abundance of common species may generally be more important (Gaston, 2010). The relative importance of biodiversity versus other drivers will determine the optimal management for ecosystem services.

In contrast to BEF research, BES studies have been mostly correlative and conducted at the landscape scale, in order to investigate how landscape management affects ecosystem services (Cardinale et al., 2012). This makes sense because policies are motivated and implemented by effects across large areas. However, it has arguably resulted in studies that are less rigorous and conclusive than in the BEF field, which encompasses a diverse range of theoretical, mathematical, field, and laboratory experiments.

Whilst there is already evidence in support of BEF/BES relationships, studies in additional systems will determine how universal and eminent these relationships are, compared to other factors. BES research could benefit by taking an increasingly experimental approach in order to isolate causative relationships, and to examine the specific mechanisms behind BES relationships. Careful experimental designs, which build upon the early mistakes of BEF research, will be important in making this possible.

Insect Pollination: An Important Ecosystem Service

Pollination is a critical ecosystem service. Animal pollinators transfer pollen between flowers, leading to fertilisation of ovules, seed set and fruit production. Pollinators mediate the reproduction of 87% of angiosperms, making them essential in the maintenance of plant diversity (Ollerton et al., 2011). Of the leading global food crops, 87 are dependent upon mutualistic relationships with animal pollinators, contributing 35% of total food production (Klein et al., 2007). Furthermore, as this food consists of fruit, vegetable, nut, seed, and oil crops, it provides the majority of essential micronutrients in the human diet (Eilers et al., 2011; Vanbergen & the Insect Pollinators Initiative, 2013). Although there is debate about how best to value pollination services (Allsopp et al., 2008; Losey & Vaughan, 2006; Winfree et al., 2011), an estimated value of €153 billion per year is widely cited (Gallai et al., 2009).

Many crops require insect pollination in order to achieve high quality and quantity yields, whilst others are entirely dependent upon insect pollination in order to produce a marketable good (Aizen et al., 2009; Klein et al., 2007). The widespread use of managed pollinators, particularly the European honeybee *Apis mellifera*, is an indicator of the reliance of agriculture upon insect pollination. The scale of this dependency is best seen in the Californian almond industry, where a recommended 2 million hives of honeybees are required every year in order to provide an adequate pollination service (Klein et al., 2012; Sumner & Boriss, 2006).

In comparison to honeybees, the role of wild pollinators is still not fully appreciated or understood. Not only are wild pollinators likely to insure against honeybee losses (Winfree et al., 2007), they can also be important for crop productivity and stability even when honeybees are abundant (Garibaldi et al., 2011; 2013; Greenleaf & Kremen, 2006; Klein et al., 2012; Mallinger & Gratton, 2015). Even non-bee pollinators are important contributors to global pollination services (Orford et al., 2015; Rader et al., 2015; Ssymank et al., 2008). Furthermore, the optimum pollinator species or community is likely to be context-specific. Whilst honeybees are usually effective pollinators, they actively remove pollen from the stigmas of some plant species (Gross & Mackay, 1998). In addition, specialised flowers with long corolla tubes, such as red clover, are better pollinated by long-tongued bumblebees. Short-tongued species such as

honeybees often engage in nectar robbing of these specialised flowers, which does not result in pollen transfer (Free, 1965). Furthermore, the presence of managed honeybees can suppress populations of wild pollinator due to competition (Lindström et al., 2016; but see Steffan-Dewenter & Tscharntke, 2000). There remains much more to understand about the best combinations of pollinators and management practices for different agricultural scenarios. However, due to the accumulating evidence that wild pollinators can benefit crop yields (Garibaldi et al., 2014; 2016), attitudes have shifted towards management that promotes wild pollinators. In the future, optimal pollinator management is likely to find a balance between supporting wild and domesticated species. This is likely to be driven by concerns of over-dependence upon single species, and by the potential benefits of diversity that BES theory predicts.

The evidence that diverse pollinator assemblages can often provide a greater pollination service than that of managed honeybees alone is expected by BES theory. If pollinator species show niche complementarity then a diverse community may perform a greater pollination service. Consequently, a greater number of resources may be accessible to the community and therefore a greater abundance of pollinators might be supported. Evidence suggests that plant-pollinator interactions exhibit a particularly high level of niche complementarity (Blüthgen & Klein, 2011). Specifically, species are often separated spatially (foraging on different plants or on different parts of the same plant), or temporally (active at different times of day, different times of year, or in different weather conditions). For example, pollinators of pumpkin differ in flower visiting height and time of day (Hoehn et al., 2008), honeybees and solitary bees differ in flower visiting height on almond trees and in minimum weather conditions for foraging (Brittain et al., 2013), social and solitary bees visit radish plants at different times of day (Albrecht et al., 2012), bees and flies visit pak choi at different times of day (Rader et al., 2013), nocturnal pollinators of lowbush blueberry are temporally separated from daytime pollinators (Cutler et al., 2012), and pollinators of watermelon crops show multiple spatial and temporal levels of niche complementarity (Pisanty et al., 2016). Complementarity may also exist more subtly. For example, if pollinators of different sizes typically visit different parts of the same flower, then visits by multiple species may lead to fertilisation of a greater proportion of ovules than

visits by a single species (Chagnon et al., 1993). The high level of diversity and specialisation of both plant and pollinator species, in terms of behaviour and morphology, provides many opportunities for complementarity.

As predicted, pollinator diversity has been shown to increase plant reproductive success (Albrecht et al., 2012) and crop yield (Hoehn et al., 2008). It has also been shown to increase and maintain the diversity of plant communities (Fontaine et al., 2006) and to buffer pollination services from changes in environmental conditions (Brittain et al., 2013; Winfree & Kremen, 2009). However, the relative importance of diversity compared to other drivers, such as abundance and composition, is unclear and these factors may have a much greater effect (Kleijn et al., 2015; Winfree et al., 2015). As with all BES studies, it is often difficult to separate the relative contribution of diversity, abundance, and composition as drivers of service. The extent to which diversity is a determinant of service governs whether management for pollination service justifies the conservation of many, or just some, pollinator species (Kleijn et al., 2015).

The value of pollinators, in terms of the service that they provide, justifies their study and protection. Pollination is an essential ecosystem service, both in agriculture and in the maintenance of plant diversity. Both managed and wild pollinators are important and the relative potential of each, and of particular pollinator species, appears to be context-specific. There are accumulating examples of a BES relationship for pollinators, suggesting that it may be a widespread phenomenon. Pollinator species may exhibit a particularly high level of niche complementarity due to their extensive morphological and behavioural diversity. Diverse pollinator communities can provide a superior service and be more resilient to environmental stress. Overall, it is clear that the diversity and abundance of pollinators must be promoted and maintained in order to continue to benefit from the valuable service that they provide.

Global Pollinator Decline & Landscape Management

In recent years, there has been a decline in the number of managed honeybee colonies in Europe (Potts et al., 2010a) and in the United States (Ellis et al., 2010). Despite a global increase of 45%, the stock of domesticated honeybees is not able to keep up with increasing agricultural demand (Aizen & Harder, 2009; Breeze et al., 2014). In Europe, there have been parallel declines in wild bees (Biesmeijer et al., 2006; Potts et al., 2010b), and other wild pollinators such as hoverflies (Keil et al., 2011). As a result, there are increasing concerns about pollination deficits (Vaissière et al., 2011).

There are multiple implicated causes of pollinator decline, including habitat loss, agrochemicals, pathogens, invasive species, and climate change, and these are likely to be acting synergistically (Brown et al., 2016; Gill et al., 2016; Goulson et al., 2015; Potts et al., 2010b; 2016; Vanbergen & the Insect Pollinators Initiative, 2013). The widespread use of agrochemicals is generally considered to have had a negative effect on pollinators (Cresswell, 2011; van der Sluijs et al., 2014). For example, exposure to neonicotinoid insecticides has been shown to impair foraging behaviour in bees, leading to reduced longevity, fecundity, and resistance to disease (Gill et al., 2012; van der Sluijs et al., 2014; Whitehorn et al., 2012). There is also evidence that neonicotinoid exposure has led to population declines and extinctions of wild bees (Rundlöf et al., 2015; Woodcock et al., 2016). Invasive pathogens are also contributing to pollinator losses. In particular, the parasitic mite, *Varroa destructor*, is a widespread problem for honeybee colonies (Le Conte et al., 2010). The mite switched from its natural host, *Apis cerana*, to *Apis mellifera* when colonies were imported around the globe. Subsequently, *V. destructor* has become a global problem for honeybees, which have little resistance having not coevolved with the parasite (Le Conte et al., 2010). Furthermore, commercial colonies appear to be a major driver of disease emergence in wild pollinators (Manley et al., 2015).

Over the coming decades, the climate is predicted to change in a variety of ways that will affect pollinators. In general, average global temperatures are predicted to increase by between 2°C and 4°C (IPCC, 2014). Species that are unable to shift their range in response to this change are likely to become threatened or extinct (Thomas et al., 2004). Furthermore, this is likely to cause changes in phenology that will disrupt plant-pollinator interactions and may

result in decoupling of dependent species (Memmott et al., 2007; 2010). In addition to these gradual changes, there is predicted to be more frequent extreme weather events, such as heatwaves, droughts, and heavy precipitation events (IPCC, 2014). Whilst there may be both benefits and costs of this average increase in temperature for plants and pollinators, extreme weather events will have almost exclusively negative consequences. For example, one of the major predicted impacts of climate change across the UK and much of Europe is increased occurrence and intensity of drought (Dai, 2013; Rahiz & New, 2013). This is likely to affect pollinators directly in terms of their ability to forage due to thermoregulatory limits (Scaven & Rafferty, 2013), and indirectly in terms of the availability of floral resources.

Landscape characteristics affect patterns of biodiversity in a complex and multidimensional way (Tscharntke et al., 2012). However, it is generally agreed that the major underlying cause of declines in wild pollinators is change in land-use, in the form of agricultural expansion and a tendency towards intensification (Brown & Paxton, 2009). This is in large part because over a third of suitable land is now utilised for agriculture (Bruinsma, 2003). Agricultural intensification, in the form of monoculture cropping and hedgerow removal, has resulted in a loss of habitat diversity. These land-use changes explain patterns of bee extinctions in the UK (Ollerton et al., 2014), and have been detrimental to pollinator communities in terms of both composition and diversity (Senapathi et al., 2015; Woodcock et al., 2014). As flowering crop species provide highly abundant floral resources, some pollinator species undoubtedly benefit from them (Holzschuh et al., 2012; Westphal et al., 2003). However, crop flowering periods are often short and outside of this, crop areas provides few resources. Non-flowering crops offer few discernible benefits to pollinators and may constitute an area of nearly zero resources. Declines of many pollinator species have been linked to loss of forage and floral resources (Baude et al., 2016; Carvell et al., 2006; Roulston & Goodell, 2011).

It is concerning that agriculture has been such a major cause of ecosystem degradation, given that many crops are dependent upon insect pollination to some degree (Klein et al., 2007), or upon other ecosystem services (Zhang et al., 2007). It is understood that landscape management can determine biodiversity and therefore promote ecosystem service provision and

conservation (Tscharntke et al., 2005), and this is true for pollinators (Kennedy et al., 2013; Senapathi et al., 2015; 2016; Vanbergen, 2014). Ecological intensification aims to enhance crop productivity by promoting beneficial ecosystem processes (Bommarco et al., 2013; Tittonell et al., 2014). Therefore, the costs of changing practices or setting aside land can be reduced or offset by the benefits that pollinators have on yield (Pywell et al., 2015).

It is clear that the presence and management of landscape features such as hedgerows, field margins, and neighbouring semi-natural habitat determine the pollinator community that can be supported. In a landscape of monoculture cropping, only the staggered flowering times of a diverse plant community can provide floral resources for pollinators throughout the entire year (Nicholls & Altieri, 2013). For example, in some cases the presence of weeds increases crop productivity because greater pollinator populations are supported, resulting in a greater pollination service (Carvalheiro et al., 2011; Holzschuh et al., 2008).

The phenomenon of pollinator ‘spill-over’, whereby resource-rich habitat provides a source of pollinators to neighbouring resource-poor habitat, is likely to be an important determinant of pollination service in agriculturally intensive landscapes. On a large scale, semi-natural habitat can provide a source of pollinating insects (Klein et al., 2012; Nayak et al., 2015; Öckinger & Smith, 2007). However, even smaller patches of semi-natural habitat, such as riparian buffer strips, can be effective (Cole et al., 2015). Similarly, the restoration of hedgerows can promote pollinator populations in neighbouring fields (Morandin & Kremen, 2013). In general, setting aside forage habitat, such as wildflower field margins, can conserve wild pollinators in otherwise resource-poor landscapes (Barbir et al., 2015; Blaauw & Isaacs, 2014; Dicks et al., 2015). Even small-scale practices can have major effects on pollination services (Garibaldi et al., 2016). However, both pollinator diversity and pollinator activity have been shown to decay rapidly with distance from natural or semi-natural habitat (Ricketts et al., 2008). Although this effect is found even when neighbouring habitat is biodiversity-rich (Carvalheiro et al., 2010), the diversity and abundance of the pollinator community that spills over is likely to be determined by the quality and quantity of neighbouring habitat.

Whilst there is a reasonable understanding of the management strategies that can benefit pollinators, further work is needed to ensure that these are taken up

at a large scale (Dicks et al., 2016; Potts et al., 2016). Furthermore, current understanding and recommendations should be developed through ongoing research (Dicks et al., 2015; Dicks et al., 2016). Agricultural management must move towards fully integrating the value of ecosystem services into decisions of agricultural practices, in order to prevent further degradation of these services. Doing so provides the opportunity to increase yields by promoting ecosystem services through ecological intensification (Bommarco et al., 2013; Pywell et al., 2015; Tittonell et al., 2014).

Conclusion

There is strong evidence to support the existence of BEF/BES relationships in a wide range of systems. Although further work is needed in order to reveal how widespread and substantial such relationships are, pollinators provide many examples. BES relationships may be particularly widespread in pollinator communities due to the morphological and behavioural diversity of pollinator species, which provides many opportunities for niche complementarity. As a result, there is accumulating evidence to suggest that management for wild pollinators can be beneficial, even when domesticated honeybees are present. As the diversity, abundance, and composition of a community may determine the pollination service that it provides, it is important to understand the function and contribution of each. Given the economic importance of pollinators, this understanding is essential in directing optimal management for pollination services. Furthermore, it will reveal whether management for pollination services is a sufficient justification for the conservation of pollinator diversity.

Unfortunately, both the diversity and abundance of pollinators are suffering global decline and pollination services are threatened systemically. This is being driven by multiple threats, which are acting synergistically. Over the coming decades, the gravity of these threats will largely depend upon how policy makers respond. However, the threat of climate change will exist to a significant degree, regardless of governmental response. Therefore, it is particularly important to explore how pollinators will be affected by climate change. Subsequently, this can direct the way in which the landscape is managed and determine the resilience of pollinator communities and pollination services to this pressure.

Chapter 2:

The flower visitors and community characteristics

driving pollination services to winter-sown oilseed rape.

At the time of submission, this chapter was in preparation for publication as part of a larger project, with the following authorship list: Shaw RF, Phillips BB, Pell JK, Doyle T, Savage J, Redhead J, Woodcock BA, Bullock JM, Osborne JL.

ABSTRACT

Insect pollinators provide a valuable ecosystem service in terms of crop pollination. The global decline of pollinators has caused concern because there is accumulating evidence that pollinator diversity is an important driver of pollination service.

This chapter explores the drivers of the pollination service to winter-sown oilseed rape (OSR), an economically important, mass-flowering crop. Invertebrate communities were sampled in fields of OSR using multiple survey methods in order to provide an overall estimate of flower visitation rate. The visitation effectiveness of flower visitors was assessed by measuring single visit pollen deposition, and this was related to pollen load (the amount of free pollen on the body). These data were combined to create a predictive model of pollination service that estimated rate of pollen deposition.

A diverse community of invertebrates (including 32 bee species and 10 fly families) was observed visiting flowers of winter-sown OSR, despite an early time of year (mid-April to mid-May). The single visit pollen deposition of nearly all flower visitors was greater than the number of pollen grains found on control stigmas. However, the pollen load of an individual did not relate closely to single visit pollen deposition. It was estimated that the majority of pollen deposition was provided by just a few groups (Anthomyiid flies, honeybees, bumblebees, and solitary bees). However, the relative contribution of each differed dramatically between sites. Overall, the estimated contribution of bees and non-bees to pollen deposition was similar. Non-bee pollinators, rather than wind pollination, may explain why OSR flowers often receive adequate pollination, even when bees are scarce. Model simulations (controlling for visitation rate) found that functional group richness had a positive effect on estimated pollen deposition, providing evidence for a BES relationship due to community structuring. However, the abundance of common species was of primary importance. Therefore, management that results in population changes of these species is likely to have the greatest impact on the pollination service to OSR.

INTRODUCTION

As the human population continues to expand, ecosystems are being rapidly degraded on a global scale (Cardinale et al., 2012). Whilst this is clearly a conservation issue, it also raises economic concerns because ecosystems provide services that have a quantifiable value (Constanza et al., 2014). Animal pollinators provide a valuable ecosystem service by transferring pollen between crop flowers, leading to fertilisation, seed set, and fruit production (Klein et al., 2007). Therefore, it is concerning that pollinator populations are in decline in the UK (Biesmeijer et al., 2006) and across the world (Potts et al., 2010b; 2016). Consequently, there is increasing concern about pollination deficits, whereby yields are limited by insufficient crop pollination (Vaissière et al., 2011).

Theoretical and empirical research into the relationship between biodiversity and ecosystem services (BES) has demonstrated that greater diversity can result in greater services (Balvanera et al., 2006; Cardinale et al., 2012; Mace et al., 2012). This suggests that diverse pollinator communities can provide a greater pollination service than that of honeybees alone. Furthermore, the stock of domesticated honeybees is not able to keep up with increasing agricultural demand (Aizen & Harder, 2009; Breeze et al., 2014). Thus, the potential importance of wild pollinators has become increasingly realised. There is already evidence that wild pollinators are important for crop productivity and stability, even when honeybees are abundant (Garibaldi et al., 2011; 2013; 2014; Greenleaf & Kremen, 2006; Mallinger & Gratton, 2015). Even non-bee pollinators are increasingly seen as substantial contributors to global pollination services, and their importance has probably been broadly overlooked (Orford et al., 2015; Rader et al., 2015; Ssymank et al., 2008).

Whilst there is little doubt that pollinator community characteristics determine the pollination service that is provided, separating the underlying mechanisms (diversity, abundance, and composition) can be problematic. Many studies have identified benefits of diversity (Albrecht et al., 2012; Garibaldi et al., 2013; 2016; Hoehn et al., 2008; Winfree & Kremen, 2009), but other factors such as the abundance of common species may be more important drivers of pollination service (Kleijn et al., 2015; Winfree et al., 2015). The mechanisms of diversity and abundance are deeply entangled for two reasons. Firstly, greater diversity may result in greater abundance due to niche complementarity,

whereby more individuals can be supported if they utilise different resources. Secondly, greater abundance may result in apparently greater diversity due to sampling effects, whereby more individuals are sampled per equivalent sampling effort. Furthermore, diverse communities may provide a greater service simply because they are more likely to contain functionally important species, rather than due to diversity *per se*. It is important to disentangle these mechanisms in order to strictly test for a BES relationship. Only by doing so will it be clear whether management for ecosystem services can justify conservation, or whether management for diversity requires additional efforts (Adams, 2014; Kleijn et al., 2015).

BES studies have often looked at the effects of diversity upon yield directly (Albrecht et al., 2012; Hoehn et al., 2008). This approach makes it difficult to understand the underlying mechanisms because species differences are ignored. Furthermore, proxies such as pollen load (the amount of free pollen on the body) and visitation rate are often used to estimate pollinator importance (Popic et al., 2013a; Stanley et al., 2013), despite evidence that such proxies are poor predictors of pollination service (Adler & Irwin, 2006; King et al., 2013). Species differ in their contribution to pollination service due to differences in visitation effectiveness (measured as single visit pollen deposition; Ne'eman et al., 2010) and visitation rate (measured as the number of flowers that are visited per unit of time). Visitation effectiveness is determined by behaviour (for example, visit duration (Fishbein & Venable, 1996; Ivey et al., 2003)) and morphology (for example, interspecific body size (Kandori, 2002) and intraspecific body size (Willmer & Finlayson, 2014)). Dramatic differences have been identified between pollinators in terms of flower-visiting behaviour (Sahli & Conner, 2007; Woodcock et al., 2013). Visitation rate is determined by abundance and spatiotemporal activity patterns. It is important to consider visitation effectiveness and visitation rate concurrently because less efficient pollinators can provide a greater pollination service due to more frequent visits (Sahli & Conner, 2007).

This study will investigate the pollination service that is provided by insect communities to winter-sown oilseed rape (OSR) (*Brassica napus* L.). OSR is a mass flowering crop that is grown as a source of vegetable oil and increasingly used as a biofuel. Pollination leads to the development of pods that contain seeds that can be processed to produce oil. It is an important break

crop that is grown as part of crop rotations with cereals. As a result, it is the most widely grown flowering field crop in the European Union (Jauker et al., 2012). In the UK in 2015, it covered 670 thousand hectares, and had a value of £706 million (DEFRA, 2016).

OSR is self-compatible (Williams, 1978; Williams et al., 1986). Wind pollination is considered to be of principal importance (Williams, 1984), particularly for winter-sown varieties because bees are relatively scarce at the time of flowering (Hayter & Cresswell, 2006). However, it also produces large amounts of nectar and is visited by a diverse pollinator community, including honeybees, bumblebees, solitary bees, and hoverflies (Chifflet et al., 2011; Garratt et al., 2014; Jauker et al., 2012; Rader et al., 2009; Stanley et al., 2013; Woodcock et al., 2013). It is also visited by non-Syrphid flies (Rader et al., 2009), and it has been suggested that further research should assess their importance (Garratt et al., 2014). Insect pollination can result in greater yield (Bommarco et al., 2012; Jauker et al., 2012; Marini et al., 2015; Sabbahi et al., 2005; Stanley et al., 2013; Steffan-Dewenter, 2003), shorter flowering period (Sabbahi et al., 2006; Williams et al., 1987), and increased market value (Bommarco et al., 2012). In one study, exclusion of pollinators resulted in a 27% decrease in the number of seeds produced, and a 30% decrease in seed weight per pod (Stanley et al., 2013). However, the relationship between cross-pollination and yield appears to vary remarkably between varieties (Hudewenz et al., 2014). It is important to understand how pollinator community characteristics relate to pollination service and yield, given that pollination demands of OSR are not currently being met (Garratt et al., 2014).

This chapter investigates how pollinator community characteristics relate to the pollination of OSR. The following hypotheses were tested:

H1: Pollen load (the quantity of free pollen on the body) determines the visitation effectiveness of an individual.

H2: Non-Syrphid Diptera are important pollinators of winter-sown OSR.

H3: The diversity, abundance, and composition of a pollinator community determine the pollination service that it provides.

A range of methods was used to measure the visitation rate, visitation effectiveness, and pollen load of each flower-visiting species, and these data were combined to produce a model that estimated pollination service (fig. 2.1). This type of modelling has been used to estimate the contribution of different pollinator species to pollination service, and to compare the pollination service of communities in time and space (Kremen et al., 2002; Winfree et al., 2007). Furthermore, this modelling approach makes it possible to disentangle the drivers of pollination service in this system. Understanding whether many or few species are required to provide an adequate pollination service will determine whether management for pollination services can be used to justify the conservation of pollinator diversity (Kleijn et al., 2015).

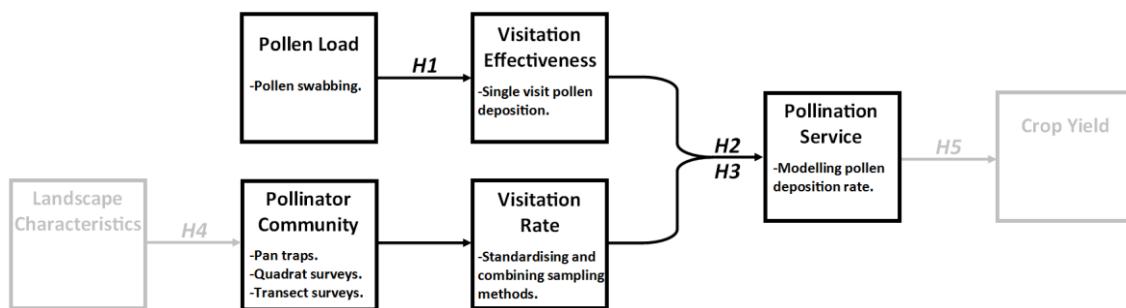


Figure 2.1. A diagrammatic overview of the study. The focus of this chapter are indicated by H1, H2, and H3. The remaining areas (H4 and H5), in grey, refer to the overall aims of BESS work package 4 and are being explored elsewhere (Shaw et al., in prep.). The subtext beneath each heading refers to the methods that were used to measure each aspect.

MATERIALS & METHODS

Field Site. Experiments were conducted within the region of the Wessex Biodiversity and Ecosystem Service Sustainability (BESS) project, a 1400 km² area in southern England (51.185988, -1.832657). The habitat in this area consists of calcareous chalk grassland, with a mixture of productive arable land, managed grassland undergoing biodiversity restoration, and ancient grassland. This served as a gradient of neighbouring land-use intensities, which provided the natural variation in pollinator communities that was necessary for this study. The effect of the surrounding landscape on the characteristics of pollinator communities in fields of OSR is being explored elsewhere (Shaw et al., in prep.). In order to capture this variation, fields were selected based upon the proportion of neighbouring natural and semi-natural habitat. Fields were at least 1 km apart in order to ensure independence of sites. Data were collected between 10th April and 17th May of each year, in three field seasons, between 2014 and 2016.

Visitation Rate. In order to estimate the visitation rate to crop flowers, invertebrate communities were sampled in fields of OSR. In both 2014 and 2015, twelve fields of OSR were selected (twenty-four fields in total). Within each field, a 58 m transect line ran from each of three sides of the field towards the centre, and three points were marked along each transect at distances of 8 m, 33 m, and 58 m from the field edge (fig. 2.2). Pollinator communities were sampled using multiple survey methods (pan traps, quadrat surveys, transect surveys), because different methods are ineffective at detecting particular species (Nielsen et al., 2011; Popic et al., 2013b). Surveys were completed only when weather conditions met the criteria described in Pollard & Yates (1993).

Pan traps. Pan traps (fluorescent yellow plastic bowls, with radius 0.075 m) were set within the flowering canopy and filled with water (to a depth of 3 cm) and a drop of non-scented detergent (to break surface tension). Pan traps were collected after 96 h and the contents were stored in 70% ethanol. In the laboratory, the contents were sorted: bees, and flies of the families Syrphidae, Bibionidae, and Empididae, were identified to species level, and other flower visitors were identified to at least family level.

Quadrat surveys. The number of receptive flowers was recorded in a 1 m² area, and then visits to OSR flowers were recorded for a duration of 5 min.

Transect surveys. The 58 m transect line was walked out and back over a ~20 min period and individuals that were observed visiting OSR flowers within a 2 m² area either side of the transect were recorded.

During quadrat and transect surveys, the proportion of flower visits with and without stigmal contact was recorded for a subset of flower visitors. Flower visitors were identified as far as possible on the wing, often to morphotype, and type specimens were collected for further identification.

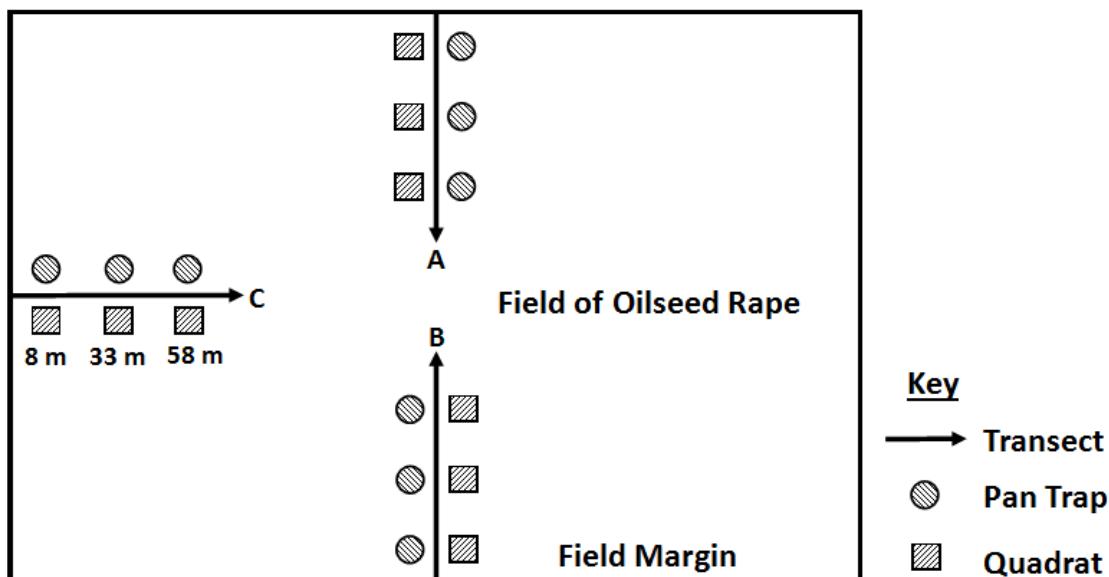


Figure 2.2. A schematic of a typical study field of OSR. The relative locations of transects (arrows), pan traps (circles), and quadrats (squares), within fields, with distances (8 m, 33 m, 58 m) in relation to the field margin.

In order to produce an overall measure of visitation rate, data from each sampling method were converted to a standardised measure of visitation rate in terms of number of visits per unit area per unit time (visits/m²/h). Sampling effort was assumed to be a function of the survey area (pan traps 0.017 m² (circle with radius 0.075 m), quadrat surveys 1 m², transect surveys 6.28 m² (semi-circle with radius 2 m)) and the survey duration (pan traps 96 h, quadrat surveys 5 min, transect surveys ~20 min (survey-specific)). However, pan traps were assumed to represent a proportionally greater area, because an area of equivalent size in the surrounding crop is only partially occupied by flowers. Whilst an individual that landed on any part within a pan trap would be caught

and recorded, only an individual that landed on a flower (a relative proportion of the total survey area) was recorded in quadrat and transect surveys. Therefore, the representative area of pan traps was corrected, based upon the receptive flower density at each transect, using the following equation:

$$A_T = \frac{1}{D_T} \cdot \frac{\pi r^2}{\emptyset^2}$$

A = Representative area of pan trap (m^2).

D = Receptive flower density (flowers/ m^2).

r = Radius of pan trap (m^2).

\emptyset = Diameter of OSR flower (m^2).

T = Transect.

As pan traps were left out overnight, an estimate of the number of hours during which pollinators were at peak activity per day was used. Day length was 14 h 46 min in the middle of the survey period and pollinators were less active during the early and late hours of the day. Therefore, it was assumed that pollinators were at peak activity for an average of 8 hours per day (6 h of peak activity, 4 h of half activity, and 4 h 46 min of no activity during daylight hours). As a result, the 96 h over which a pan trap was set out represented 32 h of peak pollinator activity.

An additional factor that needed to be accounted for was that transect surveys and pan traps measured abundance rather than visitation rate. The presence of an individual in a pan trap indicated a termination of that individual's foraging bout, and transects measured the number of individuals visiting flowers rather than the number of flower visits. Therefore, the abundance of each species was multiplied by the mean number of flower visits by an individual of that species in quadrat surveys within the 1 m^2 area. Unfortunately, there were insufficient data for some non-bee species, so the average number of visits per individual in a 1 m^2 area per 5 min was assumed to be 1 for these species. This is because non-bee flower visitors were generally observed to do one of the following in the field: (i) remain on a flower for the duration of the survey period, (ii) depart the flower and settle on a non-floral structure, or (iii) depart the flower and leave the survey area (personal observation). However, as no data were collected to support this assumption, visitation rates for non-bees may be an underestimate. Standardised visitation rates (visits/ m^2/h) were combined for points within transects in order to provide

an average visitation rate for each transect (72 transects in total, across 24 fields).

To ensure that these standardised sampling methods produced reasonable estimates, the standardised visitation rates were compared between each sampling method (table 2.1). Data from quadrat surveys were considered the most realistic because this method was only adjusted for survey duration. The estimated visitation rates of medium-sized flies from pan traps were similar to those from quadrats surveys (pan traps $36.96 \pm 4.87 SE$, quadrat surveys $33.92 \pm 6.90 SE$; table 2.1). The estimated visitation rates of small beetles were similar between pan traps and transect surveys, but underestimated in comparison to quadrat surveys (pan traps $8.56 \pm 1.14 SE$, transect surveys $9.97 \pm 1.19 SE$, quadrat surveys $33.19 \pm 2.90 SE$; table 2.1). Quadrat surveys had a very low detection rate for all other species because the survey duration was short, hence the need to combine multiple sampling methods. The number of male solitary bees that were found in pan traps was often extremely high, and resulted in visitation rates that were more than ten times greater than observed in a previous study (Woodcock et al., 2013). This may be due to males being attracted to reproductive cues of other bees in pan traps, because male bees were never observed in such abundance in the field. Furthermore, solitary bees that were observed foraging were predominantly female, probably because they have much greater foraging requirements due to nest provisioning. For these reasons, male solitary bees were excluded from further analyses. Once this had been done, the visitation rates for solitary bees were very similar between pan traps and transect surveys (pan traps $0.86 \pm 0.13 SE$, transect surveys $0.89 \pm 0.10 SE$; table 2.1). Transect surveys were the only method that consistently detected honeybees and bumblebees. The estimated visitation rates for transect surveys were very similar to those recorded in the same region in a previous study for honeybees (transect $4.03 \pm 0.87 SE$, Woodcock et al. (2013) $3.91 \pm 0.28 SE$; table 2.1) and bumblebees (transect $3.01 \pm 0.39 SE$, Woodcock et al. (2013) $2.66 \pm 0.28 SE$; table 2.1). Whilst there is some uncertainty around these estimates of visitation rate, the overlap between the different sampling methods and with published data provides reassurance that these values are field realistic.

For each species, the visitation rate was selected from the survey method that provided the greatest rate of detection (table 2.1; appendix A). For

most species, this was pan traps, because this method samples the community over the greatest duration. However, larger species were rarely detected in pan traps (*Apis mellifera*, *Bombus* spp., *Bibio marci*) so visitation rates from transect surveys were used for these species. Although this method of combining visitation rates deserves some caution when considering the absolute values, it allows for relative comparison between sites.

Table 2.1. The estimated OSR flower visitation rates (visits/m²/h) of key visitor groups from each survey method, standardised for area and time. Values are mean ± standard error, followed by the sample size in brackets. The sample size represents the number of transects (out of 72) at which each group was detected. The estimated visitation rate for solitary bees from pan traps only includes females because male counts were found to be heavily biased.

Survey Method	Mean Visitation Rate ± SE (visits/m ² /h)					
	Solitary Bee	Honeybee	Bumblebee	Medium-Sized Fly	Large-Sized Fly	Small Beetle
Pan Trap	0.86 ± 0.13 (67)	0.46 ± 0.00 (1)	0.13 ± 0.00 (1)	36.96 ± 4.87 (72)	1.21 ± 0.20 (67)	8.56 ± 1.14 (72)
Quadrat	5.00 ± 1.00 (4)	19.00 ± 7.00 (4)	36.00 ± 16.00 (2)	33.92 ± 6.90 (52)	7.11 ± 1.30 (9)	33.19 ± 2.90 (67)
Transect	0.89 ± 0.10 (31)	4.03 ± 0.87 (32)	3.01 ± 0.39 (30)	5.98 ± 0.68 (64)	0.61 ± 0.08 (20)	9.97 ± 1.19 (53)
Woodcock et al. (2013)	2.25 ± 0.34	3.91 ± 0.28	2.66 ± 0.28	-	-	-

Visitation Effectiveness. The visitation effectiveness of flower visitors was assessed by measuring single visit deposition. This is a well-established method and considered the best direct measure of pollinator effectiveness (King et al., 2013; Kremen et al., 2002; Ne’eman et al., 2010; Winfree et al., 2007).

In the field, OSR flowers were inspected (using a hand lens with x20 magnification) for ripe anthers and a receptive stigma that was absent of pollen grains. Suitable flowers were collected, taking care to minimise transfer of pollen from the anthers to the stigma. Flowers were set up using one of the following methods, depending upon the visitor species being tested: (i) Bouquet method- used for solitary bees, bumblebees, and honeybees; the flower was taped by the stem to a clear drinking straw, forming a mobile bouquet (Thomson, 1981), (ii) Plastic box method- used for beetles, flies, and sawflies; the flower was placed in a stationary 1 cm segment of straw, and covered with a small plastic container.

Flower visitors were found on OSR plants. If a solitary bee, bumblebee, or honeybee was found, the experimental flower was presented at arm’s length using the bouquet method. If a non-bee visitor was found, it was caught with a

net and placed inside the plastic container, which contained the experimental flower of the plastic box method. In both cases, the visitor was left undisturbed until it had made contact with the stigma, and subsequently left the flower. The duration of the visit and the behaviour of the visitor was recorded. The visitor was then caught for identification and for assessment of pollen load. If the flower visitor was unable to be caught, a morphotype identification was recorded. The stigma was then carefully removed from the flower using forceps, and preserved in a box of fuschin jelly (glycerine jelly mixed with basic fuschin stain, Brunel Microscopes Ltd, UK). Samples were kept in a cool box and then refrigerated in the laboratory. In order to control for the effects of handling and wind pollination, control OSR flowers were collected, prepared using one of the two methods, and the stigma extracted, without having been visited.

In the laboratory, samples were prepared onto microscope slides by cutting stigmas in half, squashing them face down, melting the jelly onto separate slides (in 2 mm cubes), and placing a cover slip over each slide. The total number of pollen grains was counted by carrying out transverses under a microscope at x20 magnification.

Pollen Loads. In order to assess the relationship between the quantity of pollen on the body of an insect and visitation effectiveness, individuals were swabbed for pollen. In this study, ‘pollen load’ refers to the quantity of free pollen that is carried on the body of an individual and is available for transfer to flowers. This excludes pollen from pollen storage areas such as the pollen baskets of bees. Individuals were swabbed on the head, top of the thorax, tarsi, and bottom of the thorax, using 2 mm cubes of fuschin jelly. Each body part was swabbed separately, and each set of swabs consisted of fifteen strokes. Each body part was swabbed with a second square of jelly to ensure that the majority of pollen had been collected. As above, cubes of jelly were prepared onto microscope slides, and the number of pollen grains was counted.

Functional Groups. In order to assess functional diversity, flower visitors were arranged into functional groups. Functional diversity is likely to be a better predictor of ecosystem functioning than species diversity, because many species provide functionally similar roles (Petchey & Gaston, 2006). The body length and intertarsal distance (ITD) were measured for at least five individuals

per species. ITD is closely related to overall size and is considered the best measure for comparison across bee genera (Cane, 1987). Functional groups were created based upon these measurements, in combination with behavioural traits: whether or not individuals were seen probing (personal observations) and specific flower visit behaviour (Woodcock et al., 2013). Flower visitors formed eleven functional groups in total, consisting of five bee groups and six non-bee groups (table 2.2; see appendix A for species list).

Table 2.2. The eleven functional groups that were used in the study, and the defining traits of each group. A complete list of the species in each functional group can be found in appendix A.

Functional Group	Description	Probes for Nectar ¹	Flower Visit Duration (secs) ²	Intertegular Distance (mm) ³	Body Length (mm) ³	Relative Size ³
1	Small Solitary Bee	✓	6+	1.00 - 1.75	6.50 - 8.50	Small
2	Medium Solitary Bee	✓	6+	1.75 - 2.50	9.50 - 12.50	Medium
3	Large Solitary Bee	✓	6+	2.50 - 4.00	11.00 - 16.00	Large
4	Honeybee	✓	3.5	2.75 - 3.25	11.00 - 14.00	Large
5	Bumblebee	✓	3	4.00 - 6.50	13.00 - 22.00	Very Large
6	Small Probing Fly	Occassionally	Variable	0.50 - 1.50	4.00 - 8.50	Small
7	Medium Probing Fly	Occassionally	Variable	1.50 - 2.00	8.00 - 10.00	Medium
8	Large Probing Fly	Occassionally	Variable	2.00 - 4.50	9.50 - 13.50	Large
9	Medium Fly	✗	Variable	1.00 - 2.00	5.00 - 7.00	Medium
10	Large Fly/Other	✗	Variable	2.00 - 4.50	9.00 - 12.50	Large
11	Small Beetles	✗	100+	0.50 - 1.00	2.40 - 2.80	Very Small

¹Field Observations (B Phillips)

²Woodcock et al. (2013)

³Trait Measurements (B Phillips & A Williams)

Model of Pollination Services. Data were combined to create a predictive model of pollination service, based upon models in Kremen et al. (2002) and Winfree et al. (2007). The model used the collected data to estimate the rate of pollen deposition, due to flower visitation, at each transect. From this, the relative contribution of each functional group could be quantified, and community characteristics could be related to estimated pollen deposition. The model was produced in MATLAB R2014b (The MathWorks, Inc., 2014, Natick, MA 01760, USA). Model parameters were (i) Visitation rate- the number of flower visits by each functional group (visits/m²/h), (ii) Proportion of visits with stigmal contact, (iii) Visitation effectiveness- the median single visit pollen deposition for each functional group, and (iv) Number of pollen grains required to fully fertilise all ovules of an OSR flower. The source of the data for

parameters (i), (ii), and (iii) has been described above. The use of median values for visitation effectiveness provides conservative estimates because data were positively skewed. Visitation rate differed between transects, representing different pollinator communities, whilst the other parameters were assumed to be constant. Parameter (iv) was retrieved from the literature: OSR flowers have 26 ovules on average (Bouttier & Morgan, 1992), and 160 pollen grains are required to fully fertilise these ovules (Mesquida & Renard, 1984). The output of the model was rate of pollen deposition (grains/m²/h), a measure of pollination service, at each transect.

The estimated rate of pollen-deposition at each transect was described in the model by the following equation:

$$D_T = \sum_{G=1}^n R_{G_T} \cdot P_G \cdot (E_G - C) \quad (E_G - C) = \begin{cases} F, & (E_G - C) > F \\ (E_G - C), & (E_G - C) \leq F \end{cases}$$

D = Estimated rate of pollen deposition (grains/m²/h).

R = Visitation rate (visits/m²/h).

P = Proportion of visits that result in stigmal contact.

E = Median visitation effectiveness (grains/visit).

C = Median number of pollen grains on control stigmas.

F = Number of pollen grains required for full fertilisation of OSR flower ovules.

G = Functional group.

n = Number of functional groups.

T = Transect.

Real communities. The contribution of each functional group to estimated pollen deposition was compared overall and between transects. The contribution of bees and non-bees to estimated pollen deposition was compared by summing the contribution of functional groups (bees: functional groups 1 to 5, non-bees: functional groups 6 to 11). The rate of pollen accumulation on stigmas of OSR flowers, due to flower visitors, was calculated at each transect based upon the estimated rate of pollen deposition and the receptive flower density.

Simulated communities. Communities were simulated in order to isolate the effect of diversity as a driver of pollination service. For the first analysis, the community at each transect was resampled, keeping visitation rate constant. A mean was taken from fifty iterations of resampling for each transect. For the

next analysis, simulated communities were created in the following way: (i) Species were selected based upon the proportion of transects at which they were encountered. (ii) Visits were assigned one-at-a-time to the selected species based upon the relative visitation rate of the species across all transects where they were encountered. Visitation rate was made constant for each community, and an equal number of communities were simulated for each value of functional group richness.

Statistical Analyses. All statistical analyses were carried out in R (R Core Team, 2015). Analyses were completed using linear models in the inbuilt R function, and linear mixed effects models in the nlme package (Pinheiro et al., 2016). Where necessary, response variables were transformed with square root or log transformations in order to meet the assumptions of statistical tests. Random models were used for the analysis of the community-level data to account for multiple transects being within the same field. Full details of the statistical analyses can be found in appendix B.

RESULTS

Across the 24 fields of winter-sown OSR, 32 species of bee were sampled, consisting of 17 *Andrena* spp., 5 *Lasioglossum* spp., 2 *Nomada* spp., 1 *Osmia* spp., 6 *Bombus* spp., and *Apis mellifera*. Many non-bee groups were also encountered that were suspected to be transferring pollen between OSR flowers. These included ten fly families (Anthomyiidae, Bibionidae, Bombyllidae, Calliphoridae, Conopidae, Empididae, Muscidae, Sarcophagidae, Scathophagidae, and Syrphidae), one sawfly family (Tenthredinidae), and beetles, primarily of the species *Meligethes aeneus* and *Ceutorrhynchus assimilis* (appendix A). The single visit pollen deposition was measured for 197 individuals across these groups.

Pollen Load & Visitation Effectiveness

Functional group was a significant predictor of single visit pollen deposition ($F_{11,216} = 16.443, P < 0.001$; fig. 2.3a). Control stigmas contained a median of 14 pollen grains, demonstrating that the majority of pollen grains found on stigmas after a single visit were the result of the behaviour of the visitor, rather than due to the effects of wind pollination or handling by the experimenter. Large solitary bees, medium solitary bees, bumblebees, and honeybees had by far the greatest single visit pollen deposition. However, the single visit pollen deposition of all functional groups was greater than the number of pollen grains that were found on control stigmas, except for small beetles (FG11; $t = -1.002, n = 228, P = 0.317$). Small beetles were therefore excluded from subsequent analyses.

Functional group was a significant predictor of pollen load ($F_{10,94} = 11.903, P < 0.001$; fig. 2.3b). Functional groups showed a similar pattern in terms of single visit pollen deposition and pollen load, with groups that had the greatest single visit pollen deposition (large solitary bees, medium solitary bees, bumblebees, and honeybees) also having the greatest pollen loads. This was supported statistically, with pollen load being a significant predictor of single visit pollen deposition ($F_{1,84} = 10.659, P = 0.002$; fig. 2.3c). However, the amount of variation that was explained when data for individuals were plotted was low ($R^2 = 0.102$).

Pollinator Communities & Pollination Services

The vast majority of flower visits were by flies of the family Anthomyiidae (86% of total visits). Only 4% of flower visits were by honeybees, 3% by bumblebees, and 2% by solitary bees. Other non-bee functional groups made up the remaining 5% of flower visits (fig. 2.4a).

The proportion of flower visits that resulted in stigmal contact, and were therefore likely to result in pollen transfer, differed dramatically between bee and non-bee groups. Whilst visits by bees always resulted in stigmal contact, only 30% of observed flower visits by non-bees did (fig. 2.4b).

Although there were large differences between groups in single visit pollen deposition, these were restricted in the model by an upper limit of 160, which has been identified as the number of grains required for fertilisation of all ovules (Mesquida & Renard, 1984) (fig. 2.4c). As a result, the number of pollen grains that were utilised per visit in the model was relatively similar between functional groups. Visits by individuals of all groups sometimes resulted in sufficient pollen deposited for full fertilisation of ovules. However, as median values were used for each group in the model, flowers required multiple visits by non-bee groups, but only a single visit by bee groups (except for small solitary bees), in order to achieve full fertilisation of ovules.

Overall, the majority of the total estimated pollen deposition was by Anthomyiid flies (49%), honeybees (21%), bumblebees (15%), and solitary bees (9%). Other non-bee groups made up the remaining 6% of total estimated pollen deposition (fig. 2.4d). Across all transects, the total estimated contribution of bees (functional groups 1 to 5) and non-bees (functional groups 6 to 10) to pollen deposition was similar (bees 45%, non-bees 55%; fig. 2.5).

Although, the model estimated that pollen deposition was dominated by a few functional groups, the relative contribution of each varied dramatically between transects (fig. 2.6). The model estimated that flower visitors resulted in an accumulation of pollen on stigmas at a mean rate of 2.06 ± 0.18 SE grains/hour.

Visitation rate, one of the fundamental parameters of the model, was a strong predictor of estimated pollen deposition ($F_{1,45} = 175.366$, $P < 0.001$; fig. 2.7a). Functional group richness was also a significant predictor of pollen deposition, even when visitation rate was accounted for in the statistical model

($F_{1,45} = 37.867$, $P < 0.001$; fig. 2.7b). However, there was a significant relationship between visitation rate and functional group richness ($F_{1,47} = 5.228$, $P = 0.027$; fig. 2.7c).

Simulated Communities

When the communities at each transect were resampled using the model (keeping visitation rate constant), functional group richness had a positive effect on pollen deposition ($F_{1,47} = 89.762$, $P < 0.001$; $R^2 = 0.772$; fig. 2.8).

When artificial communities were simulated (again, keeping visitation rate constant), based on the overall probability of encounter across transects and the overall visitation rate of each functional group, functional diversity was found to have a stabilising effect on estimated pollen deposition (fig. 2.9).

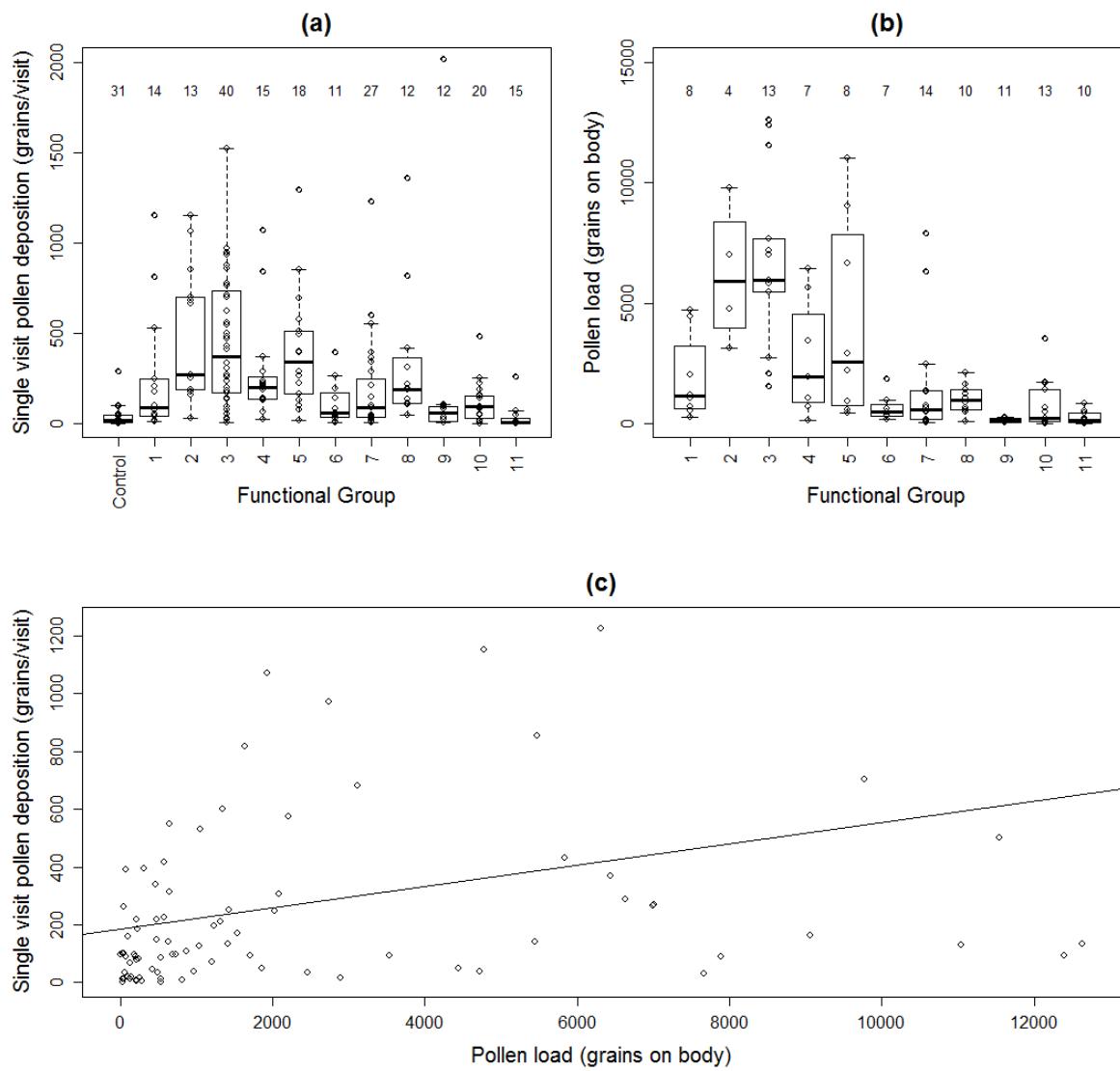


Figure 2.3. (a) The single visit pollen deposition of individuals of each functional group. Values above boxplots are sample sizes. (b) The pollen load of individuals of each functional group. Values above boxplots are sample sizes. (c) The relationship between pollen load and single visit pollen deposition for each individual. Functional groups are 1. Small solitary bee, 2. Medium solitary bee, 3. Large solitary bee, 4. Honeybee, 5. Bumblebee, 6. Small probing fly, 7. Medium probing fly, 8. Large probing fly, 9. Medium fly, 10. Large fly/other, 11. Small beetle.

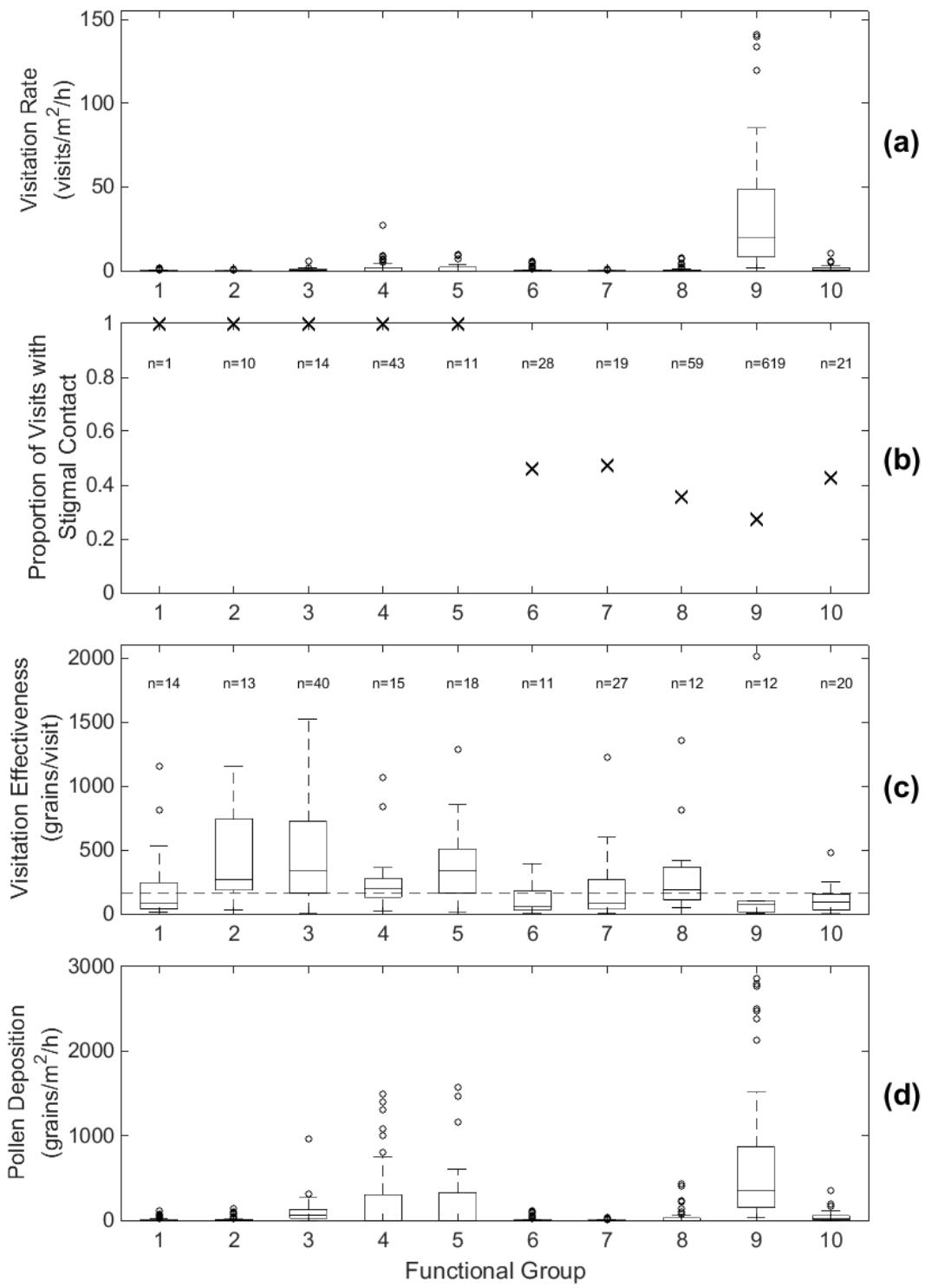


Figure 2.4. The model parameters (a-c) and the resulting output (d): (a) Visitation rates ($\text{visits}/\text{m}^2/\text{h}$) for each functional group at each of the 72 transects. (b) The proportion of total visits that resulted in stigmal contact for each functional group. (c) The visitation effectiveness (single visit pollen deposition) for each functional group. Median values were used and the dashed line represents an upper limit as this is the number of pollen grains required to fully fertilise all OSR flower ovules (Mesquida & Renard, 1984). (d) Estimated rate of pollen deposition ($\text{grains}/\text{m}^2/\text{h}$) due to each functional group, at each of

the 72 transects. Functional groups are 1. Small solitary bee, 2. Medium solitary bee, 3. Large solitary bee, 4. Honeybee, 5. Bumblebee, 6. Small probing fly, 7. Medium probing fly, 8. Large probing fly, 9. Medium fly, 10. Large fly/other.

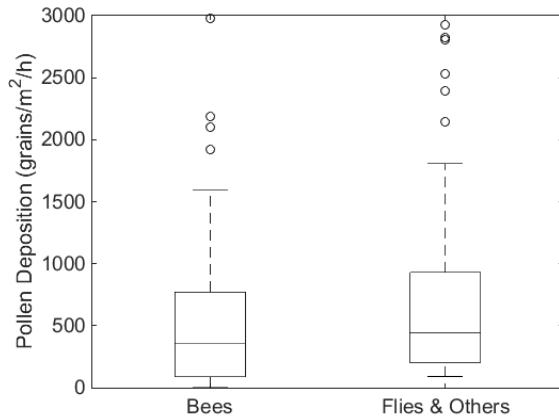


Figure 2.5. The estimated pollen deposition due to flower visits by bees (functional groups 1 to 5) and non-bees (functional groups 6 to 10) at each of the 72 transects.

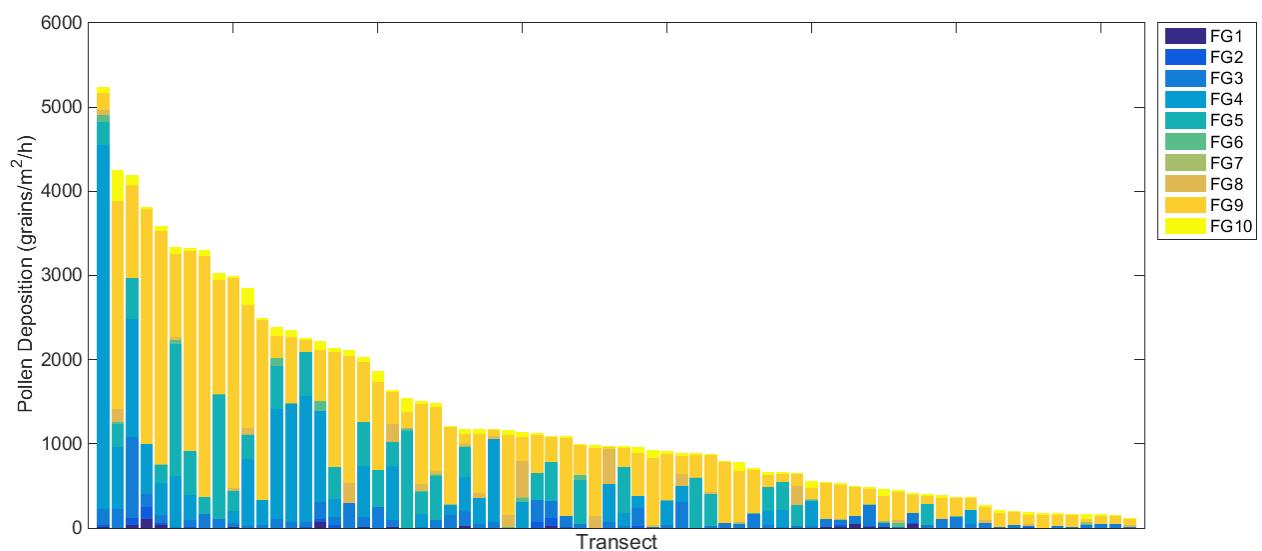


Figure 2.6. A comparison of the relative contribution of each functional to estimated pollen deposition at each of the 72 transects, ordered from highest to lowest in terms of estimated pollen deposition. FG refers to functional group.

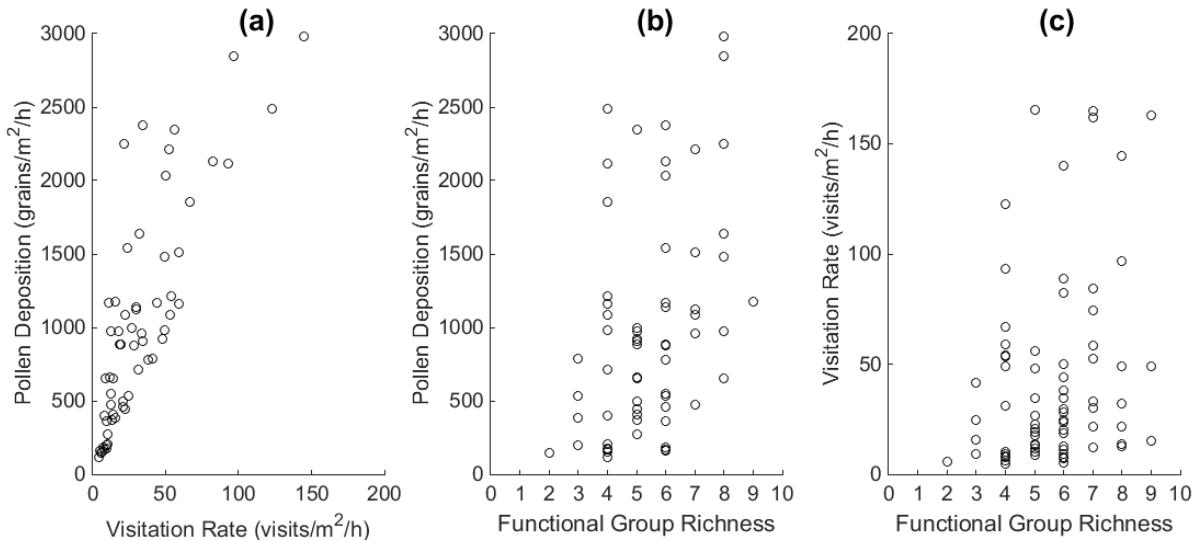


Figure 2.7. The possible drivers of differences in estimated pollen deposition

(a-b) and the correlation between them (c). (a) The relationship between visitation rate and estimated pollen deposition at each transect. (b) The relationship between functional group richness and estimated pollen deposition at each transect. (c) The relationship between functional group richness and visitation rate at each transect.

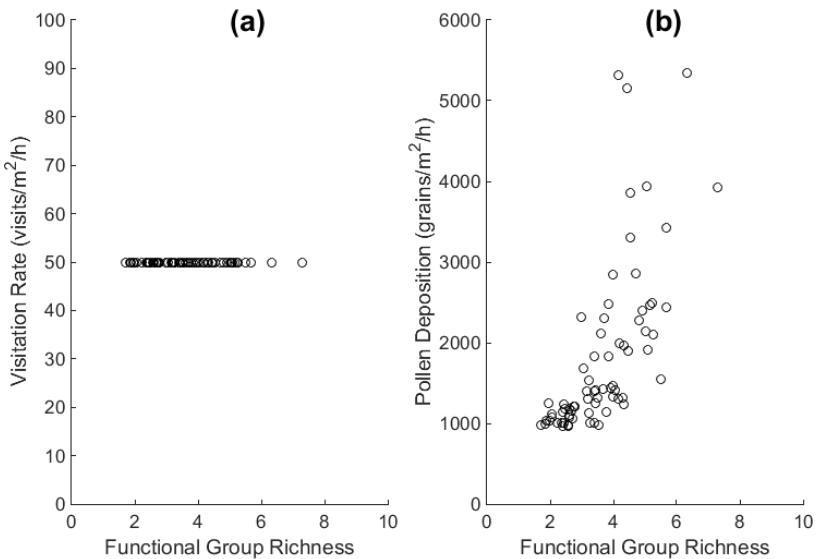


Figure 2.8. The resampled communities at each of the 72 transects. (a) Visitation rate was kept constant in order to isolate the effect of functional diversity. (b) The relationship between functional group richness and estimated pollen deposition at each transect. Points represent the mean of fifty iterations of resampling at each transect.

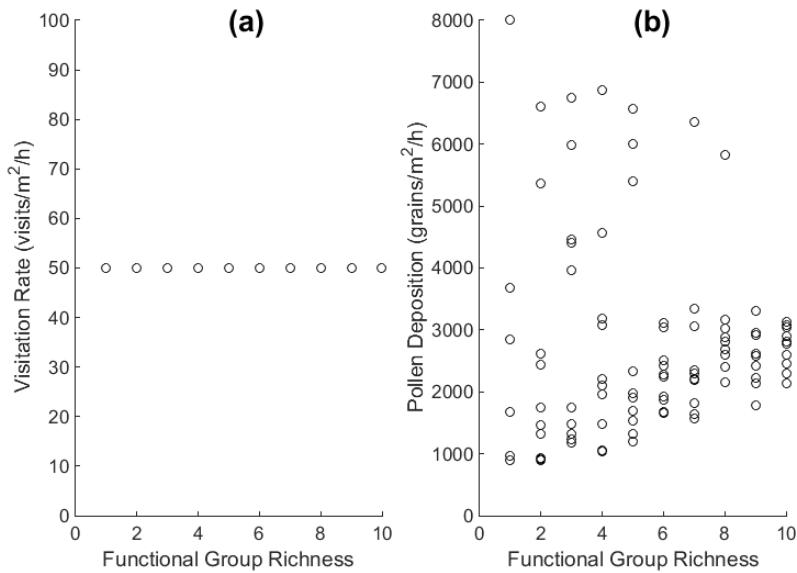


Figure 2.9. The simulated pollinator communities. (a) Visitation rate was kept constant in order to isolate the effect of functional diversity. (b) The relationship between functional group richness and estimated pollen deposition for each of the 100 communities.

DISCUSSION

A diverse community of invertebrates was observed visiting flowers of winter-sown OSR, despite a relatively early time of year (mid April-mid May). As well as many wild bee species, many non-bee species were observed foraging on OSR flowers. Non-bee species were even regularly observed probing into flowers for nectar, particularly flies of the families Bibionidae, Empididae, and Syrphidae. Furthermore, many additional non-bee species were found in the crop and, even if not actively foraging for nectar or pollen, were often observed in contact with the reproductive parts of OSR flowers, and are therefore likely to be transferring pollen.

Pollen Load & Visitation Effectiveness

Visits by almost all functional groups resulted in significantly more pollen on stigmas than was found on control stigmas. This included probing flies of the families Empididae and Bibionidae, as well as medium and large sized flies that were not regularly observed probing, including flies of the families Anthomyiidae, Calliphoridae, Canopidae, Muscidae, Scathophagidae, and Sarcophagidae. This provides quantitative evidence that incidental flower visits that were regularly observed in the field result in pollen transfer, even though individuals were not actively foraging for nectar or pollen. As it has been argued that single visit pollen deposition should be measured in order to determine whether individuals are ‘pollinators’ or ‘flower visitors’ (King et al., 2013), this justifies describing many of these non-bee visitors of OSR as ‘pollinators’.

Whilst visits by almost all functional groups resulted in pollen transfer, there were clear differences in terms of visitation effectiveness. As expected, bees were by far the most effective visitors, in terms of single visit pollen deposition. On average, bees deposited enough pollen grains per visit to fertilise all ovules of an OSR flower, whereas flies did not. Therefore, flowers are likely to require multiple visits by non-bee groups in order to achieve full fertilisation. However, this also suggests that only a fraction of the pollen grains that are deposited by bees are utilised by the flower in terms of fertilisation of its ovules. In this respect, bee visitors are ‘over-pollinating’. Therefore, if using only visitation effectiveness as a predictor of pollinator importance for OSR, the relative importance of bees would be overemphasised. The actual difference

between bees and non-bees, in terms of fertilisation of ovules per visit and subsequent seed production, is likely to be much smaller than suggested by single visit pollen deposition alone.

Although there were similar trends for each functional group in terms of pollen load and single visit pollen deposition, the number of pollen grains that were swabbed from an individual did not relate closely to the number of pollen grains that it deposited on a stigma. There are two likely explanations for this result. Firstly, differences in behaviour and morphology between functional groups may be more important in determining single visit pollen deposition. Even within functional groups, behavioural differences such as whether an individual probed for nectar, collected pollen, or simply crawled across a flower, may be of greater importance than the amount of pollen on the body of the insect. For instance, if an individual contacts the stigma with only a tarsus, then few pollen grains will be transferred even if the body is laden. Secondly, a substantial amount of pollen may be transferred from the anthers to the stigma of the same flower, even if the visitor is carrying little or no pollen. Given that the number of pollen grains on the anthers of an OSR flower can exceed 100,000 (Hayter & Cresswell, 2006), the observed number of grains on the stigma after a flower visit could be very high if the visit resulted in direct or indirect contact between the reproductive parts of the same flower. This is observed regularly in the field (personal observation), and would result in single visit pollen deposition that is irrespective of the pollen load of the visitor. Support for this is provided by Cresswell (1999), who found that the single visit pollen deposition of bumblebees was three times lower when the anthers of the flower had been brushed to remove pollen. This suggests that a substantial proportion of pollen that is found on an OSR stigma after a visit by a pollinator is self-pollen, rather than from the body of the forager. As OSR is self-compatible, self-pollen results in at least moderate fertilisation of ovules, seed set, and yields for many varieties (Williams, 1978; Williams et al., 1986). However, cross-pollination can provide further benefits in terms of yield (Bommarco et al., 2012).

Overall, the finding that pollen load does not strongly relate to single visit pollen deposition (see also Adler & Irwin, 2006) is important, given that some studies use the presence or quantity of pollen on the body as a proxy for pollinator quality or importance (Popic et al., 2013a; Stanley et al., 2013). As there were similar general trends in terms of pollen load and visitation

effectiveness, it may still be reasonable to draw very general relationships across pollinator groups. However, it should be understood that this is not a mechanistic relationship. A measure of pollinator effectiveness through studies of single visit pollen deposition is the most reliable assessment (King et al., 2013; Kremen et al., 2002; Ne’eman et al., 2010; Winfree et al., 2007).

Pollinator Communities & Pollination Services

The vast majority of visits to OSR flowers were by medium-sized flies of the family Anthomyiidae. However, visits by this group infrequently made stigmal contact, and resulted in the deposition of relatively few pollen grains when they did. By comparison, visits by bees always made stigmal contact, and generally resulted in the deposition of a much greater number of pollen grains. As a result, solitary bees, bumblebees, and honeybees each made a substantial contribution to pollen deposition despite relatively low visitation rates. Overall, the contribution of non-bee groups to pollen deposition was estimated to be greater, though similar, to that of bees. This is in accordance with a recent literature synthesis, which concluded that non-bees performed, on average, 25-50% of total flower visits (Rader et al., 2015).

The model estimated that visits to flowers by invertebrates resulted in pollen accumulating on OSR stigmas at a mean rate of 2.06 ± 0.18 SE grains/hour. By comparison, Hayter & Cresswell (2006) measured the mean rate of pollen accumulation on stigmas of winter-sown OSR flowers to be 3 grains/hour. They attribute the majority of this pollen deposition to wind pollination, because only 10% of flowers were ever visited by a bee in their study. Furthermore, there are often considerable quantities of air-borne OSR pollen during flowering (Williams, 1984). However, the model estimate, which does not include the contribution of wind pollination, suggests that a substantial proportion of pollen accumulation on stigmas could be the result of flower visits by non-bee groups, particularly Anthomyiid flies. Overall, this finding provides further support to the growing body of evidence that non-bee invertebrates are important crop pollinators (Orford et al., 2015; Rader et al., 2015; Ssymank et al., 2008).

The visitation rate and composition of functional groups varied dramatically between transects and the relative importance of each functional groups was therefore transect-specific. Although our study represents a

snapshot in time, it is likely that community composition also varies from year-to-year due to changes in environmental and ecological conditions. For example, Kremen et al. (2002) found that pollinator diversity was important in maintaining pollination services due to yearly variation in community composition. Therefore, the relative importance of species is likely to vary in both time and space, and diversity of pollinators may be a stabilising factor of pollination services.

The estimated pollen deposition at each transect was closely related to flower visitation rate, particularly because visitation effectiveness was similar between functional groups once the number of pollen grains required for full fertilisation had been taken into account. However, functional diversity remained a significant predictor of pollen deposition, even when visitation rate was controlled. This provides evidence for a BES relationship in this system. Because of the parameters of the model, this can only be the result of non-random community structuring, meaning that functionally diverse communities are disproportionately likely to contain functionally important groups (those with the greatest single visit pollen deposition, in this case, bees). This effect is over and above the mathematical phenomenon that was the source of much criticism of early BEF studies, whereby randomly composing communities of species leads to a greater probability of functionally important species being present (Huston, 1997; Loreau, 2000).

There are further possible sources of diversity benefits that were not encapsulated in the model. For example, the observed correlation between visitation rate and functional diversity may not exclusively be the result of sampling effects, but may also be a consequence of niche complementarity, whereby differences in resource use result in a greater abundance of individuals being supported. Unfortunately, it is not possible to disentangle these effects due to the relatively short duration of this study. Furthermore, there is a variety of other possible sources of niche complementarity. For example, different species may typically pollinate flowers on different parts of the plant, contact different parts of the same stigma, or be active in different weather conditions (Blüthgen & Klein, 2011). The BES relationship that has been identified in this study provides a baseline of the benefits of pollinator diversity in this system. Future studies can incorporate these potential effects into the model that has been provided here.

There is some uncertainty in the model from the visitation rate estimates that were required in order to combine sampling methods. As this study aimed to assess the contribution of a wide range of flower visitors, this method was necessary because pan traps were ineffective at detecting large species, and transects were less effective at detecting smaller species and provided coarse taxonomic resolution. However, the overlap in the estimates between sampling methods, and with published data (Woodcock et al., 2013) suggests that these estimates are reasonable. If anything, this method is likely to have underestimated the contribution of non-bee species because a lower reasonable estimate was used for one of the correction factors due to a lack of available data. The use of median values for visitation effectiveness will have further underestimated the contribution of non-bee groups compared to bee groups. This is because the median single visit pollen deposition for bee groups was greater than the upper limit parameter, so only non-bee groups were affected by the use of median values rather than means.

The main source of uncertainty in the model comes from the parameter for the number of pollen grains required to fully fertilise an OSR flower. In particular, the value used for this parameter has a large effect on the estimated contribution of bee species because it provides an upper limit on single visit pollen deposition. Consequently, it is worth discussing the reliability of this model parameter. It is likely that the number of pollen grains required for full fertilisation of ovules differs between varieties of OSR. As OSR flowers have 26 ovules on average (Bouttier & Morgan, 1992), this puts a lower limit on the number of pollen grains required. The value that was used (160, from Mesquida & Renard, 1984) may be unrepresentative of typical values for modern OSR varieties for multiple reasons. Firstly, the dependency upon cross-pollination is highly variable between varieties (Hudewenz et al., 2014). Secondly, the average requirement of modern varieties may be lower, because plant breeding attempts to maximise yield. If pollination is often a limiting factor upon yields, then varieties with lower pollination requirements may have been inadvertently selected. On the other hand, there is evidence to suggest that the actual number of pollen grains required under field conditions is much higher than under controlled conditions. For example, Winfree et al. (2007) used a similar modelling approach and found that the number of pollen grains on open pollinated stigmas was consistently less than half the number that their model

predicted. This suggests that a high proportion of pollen grains that are deposited during a visit do not fully adhere to the stigma. Although this value has a large effect upon the relative contributions of each functional group, it would not change the overall conclusions of the study.

In this study, pollen deposition has been used as an approximation for pollination service rather than crop yield. The next logical step is to relate estimated pollen deposition to yield data. Although OSR has a relatively low dependency upon insect pollination (Williams, 1978; Williams et al., 1986), there is likely to be some relationship because many studies have found discernible benefits (Bommarco et al., 2012; Jauker et al., 2012; Lindström et al., 2016; Marini et al., 2015; Sabbahi et al., 2005; Steffan-Dewenter, 2003; Stanley et al., 2013). In the model, a higher rate of pollen deposition is likely to translate into flowers being pollinated more quickly in the field. Fields in which flowers are pollinated more quickly may benefit two-fold. Firstly, a flower must receive sufficient pollination within its lifetime in order to achieve its potential yield. Secondly, fields in which flowers are pollinated more quickly are likely to finish flowering earlier (Williams et al., 1987), and subsequently produce pods that ripen more evenly across the field. As plants are harvested in a destructive manner, harvest occurs at peak production rather than continuously. If pods ripen simultaneously, this will increase the harvestable yield. Relating estimated pollen deposition to yield is beyond the scope of this chapter and is being explored elsewhere (Shaw et al., in prep.).

Conclusion

In this system, non-bee invertebrates are significant pollinators and may have a combined contribution to pollination services that is greater than that of bees. It has long been assumed that OSR has a low dependency upon insect pollination because it achieves considerable yields through self-pollination (Williams, 1978; Williams et al., 1986). In particular, wind pollination has been considered to be of primary importance for winter-sown OSR, because it flowers early in the year when bees are scarce (Hayter & Cresswell, 2006; Williams, 1984). However, this study suggests that non-bees, which have often been neglected in pollination studies (Orford et al., 2015), may provide much of this pollen transfer. Overall, this finding supports the growing body of evidence that non-

bee invertebrates are important contributors to pollination services (Orford et al., 2015; Rader et al., 2015; Ssymank et al., 2008).

This study has provided evidence for a BES relationship because functionally diverse communities were more likely to contain functionally important groups. Given that the importance of key species varied dramatically between sites, diversity may also be important in maintaining pollination services across a range of local conditions. Furthermore, pollinator diversity may provide resilience to environmental change and a range of other benefits, due to niche complementarity, that were not explored in this study. However, in this situation, the abundance of common species was the primary determinant of pollination service, as has been found elsewhere (Gaston, 2010; Kleijn et al., 2015; Winfree et al., 2015). The majority of the functional groups and species that were observed on crop flowers were estimated to constitute a small proportion of the pollination service. In this case, management for pollination services does not sufficiently justify the conservation of many pollinator species (Adams, 2014; Kleijn et al., 2015). However, general measures to support populations of common species, such as providing additional floral or nesting resources, may also benefit populations of less functionally important species. Nonetheless, conservation of pollinator diversity requires additional efforts in this landscape because pollination services are provided by a subset of available species.

Chapter 3:

**Impacts of drought on floral resources and
pollinators in an experimental grassland system.**

At the time of submission, this chapter was in preparation for publication, with the following authorship list: Phillips BB, Shaw RF, Fry EL, Bardgett RD, Bullock JM, Osborne JL.

ABSTRACT

Climate change is expected to result in widespread environmental stress upon ecosystems. One of the major impacts, in the UK and across Europe, is predicted to be increased occurrence and intensity of drought. Drought is likely to reduce plant fitness, and subsequently may affect the floral resources that are available to pollinators.

In this chapter, rain shelters were used to investigate the impacts of drought stress on floral resources and visits to flowers by invertebrates in a field experiment in calcareous grassland, an important habitat for pollinators. The effects of drought were explored at multiple scales. Firstly, the effects of drought stress upon flowers and plants were investigated by measuring nectar production, using three wild flower species as indicators. Secondly, the effects of drought stress upon the community as a whole were investigated by measuring the diversity and abundance of floral resources, and relating this to flower visitor activity. Flowers in the drought treatment were less likely to contain nectar and racemes had fewer flowers overall, resulting in lower sugar production per raceme. At the community scale, there were substantially fewer flowers in the drought treatment, but flower visitation rate was unaffected.

These findings suggest that drought has a substantial impact upon the availability of floral resources in calcareous grassland, and that climate change will result in more frequent periods of floral resource scarcity. This will have negative consequences for pollinators and the pollination service that they provide. This highlights the importance of managing habitats and landscapes in a way that maximises ecological resilience to climate change.

INTRODUCTION

The climate is changing and human activity is almost certainly the primary driver (IPCC, 2014). This is predicted to cause unprecedented environmental stress upon ecosystems. The consequences of this for individuals, populations, and communities, will depend upon resilience and the ability to adapt to changing conditions. Whilst much ecological climate change research has focused on species in terms of range shifts, there has been comparably little focus on the impacts upon interactions. In particular, it is important to understand how climate change will affect ecosystem services as these are of profound social and economic importance (Millennium Ecosystem Assessment, 2005). Only by doing so can ecosystems be managed effectively to minimise undesirable consequences.

Insect pollinators will be affected by climate change in a variety of ways. Desynchronization of species in time and space will cause the loss of plant-pollinator interactions and species (Hegland et al., 2009; Memmott et al., 2007; Miller-Struttmann et al., 2015). Changes in environmental conditions will have a negative effect upon fitness at multiple scales, for example due to loss of local adaptation and loss of genetic diversity resulting from directional selection (Bellard et al., 2012). In addition, climate change will affect pollinators indirectly because plant fitness determines the availability of floral resources. Fitness effects will be species-specific and will depend upon the optimum conditions for each species. However, whilst a general increase in temperature may benefit some plant species, extreme weather events will have mostly negative consequences. Furthermore, the effects of climate change will be acting in combination with existing drivers of pollinator decline, such as habitat loss, agrochemicals, pathogens, and alien species (Potts et al., 2010b; 2016).

One of the major impacts of climate change is predicted to be increased occurrence and intensity of drought across much of Europe (Dai, 2013; Forzieri et al., 2014), including in the UK (Rahiz & New, 2013). There is evidence that this is already happening and this trend is projected to continue for many decades (Boko et al., 2007). A recent horizon scan of future threats to pollinators and pollination identified the increased risk of heatwaves and drought due to climate change as a high priority issue (Brown et al., 2016). Despite this, there has been relatively little research into the matter.

Drought is likely to have both direct and indirect consequences for pollinators. Pollinators will be affected directly due to associated increases in temperature, which will affect behaviour. Principally, the ability of an individual or species to forage at high temperatures is determined by its thermoregulatory limits (Corbet et al., 1993; Scaven & Rafferty, 2013; Willmer & Stone, 2004). The accumulation of negative short-term behavioural changes are likely to scale up to long-term population effects. However, drought *per se* may have even greater indirect effects upon pollinators because the impact upon plants will affect the production of floral resources upon which pollinators rely.

At the community scale, drought may affect the diversity of floral resources that are available. Floral diversity is important because it is likely to determine the diversity of pollinator species that can be supported and the pollination service that is provided (Ghazoul, 2006). Both plant and pollinator diversity may also increase the resilience of communities and ecosystem functions (Oliver et al., 2015). Furthermore, floral diversity allows individuals to select specific pollen and nectar sources to meet their energy and nutrient requirements (Vaudo et al., 2015). Despite this, there are few studies investigating the effects of drought upon floral diversity.

The availability of floral resources may be affected in multiple ways. Drought has been shown to reduce flower quality, in terms of flower size, as well as the number of flowers per plant (Burkle & Runyon, 2016). Within flowers, water availability has been shown to affect pollen in terms of quantity and viability (Al-Ghzawi et al., 2009), and nectar in terms of volume and sugar concentration (Zimmerman & Pyke, 1988; Wyatt et al., 1992). This is likely to affect pollinator foraging behaviour, because bees select flowers based upon subtle differences in nectar volume and sugar concentration (Cnaani et al., 2006). Furthermore, changes in nectar quality will affect the energy intake rate of pollinators (Schweiger et al., 2010), which is optimised at intermediate sugar concentrations (Borrell, 2007). Whilst few studies have related the effects of drought upon floral resources to visits by pollinators, one study found that it resulted in fewer visits by bees (Al-Ghzawi et al., 2009). However, the way in which drought has been found to affect floral resources and pollinators is quite variable between studies. For example, both Villarreal & Freeman (1990) and Carroll et al. (2001) found that nectar volume, but not sugar concentration was affected by drought stress, and Lee & Felker (1992) did not identify an effect

upon nectar quality or quantity. Burkle & Runyon (2016) found that the response of pollinators was dependent upon the species of pollinator, with some species increasing in visitation rate. These results suggest that the response to drought is highly context-specific, and is determined by the plant species, pollinator species, habitat of study, and the intensity of the drought stress.

The majority of existing studies have been conducted in arid and semi-arid regions where drought events are relatively common (Al-Ghzawi et al., 2009; Lee & Felker, 1992; Takkis et al., 2015). However, there is a need to expand studies into regions where drought is becoming an increasing risk due to climate change, such as the UK (Rahiz & New, 2013). Habitats in these regions may be more severely affected because drought has not been a prominent evolutionary pressure. In order to produce a representative model of the impacts of drought over the coming decades, it is necessary to repeat such experiments in these regions, and across a range of habitats.

This chapter investigates the impacts of drought stress on floral resources and visits to flowers by invertebrates, in an experimentally sown field set up. These effects were explored in calcareous grassland, one of the most important habitat types for pollinators in terms of nectar production (Baude et al., 2016). The following hypotheses were tested, each focusing on measuring effects at different scales:

Flower Scale

H1: Flowers that are subjected to drought stress have a lower volume and higher sugar concentration of nectar, but a lower weight of sugar overall.

Raceme Scale

H2: Racemes that are subjected to drought stress have fewer flowers and a lower total weight of sugar in nectar.

Community Scale

H3: Plant communities that are subjected to drought stress have a lower diversity and abundance of flowers and flower visitors.

The results of this study provide insight into the likely impacts of climate change-induced drought upon pollinator communities in the UK.

MATERIALS & METHODS

Experimental Design. The experiment was conducted between May and August 2016, on Cranborne Chase Area of Outstanding Natural Beauty, in southwest Wiltshire (50.991207, -2.069834). The experiment utilised the field site of an existing study, which formed part of the Wessex Biodiversity and Ecosystem Service Sustainability (BESS) project. The existing study, designed and conducted by Dr Ellen Fry and Prof Richard Bardgett of the University of Manchester, investigated how plant and soil biodiversity affect climate regulation services, including resilience to drought stress (Fry et al., *in prep.*).

Existing Setup (*Fry et al., in prep.*). Three functional groups of plants were created using trait data in a cluster analysis (see appendix C for species list). Functional group 1 (FG1; 16 species) consisted of species with variable longevity, deep tap roots, stoloniferous roots, and large, thin leaves. Functional group 2 (FG2; 15 species) consisted of long-lived species with a shallow tap root and small rosettes. Functional group 3 (FG3; 20 species) consisted of long-lived species with shallow, fibrous roots, thick, fleshy leaves, and high activity and turnover. Plant communities consisted of all combinations of FGs (seven communities; FG1, FG2, FG3, FG1+2, FG1+3, FG2+3, FG1+2+3), providing plant communities that differed in functional diversity. There were six replicate plots of each community (8 m x 8 m, separated by 2 m guard rows), a total of 42 plots. The site was divided into six rows with each plant community sown once in each row, in random order, in order to control for spatial, edge, and neighbouring effects. Plots were seeded in May 2013, prior to which they contained little vegetation. The number of seeds that were applied to each plot was determined by the mean seedbank density for each species (from the LEDA Traitbase; Kleyer et al., 2008) and the mean seed weight for each species (from the Kew SID; Royal Botanic Gardens Kew, 2016), and scaled for number of functional groups per plot. In subsequent years (though not in the year of the study), a small number of plant species were hand weeded from plots in order to maintain the desired plant communities.

Each plot contained three subplots (1 m x 1.5 m) and subplots were at least 1 m from each other and from the edge of the plot. In each plot, the three subplots contained the three treatments: (i) Drought (D), covered with a clear corrugated PVC roof to exclude rain, simulating drought, (ii) Roofed Control (R),

covered with the same PVC roof, but with 3 cm holes drilled into it, allowing rain to enter, but controlling for additional microclimatic effects such as increased temperature and decreased light intensity, and (iii) Control (C), not covered with a roof. There were 42 subplots of each treatment, a total of 126 subplots.

Rooves were in place for six weeks, from 6th June to 13th July. Soil moisture content (SMC) was measured at the end of the drought period in order to quantify the effectiveness of the rooves.

Modified Setup. In this study, data were only collected from plant communities FG1, FG2, FG3, and FG1+2+3. Therefore, the full range of plant species at the site were included, but the number of plots to be sampled was reduced in order to make data collection plausible with the available resources. All data were collected at least 48 h after experimental rooves had been removed and within seven days following this. During this time, there was little rainfall input.

Flower & Raceme Scale. Three plant species (*Lathyrus pratensis*, *Onobrychis viciifolia*, and *Prunella vulgaris*; fig. 3.1) were selected based on abundance, coverage, floral traits, flowering period, and ease of nectar extraction. Racemes of these species were randomly selected in subplots and covered with a fine mesh bag for 24 h to exclude invertebrate flower visitors. After 24 h, bags were removed and the number of flowers on the raceme was recorded. Up to three flowers per raceme (if available) were then randomly selected in order to measure nectar volume and sugar concentration, following standard protocol (Corbet, 2003). As nectar was not removed from flowers before applying bags, the amount of nectar in flowers after 24 h represented a combination of standing crop and 24 h accumulation. Each raceme was sampled only once. Full details of the number of racemes and flowers that were sampled for each species can be found in table 3.1. Glass microcapillary tubes (sizes 0.5, 1, 2, and 5 µl microcaps, Drummond Scientific, Broomall PA, USA) were used to take up nectar by capillary action and the volume of nectar in the tube was measured to the nearest 0.5 mm using a ruler. Nectar sugar concentration was measured to the nearest 0.5% using a hand-held refractometer modified for small volumes (Eclipse, Bellingham & Stanley, Tunbridge Wells, UK). When the volume of nectar per individual flower was insufficient to provide a reading on the refractometer, nectar was pooled from multiple flowers on the same raceme in

order to provide an average value. Despite this, it was not always possible to produce a reading. Nectar volume (μl), was calculated using the following equation:

$$\text{Nectar Volume } (\mu\text{l}) = \frac{\text{Nectar Volume } (\text{mm})}{\text{Microcap Length } (\text{mm})} \cdot \text{Microcap Size } (\mu\text{l})$$

In order to calculate the amount of sugar in nectar (mg), the refractometer reading of percentage weight of sucrose equivalents per weight of solution (mg/mg) was converted to percentage weight of sucrose equivalents per volume of solution (mg/ μl), using the following equation (from Prŷs-Jones & Corbet, 1991):

$$\text{Concentration } \left(\frac{\text{mg}}{\mu\text{l}} \right) = \frac{(0.0037291C + 0.0000178C^2 + 0.9988603)C}{100}$$

where C is Concentration (mg/mg), as read on the refractometer. Weight of sugar in nectar (mg) was then calculated using the following equation:

$$\text{Sugar in Nectar } (\text{mg}) = \text{Nectar Volume } (\mu\text{l}) \cdot \text{Concentration } \left(\frac{\text{mg}}{\mu\text{l}} \right)$$

When a concentration reading was absent because nectar volume was too small (144 out of 703 cases), a mean species value was used. Smaller volumes of nectar are likely to contain more concentrated sugar (Wyatt et al., 1992), making this a conservative estimate. The total weight of sugar in nectar for each raceme was estimated using the following equation:

$$\text{Total Sugar } (\text{mg}) = \text{Mean Sugar per Flower } (\text{mg}) \cdot \text{No. Floral Units}$$

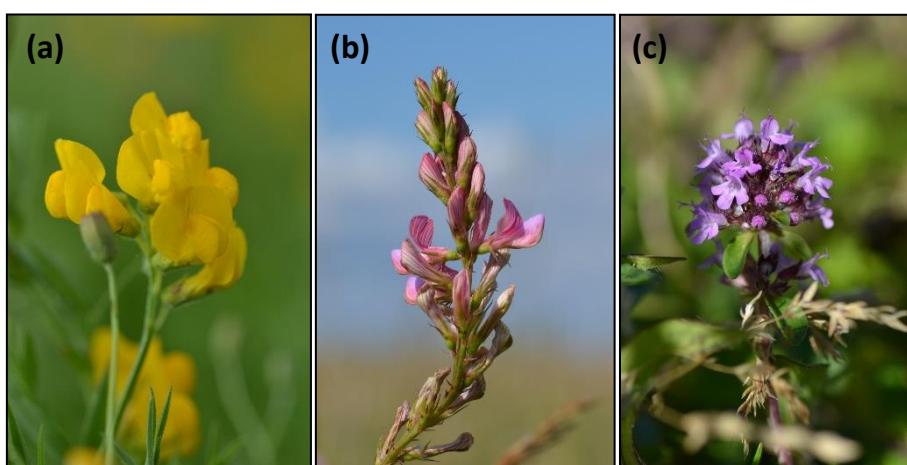


Figure 3.1. Photographs of the plant species that were the focus of the flower and raceme scale study: (a) *Lathyrus pratensis*, (b) *Onobrychis viciifolia*, and (c) *Prunella vulgaris*.

Table 3.1. Sample sizes for the flower and raceme scale study. i) the number of racemes that were selected, ii) the number of racemes from which nectar was sampled (less than the number that were selected because some racemes had no flowers remaining after 24 hours), and iii) the number of flowers from which nectar was sampled, for each plant species, in each treatment.

	<i>Lathyrus pratensis</i>			<i>Onobrychis viciifolia</i>			<i>Prunella vulgaris</i>			Total
	C	R	D	C	R	D	C	R	D	
No. Racemes Bagged	48	46	44	45	46	45	52	54	57	437
No. Racemes Nectar Sampled	44	42	42	40	41	37	44	41	41	372
No. Flowers Nectar Sampled	107	84	88	111	104	87	112	113	100	906

Community Scale. Surveys were carried out in 1 m² quadrats, placed in the centre of each subplot. All flowering plants were identified to species level, and the number of floral units of each species was recorded (a floral unit was defined as one or multiple flowers that can be visited by an insect without flying; Baldock et al., 2015; Baude et al., 2016; appendix D). Quadrats were observed for 10 min, during which time all flower visit activity was recorded including flower visitor identity, plant species visited, and the number of floral units visited. Flower visitors were identified as far as possible on the wing, often to morphotype, and type specimens were collected for further identification. Surveys were completed at each subplot on two occasions, on different days, separated by a maximum of four days. The survey order of both plots and subplots was randomised to control for the effects of treatment order and plot position. Pollinator surveys were carried out between the hours of 1030 and 1600, and only when weather conditions met the criteria described in Pollard & Yates (1993).

Statistical Analyses. All statistical analyses were carried out in R (R Core Team, 2015). Analyses were completed using generalised linear models in the inbuilt R function, and linear mixed effects models in the ‘nlme’ package (Pinheiro et al., 2016). Where necessary, response variables were transformed with square root or log transformations in order to meet the assumptions of statistical tests. Random models were used in the analyses to account for multiple flowers being sampled per raceme, for subplots being contained within plots, and for plot position. Full details of the statistical analyses can be found in table 3.2.

Table 3.2. The statistical analyses that were used to test the outlined hypotheses. Treatment refers to Control (C), Roofed control (R), and Drought (D) and FG refers to functional group. Pairwise comparisons between levels are only included when the fixed effect had a significant overall effect.

Description	Statistical Model	Transformation	Fixed Effects	Random Effects	Effect	Test Statistic	Value	df	P-value
Soil Moisture Content (%)	LME	-	Treatment	Row/Plot	Treatment	F	58.703	2,82	< 0.0001
					C-D	t	-10.262	82	< 0.0001
					C-R	t	-2.118	82	0.0372
					D-R	t	-8.144	82	< 0.0001
Prop. of Flowers with Nectar (%)	GLMM (binomial)	-	Treatment * Plant Species	Plot	Treatment	χ^2	25.033	2,421	< 0.001
					C-D	Z	2.672	423	0.0075
					C-R	Z	1.000	423	0.3173
					D-R	Z	1.594	423	0.1110
Nectar Volume (μ l)	LME	log	Treatment * Plant Species	Plot/Plant	Plant Species	χ^2	9.154	2,419	0.0103
					Treatment:Plant Species	χ^2	1.451	415	0.8353
					Treatment	F	1.774	2,274	0.1716
					Plant Species	F	224.080	2,274	< 0.0001
Nectar Sugar Concentration (%)	LME	-	Treatment * Plant Species	Plot/Plant	Treatment:Plant Species	F	0.775	4,274	0.5423
					Treatment	F	0.227	2,216	0.7971
					Plant Species	F	6.343	2,216	0.0021
					Treatment:Plant Species	F	0.443	4,216	0.7772
Nectar Sugar Weight (mg)	LME	log	Treatment * Plant Species	Plot/Plant	Treatment	F	2.262	2,274	0.1061
					Plant Species	F	178.911	2,274	< 0.0001
					Treatment:Plant Species	F	1.096	4,274	0.3587
					Treatment	F	4.552	2,388	0.0111
Flowers per Raceme	LME	sqrt	Treatment * Plant Species	Plot	C-D	t	-1.127	388	0.2604
					C-R	t	-1.507	388	0.1326
					D-R	t	-0.359	388	0.7197
					Plant Species	F	8.308	2,388	0.0003
Sugar per Raceme (mg)	LME	sqrt	Treatment * Plant Species	Plot	Treatment:Plant Species	F	0.978	4,388	0.4196
					Treatment	F	10.018	2,379	0.0001
					C-D	t	-2.842	379	0.0047
					C-R	t	-1.971	379	0.0495
Number of Floral Units	LME	log	FG + Treatment	Row/Plot	D-R	t	0.634	379	0.5269
					Plant Species	F	30.645	1,379	< 0.0001
					Treatment:Plant Species	F	1.017	2,379	0.3626
					FG	F	5.204	3,15	0.0116
					Treatment	F	10.061	2,46	0.0002
					C-D	t	-4.472	46	0.0001
					C-R	t	-1.933	46	0.0594
					D-R	t	2.539	46	0.0146

Chapter 3

Description	Statistical Model	Transformation	Fixed Effects	Random Effects	Effect	Test Statistic	Value	df	P-value
Floral Species Richness	LME	sqrt	FG + Plant Species Richness + Treatment	Row/Plot	FG	F	5.862	3,15	0.0074
					Plant Species Richness	F	2.712	1,45	0.1066
					Treatment	F	7.613	2,45	0.0014
					C-D	t	-3.072	45	0.0036
					C-R	t	-3.539	45	0.0009
					D-R	t	-0.335	45	0.7394
Visitation Rate	GLM (quasipoisson)	sqrt	No. Floral Units + FG + Visitor Identity * Treatment + No. Floral Units:FG + No. Floral Units:Treatment + FG:Visitor Identity + FG:Treatment	-*	No. Floral Units	F	1.1844	1,82	0.2817
					FG	F	2.440	1,81	0.1246
					Visitor Identity	F	12.855	6,75	< 0.0001
					Treatment	F	0.093	2,73	0.9115
					Visitor Identity:Treatment	F	0.915	12,61	0.5387
					No. Floral Units:FG	F	0.009	1,60	0.9252
					No. Floral Units:Treatment	F	1.009	2,58	0.372
					FG:Visitor Identity	F	0.9026	6,52	0.5006
					FG:Treatment	F	0.2835	2,50	0.7543
*Data were combined for subplots of the same functional group meaning that a random model for row/plot was not necessary.									
Flower Visitor Taxonomic Richness	LME	-	FG + Floral Diversity * Treatment	Row/Plot	FG	F	3.186	3,15	0.0544
					Floral Diversity	F	2.725	1,43	0.1061
					Treatment	F	0.060	2,43	0.9423
					Floral Diversity:Treatment	F	1.579	2,43	0.2179

RESULTS

Soil moisture content (SMC) was significantly lower in the drought treatment ($F_{2,82} = 58.703, P < 0.001$; control $33.05 \pm 0.50 \text{ SE}$; roofed control $31.09 \pm 0.77 \text{ SE}$; drought $23.55 \pm 0.93 \text{ SE}$; fig. 3.2; table 3.2).

Flower & Raceme Scale

Across the three plant species, 437 racemes were selected and of these, 372 had flowers remaining after 24 h (table 3.1). Nectar was collected from the flowers of between 37 and 44 racemes per plant species per treatment (table 3.1) and between 87 and 113 flowers were sampled per plant species per treatment (table 3.1).

The proportion of flowers containing nectar differed between plant species ($F_{2,419} = 9.154, P = 0.010$) and was significantly lower in the drought treatment than in the control treatment for all plant species ($F_{2,421} = 25.033, P < 0.001$) (fig. 3.3; table 3.2).

There were dramatic differences between plant species in terms of nectar volume ($F_{2,274} = 1.774, P < 0.001$), nectar sugar concentration ($F_{2,216} = 6.343, P = 0.002$), and weight of sugar in nectar ($F_{2,274} = 178.911, P < 0.001$) (fig. 3.4; table 3.2). Flowers of *L. pratensis* had by far the greatest volume of nectar ($1.325 \pm 0.095 \text{ SE}$), followed by *P. vulgaris* ($0.257 \pm 0.016 \text{ SE}$) and *O. viciifolia* ($0.139 \pm 0.010 \text{ SE}$). However, for flowers that contained nectar, there was no significant effect of treatment on nectar volume, nectar sugar concentration, or weight of sugar in nectar for any of the three plant species (fig. 3.4; table 3.2).

Plant species differed in the number of flowers per raceme ($F_{2,388} = 8.308, P < 0.001$). Racemes of *P. vulgaris* had the greatest number of flowers ($5.188 \pm 0.192 \text{ SE}$), followed by *O. viciifolia* ($4.412 \pm 0.140 \text{ SE}$) and *L. pratensis* ($2.990 \pm 0.090 \text{ SE}$). Racemes of all species in the drought treatment had significantly fewer flowers ($F_{2,388} = 4.552, P = 0.011$) and a lower weight of sugar per raceme than in the control treatment ($F_{2,379} = 10.018, P < 0.001$) (fig. 3.5; table 3.2).

Community Scale

Across all surveys, flowers of 46 different species of plant were identified. The three study plant species constituted 40% of all floral units, whilst the top ten most florally abundant plant species constituted over 88% of all floral units (fig. 3.6).

Subplots in the drought treatment had a significantly lower floral species richness ($F_{2,45} = 7.613, P = 0.001$; fig. 3.7a) and substantially fewer floral units than in the control treatment ($F_{2,46} = 10.061, P < 0.001$; fig. 3.7b) (table 3.2).

Across all surveys, 986 flower visits were observed to 25 different species of plant. Of all flower visits, 25% were by hoverflies (Syrphidae), 19% were by other fly species (non-Syrphid Diptera), 19% were by bumblebees (*Bombus lapidarius* workers), and 18% were by pollen beetles (Nitidulidae). However, the vast majority of visits were to relatively few plant species, with 75% of visits to only three species (*Daucus carota* 32%, *Crepis capillaris* 22%, *Onobrychis viciifolia* 20%). Furthermore, the majority of visits by each taxonomic group of visitor was to a single plant species. For example, 74% of bumblebee visits were to *O. viciifolia*, and 66% of hoverfly visits were to *Daucus carota*.

Overall, there was no significant effect of treatment on the taxonomic richness of flower visitors ($F_{2,43} = 0.060, P = 0.942$; fig 3.8a) or on the visitation rate to subplots ($F_{2,73} = 0.093, P = 0.912$; fig 3.8b) (table 3.2). There was no significant effect of the number of floral units on the visitation rate to subplots ($F_{1,82} = 1.184, P = 0.282$; table 3.2).

Of the three study plant species, very few visits were observed to flowers of *L. pratensis* (5 visits observed) or *P. vulgaris* (14 visits observed). In contrast, flowers of *O. viciifolia* received 20% of all visits, including 74% of those by bumblebees. Sixty-two bumblebee visits were observed to flowers of *O. viciifolia* in the control treatment, 49 in the roofed control treatment, and only 6 in the drought treatment. Although there were also associated differences in the number of *O. viciifolia* flowers in subplots of each treatment type (a total of 2827 flowers in control subplots, 1875 in roofed control subplots, 463 in drought subplots), differences were maintained even when this was controlled for by calculating visitation rate per flower for each survey (control 0.020 ± 0.009 SE, roofed control 0.014 ± 0.006 SE, drought 0.009 ± 0.008 SE).

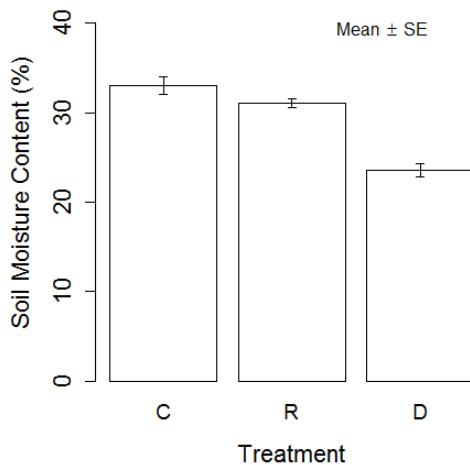


Figure 3.2. The mean SMC (%) \pm standard error, measured within two days of rooves being removed, in the 42 subplots of each treatment. Treatment refers to Control (C), Roofed control (R), and Drought (D). From Fry et al. (in prep.).

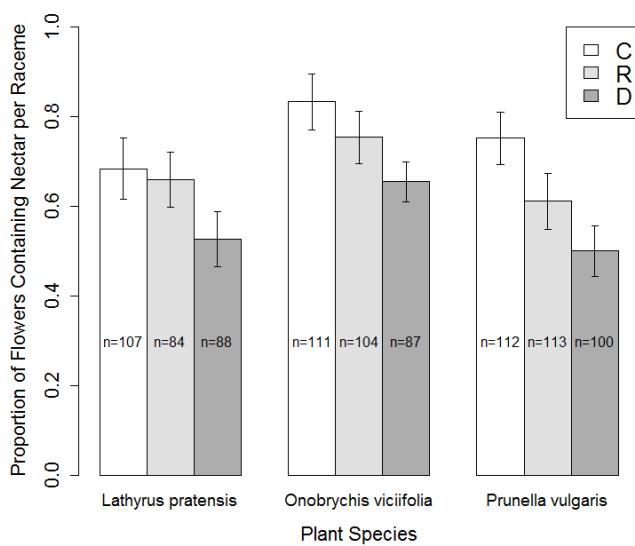


Figure 3.3. The mean proportion of flowers per raceme that were found to contain nectar \pm standard error, 24 h after bagging, for each plant species, in each treatment. Treatment refers to Control (C), Roofed control (R), and Drought (D).

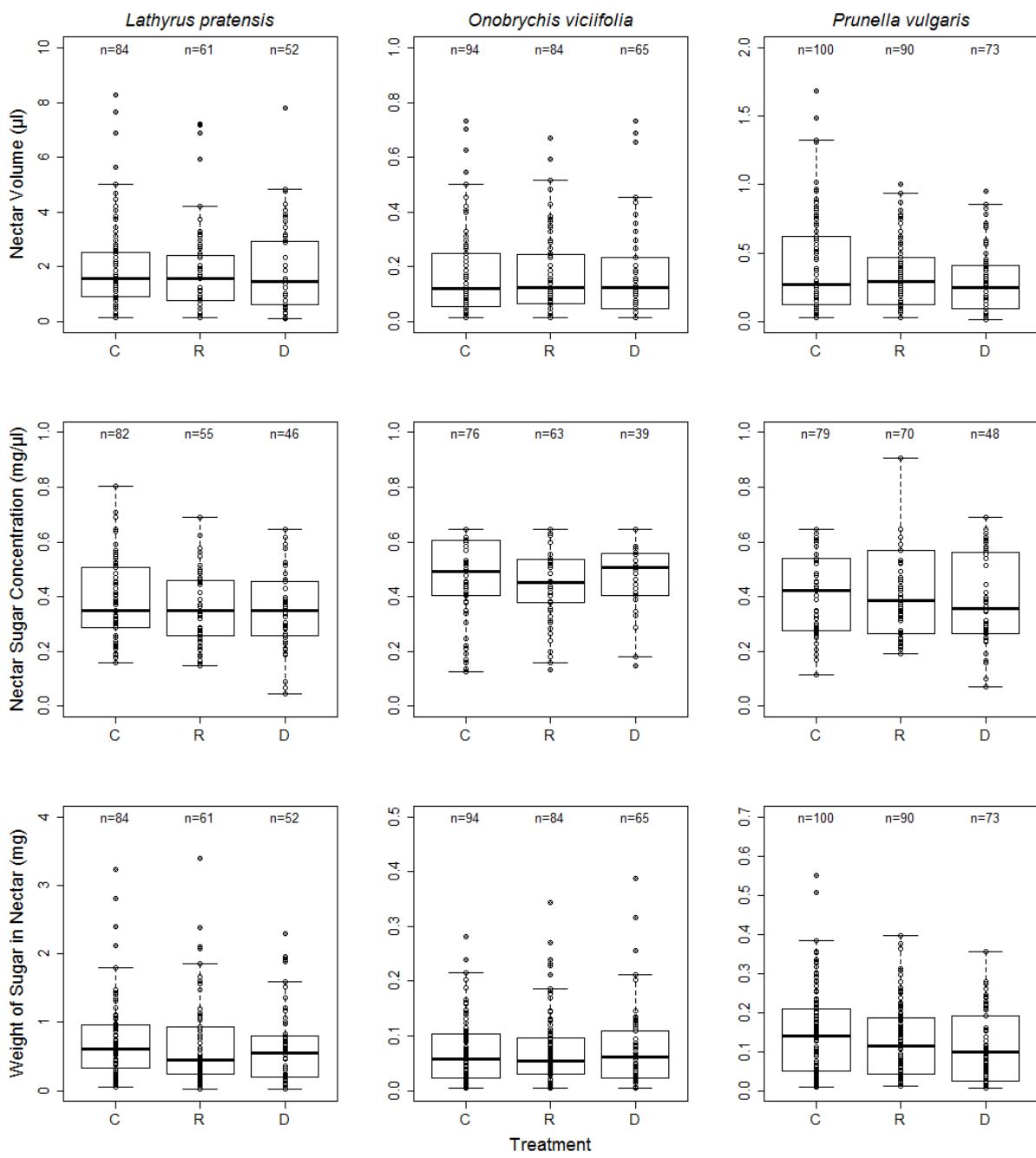


Figure 3.4. The volume, sugar concentration, and total weight of sugar in nectar from flowers, 24 h after bagging, for each plant species, in each treatment. Flowers that contained no nectar (volume = 0) have been excluded. The scale of the y-axis is different for each plant species in order to allow the data to be visualised. Treatment refers to Control (C), Roofed control (R), and Drought (D).

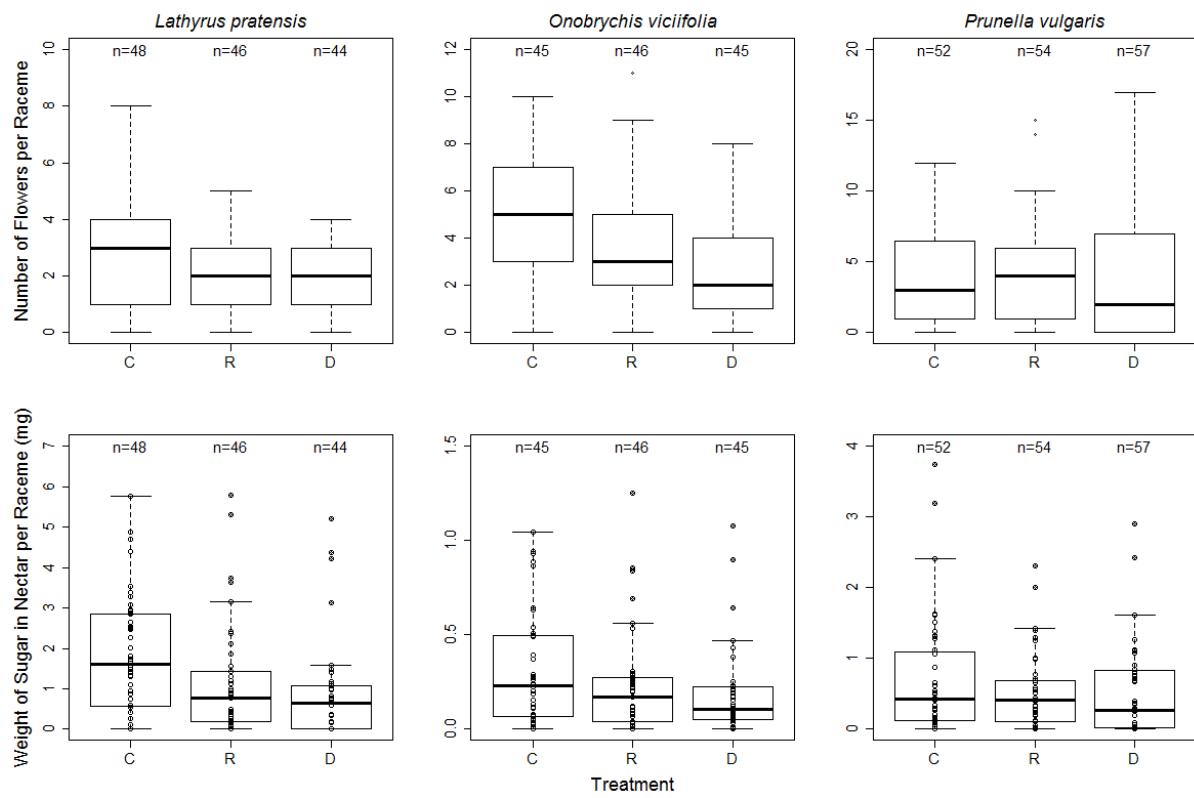


Figure 3.5. The number of flowers per raceme, and the total weight of sugar in nectar per raceme (mg), 24 hours after bagging, for each plant species, in each treatment. The scale of the y-axis is different for each plant species in order to allow the data to be visualised. Treatment refers to Control (C), Roofed control (R), and Drought (D).

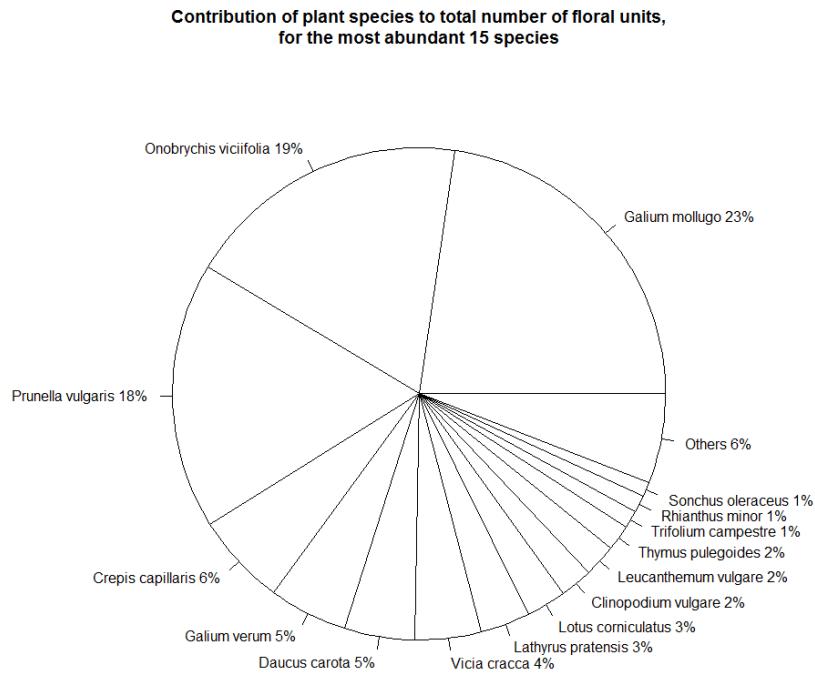


Figure 3.6. The proportion of floral units of each plant species across all subplots. Only the 15 most florally abundant species are shown. The remaining 6% of floral units consist of a further 31 plant species.

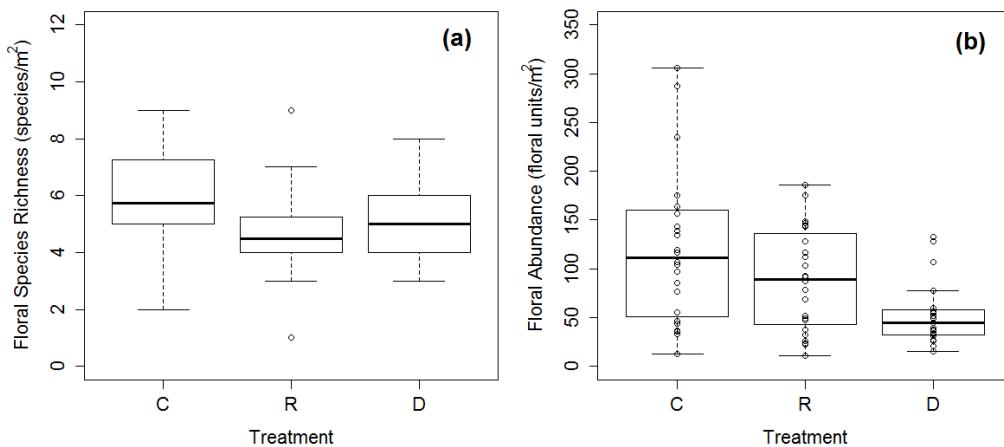


Figure 3.7. (a) Floral species richness, and (b) Floral abundance, in the 24 subplots of each treatment. Treatment refers to Control (C), Roofed control (R), and Drought (D).

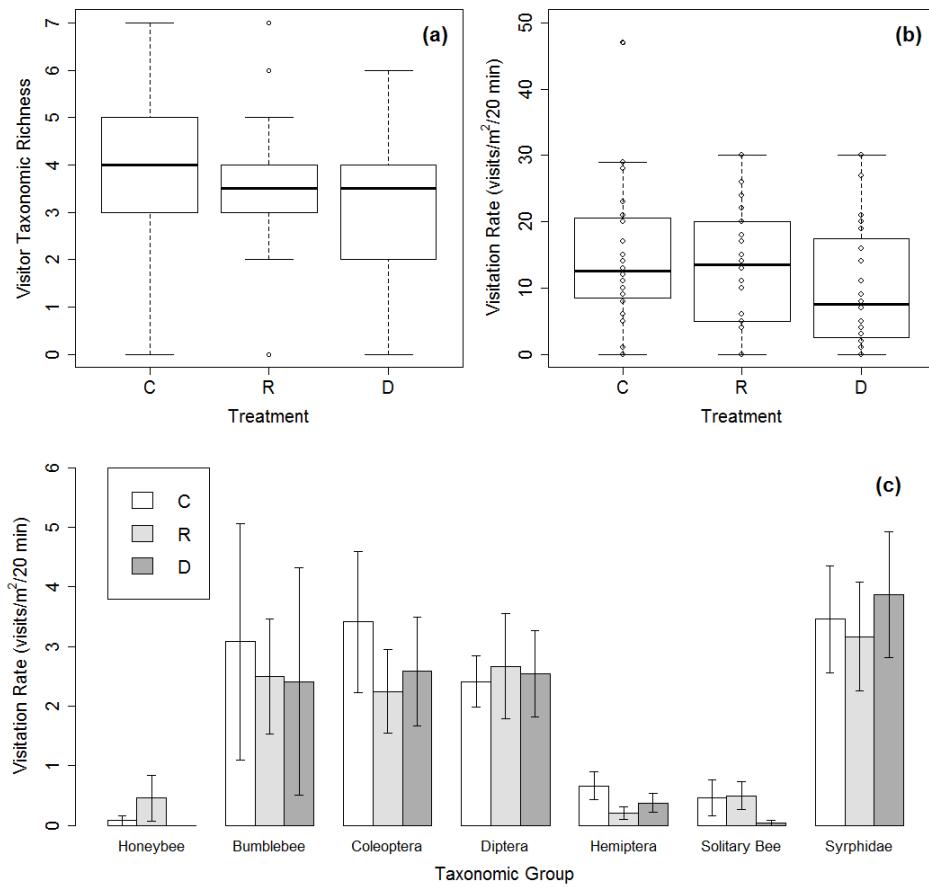


Figure 3.8. (a) Visitor taxonomic richness (number of groups from (c) that were present), (b) Total flower visitation rate, for the sum of the two 10 min surveys at each subplot, and (c) Mean visitation rate \pm standard error for each taxonomic group of flower visitor. Each bar/boxplot represents the 24 subplots of one treatment type. Treatment refers to Control (C), Roofed control (R), and Drought (D).

DISCUSSION

Experimental drought resulted in substantially fewer floral resources in the community, and this was the accumulation of effects at multiple scales. Flowers in the drought treatment were less likely to contain nectar, although nectar volume and sugar concentration were unaffected. Racemes in the drought treatment contained fewer flowers, and these effects accumulated in a lower total weight of sugar in nectar per raceme. Overall, plant communities in the drought treatment contained substantially fewer floral units, and this is expected to relate closely to the overall availability of nectar and pollen in the community.

Although the drought treatment reduced water availability to subplots, SMC remained relatively high (23%). By comparison, permanent wilting point (the SMC at which plants begin to wilt) is approximately 6-8% in grassland soils (E. L. Fry, personal communication). In addition, treatments did not have a visible effect on plants in the field, and all plants appeared to be in reasonable condition (personal observation). There are two possible explanations for this: i) The habitat is relatively drought tolerant and able to maintain a reasonable SMC with little rainfall input, or ii) The drought treatment was not completely effective at excluding water due to runoff, rain getting in through the side of the roof, or because plants were accessing water through their root systems when they extended outside of the covered area. As a result, water availability to the plant communities in the drought treatment was reduced, but plants may not have been completely deprived of water. Therefore, these results may be conservative of the effects of an actual drought. Despite this, the drought treatment had a significant, and often substantial effect upon many of the variables that were measured.

The roofed control treatment also had a lower SMC than the control treatment. As this difference was quite small, it suggests that the holes in the rooves were mostly, but not absolutely, effective at channelling rainwater through. Therefore, the roofed control treatment actually resulted in a slightly reduced level of water availability, rather than solely controlling for undesired microclimatic effects of the roof. Nonetheless, it is important to consider the possible microclimatic effects of the roof, such as increased temperature and decreased light intensity, when interpreting the results of this study.

Flower & Raceme Scale

A proportion of flowers on racemes had no nectar (25%) and this proportion increased for racemes in the drought treatment (45%). Bell et al. (1986) predicted that plants should produce a proportion of ‘empty flowers’ that cheat pollinators in order to maximise the number of pollinator visits that the plant receives per unit of investment. The proportion of empty flowers should depend upon the cost to pollinators of discriminating between cheat and non-cheat flowers, and this prediction is supported empirically (Gilbert et al., 1991). There is also evidence that some plant species that do not produce empty flowers under normal conditions may begin to do so under temperature stress (Petanidou & Smets, 1996; but see Takkis et al., 2015). In this case, producing empty flowers may be a way of conserving resources without dramatically reducing reproductive potential.

In a natural setting, flowers are likely to be visited regularly and therefore the number of empty flowers may be high. By comparison, the data in this study were collected from flowers that had been protected from visitation for 24 hours. Many insect pollinators exhibit ‘flower constancy’, whereby individuals exclusively visit one or few plant species at a time (Waser, 1986). The foraging success of a pollinator, such as the energetic rate of gain, is likely to determine whether a pollinator continues to forage upon a particular plant species. Assuming that the number of empty flowers is naturally quite high, it may be more important to sustain nectar volume and sugar concentration in fewer flowers, in order to maintain pollinator visitation. Therefore, an increase in the number of empty flowers may allow nectar volume and sugar concentration to be maintained in others, as was found in this study. However, it is worth noting that environmental conditions have had a measured effect on nectar volume and sugar concentration in other studies (Carroll et al., 2001; Villareal & Freeman, 1990; Wyatt et al., 1992; Zimmerman & Pyke, 1988). The result in this experiment may be due to species-specific differences, though as all three of the focus species responded in the same way, it is more likely due to some general effect of the drought treatment. One suggestion is that the proportion of empty flowers is achieved through flower age or condition (Gilbert et al., 1991). If this is the case for these plant species, then this result may relate to flowers in the drought treatment being in poorer condition.

There were strong effects of species identity upon nectar provision per flower. Even though flowers of *L. pratensis* and *O. viciifolia* are of similar size, flowers of the former contained roughly ten times as much nectar after 24 hours. However, *L. pratensis* had nearly twice the proportion of empty flowers (32%) compared to *O. viciifolia* (17%). These may be strategical differences between the two plant species, whereby *L. pratensis* produces flowers with a greater reward but with lower consistency, and *O. viciifolia* produces flowers with a lower reward but with greater consistency. Again, it is worth remembering that these values represent 24 hour nectar accumulation (though possibly confounded due to nectar reabsorption; Corbet, 2003), and are not necessarily representative of the amount of nectar that is typically available during a visit by a pollinator. Additionally, some plant species are stimulated to replenish nectar after removal at a greater rate than suggested by 24 hour nectar accumulation (Luo et al., 2014). For this reason, it is possible that the amount of nectar that is available during a typical visit to *L. pratensis* and *O. viciifolia* flowers is similar, but that the former replenishes this nectar more quickly (indicated by a greater 24 hour nectar accumulation).

Racemes in the drought treatment had a lower total weight of sugar in nectar. This is likely to be the consequence of there being fewer flowers per raceme, and of flowers being less likely to contain nectar. Overall, this suggests that drought-stressed plants are diverting resources away from reproduction.

Community Scale

The most prominent effect of the drought treatment was on the number of floral units in plant communities. This is likely to be due to plants producing fewer flowers, as was observed for the three study species, because vegetative cover is not likely to have changed so dramatically over the six-week period of the experimental treatment.

The majority of visits by each pollinator group were to a single plant species. Bumblebees were mostly visiting *O. viciifolia*, and hoverflies were mostly visiting *Daucus carota*. As the three study plant species made up 40% of all floral units, the measured effects at the flower and raceme scale will have undoubtedly resulted in a considerable impact at the community level in terms of the amount of nectar available, even if other plant species did not respond in the same way. However, it was not possible to relate these effects to

differences in flower visits to *L. pratensis* or *P. vulgaris* because very few visits were observed to either species. In contrast, *O. viciifolia* received 20% of all flower visits and 74% of all bumblebee visits. Whilst there was no overall effect of treatment on flower visitation rate, there were fewer visits to *O. viciifolia* flowers by bumblebees in the drought treatment. Despite associated differences in the numbers of *O. viciifolia* flowers per treatment, this effect was upheld when floral abundance was controlled. This suggests that bumblebees may have been preferentially visiting *O. viciifolia* flowers in non-drought subplots, perhaps because they contained fewer empty flowers, or because they were being attracted by greater flower density. However, further replication of surveys would be required in order to test this statistically, because visits were observed on relatively few occasions.

It is important to consider these results in a wider ecological context. Due to the relatively small scale of this study, the flower visitation aspect is a measure of the impact of drought on pollinator behaviour. In order to measure population-level effects on pollinators, it would be necessary to simulate drought across the foraging range of pollinator species, and to follow this through multiple years. In reality, this is only plausible by using real drought events, or through modelling of population dynamics using models such as BEEHAVE (Becher et al., 2014; Horn et al., 2015). This limitation highlights one of the major difficulties of taking an experimental approach to studying the ecological effects of climate change.

Conclusion

Overall, this study provides strong evidence that drought stress has a substantial impact upon the availability of floral resources in calcareous grassland. Effects were found at multiple scales, ranging from plants producing a greater proportion of nectarless flowers, to there being fewer flowers in the community overall. This is likely to have detrimental consequences for pollinators, given that floral resources are generally a limiting factor upon pollinator populations (Baude et al., 2016; Carvell et al., 2006; Roulston & Goodell, 2011).

The impacts of drought on pollinators may be particularly evident in the study habitat because calcareous grassland is one of the most important habitat types for pollinators in the UK due to its high nectar productivity and diversity

(Baude et al., 2016). On the other hand, this diversity and abundance of resources may provide a buffer against environmental stress, compared to habitats that contain few resources to begin with. It is necessary to repeat such experiments in additional habitats in order to produce a clear understanding of the likely effects of drought on floral resources and pollinators in the UK.

Although this study constitutes just a snapshot of the flowering season, the effects of such environmental stress may have consequences that persist further into the year and into following years. Future research should aim to look at the recovery of plant communities following drought stress, and possible long-term legacy effects upon both populations and communities of plants and pollinators.

To conclude, this study provides evidence that increased occurrence and intensity of drought due to climate change will lead to more regular periods of floral resource scarcity. The consequences of this for pollinator communities will affect the pollination service that they provide, and may ultimately affect plant reproductive success and crop yields. In response, it will become increasingly important to protect and restore suitable habitat for pollinators. Furthermore, this study highlights the importance of understanding mechanisms for resilience (of ecosystems and of ecosystem processes and services) against the environmental stresses that are likely to result from climate change. Promoting diversity of both plant and pollinator communities is likely to be one such mechanism (Elmqvist et al., 2003; Ives & Carpenter, 2007; McCann, 2000; Oliver et al., 2015; Tilman, 1996). Additionally, it will be beneficial to identify drought-tolerant flowering plant species that can be incorporated into wildflower margins and seed mixes. Simple measures such as this can provide insurance in the face of extreme weather events. Understanding the likely effects of climate change early and preparing ecosystems accordingly, is the best way to limit undesirable consequences.

Chapter 4: General Discussion & Conclusion

This thesis has explored, through case studies, how pollinator community relates to pollination service, and how pollinator communities will be affected in the future by climate change. The first study has investigated how pollinator community characteristics relate to the pollination service that is provided to OSR, an economically important crop. The study has assessed the contribution of each flower-visiting species and demonstrated that the composition, diversity, and abundance of pollinators all affect the pollination service that is provided. The second study, looking at the impacts of drought events, has demonstrated that (all other things being equal) climate change will provide an additional limitation on the availability of floral resources. This is likely to have an effect on each of the community characteristics that have been described above, and will ultimately affect pollination services.

The study in chapter 2 has utilised a reductionist framework, which allows the mechanistic components that determine pollination services to be understood (Kremen et al., 2002). The results of this study highlight the importance of re-evaluating current understanding based upon new evidence. Although OSR is a well-studied system in terms of insect pollinators, the vast majority of studies have ignored the presence of non-Syrphid Diptera (Hayter & Cresswell, 2006; Jauker et al., 2012; Garratt et al., 2014; Stanley et al., 2013). At the time of writing, there appeared to be only one case in which non-Syrphid Diptera were included in an OSR pollinator study (Rader et al., 2009), and this was not in the UK or in Europe. This is concerning, given that the inclusion of non-Syrphid Diptera in this study dramatically alters both the relative importance of each species, and the total estimated pollination service that is provided by the community. This finding should encourage caution when dismissing species as unimportant. Quantitative evidence of the direct effects of pollination should be used in order to determine whether an individual is involved in pollen transfer (King et al., 2013). This neglect of non-bee flower visitors has probably led to error in many other systems, given that the extent of their importance as pollinators is increasingly being realised (Orford et al., 2015; Rader et al., 2015; Ssymank et al., 2008).

One of the potential caveats in the study is the use of combining different sampling methods in order to estimate the rate of flower visitation. Despite the assumptions that are involved in doing so, the values that resulted were

comparable to visitation rates in the literature (where available). However, a superior method would have been to use quadrat surveys over a much greater duration (on the scale of hours or days, rather than minutes). This would have increased the detection rate of functionally important species that have a low visitation rates (in this case, bees). Furthermore, this would have provided a more reliable assessment because direct observation has fewer of the problems of bias that are associated with sampling methods such as pan traps.

Unfortunately, this method is made impractical by the vast number of hours that would be required in order to make such observations. However, it may become plausible in the near future, because advances in technology (particularly increased battery life, memory storage, and reduced cost) are making it possible to use video or time-lapse photography to record flower visitation (Edwards et al., 2015). Although this adds the additional time requirement of analysing the recorded data (though probably considerably less than observation in the field), it seems well within the limits of technology to automate this process. This would allow visitation to OSR flowers to be recorded over the lifetime of flowers (as in Kremen et al., 2002), even for crop species that have flowers that remain open over multiple days such as OSR. Having a complete record of visitation history to a flower would allow an understanding of the relationship between visitation and seed set or yield, in a natural setting. Furthermore, this technology would provide additional benefits over traditional methods, such as reduced disturbance, which would increase the legitimacy of results.

Future research should continue to quantify the relative contribution of diversity, abundance, and composition as drivers of ecosystem services, and would benefit from the establishment of a quantitative and standardised protocol of doing so (such as the Price equation; Fox, 2006; Winfree et al., 2015). If, as has been suggested, the abundance of common species is often the most important driver of ecosystem services (Adams, 2014; Gaston, 2010; Kleijn et al., 2015), then it will be important to understand how to support these key species and how to promote the resilience of their populations. In addition, it will be necessary to assess whether functionally less important species benefit from similar management, or whether they require a different set of measures.

The study in chapter 3 has demonstrated that small-scale experimental designs can be used to provide valuable insight into the impacts of environmental change. Doing so can be a useful method of studying the effects of such change when natural variation in conditions is not available. Furthermore, it can be more beneficial to use experimental manipulations because of the ability to replicate and control for confounding effects. However, the study also highlights the difficulty in investigating the indirect and long-term effects of environmental change in the field. In this study, it was only possible to infer the subsequent impacts of limited floral resources upon pollinators. Although this inference is probably qualitatively accurate, it is often important to quantify the subsequent and long-term effects. These effects can best be explored by incorporating the small-scale effects that are measured in field experiments into large-scale models that are able to simulate long-term and population-level dynamics (such as BEEHAVE; Becher et al., 2014; Horn et al., 2015).

The study has also demonstrated that valuable data can be acquired from an experimental design, even if it was not necessarily designed with that study system in mind. In this study, useful data on floral resources and pollinators were collected from an experimental site that was designed to study carbon sequestration. If expensive large-scale experiments are utilised as a way of exploring climatic effects, then funding could be most effectively used through collaborative research that allows the effects on multiple communities (for example, pollinators, soil invertebrates), and multiple scales (individual, population, community), to be explored simultaneously (such as the CLIMAITE project; Mikkelsen et al., 2008). Such collaborative research is probably also more biologically applicable, because these different systems and scales of study exist holistically in the real world.

This study has provided early insight into the impacts of extreme weather events upon pollinators, where little existing research is available. Future research should look to expand this into additional regions and habitats, investigate the effects of different severities of drought stress, and explore the specific mechanisms that result in the effects on floral resources that have been measured in this study. Understanding the effects of extreme weather events across a wider range of systems, and the mechanisms behind them, will elucidate how best to promote resilience of ecosystems and of ecosystem services.

This thesis has demonstrated that the composition, diversity, and abundance of pollinators all have an effect on the pollination service that is provided. Therefore, management for pollinators needs to take all of these factors into account because management for each is likely to require a different approach. For example, pollinator abundance is likely to be determined by the quantity of floral resources, whilst pollinator diversity is more likely to be affected by plant or habitat diversity. Furthermore, specific management may be needed to promote key species and determine community composition, such as providing bare earth as nest sites for solitary bee species. If the primary aim of management is to maximise pollination services, then studies need to explore the contribution of other flower-visiting invertebrates, such as non-Syrphid Diptera, because their importance is probably still not fully accounted for (Orford et al., 2015; Rader et al., 2015; Ssymank et al., 2008).

Overall, a more complete understanding of pollination services is needed in order for management to be as effective as possible. Not only should land managers and policy makers continue to tackle existing threats (pesticides, habitat loss, pathogens), but they will also need to prepare to contend with additional threats that are likely to arise, such as those resulting from climate change (Dicks et al., 2016; Potts et al., 2010b; 2016). There is clearly more to be understood at every stage, in terms of (i) which species are important in each system, (ii) the mechanisms that determine the pollination service at the community level, and (iii) how communities determine large-scale provision and resilience of pollination services at the landscape level. This information, in combination with an understanding of future threats, will result in management that can support pollinators and pollination services over the coming decades.

APPENDICES

Functional Group	Description	Species	Survey Method
1	Small Solitary Bee	<i>Andrena chrysosceles</i> <i>Andrena minutula</i> <i>Lasioglossum calceatum</i> <i>Lasioglossum fulvicorne</i> <i>Lasioglossum malachurum</i> <i>Lasioglossum morio</i> <i>Lasioglossum smethmanellum</i>	Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap
2	Medium Solitary Bee	<i>Andrena bicolor</i> <i>Andrena dorsata</i> <i>Andrena praecox</i> <i>Andrena wilkella</i> <i>Nomada goodeniana</i> <i>Nomada leucophthalma</i> <i>Osmia bicolor</i>	Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap
3	Large Solitary Bee	<i>Andrena apicata</i> <i>Andrena flavipes</i> <i>Andrena haemorrhoa</i> <i>Andrena nitida</i> <i>Andrena nigroaenea</i> <i>Andrena cineraria</i> <i>Andrena fulva</i> <i>Andrena carantonica</i>	Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap
4	Honeybee	<i>Apis mellifera</i>	Transect
5	Bumblebee	<i>Bombus hypnorum</i> <i>Bombus lapidarius</i> <i>Bombus lucorum</i> <i>Bombus pascorum</i> <i>Bombus pratorum</i> <i>Bombus terrestris</i> <i>Bombus bohemicus</i> <i>Bombus vestalis</i>	Transect Transect Transect Transect Transect Transect Transect Transect
6	Small Probing Fly	<i>Bibionidae: Bibio johannis</i> <i>Bibionidae: Dilophus febrilis</i> <i>Bibionidae: Dilophus femoratus</i> <i>Empididae: Platypalpus spp.</i> <i>Empididae: Empis small spp.</i>	Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap
7	Medium Probing Fly	<i>Empididae: Rhamphomyia spp.</i> <i>Empididae: Empis femorata</i> <i>Empididae: Empis opaca</i> <i>Syrphidae: Platycheirus spp.</i>	Pan Trap Pan Trap Pan Trap Pan Trap
8	Large Probing Fly	<i>Empididae: Empis tessellata</i> <i>Syrphidae: Dasysyrphus spp.</i> <i>Syrphidae: Eupodes spp.</i> <i>Syrphidae: Cheilosia spp.</i> <i>Bibionidae: Bibio Marci</i> <i>Syrphidae: Eristalis spp.</i> <i>Syrphidae: Rhingia spp.</i>	Pan Trap Pan Trap Pan Trap Pan Trap Transect Pan Trap Pan Trap
9	Medium Fly	<i>Anthomyiidae</i>	Pan Trap
10	Large Fly + Others	<i>Muscidae</i> <i>Scathophagidae</i> <i>Conopidae: Myopa</i> <i>Tenthredinidae</i> <i>Calliphoridae</i> <i>Sarcophagidae</i>	Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap Pan Trap
11	Small Beetles	<i>Ceutorhynchus spp.</i> <i>Meligethes spp.</i>	Pan Trap Pan Trap

Appendix A. A list of the species (or taxonomic groups) that visited OSR flowers during the study in chapter 2. Information is shown about the functional group that each species was allocated to and the survey method that was used to provide the visitation rate for each species.

Description	Statistical Model	Transformation	Fixed Effects	Random Effect	Effect	Test Statistic	Value	df	P-value
Single Visit Pollen Deposition	LM	log	Functional Group	-	Functional Group	F	16.443	11,216	< 0.0001
					Control-FG1	t	4.814	216	< 0.0001
					Control-FG2	t	7.390	216	< 0.0001
					Control-FG3	t	9.573	216	< 0.0001
					Control-FG4	t	6.380	216	< 0.0001
					Control-FG5	t	7.688	216	< 0.0001
					Control-FG6	t	3.276	216	0.0012
					Control-FG7	t	5.241	216	< 0.0001
					Control-FG8	t	6.131	216	< 0.0001
					Control-FG9	t	2.628	216	0.0092
					Control-FG10	t	4.094	216	< 0.0001
					Control-FG11	t	-1.002	216	0.3173
Pollen Load	LM	log	Functional Group	-	Functional Group	F	11.903	10,94	< 0.0001
SVPD - Pollen Load	LM	log	Functional Group	-	Pollen Load	F	10.659	1,84	0.0016
Pollen Deposition Rate	LME	log	Visitation Rate * FG Richness	Field ID	Visitation Rate	F	175.366	1,45	< 0.0001
					FG Richness	F	37.867	1,45	< 0.0001
					Interaction	F	5.312	1,45	0.0258
Pollen Deposition Rate (Rarefied)	LME	log	FG Richness	Field ID	FG Richness	F	89.762	1,47	< 0.0001

Appendix B. The statistical analyses that were used in order to test the outlined hypotheses in chapter 2. SVPD refers to single visit pollen deposition, and FG refers to functional group.

Functional Group (FG)	Species	Family
1	<i>Achillea millefolium</i>	Asteraceae
	<i>Agrimonia eupatoria</i>	Rosaceae
	<i>Carlina vulgaris</i>	Asteraceae
	<i>Centaurea scabiosa</i>	Asteraceae
	<i>Daucus carota</i>	Apiaceae
	<i>Galium verum</i>	Rubiaceae
	<i>Helianthemum nummularium</i>	Cistaceae
	<i>Leontodon hispidus</i>	Asteraceae
	<i>Linum catharticum</i>	Linaceae
	<i>Lotus corniculatus</i>	Fabaceae
	<i>Phleum pratense</i>	Poaceae
	<i>Pimpinella saxifraga</i>	Apiaceae
	<i>Plantago lanceolata</i>	Plantaginaceae
	<i>Poa pratensis sens lat</i>	Poaceae
	<i>Sanguisorba minor</i>	Rosaceae
2	<i>Scabiosa columbaria</i>	Caprifoliaceae
	<i>Briza media</i>	Poaceae
	<i>Campanula rotundifolia</i>	Campanulaceae
	<i>Carex flacca</i>	Cyperaceae
	<i>Clinopodium vulgare</i>	Lamiaceae
	<i>Festuca rubra agg</i>	Poaceae
	<i>Filipendula vulgaris</i>	Rosaceae
	<i>Hieracium pilosella</i>	Asteraceae
	<i>Hippocrepis comosa</i>	Fabaceae
	<i>Lathyrus pratensis</i>	Fabaceae
	<i>Plantago media</i>	Plantaginaceae
	<i>Potentilla reptans</i>	Rosaceae
	<i>Primula veris</i>	Primulaceae
	<i>Prunella vulgaris</i>	Lamiaceae
	<i>Trifolium pratense</i>	Fabaceae
	<i>Viola hirta</i>	Violaceae
3	<i>Anthyllis vulneraria</i>	Fabaceae
	<i>Avenula pratensis</i>	Poaceae
	<i>Avenula pubescens</i>	Poaceae
	<i>Bromopsis erecta</i>	Poaceae
	<i>Campanula glomerata</i>	Campanulaceae
	<i>Centaurea nigra</i>	Asteraceae
	<i>Cynosurus cristatus</i>	Poaceae
	<i>Danthonia decumbens</i>	Poaceae
	<i>Festuca ovina</i>	Poaceae
	<i>Knautia arvensis</i>	Caprifoliaceae
	<i>Koeleria macrantha</i>	Poaceae
	<i>Leucanthemum vulgare</i>	Asteraceae
	<i>Onobrychis viciifolia</i>	Fabaceae
	<i>Ranunculus bulbosus</i>	Ranunculaceae
	<i>Rumex acetosa</i>	Polygonaceae
	<i>Succisa pratensis</i>	Caprifoliaceae
	<i>Thymus praecox</i>	Lamiaceae
	<i>Tragopogon pratensis</i>	Asteraceae
	<i>Trisetum flavescens</i>	Poaceae
	<i>Vicia cracca</i>	Fabaceae

Appendix C. A list of the plant species that formed each functional group in chapter 3. From Fry et al. (in prep.).

Floral Unit definition	Plant taxa
Single flower	<i>Alstroemeria</i> spp., all Amaranthaceae, <i>Allium</i> spp., <i>Vinca</i> spp., <i>Ilex</i> spp., <i>Zantedeschia</i> spp., <i>Hedera</i> spp., <i>Hyacinthoides</i> spp., <i>Impatiens</i> spp., <i>Berberis</i> spp., <i>Mahonia</i> spp., all Boraginaceae, all Brassicaceae, all Campanulaceae, all Caprifoliaceae (apart from <i>Sambucus</i> spp.), all Caryophyllaceae, <i>Euonymus</i> spp., all Cistaceae, all Convolvulaceae, <i>Sedum</i> spp., <i>Dipsacus fullonum</i> , <i>Eleagnus</i> spp., all Ericaceae (apart from <i>Calluna vulgaris</i>), <i>Escallonia</i> spp., all Fabaceae (apart from <i>Medicago</i> spp. and <i>Trifolium</i> spp.), all Fumariaceae, all Geraniaceae, <i>Hydrangea</i> spp., <i>Hypericum</i> spp., <i>Crocosmia</i> spp., all Lamiaceae (apart from <i>Lavandula</i> spp.), <i>Laurus nobilis</i> , <i>Hemerocallis</i> spp., <i>Linum</i> spp., all Malvaceae, <i>Narthecium ossifragum</i> , all Oleaceae, all Onagraceae, all Orchidaceae, all Orobanchaceae, <i>Oxalis</i> spp., all Papaveraceae, <i>Mimulus</i> spp., <i>Plantago</i> spp., <i>Armeria</i> spp., <i>Phlox</i> spp., <i>Polygala</i> spp., all Polygonaceae, <i>Claytonia</i> spp., all Primulaceae, all Ranunculaceae, all Rosaceae (apart from <i>Spiraea</i> spp. and <i>Prunus lusitanica</i>), all Rubiaceae, <i>Choisya</i> spp., all Scrophulariaceae (apart from <i>Buddleja</i> spp., <i>Veronica pimeleoides</i> , <i>Veronica</i> spp. (subgenus <i>Pseudoveronica</i>), <i>Veronica speciosa</i> , all Solanaceae, <i>Tropaeolum</i> spp., <i>Valerianella locusta</i> , <i>Viola</i> spp.
Single capitulum	All Asteraceae (except <i>Solidago canadensis</i>), <i>Knautia arvensis</i>
Single branch of capitulas	<i>Solidago canadensis</i>
Part of panicle	<i>Spiraea</i> spp. (apart from <i>Spiraea douglasii</i>)
Secondary umbel	All Apiaceae
Single compound cyme	All Valerianaceae (apart from <i>Valerianella locusta</i>)
Single corymb	<i>Cornus</i> spp., <i>Sambucus</i> spp.
Single cyme	<i>Euphorbia</i> spp.
Single panicle	<i>Buddleja</i> spp., <i>Spiraea douglasii</i>
Single raceme	<i>Calluna vulgaris</i> , <i>Medicago</i> spp., <i>Prunus lusitanica</i> , <i>Trifolium</i> spp., <i>Veronica pimeleoides</i> , <i>Veronica</i> spp. (subgenus <i>Pseudoveronica</i>), <i>Veronica speciosa</i>
Single spike	<i>Callistemon</i> spp., <i>Lavandula</i> spp.
Single thyrsse	<i>Ceanothus</i> spp.

Appendix D. “Table 2.1 How ‘Floral units’ were defined for all plant taxa sampled in the study” from Baldock et al. (2015) supplementary information.
How ‘floral units’ were defined in the study in chapter 3.

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