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Pollination in a changing world: function and resilience

Jamie Regan Stavert

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biological Sciences, the University of Auckland, 2018.
Abstract

Human activities have far-reaching impacts on ecosystems worldwide. Agricultural expansion and intensification is a primary driver of terrestrial biodiversity loss, disrupting important ecosystem functions. Paradoxically, agriculture relies strongly on these same ecosystem functions. Animal-mediated pollination is critical for the functioning of natural and production systems, but is strongly affected by agriculture, largely due to impacts on wild pollinators that require natural habitat. However, although more biodiverse communities often provide greater functional performance and stability, specific effects of agriculture on pollination is poorly understood.

In this thesis, I used landscape and small-scale experimental studies to investigate the impacts of agricultural intensification on insect pollinator communities and pollination function. I investigated (1) whether pollinators show response diversity (differential changes in abundance) to agricultural intensification by collecting pollinator species from flowering fields along an agricultural gradient. I also quantified functional redundancy (species with similar functional roles) by measuring pollinator functional traits. I measured (2) changes to pollination services with agricultural intensification by quantifying pollen deposition rates. I then investigated (3) how plant and pollinator numerical evenness affects pollination function, using a mesocosm experiment. I developed (4) a method for measuring pollinator hairiness and investigated its predictive power for pollinator effectiveness.

In my landscape study, I found that (1) although there was strong community-wide response diversity to agricultural intensification, functionally redundant pollinator species mostly lacked response diversity. Exotic species enhanced response diversity but did not functionally replace natives. Furthermore, (2) pollination services from native species declined with agricultural intensification, whereas pollination from exotic species increased, which increased overall pollination services. However, agriculture reduced evenness of pollination service delivery
from different species. In mesocosms (3) seed production was highest where a few plant and pollinator species dominated interactions, but where interaction frequency was relatively equal. Hairiness (4) strongly predicted pollinator effectiveness, with hairier species depositing more pollen.

Thus, agricultural intensification can drastically alter pollinator communities, and their robustness to future anthropogenic disturbances. In high intensity landscapes, exotic species provide compensatory pollination services, but native species declines likely increase vulnerability to future disturbances. As anthropogenic modification of the biosphere continues, progressing our understanding of how these changes impact ecosystems is imperative.
Acknowledgements

There are countless people who have helped me throughout this journey. In fact, there are so many it’s inevitable I’ll forget someone…

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Chapter 4 - Changes to plant and pollinator numerical evenness alter network structure and seed production.

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Chapter 5 - Hairiness: the missing link between pollinators and pollination. Published in PeerJ.

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Last updated: 19 October 2015
Chapter 1 – General introduction

1.1 Drivers and consequences of biodiversity loss

Human land-use, predominantly agriculture, now covers approximately 40% of the Earth’s ice-free terrestrial surface (Ellis et al., 2010). In consequence, agricultural intensification is the primary driver of habitat destruction and degradation, causing biodiversity decline and the disruption of critical ecosystem functions (Maxwell et al., 2016; Pereira et al., 2010; Sala et al., 2000). It is predicted that agricultural land cover will increase a further 10% by 2030, having far-reaching impacts on ecosystems (Haines-Young, 2009). However, an intriguing paradox is that agricultural systems depend upon the ecosystem services that they negatively impact (Foley et al., 2011; Tscharntke et al., 2005).

Biodiversity loss causes reduced performance and stability of ecosystem functions (Hooper et al., 2012). The effect of biodiversity loss on ecosystem functioning is typically nonlinear and saturating (Cardinale et al., 2011; Larsen et al., 2005). Initial biodiversity losses have a relatively small influence on ecosystem function but, as extinction increases, the rate of function loss accelerates. In pollination systems, land-use change causes declines in species richness (e.g., Klein, 2009; Steffan-Dewenter, 2003), abundance (e.g., Ricketts et al., 2008; Winfree et al., 2009) and shifts in community composition (e.g., Andersson et al., 2013; Sjödin et al., 2008). However, most studies assessing pollinator responses to anthropogenic disturbance have been conducted under extreme land-use change scenarios (i.e., strongly human-modified landscapes) (Winfree et al., 2009; Winfree et al., 2011). Subsequently, there is limited understanding of how less intensive land-use alters pollinator communities and pollination function. Where anthropogenic disturbance is moderate, pollinator species’ responses are often varied and sometimes even positive (e.g., Cariveau et al., 2013; Winfree & Kremen, 2009). Thus, investigation of how human land-use change affects pollination function over broader land-use gradients is required to establish a more predictive understanding of anthropogenic disturbance impacts.
1.2 The biodiversity - ecosystem function relationship

There is clear agreement from hundreds of biodiversity-ecosystem function experiments that increasing biodiversity positively affects the performance and stability of ecosystem function (Cardinale et al., 2012; Ives & Carpenter, 2007). The positive relationship between richness and functional performance typically begins linearly and asymptotes at moderate richness levels (Hooper et al., 2005). Across space and time, species richness increases ecosystem function stability, mainly by the complementary responses that occur between species in response to environmental variation (Ives & Carpenter, 2007).

There are two predominant mechanisms that explain the positive biodiversity-ecosystem function relationship; selection effects and complementarity (Reiss et al., 2009). Selection (or sampling) effects arise when a species that is functionally effective is also competitively dominant. The probability of functionally effective species occurring in a community increases with species richness (Loreau & Hector, 2001; Tilman et al., 1997b). In this way, selection effects are more of a statistical rather than biological phenomenon. Accordingly, in small-scale biodiversity-ecosystem function experiments, the probability of including a functionally effective species increases with species richness and thus often drives the positive richness-function relationship. Selection effects account for approximately half of all positive biodiversity-ecosystem function relationships in terrestrial experiments, but only a few in aquatic experiments (Cardinale et al., 2011). The prevalence of selection effects is probably an artefact of small-scale experiments and is less likely to occur in real-world ecosystems. Furthermore, terrestrial biodiversity-ecosystem function experiments have mostly focused on primary production and nutrient acquisition, which are functions driven mostly by competitively dominant species (Jiang et al., 2008). This may explain the disparity in the importance of selection effects for driving terrestrial vs. aquatic functions. However, for multitrophic functions, such as pollination, there is no reason to expect an association between species’ functional performance and dominance because a species’ ability to transfer pollen is in no way linked to its competitive capability.
CHAPTER 1. GENERAL INTRODUCTION

Functional complementarity explains around half of all positive biodiversity-ecosystem function relationships in terrestrial systems and most positive biodiversity-ecosystem function relationships aquatic systems (Cardinale et al., 2011). Species are functionally complementary when they occupy different portions of the functional niche space, so their combination determines community-level function. Thus, where species are complementary, community-level function is higher when species occur in combination in comparison to any one species in isolation (Loreau & Hector, 2001; Loreau et al., 2001). Where species display functional complementarity, community-level functional performance increases linearly with increasing species richness (Blüthgen & Klein, 2011). Complementarity can occur via additive or non-additive effects. For example, in pollinator communities where each species transfers the same quantity of pollen, pollinator complementarity effects are additive regardless of how many or what species are present (e.g., Brittain et al., 2013; Hoehn et al., 2008). Complementarity is non-additive if the performance of one species depends on positive (facilitative) or negative (inhibitory) interactions with another species (Cardinale et al., 2002). For example, in sunflower crops, flower visitation by native bees causes honeybees, Apis mellifera, to switch between flowers more frequently, enhancing pollination success (Greenleaf & Kremen, 2006).

In pollinator communities, species partition the functional niche space using a variety of mechanisms including floral morphology, visual and olfactory signals and temporal visitation preferences (Blüthgen & Klein, 2011). Floral morphological features determine which pollinator species can access which flowers. For example, pollination success is higher in experimental plant communities with two pollinator groups with very different morphologies (bumblebees and syrphid flies) compared to when either pollinator group is present in isolation (Fontaine et al., 2006). Pollinators often show temporal complementarity, which can occur over various time scales. Temporal complementarity can occur when different pollinators visit a target plant species at different times of the day (e.g., Fründ et al., 2013; Hoehn et al., 2008; Rader et al., 2013a) or during the day vs. night (e.g., Fleming et al., 2001). At larger temporal scales, different pollinator species are often active over different seasons, which is important for plants that flower for long periods (Bartomeus et al., 2013b). Over even longer time periods,
the identity of the most important pollinator species can vary between years (Kremen et al., 2002) and interaction partners in plant–pollinator networks change substantially over time (Alarcón et al., 2008; Petanidou et al., 2008).

The relative importance of complementarity and selection effects for biodiversity-ecosystem function relationships can be tested in small-scale experiments by comparing the functional performance of individual species versus a community of species at an equivalent density (Blüthgen & Klein, 2011). This approach has been conducted mostly in plant communities, by comparing the performance of monocultures to polycultures (Loreau & Hector, 2001). However, this approach probably underestimates the true importance of biodiversity for ecosystem functioning because interspecific competition is typically lower than intraspecific competition. Coexisting species can reach higher collective abundance than typically possible for a single species, and higher overall density often translates to greater functioning. Further, the strength of the biodiversity-ecosystem function relationship is likely to be even greater when additional niche dimensions (other than the functional niche) are considered or where studies are conducted at larger spatial scales.

1.3 Dominance – evenness effects on ecosystem function

Most biodiversity-ecosystem function studies focus on a single metric of biodiversity, species richness, and typically use communities with high species evenness, maximising richness effects (Dangles & Malmqvist, 2004). Consequently, little is known about the importance of other biodiversity components for ecosystem functioning, e.g., species evenness (Chapin et al., 2000; Hillebrand et al., 2008; Lewandowska et al., 2016). A universal characteristic of ecological communities is the skewed species abundance distribution (SAD) wherein only a few species are common and the rest are rare (McGill et al., 2007). In real-world ecosystems, changes in species evenness due to environmental disturbance is probably an important driver of fluxes in ecosystem functioning (Hillebrand et al., 2008). This is because changes in species evenness typically alters species interactions long before extinction occurs. Considering this, current understanding of mechanisms that mediate the biodiversity-ecosystem function
relationship (i.e., between species richness and functioning) may not be directly transferable to the real-world. For example, in pollination systems, fluctuations in the abundances of common species are more important for driving changes in ecosystem functioning than species richness and composition (Winfree et al., 2015). Further, fruit production increases with pollinator species richness, but only when pollinator species evenness also increases simultaneously (Garibaldi et al., 2015). In contrast, increased dominance of decomposer species enhanced decomposition rate (Dangles & Malmqvist, 2004). Therefore, the importance of dominance/evenness for ecosystem functioning is unclear and few studies to date have attempted to disentangle this biodiversity metric from other diversity measures. Understanding evenness-function relationships is likely critical for informing decisions on how to conserve and restore ecosystem functions in the real-world (Kleijn et al., 2015).

1.4 Biodiversity mechanisms that stabilise ecosystem functioning

There is evidence that biodiversity buffers ecosystems against functional loss through space and time (Cardinale et al., 2012). However, little is known about the mechanisms that drive this relationship and current understanding is mostly from small-scale experiments (Ives & Carpenter, 2007; Kremen, 2005). Research is now beginning to focus on stabilizing mechanisms that operate at larger scales, and recent studies have investigated the importance of mechanisms that work at a landscape level (Winfree, 2013; Winfree & Kremen, 2009). Key stabilizing mechanisms that could buffer ecosystem functioning against biodiversity loss include functional redundancy, response diversity and compensation.

1.4.1 Functional redundancy

Functional redundancy occurs when different species occupy the same functional niche and are therefore mutually suitable to fulfil a particular functional role (Blüthgen & Klein, 2011). The concept of functional redundancy is an extension of the biodiversity insurance hypothesis, which proposes that ecosystem function is more stable in species rich communities where multiple species contribute to ecosystem processes (Lawton & Brown, 1993; Naeem, 1998; Walker, 1992; Yachi & Loreau, 1999). Where species are redundant, equal functioning will
occur regardless of which species are present. For example, where all species in a pollinator community are functionally redundant, the reproductive output for a particular plant species (i.e., seed production) would be equal regardless of whether it was pollinated by species A, B or C, or all species together.

There are varying degrees of redundancy in ecosystems. Some studies have shown ecosystem functioning is mostly retained despite biodiversity loss (i.e., high redundancy: Srinivasan et al., 2007), while others have found that ecosystem function has a linear relationship with biodiversity (i.e., low redundancy: Larsen et al., 2005). In many studies, rapid saturation could be an artefact of limited species pools, a short time scale or restricted spatial diversity (Duffy, 2008). However, in most biological communities it is predicted that redundancy is intermediate, resulting in a saturating biodiversity-ecosystem function curve (Gómez et al., 2007), although the shape of this curve will vary depending on the order of species gains and losses (Larsen et al., 2005).

1.4.2 Response diversity

Complementarity and redundancy can vary independently across different niche dimensions so that species may be complementary in one niche dimension, but redundant in another (Blüthgen & Klein, 2011). For example, although pollinator species A responds negatively to land-use change, whereas species B responds positively, they may still pollinate plants in a very similar way. When sympatric species differ in their responses to environmental change, this is termed “response diversity” (Elmqvist et al., 2003) and is an extension of the insurance hypothesis (Loreau et al., 2001; Valone & Barber, 2008; Yachi & Loreau, 1999). Response diversity occurs because species vary in their environmental niche.

Both experimental studies (e.g., Leary & Petchey, 2009; Romanuk et al., 2010) and observational studies (e.g., Bartomeus et al., 2013a; Cariveau et al., 2013; Rader et al., 2013b; Thibaut et al., 2011; Winfree & Kremen, 2009) have detected response diversity in ecological communities. Most studies probably underestimate true response diversity because they use
controlled experimental conditions, or because they focus on a single environmental disturbance (Mori et al., 2013). Instead, response diversity likely occurs over large spatial scales and in response to multiple disturbances simultaneously. Response diversity in pollination systems has been explored as a stabilizing mechanism in few studies. For example, in North America, native bee species display response diversity by having differential changes in abundance with land-use change (Cariveau et al., 2013; Winfree & Kremen, 2009). Further, potential response diversity to climate change in pollinator communities could help stabilize pollination services (Rader et al., 2013b). For instance, under the most extreme Intergovernmental Panel on Climate Change (IPCC) scenario, pollination services from honeybees to watermelon are predicted to decline while pollination from wild insects increases, resulting in increased aggregate pollinator services. Thus, in real world ecosystems, response diversity can potentially stabilize ecosystem function against environmental change. Communities that have functionally redundant species which also display response diversity should provide more stable ecosystem function across space and time. Understanding how response diversity operates across larger spatial scales is required to inform predictions on how environmental change could alter ecosystem functions and services.

1.4.3 Compensation

Compensation is another potentially important stabilizing mechanism for ecosystem function (Winfree & Kremen, 2009). In the literature, compensation is typically defined as “density” or “numerical” compensation, where abundances of species that contribute to an ecosystem function negatively covary with other species contributing to the same function (Klug et al., 2000; Naeem & Li, 1997; Solan et al., 2004). In pollination systems, compensation can occur either due to increased plant–pollinator interaction rate or pollination efficiency of the remaining species (e.g., Cariveau et al., 2013; Hallett et al., 2017; Winfree & Kremen, 2009), or by the addition of new species (e.g., Pattemore & Wilcove, 2012; Sanguinetti & Singer, 2014). In natural ecosystems, exotic pollinators can compensate by pollinating plants that have lost their native pollinators. For example, native orchids in the Southern Andes are pollinated by exotic *Apis* and *Bombus* species (Sanguinetti & Singer, 2014) and in New Zealand, invasive
ship rats, *Rattus rattus*, pollinate some native plants where native pollinators are extinct (Pattemore & Wilcove, 2012).

Exotic species may also provide compensation in landscapes where native species have declined or are extinct due to land-use change, but this has not been investigated to date (Morales *et al.*, 2017). Native species typically decrease in abundance with anthropogenic disturbances (Steffan-Dewenter *et al.*, 2002), yet exotic species are often well adapted to human-modified environments and are frequently associated with human activities (Alpert, 2006; Sax & Brown, 2000). Accordingly, some exotic species may benefit from anthropogenic disturbance, and compensate for ecosystem services lost from native species. However, very few studies have investigated whether exotic species make positive contributions to stabilizing mechanisms (Morales *et al.*, 2017).

The concepts of response diversity and compensation essentially extend species niche theory (and the associated concept of complementarity) to a landscape level, whereby species differentiate niche space across broad spatial and temporal scales (Winfree, 2013). In real-world landscapes, the extent that stabilizing biodiversity mechanisms actually buffer ecosystem functions is unknown. To address this we must identify the relative importance of different stabilizing mechanisms at large spatial scales.

### 1.5 Functional traits for predicting ecosystem function

Although species richness is the most widely used metric of biodiversity, there are many other measures with potentially greater predictive power. Functional traits are an important component of biodiversity that may better predict functional performance compared to species richness (Gagic *et al.*, 2015). Functional traits are the morphological, physiological and behavioural characteristics of an organisms’ phenotype that determine its effect on ecosystem processes (effect traits) (Petchey & Gaston, 2006) and response to environmental change (response traits) (Naeem & Wright, 2003). Thus, functional traits ultimately determine an organism’s functional role in an ecosystem across space and time (Violle *et al.*, 2007).
Increased functional trait diversity should enhance ecosystem functioning, but only where species display some degree of complementarity along the functional niche axis (Ebeling et al., 2014).

Biodiversity-ecosystem function research is evolving from a focus on taxonomic richness to a more functional, trait-based approach (Petchey & Gaston, 2006; Reiss et al., 2009). Instead of simply assessing the effect of species richness on ecosystem function, trait-based biodiversity-ecosystem function research addresses the functional roles of organisms within communities and the diversity of traits required to maintain a given level of ecosystem functioning. This has been driven by the growing body of work suggesting species richness has a limited effect on many real-world ecosystem functions (Dang et al., 2005; Levine & HilleRisLambers, 2009; Thompson & Starzomski, 2007; Winfree et al., 2015).

Fluxes in ecosystem functions are strongly influenced by the gain and/or loss of traits (Hooper et al., 2005). Predicting the consequences of species gains and losses for ecosystem functioning requires understanding of which response traits predispose species to invasion or extinction, and how these covary with the effect traits that drive functioning (Berg & Ellers, 2010; Larsen et al., 2005; Suding et al., 2008). If species with traits that are important for ecosystem functions are disproportionately lost from communities, then loss of ecosystem function should be even greater than that predicted by many biodiversity-ecosystem function studies (Wardle et al., 2011). Indeed, traits that predispose species to extinction also often drive ecosystem functions. For example, in pollinator and decomposer communities, native bees and dung beetles with greater body size contribute more to pollination and dung removal, but are also more vulnerable to anthropogenic disturbance (Larsen et al., 2005). Similarly, mammals that have a large body size, slow growth rate and occupy a high trophic position have strong influences on ecosystem processes, but are more susceptible to extinction (Purvis et al., 2000). Yet we lack understanding of key response traits for most species, because we know little about which traits are linked to disturbance responses (Bartomeus et al., 2016a). Accordingly, it is
probably more tractable to quantify species’ actual responses (i.e., changes in abundance) to disturbances and link these responses to species’ functional roles.

The best approach for assessing functional diversity remains uncertain; studies use both continuous and discrete measures. Previous studies quantify functional diversity by assigning species to discrete functional groups (e.g., Naeem & Wright, 2003), assessing dissimilarity by plotting species in multidimensional trait space (e.g., Heemsbergen et al., 2004), and evaluating dendrogram length generated from cluster analysis (e.g., Petchey et al., 2004). Direct measures of functional trait composition more accurately describe true functional diversity (Díaz & Cabido, 2001) and studies are increasingly using continuous trait based measures and multivariate statistical approaches (Petchey & Gaston, 2006). Studies (e.g., Laliberté & Legendre, 2010; Mouchet et al., 2010; Villéger et al., 2008) have developed various multidimensional functional diversity metrics and have assessed their predicative power for multiple ecosystem functions. These new analytical approaches provide a powerful pathway for assessing biodiversity-ecosystem function relationships in both natural and production systems (Wood et al., 2015). Nevertheless, the strength of functional trait approaches is dependent on researchers identifying traits that have good predictive power for functional performance and/or species responses to environmental change (McGill et al., 2006; Violle et al., 2012).

1.6 Pollination as a model system for investigating biodiversity effects on ecosystem function and stability

Globally, animal-mediated pollination is a critical ecosystem function in natural and production ecosystems (Potts et al., 2010). Approximately 85% of wild plant species (Ollerton et al., 2011) and 75% of agricultural crops (Klein et al., 2007b) require animal pollinators to some degree. Further, around 35% of food consumed by humans can be attributed to pollination services from animals. Globally, wild insects are more important than honeybees for pollination services to a range of crop species (Garibaldi et al., 2013; Kleijn et al., 2015). Wild insects can
provide insurance against honeybee declines because they are not susceptible to the processes and/or diseases that negatively affect honeybees (Garibaldi et al., 2013; Rader et al., 2016). However, pollination is threatened in many parts of the world, although studies have predominantly been conducted in Europe and North America (Potts et al., 2016). From studies to date, reduction of pollination function is largely due to the decline of wild pollinators due to habitat loss driven by agricultural intensification (Garibaldi et al., 2011; Winfree et al., 2009). There is currently limited understanding of how global change affects most wild pollinator species and what the potential implications are for pollination function, especially in countries outside of Europe and North America (Kennedy et al., 2013; Potts et al., 2016).

Animal-mediated pollination presents a unique opportunity to investigate biodiversity-ecosystem function questions across large spatial and temporal scales and is therefore a valuable model system for advancing biodiversity-ecosystem function research (Winfree, 2013). Most biodiversity-ecosystem function research has focused on functions that involve one trophic level (e.g., plant biomasses; Balvanera et al., 2006). In contrast, pollination is a mutualistic function, involving multiple trophic levels (Schleuning et al., 2015). Further, pollination is well suited for addressing biodiversity-ecosystem function questions with alternative biodiversity metrics (e.g., functional traits, species evenness) since it involves diverse ecological communities (Wood et al., 2015).

1.7 Research aims

The overarching aim of this PhD thesis is to understand how different biodiversity mechanisms drive and stabilize pollination function. In this thesis I seek to disentangle the importance of biodiversity mechanisms for driving animal-mediated pollination function and stability using multiple experiments across different spatial scales. I ask four specific questions: 1) How do different biodiversity mechanisms stabilise pollinator communities in response to anthropogenic disturbance? 2) Does anthropogenic disturbance alter pollination services? 3) Are other measures of biodiversity, specifically species evenness, important for driving
pollination function? 4) Are there components of biodiversity, specifically functional traits, which strongly predict pollinator functional performance?

1.8 Thesis outline
This thesis contains four data chapters (chapters 2-5) that can be read as standalone research papers. Published chapters have been reformatted so that formatting is consistent throughout the thesis. The content of chapters has not been altered from the published form. The final chapter (chapter 6) is a synthesis of the results from this study and poses important future research avenues. I have aimed to give the best possible continuity between chapters throughout the thesis. However, because all chapters are encompassed by the broader themes of pollinator biodiversity and ecosystem functioning, some repetition between chapters is inevitable.

Chapter 2 investigates whether two important stabilizing mechanisms (response diversity and functional redundancy) operate in concert with land-use intensification. I predicted that the degree of response diversity would vary between functional groups. Further, I expected that where functionally redundant species showed response diversity, this would stabilize abundance within functional groups. Lastly, I predicted that exotic species would enhance response diversity to land-use change by increasing the range of positive, negative and neutral responses. Exploring these community-level mechanisms sets the scene for understanding how anthropogenic disturbance could alter pollination function. This chapter was published in *Proceedings of the Royal Society B: Biological Sciences*.

Chapter 3 quantifies changes in pollination service delivery from wild insects across a land-use intensification gradient. I expected that aggregate pollination services would decline with agricultural intensification, primarily due to loss of pollination from native species. However, I predicted that pollination from exotic species would increase with agricultural intensification, providing some degree of pollination service compensation. In consequence, I expected that there would be a temporal shift in pollination service delivery and a reduction in evenness of
pollination services provided by different species. This chapter addresses how pollination
service delivery can be altered by anthropogenic disturbance. This chapter was published in
the *Journal of Applied Ecology*.

**Chapter 4** investigates how pollinator numerical evenness affects plant–pollinator interaction
networks and subsequently, pollination function. I use a mesocosm experiment, wherein plant
and pollinator abundances are manipulated, to simulate different evenness scenarios. I
predicted that increased plant and pollinator numerical evenness would enhance
complementary interactions within networks. Consequently, I expected that species-level and
community-level seed production would increase with increased evenness due to greater
complementarity effects. This study provides a highly controlled experimental assessment of
how changes in species evenness (as shown in chapters 3 and 4 for real-world communities)
drives changes in function. This chapter has been formatted for submission to *Functional
Ecology*.

**Chapter 5** presents a new methodology for quantifying hairiness, an important pollinator
functional trait as it determines the amount of pollen an insect can collect and deliver in floral
visits. I measured the hairiness of various native and exotic insect pollinators by quantifying
the degree of entropy (variability) for different body-surface regions. I then assessed the
relationship between entropy and pollination effectiveness for plant species with different floral
morphologies. I expected that hairier insects would carry larger pollen loads and deposit a
greater number of pollen grains onto stigmas. However, I expected this relationship would be
specific to different body regions, determined by flower–pollinator trait matching. This chapter
was published in *PeerJ*.

**Chapter 6** is the conclusion, which integrates results from all chapters and highlights the
importance of this work for the broader biodiversity-ecosystem function research field.
Recommendations for important future research avenues are also provided.
Chapter 2 – Exotic species enhance response diversity to land-use change but modify functional composition

Abstract
Two main mechanisms may buffer ecosystem functions despite biodiversity loss. First, multiple species could share similar ecological roles, thus providing functional redundancy. Second, species may respond differently to environmental change (response diversity). However, ecosystem function would be best protected when functionally redundant species also show response diversity. This linkage has not been studied directly, so we investigated whether native and exotic pollinator species with similar traits (functional redundancy) differed in abundance (response diversity) across an agricultural intensification gradient. Exotic pollinator species contributed most positive responses, which partially stabilized overall abundance of the pollinator community. However, although some functionally redundant species exhibited response diversity, this was not consistent across functional groups and aggregate abundances within each functional group were rarely stabilized. This shows functional redundancy and response diversity do not always operate in concert. Hence, despite exotic species becoming increasingly dominant in human-modified systems, they cannot replace the functional composition of native species.

Publication status: Published
2.1 Introduction

Globally, agricultural intensification is the primary driver of habitat loss and subsequently, biodiversity decline, which often has cascading effects through ecosystems (Sala et al., 2000). However, two key mechanisms, functional redundancy and response diversity, may buffer ecosystem functions from the negative impacts of biodiversity loss (Elmqvist et al., 2003; Walker, 1992). The concept of functional redundancy arises from the biodiversity insurance hypothesis, which proposes that ecosystem function is more stable in species rich communities in which multiple species contribute to, and therefore safeguard, each ecosystem process (Naeem, 1998; Walker, 1992; Yachi & Loreau, 1999). Functional redundancy can be inferred by categorizing species according to their functional traits, which are the morphological, physiological and phenotypical characteristics that determine their functional role in an ecosystem (Lavorel & Garnier, 2002). Species are considered functionally redundant when they share many traits and are therefore equally capable of fulfilling a particular functional role (Blüthgen & Klein, 2011; Mori et al., 2013). In systems with high functional redundancy, ecosystem function is mostly retained despite species loss (Petchey et al., 2007; Srinivasan et al., 2007), whereas in systems with low functional redundancy, functional decline accelerates as species become extinct (Laliberté et al., 2010; Larsen et al., 2005).

The second mechanism that could buffer ecosystems against biodiversity loss is response diversity, which is the extent that species contributing to the same ecosystem function differ in their responses to an environmental disturbance (Elmqvist et al., 2003; Walker et al., 1999). Response diversity can be quantified by determining whether species abundances increase, decrease, or remain similar in response to environmental change (Cariveau et al., 2013; Elmqvist et al., 2003), although other studies have used different approaches (e.g., quantifying response trait dispersion in communities; Laliberté et al., 2010). Response diversity in abundance occurs in native North American bee species, which show differential changes in abundance in response to habitat loss (Winfree & Kremen, 2009) and aquatic micro-organisms vary in population growth rate in response to temperature change (Leary & Petchey, 2009). Response diversity is reported in both experimental studies (e.g., Leary & Petchey, 2009;
Romanuk et al., 2010) and observational studies (e.g., Bartomeus et al., 2013a; Cariveau et al., 2013; Rader et al., 2013b; Thibaut et al., 2011; Winfree & Kremen, 2009) but the approach used to quantify it varies widely. Whether response diversity can buffer ecosystem functions against disturbances is unclear (Winfree & Kremen, 2009), although one study shows that bee species that respond positively to disturbance by increasing in abundance can reduce the loss of pollination function (Cariveau et al., 2013). Response diversity is likely a key buffering mechanism because changes in species abundances are more important for driving fluxes in ecosystem functions than other components of biodiversity, such as species richness (Winfree et al., 2015).

In complex, real-world ecosystems, functional redundancy and response diversity would stabilize ecosystem functions most effectively if they operate in concert. For example, if a set of functionally redundant species all respond negatively to disturbance by decreasing in abundance, there would be greater loss of function than if they had a more complex range of positive, neutral, and negative responses (Figure 1). This requires species that are redundant in one niche dimension (e.g., the functional niche, such as their role as pollinators) to be complementary in another (e.g., the environmental niche, such as climatic foraging preferences) (Blüthgen & Klein, 2011; Loreau & Hector, 2001). Only one study has assessed the relationship between functional redundancy and response diversity (Laliberté et al., 2010); both functional redundancy and response diversity decreased with land-use intensification, indicating a positive association between these two mechanisms. However, this study inferred response diversity indirectly from the species’ response traits (e.g., age of reproduction in plants). This assumes that response traits are directly linked to species’ changes in abundance in response to environmental change, but in most systems, we rarely know what traits are linked to disturbances (Bartomeus et al., 2017). In addition, species that share response traits often respond differently to disturbance. For example, bumble bee species mostly share large body size, but some species increase in abundance in response to environmental disturbance while others become rarer (Bartomeus et al., 2013a; Cameron et al., 2011). Moreover, this approach does not allow the quantification of how changes in the abundances of individual species affect
overall aggregate community abundance. This makes it difficult to identify the potential buffering capacity of response diversity because actual changes in relative abundance are not quantified.

**Figure 1.** Cartoon illustrating different scenarios for the interaction between functional redundancy and response diversity. Lines represent the change in abundance of two functionally redundant species in response to an environmental disturbance. Function is lost when redundant species respond negatively to disturbance (a), but is gained when species respond positively (b), and does not change when species have neutral responses (c) or show response diversity (d).
Most species adapted to their natural ecosystems are expected to show negative responses to habitat modification and become rarer (Steffan-Dewenter et al., 2002). However, opportunist species that are well-suited to human-modified environments could benefit from disturbance. For example, exotic species are often well-adapted to human-modified environments, and are generalist in their foraging and habitat preferences (Alpert, 2006; Sax & Brown, 2000). Association with human activity enhances the chance of assisted dispersal to new regions, to which exotic species are already pre-adapted to thrive (Alpert, 2006). However, there is debate about the extent to which exotic species can occupy similar functional niches as natives (i.e., matching vs filling hypotheses; Ordonez et al., 2010). If exotic species differ in their responses while fulfilling the same functional niches as natives, then they provide both response diversity and functional redundancy, potentially buffering against the loss of ecosystem function in highly disturbed ecosystems.

Here, we investigate whether functionally redundant species show response diversity by grouping species based on traits that are important for pollination of a wide variety of plant species, and quantifying changes in their abundances across a land-use intensification gradient. Animal-mediated pollination is a critical ecosystem function; ~85% of wild plant species (Ollerton et al., 2011) and ~75% of agricultural crops (Klein et al., 2007b) require animal pollinators to some degree. Insect pollinator communities are well-suited for investigating how functional redundancy and response diversity affect ecosystem functions because pollinator traits are tractable measures of their functional roles (Garibaldi et al., 2015; Hoehn et al., 2008; Stavert et al., 2016), and pollinators often have strong and species-specific changes in abundance in response to land-use intensification (Cariveau et al., 2013; Winfree & Kremen, 2009). We use a landscape scale experiment to test whether: (i) functionally redundant species show response diversity (differential changes in abundance) to land-use intensification; (ii) exotic species enhance response diversity within functional groups and the wider pollinator community and; (iii) whether response diversity stabilizes aggregate abundance within functional groups and the wider pollinator community. First, we identify groups of functionally redundant pollinator species using a set of functional traits that are important for pollination.
Then, we quantify response diversity within these functional groups by measuring the relative changes in species’ abundances at field sites across a gradient of increasing land-use intensification (Cariveau et al., 2013; Winfree & Kremen, 2009).

### 2.2 Materials and methods

#### 2.2.1 Study system

We selected 12 study sites that represented a gradient of land-use intensification in an 80 x 60 km area within the Waikato Region, New Zealand. Sites were separated by at least 3 km (range, 3-20 km) to ensure that we sampled separate pollinator communities. All sites were contained within conventional farms to minimize inter-site variation in insecticide use, which can affect insect pollinator communities (Tuell & Isaacs, 2010).

In order to standardize the attractiveness of the focal sampling site, we planted 25 x 25 m plots of pak choi (*Brassica rapa*), which has an open access flower that is attractive to a wide variety of insect pollinators in New Zealand (Rader et al., 2013a). All *B. rapa* fields were cultivated within two weeks of each other in mid-spring (October) 2014, to ensure flowering synchrony among sites and reduce the likelihood of detecting temporal rather than spatial variation in pollinator communities.

#### 2.2.2 Landscape analysis

To classify and measure the area of different land-use types in the landscape surrounding our study sites, we used the Land Cover Data Base version 4.1 (Land Resource Information Systems) with the most recent thematic classification of New Zealand’s land-use cover. We analysed these data using ArcGIS 10.3.1 (ESRI, 2011). For initial site selection we used the ArcGIS Spatial Analyst tool to calculate the total area occupied by different land-use types at different scales (500 m and 2,000 m). We used 500 m and 2,000 m radii because these scales are important for different insect pollinator taxa (Benjamin et al., 2013; Greenleaf et al., 2007;
Rader et al., 2011). Study sites were then selected that had a strong correlation in the proportion of agriculture at 500 m and 2,000 m (Pearson’s \( r = 0.93; P < 0.0001 \)), to ensure that subsequent analyses were scale independent (e.g., Benjamin et al., 2013). This yielded 12 sites ranging from 42–99% agriculture. Land-use intensity for further analyses was defined as the proportion of agriculture at a 2,000 m radius surrounding the center of each sampling site. We focus on the conversion of natural and semi-natural habitat to agriculture as our primary environmental disturbance because this is the leading cause of biodiversity loss worldwide (Pereira et al., 2010). In our study, agricultural land-use was strongly inversely correlated with semi-natural habitat (defined as broadleaved indigenous hardwood, deciduous hardwood, exotic forest, *Ulex europaeus* (Fabaceae) and/or *Cytisus scoparius* (Fabaceae), indigenous forest and *Leptospermum scoparium* (Myrtaceae) and/or *Kunzea* spp. (Myrtaceae) at 2,000 m radius; Pearson’s \( r = -0.93; P < 0.0001 \)).

### 2.2.3 Pollinator sampling

We sampled pollinator communities in summer, from December 2014 to January 2015. Each site was sampled on two separate days and only when the weather was sunny or partly cloudy with wind speeds <5 m/sec and temperature >15°C. We collected insects at standardized 2-hour intervals from 08:00 to 20:00 at each site, resulting in 7 collection periods per sampling day. To measure abundance, insects were collected by sweep netting along fixed transects that began 5 m from the edge of the flower patch and ran through the center of the plot. Each sweep netting session lasted for a total of 12 mins and timing was paused whenever an insect was captured and transferred into a collection vial. We did not include honeybees, *Apis mellifera*, in our study as nearly all colonies in New Zealand are managed hives and therefore, are not directly influenced by surrounding land cover (Newstrom-Lloyd, 2013). Insects were killed immediately by freezing and stored at -18°C. All collected specimens were identified using identification keys and voucher specimens in existing collections with assistance from expert taxonomists.
CHAPTER 2. LINKING RESPONSE DIVERSITY WITH REDUNDANCY

2.2.4 Effect trait selection and measurement

To define groups of functionally redundant species, we used a set of six functional effect traits that are important for pollination function to wild plants. Effect traits determine a species’ contribution to functional diversity (Laliberté et al., 2010) and their functional role within an ecosystem (Lavorel & Garnier, 2002). Thus species with similar functional effect trait composition are expected to fulfil equivalent functional roles. The diversity of functional traits in a community has been used to accurately predict ecosystem functioning (e.g., Gagic et al., 2015; Reiss et al., 2009; Scherer-Lorenzen, 2008; Tilman et al., 1997a) and ecosystem multifunctionality (Mouillot et al., 2011).

We determined body size (trait 1) by measuring body length, body width, head length, head width, head depth, foreleg length and hind leg length. Body size affects an insects’ relative ability to pollinate flowers with different structures i.e., trait matching (Garibaldi et al., 2015; Hoehn et al., 2008; Stang et al., 2009). We measured mouthpart length (trait 2) including the glossa and the prementum (Cariveau et al., 2016) because this often affects pollination by matching with corolla depth (Fontaine et al., 2006; Garibaldi et al., 2015; Junker et al., 2013). We measured hairiness (trait 3) on 7 pollinator body regions (face, thorax dorsal, abdomen dorsal, thorax ventral, abdomen ventral, head ventral and foreleg). Hairiness of different body parts is a good predictor of single visit pollen deposition for multiple flower types (Stavert et al., 2016). Hairiness was quantified using image entropy analysis, which provided average entropy values for different body regions (see ref. Stavert et al., 2016 for further details). For entropy analyses we used ventral, dorsal and frontal shots with clear illumination to minimise reflection from shiny insect body surfaces. High-resolution imagery (Visionary Digital Passport portable imaging system coupled with a Canon EOS 5D Mark II digital camera) was used to obtain precise linear morphological measurements. Morphological trait values were calculated from the mean measurements of 8-10 representative specimens for each species.

We included pollen carrying structure (trait 4) as a categorical trait because it affects how pollinators collect pollen from flowers and consequently, how they interact with floral
reproductive structures (Parker et al., 2015; Thorp, 2000). Some bees in the family Apidae have a concave plate-like structure on the hind tibiae (corbiculae), which they use to compress pollen grains into dense pellets for transport back to the colony (Thorp, 2000). Non-corbicula bees have brush-like structures, either on the hind tibiae or ventral surface of the abdomen (scopae), that they use to collect pollen for larval provisioning. Flies lack specialized collection structures and tend to collect pollen passively on the body surface (Holloway, 1976).

We measured temporal activity (trait 5) as the proportion of individuals of each species collected across all sites at selected times during the day (08:00; 10:00; 12:00; 14:00; 16:00; 18:00 and 20:00). Temporal activity interacts with pollen deposition and stigma receptivity, which is important for plant reproductive success (Hoehn et al., 2008; Potts et al., 2001). Lastly, we measured phenology (trait 6) using museum collection records. We defined phenology as the proportion of specimens collected in each month for each species throughout the calendar year. Pollinator phenology is important for determining the degree of matching between pollinators and plant flowering periods (Bartomeus et al., 2011; Blüthgen & Klein, 2011).

Multiple trait measurements related to four overall traits: body size (7 measures), hairiness (7 measures), temporal activity (7 measures), and phenology (12 measures). To account for this, we assigned relative weighting for each measure (weights = 0.143 for body size measurements; 0.143 for hairiness measurements; 0.143 for temporal activity measurements; 0.083 for phenology measurements). Subsequently, all traits used in our functional redundancy analysis had an equal overall weighting of 1.

### 2.2.5 Functional redundancy

For functional redundancy and response diversity analyses, we only included species that were present at three or more sites and for which we collected 15 or more individuals (\( n = 3,653 \))...
specimens from 22 species, representing 97.5% of the total number of specimens collected; see Table A1 in Appendix A).

We categorized species into functional groups based on their functional trait similarity (Laliberté et al., 2010). This allowed us to define groups of functionally redundant species based on traits that are important for pollination function, independent of exotic/native identity and taxonomic relatedness. To identify functional groups, a Gower dissimilarity matrix was computed in the FD package using weighted trait values for each species (Laliberté & Shipley, 2010). We then computed the KGS penalty function to determine the optimal number of functional groups using the maptree package (White & Gramacy, 2012). The number of groups with the minimum KGS penalty value is suggested as the optimal number of clusters (Kelley et al., 1996). We then identified functional groups using Ward’s minimum variance clustering on the Gower dissimilarity matrix (Legendre & Legendre, 1998). The consistency of each species’ membership to its assigned functional group was validated using silhouette plots (Rousseeuw, 1987) in the cluster package (Maechler et al., 2016).

2.2.6 Response diversity

Here, we define response diversity as the differential change in pollinator species abundances with increasing land-use intensification (Cariveau et al., 2013; Winfree & Kremen, 2009). We firstly quantified response diversity within each functional group. We then additionally tested for response diversity in other species sets (natives only; exotics only; bees only; flies only; full pollinator community). To test for response diversity, we used generalised linear mixed effects models (GLMMs) with a negative binomial distribution and a log link function because our data were counts and were overdispersed for a Poisson distribution. The negative binomial error structure allowed us to account for overdispersion in the data, caused by a large number of zero abundance observations. All GLMMs were constructed using the glmmADMB package (Fournier et al., 2012). In all models, the response variable was the number of pollinator individuals collected per species, and the predictors were pollinator species (categorical), the
proportion of land used for agriculture (continuous) and the pollinator species : proportion agriculture interaction. The response variable (pollinator abundance per species) was pooled across all sampling sessions and all days for each site. Site was included as a random effect in all models. Fitted models were validated by examining the distribution of residuals plotted against fitted values (Crawley, 2002; Zuur et al., 2009). We assessed spatial autocorrelation of both the data and model residuals using Moran’s index in the spdep package (Bivand et al., 2011). Moran’s index value was low (I = –0.004) and therefore spatial autocorrelation was not taken into account in further analyses.

We determined the strength of response diversity within species groups by calculating the relative importance of the species : proportion agriculture interaction in our models. The interaction term indicates whether responses of the pollinator species (change in abundance) to land-use intensification varied (Winfree & Kremen, 2009). Specifically, we calculated the difference in ΔAICc and evidence ratios between additive models (abundance ~ species + agriculture) and interaction term models (abundance ~ species + agriculture + species : agriculture). ΔAICc values were calculated by subtracting the AICc values for additive models from the AICc values for interaction models (Richards et al., 2011). Evidence ratios were calculated by dividing AICc weights of interaction models by AICc weights of additive models (Burnham et al., 2011). Higher ΔAICc and evidence ratio values indicate greater importance of the species : agriculture interaction and consequently, response diversity within each species set. In particular, evidence ratios give an easily interpretable metric of how the data support the two competing models (Burnham et al., 2011; Richards et al., 2011). For example, if the interaction model has an evidence ratio of 20, there is 20 times stronger evidence that it is a better model than the additive model. All statistical analyses were conducted in R version 3.2.4 (R Development Core Team, 2014).

### 2.3 Results

In total, we analysed 3,653 insect pollinators from 22 species and 10 families that were present at three or more sites and for which we collected 15 or more individuals (see Table S1). This
included eight species of bees (seven native and one exotic) and 14 species of flies (five native and nine exotic). Given that New Zealand has a high proportion of generalist insect pollinator species and a relatively depauperate pollination fauna (Newstrom & Robertson, 2005), the sampled communities are representative of the local species pool.

2.3.1 Functionally redundant species

Hierarchical clustering of species functional effect traits yielded six functional groups that ranged in size from 1-5 species (Figure 2). These included 2 groups of native species only, 1 group of exotics species only, and 3 groups of exotics and natives. Functional group 1 was comprised of large native and exotic Syrphid, Stratiomyid and Calliphorid flies; Functional group 2 comprised medium sized native and exotic flies from multiple families; Functional group 3 comprised medium sized native bees in the genus *Leioproctus*; Functional group 4 comprised small exotic Anthomyiid and Stratiomyid flies and a small native Syrphid fly; Functional group 5 only included the exotic bumble bee *Bombus terrestris* and Functional group 6 comprised two small native bee species in the genus *Lasioglossum* (see Table S1).
2.3.2 Community-wide response diversity

When taking into account the full pollinator community, we found strong response diversity (i.e., considerable changes in relative abundance for each species; Table 1) and a moderate 29% decrease in overall aggregate pollinator abundance across the agricultural intensification gradient (Figure 3). However, removing exotic species from the data set resulted in an 87% decrease in pollinator abundance across the intensification gradient. In contrast, exotic species abundance increased by 150% across the intensification gradient. Both native and exotic species displayed moderate response diversity (Table 1). Remarkably, only two out of 13 native species responded positively to intensification, compared with six out of nine exotic species (see Figure A1 in Appendix A). Only two bee species, *Lasioglossum cognatum* and *Leioproctus*
boltoni, had non-negative responses to land-use intensification, which resulted in a decrease in aggregate abundance of 90% among all bee taxa (see Figure S1). In contrast, seven of the 14 fly species displayed positive responses to intensification, which resulted in an overall increase in abundance of 95%.

![Figure 2.](image)

**Figure 2.** The relationship between aggregate pollinator abundance and the proportion of agriculture for exotic and native species, and all species combined. Coloured lines are the changes in abundance for exotic vs native species. The dashed black line is the change in overall aggregate pollinator abundance.

### 2.3.3 Response diversity and abundance changes among functionally redundant species

We found that species in 2 out of the 6 functional groups showed response diversity, but total abundance was only stabilized, to some degree, in functional group 6 (native *Lasioglossum* bees) (Figure 4; Table 1). Species showing response diversity were in Functional group 4 (small
native and exotic flies; $\Delta AIC_C = 14.52$) and Functional group 6 (native *Lasioglossum* bees; $\Delta AIC_C = 5.14$), but total abundance in Functional group 4 increased by 241% whereas total abundance in Functional group 6 decreased by 32%. In contrast, Functional group 1 (composed mainly of large native and exotic flies) did not show response diversity and total abundance increased by 71%, which was driven by the exotic fly *Eristalis tenax*. We also detected no response diversity in Functional groups 2 (medium native and exotic flies) and 3 (medium native *Leioproctus* bees) and consequently, total abundance decreased by 38% and 99% respectively in these groups.

**Figure 3.** The relationship between pollinator species abundance and the proportion of agriculture. Each pane contains a set of functionally redundant pollinator species as determined by multivariate functional trait analysis. Plotted lines are the responses of individual species; purple lines are exotic species and green lines are native species. Black dashed lines represent the change in aggregate abundance within each functional group.
Table 1. $\Delta$AIC$_c$ and evidence ratio values for additive models (abundance $\sim$ species + agriculture) vs interaction models (abundance $\sim$ species + agriculture + species : agriculture). Evidence ratios are the ratio of the interaction model weight to the additive model weight. Higher $\Delta$AIC$_c$ and evidence ratio values indicate greater importance of the interaction term and consequently, response diversity. Evidence of response diversity is denoted by ‘*’. NAs are given where it was not possible to test for response diversity due to the presence of only a one species in a functional group.

<table>
<thead>
<tr>
<th>Species group</th>
<th>$\Delta$AIC$_c$</th>
<th>Evidence ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional group 1</td>
<td>-6.62</td>
<td>0.04</td>
</tr>
<tr>
<td>Functional group 2</td>
<td>-3.58</td>
<td>0.17</td>
</tr>
<tr>
<td>Functional group 3</td>
<td>-3.76</td>
<td>0.15</td>
</tr>
<tr>
<td>Functional group 4</td>
<td>14.52*</td>
<td>1425.98*</td>
</tr>
<tr>
<td>Functional group 5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Functional group 6</td>
<td>5.14*</td>
<td>13.08*</td>
</tr>
<tr>
<td>Bees only</td>
<td>3.26*</td>
<td>5.10*</td>
</tr>
<tr>
<td>Flies only</td>
<td>11.42*</td>
<td>302.08*</td>
</tr>
<tr>
<td>Natives only</td>
<td>5.11*</td>
<td>12.88*</td>
</tr>
<tr>
<td>Exotics only</td>
<td>6.29*</td>
<td>23.23*</td>
</tr>
<tr>
<td>Full community</td>
<td>24.14*</td>
<td>174199.90*</td>
</tr>
</tbody>
</table>

2.4 Discussion

We detected response diversity to land-use intensification in only two out of six functional groups. Changes in total abundance differed substantially between functional groups and abundance was partially stabilized in only one group (Functional group 6, native *Lasioglossum* bees). Interestingly, exotic species contributed most positive and neutral responses to agricultural intensification and subsequently, largely maintained community-wide aggregate pollinator abundance, resulting in a 29% drop with maximum agricultural land-use. From a
taxonomic perspective, flies contributed most positive and neutral responses to intensification and only two bee species had positive and/or neutral responses.

Both native and exotic species displayed moderate response diversity, but when grouped together (i.e., the full pollinator community) we detected strong response diversity. This is because most exotic species had positive responses, whereas native species mostly had negative responses. Thus contrasting responses between exotic and native species enhanced response diversity in the full pollinator community, which partially stabilized aggregate abundance. However, this was not the case within functional groups where there was considerable variation in how total abundance changed with land-use intensification. For example, functional groups 4 (small native and exotic flies) and 6 (native *Lasioglossum* bees) both showed response diversity, but total abundance in Functional group 4 increased by 241%, whereas total abundance in Functional group 6 decreased by 32%. In Functional group 4, three of five species had strong positive responses, resulting in a large cumulative increase in abundance. Species in Functional group 6 had contrasting positive and negative responses, leading to relative stabilization of total abundance. Where functional groups lacked response diversity, change in total abundance was often driven by the magnitude and direction of dominant species responses. For example, Functional group 1 (large native and exotic flies) showed a 71% increase in total abundance, which was driven by one species (*Eristalis tenax*) that had a strong positive response and was the most abundant species in the group. In contrast, the most abundant species in Functional group 3 had strong negative responses, leading to a 99% decrease in total abundance. Although we did not measure pollination services or seed production in wild plants, non-ubiquitous changes in aggregate abundance between functional groups are likely to markedly alter the functional architecture of the pollinator community (Fründ *et al.*, 2013; Vázquez *et al.*, 2005). This could have serious implications for pollination function of wild plants.

Other studies have shown that response diversity does not confer increased ecosystem function stability. For example, response diversity to land-use intensification in native bee communities
was detected in three different crop systems, but differential responses were only associated with increased pollination service stability in one system (Cariveau et al., 2013). This is because the capacity of response diversity to stabilize pollination services is dependent on the distribution of species responses within the community. Although we did not find that response diversity stabilized aggregate abundance within functional groups, we only quantified changes in species abundances to a single disturbance, over one season. If species responses to multiple disturbances over a longer time period were measured, response diversity may have had a greater stabilizing effect. Our approach is also limited as it assumes species within functional groups are absolutely equivalent, and are also absolutely unique compared to species in other functional groups. Yet in reality, species occur along a functional redundancy continuum within multidimensional trait space. Here we present a likely simplified version of true functional redundancy-response diversity relationships (Carmona et al., 2016), but our scenario allows precise control of the degree of land-use change, while permitting analysis of multispecies responses at a landscape scale.

Exotic and native species responded very differently to increasing land-use intensification, even when categorised within the same functionally redundant group. This suggests that exotics and natives sometimes occupy different environmental niches despite being functionally similar. It could be interpreted that our results contradict recent studies showing exotic plants are less likely to establish in communities with functionally equivalent natives (e.g., Ordonez, 2014; Ordonez et al., 2010). However, we only explored the trait space related to pollination function to classify redundant species. Exotics that are equivalent to natives regarding traits that are important for pollination function may differ in other portions of the trait space (i.e., traits important to non-floral resource use). Native species that have limited resource acquisition potential (i.e., floral specialists) are unlikely to survive in highly disturbed environments, whereas exotics with generalist resource acquisition traits can persist (Crowder & Snyder, 2010; Tilman, 2004).
Our analysis does not allow us to determine whether the increase in exotic dominance was due to non-interactive factors, such as land-use intensification, or interactive factors, such as competitive exclusion (i.e., the passenger vs driver model; see Didham et al., 2005; MacDougall & Turkington, 2005). This is because anthropogenic habitat modification often strongly covaries with increased invasive species dominance (e.g., Gurevitch & Padilla, 2004). However, non-interactive rather than interactive factors are often more important for driving exotic dominance in human-modified systems (e.g., MacDougall & Turkington, 2005). In our system, increased exotic dominance is unlikely to be driven by species interactions, but rather, non-interactive processes (i.e., land-use intensification), which have greater impacts on native species (Didham et al., 2005). Native pollinators are reliant on natural habitat for nesting and floral resources, including undisturbed nesting substrate, diverse floral resources and particular larval host plants (Winfree et al., 2011). This is not the case for many exotic species, which can use a broader range of resources in agricultural landscapes. For example, *E. tenax*, which responded very positively to agricultural intensification in our study, reproduces in effluent pits, which are used to dispose cow excrement on dairy farms. The shift to dairy farming from less intensive agricultural practices, such as sheep and beef farming and cropping, is the primary driver of land-use intensification in our study region. Other exotic flies (e.g., Anthomyiid species and *Oxysarcodexia varia*) readily use resources in agricultural landscapes (Finch, 1989; Mulieri et al., 2010). The association between increased exotic abundance and agricultural intensification is unsurprising, as human-mediated dispersal pathways inadvertently select for species with characteristics that are strongly linked with survival in environments subject to anthropogenic disturbance (Alpert, 2006). For example, in other systems, introduced plants in parts of Europe have a common set of traits that allow them to grow in highly disturbed habitats (Thompson et al., 1995) and tolerance to poor water quality is associated with the spread of introduced fish in California (Marchetti et al., 2004).

Flies, rather than bees, are likely to play a key role in buffering pollination function against environmental disturbance. The resilience and adaptability of many fly species to modified habitats is reflected in our data where they represent 7 out of the 9 positive and/or neutral
responses to intensification. The agricultural matrix is more permeable to flies than bees, which is probably driven by behavioural differences between these taxa (Rader et al., 2016) and greater endemism amongst bees than flies in our study. Flies have no central nest location and can often forage and use resources in highly modified agricultural landscapes (Orford et al., 2015; Rader et al., 2016). In contrast, bees are central place foragers that typically travel only short distances, so they are directly affected by the surrounding land-use (Greenleaf et al., 2007; Rader et al., 2011). Consistent with this, some solitary bees respond strongly to changes in plant diversity, whereas flies are less affected (Weiner et al., 2011). Many solitary bees require untilled and sparsely vegetated ground for nesting, and reliable, long-term pollen and nectar sources (Winfree et al., 2011). These disappear with agricultural modification.

We found only two bee species had positive and/or neutral responses to intensification, (*Lasioglossum cognatum* and *Leioproctus boltoni*). Interestingly, *Lasioglossum cognatum* was the only bee species to show a positive response and was one of only two non-endemic bees collected (in addition to *Bombus terrestris*). *Lasioglossum cognatum* occurs in New Zealand and Australia and is therefore more widespread than the endemic *Lasioglossum* species. Native species with large natural ranges likely have broader environmental tolerances and better survival in modified environments (Sax & Brown, 2000). The neutral response of endemic *Leioproctus boltoni* is also intriguing, and future work should test which traits allow persistence with agricultural modification. Despite the neutral response of *Leioproctus boltoni*, aggregate abundance within Functional group 3 decreased dramatically. This is concerning given that *Leioproctus* species are important pollinators in natural New Zealand ecosystems, and have specialist interactions with native plants that are unlikely to be fulfilled by exotic pollinators (Newstrom & Robertson, 2005; Pattemore & Wilcove, 2012; Robertson et al., 2005).

### 3.4.1 Conclusion

In conclusion, species that are functionally redundant display varying degrees of response diversity to land-use intensification in a real-world ecosystem. This suggests that functionally
redundant species sometimes occupy different environmental niches (Coux et al., 2016). Exotic species contributed most of the positive responses to agricultural intensification and this enhanced response diversity within the wider pollinator community. Yet, although response diversity partially stabilized community-wide abundance with agricultural intensification, it did not stabilize abundance within functional groups. Further, some functional groups were far more vulnerable to land-use change than others. This could have serious implications for pollination function in natural and production systems. We only assessed response diversity to a single disturbance (agricultural intensification), so we likely underestimated true response diversity, which can occur in regard to many environmental variables (Mori et al., 2013; Rader et al., 2013b). The relationship between species’ functional and environmental niches could be fundamental for driving fluxes in ecosystem functions. Quantifying changes in total abundance of functionally redundant species, rather than response diversity per se, is a promising approach for assessing the vulnerability of ecological communities and associated functions in a rapidly changing world. Our approach provides an important step toward understanding how species functional roles are linked to their responses to environmental change.
Chapter 3 – Exotic flies maintain pollination services as native pollinators decline with agricultural intensification

Abstract
Globally, agricultural intensification is a primary driver of declines in critical ecosystem services such as pollination. However, exotic species are often well-adapted to human-modified environments and could compensate for ecosystem services that are lost when native species decline. We measured pollination services (pollen delivery to stigma) provided by wild insects to a mass flowering crop, pak choi (Brassica rapa) at 12 sites across an agricultural intensification gradient in New Zealand. We found that pollination services increased as agricultural land-use intensified; pollination from exotic fly species exceeded the loss of pollination from native species. However, pollination service delivery became increasingly dominated by a few exotic fly species that were active throughout the day, compared to native species which had more constrained activity patterns. Exotic pollinators can play a key role in stabilising pollination services in highly modified agricultural systems. Despite the often far-reaching negative impacts of exotic species, we suggest positive contributions are possible, especially where anthropogenic disturbance has caused native species to decline. However, diverse native communities are likely to provide important insurance, stabilising ecosystem services against further environmental disturbance.

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3.1 Introduction

Ecosystem services are the subset of ecological functions that provide benefits to human society (Daily, 1997). Numerous studies have shown that ecosystem services are enhanced by natural habitats and the native species that use those habitats (Chaplin-Kramer et al., 2011; Garibaldi et al., 2011). One key ecosystem service is animal-mediated crop pollination, for which diverse wild species are important (Garibaldi et al., 2013; Rader et al., 2016). Globally, around 75% of crops benefit from animal pollinators to some degree and 35% of food consumed by humans can be attributed to pollination services provided by animals (Klein et al., 2007b). Paradoxically, although agriculture relies on ecosystem services such as pollination, agricultural intensification is a key threat to their maintenance (Foley et al., 2011; Tscharntke et al., 2005).

Agricultural intensification impairs pollination services because the associated loss of natural habitat causes declines in wild pollinator species (Potts et al., 2016; Winfree et al., 2009). However, some pollinators may be opportunists that actually benefit from agricultural intensification. If these species are effective crop pollinators, they could stabilize pollination services despite general declines in biodiversity (Cariveau et al., 2013; Stavert et al., 2017). Opportunist species are well adapted to human-modified environments and consequently, could sustain pollination services in landscapes subject to strong land-use change (Alpert, 2006; Pejchar & Mooney, 2009; Sax & Brown, 2000). Exotic species are often opportunists that are preadapted or adapt rapidly to novel ecological conditions, which are unfavourable for natives (Alpert, 2006; Byers, 2002). This is because human-mediated dispersal pathways inadvertently select for species with traits that facilitate survival in highly disturbed environments (introduced fish in California: Marchetti et al., 2004; e.g., introduced plants in Europe: Thompson et al., 1995).

If exotic species respond positively to agricultural intensification, they could maintain ecosystem services by compensating for declines in native species that are dependent on natural habitat. In pollination systems, compensation can occur either as a result of increased plant-
pollinator interaction rate or enhanced pollination efficiency of the remaining pollinator species (e.g., Cariveau et al., 2013; Hallett et al., 2017; Winfree & Kremen, 2009), or by the recruitment of novel species (e.g., Pattemore & Wilcove, 2012; Sanguinetti & Singer, 2014). In native ecosystems, exotic insects can pollinate plants whose native pollinators are now extinct or have declined (e.g., pollination of canopy trees in Amazonian forest fragments by African honeybees, Apis melifera scutellata: Dick, 2001; pollination of native orchids by exotic Apis and Bombus species in the Southern Andes: Sanguinetti & Singer, 2014). However, exotic pollinators can also reduce plant reproduction if they have lower pollination efficiency than native pollinators, due to poor trait matching. For example, in Japan, introduced Bombus terrestris have shorter tongues than native bumblebees, and are poorer pollinators of native plants (Kenta et al., 2007). Exotic pollinators can also have indirect negative effects via their interactions with native pollinators, as when honeybees, Apis mellifera, remove pollen previously deposited on stigmas by native bees (Gross & Mackay, 1998). Despite these few examples, the impact of exotic pollinators on pollination services is poorly understood since most studies have focused on the importance of native species, typically bees (Morales et al., 2017).

For both native and exotic pollinators, the effect of agricultural intensification can vary between taxonomic groups, potentially altering patterns of pollination service delivery. For instance, flies are often resilient to land-use change (Rader et al., 2016), whereas bees generally respond negatively (Garibaldi et al., 2011). Flies can forage at lower temperatures compared to bees and therefore, have broader temporal activity ranges (Rader et al., 2013a). Thus, if agricultural intensification benefits certain taxa over others, it could also alter the temporal patterns of pollination service delivery. This can affect seed production because flowers are often receptive at different times of the day (Potts et al., 2001; Spira et al., 1996).

Additionally, agricultural intensification is likely to reduce the evenness of pollination service delivery, as few pollinator species provide a greater contributions to pollination. This is because anthropogenic habitat modification typically favours the subset of species able to exploit novel
resources (Hillebrand et al., 2008). However, higher evenness allows communities to adapt more rapidly to new environmental conditions and consequently, sustain ecosystem functions. Therefore, although compensation by exotic species could buffer ecosystem services against land-use change, when it is accompanied by reduced evenness, this may diminish resilience to future environmental disturbance.

Here, we assessed pollination services across a gradient of agricultural intensification using a model crop, pak choi, *Brassica rapa* L. ssp. *chinensis* L. (Hanelt.). We focus on the conversion of natural habitat to agriculture because it is the leading cause of biodiversity loss worldwide (Pereira et al., 2010). *B. rapa* is an important global food crop and is visited by a diverse suite of wild pollinator species that vary in their ability to transfer pollen (Rader et al., 2012). Hence, we concentrated on four questions: (i) How does agricultural intensification affect aggregate pollination services provided by wild insects? (ii) Do exotic species compensate for any losses in pollination services provided by native pollinators with agricultural intensification? (iii) Does agricultural intensification cause a temporal shift in pollination service delivery? (iv) Does agricultural intensification cause a change in the evenness of species’ contributions to pollination services? We expected that agricultural intensification would cause a decline in aggregate pollination services, due to loss of pollination from native species. However, we predicted pollination services from exotic species would increase, providing some degree of compensation. Subsequently, we expected a temporal shift in pollination service delivery and a reduction in evenness of pollination services provided by different species with agricultural intensification.

### 3.2 Material and Methods

#### 3.2.1 Study system

We studied pollination services to a mass flowering crop “pak choi” *Brassica rapa* var. *chinensis* (Brassicaceae) that we planted on 12 selected farms along an agricultural intensification gradient, within an 80 x 60 km area in the Waikato Region, New Zealand. We
planted one 25 x 25 m plot of B. rapa at each farm. Plots were at least 3 km apart (range: 3-20 km) to ensure we sampled different pollinator communities (Greenleaf et al., 2007; Rader et al., 2011). Plots were planted on conventional farms to minimise inter-site variation in insecticide use, which can affect insect pollinator communities (Tuell & Isaacs, 2010). Planting at all sites occurred within two weeks in mid-Spring (October) 2014 to ensure flowering synchrony and reduce temporal variation in pollination services between sites.

We used B. rapa because it has an open access yellow flower, with six stamens, a single stigma and epigynous nectaries. B. rapa is an important mass flowering global food crop that shows increased seed production in the presence of insect pollinators (Rader et al., 2009) and is visited by a diverse assemblage of insects that vary widely in their pollen transfer ability (Rader et al., 2013a).

3.2.2 Landscape analysis

To classify and measure the proportion of agriculture (defined as pasture, orchards, vineyards and croplands) in the landscape surrounding our B. rapa plots, we used Land Cover Data Base version 4.1 (Land Resource Information Systems), with the most recent thematic classification of New Zealand’s land-use cover. We analysed these data using ArcGIS 10.3.1 (ESRI, 2011). We used agriculture as our primary disturbance because it was strongly inversely correlated with semi-natural habitat (defined as broadleaved indigenous hardwood, deciduous hardwood, exotic forest, Ulex europaeus (Fabaceae) and/or Cytisus scoparius (Fabaceae), indigenous forest and Leptospermum scoparium (Myrtaceae) and/or Kunzea spp. (Myrtaceae); Pearson’s r –0.93; P < 0.0001). For initial site selection, we used the ArcGIS Spatial Analyst tool to calculate the total area occupied by different land-use types at different scales (500 m and 2,000 m). We then preselected sites with a strong correlation in the proportion of agriculture at different radii (500 m and 2,000 m; Pearson’s r 0.93; P < 0.0001). We used 500 m and 2,000 m radii because these scales are important for different insect pollinators (Benjamin et al., 2013; Greenleaf et al., 2007; Rader et al., 2011) and thus, we were able to minimize the effects
of scale on species’ responses to agricultural intensification. Land-use intensity for subsequent analyses was defined as the proportion of agriculture at a 2,000 m radius surrounding the centre of each *B. rapa* plot, which resulted sites ranging from 42–99% agriculture.

### 3.2.3 Pollinator sampling

We sampled pollinator communities in summer, from December 2014 to January 2015. Each site was sampled on two separate days and only when the weather was sunny or partly cloudy with wind speeds < 5 m/sec and temperature > 15°C. Sites were sampled when the crop was nearing its peak bloom period (mean ± SE = 643.18 ± 4.13 flowers per m²).

To determine the visitation rate of wild insect pollinator species to *B. rapa* flowers, we conducted three 5 min focal observations along a fixed transect that ran through the centre of each plot, commencing 5 m from the plot edge. For each focal observation, we counted the number of open *B. rapa* flowers and the number of pollinator visits within a 0.25 m² quadrat. Pollinators were identified to species where possible but some taxa could not be identified to species level on the wing (native bee species in the genera *Leioproctus* and *Lasioglossum*). Therefore, species in these genera were grouped, and for brevity we use the term “species” to refer to the lowest taxonomic level that was discernable in the field.

We censused each plot on two separate days at standard times: 08:00, 10:00, 12:00, 14:00, 16:00, 18:00 and 20:00. This was to measure visitation from the full diurnal pollinator community and to quantify shifts in pollinator services from different taxa throughout the day. We recorded visits by honeybees, *Apis mellifera*, in our focal observations but omitted honeybees from community-level pollination service analyses because nearly all colonies in New Zealand are managed hives and therefore, not directly influenced by the surrounding land-use (Newstrom-Lloyd, 2013).
3.2.4 Single visit pollen deposition (SVD)

We used single visit pollen deposition (SVD) values for insect pollinator species to *B. rapa* in New Zealand as reported in Rader *et al.* (2009) and Howlett *et al.* (2011); a brief description of their methods follows.

The efficiency of different pollinator species was estimated by counting the number of pollen grains deposited on stigmatic surfaces by an individual insect in a single visit to a virgin flower. Unopened, virgin *B. rapa* inflorescences were covered with pollinator exclusion bags. Once flowers had opened, the bag was removed and flowers were observed until an insect visited and contacted the stigma. The stigma was then removed and stored in gelatine-fuchsin and the insect was captured for later identification. SVD was quantified by counting all *B. rapa* pollen grains on the stigma.

In our study, SVD values for three pollinator species that were frequent visitors (*Anthomyia punctipennis*, *Delia platura* and *Oxysarcodexia varia*), were not reported in Rader *et al.* (2009) or Howlett *et al.* (2011), and we were not able to collect adequate SVD sample sizes. Therefore, we used hairiness (body surface entropy) to calculate predicted SVD values for these species. Hairiness on the face and thorax regions is strongly predictive for SVD to *B. rapa* for insect pollinators in New Zealand (Stavert *et al.*, 2016). The best model for determining SVD to *B. rapa* includes the face and thorax dorsal regions as predictors. Accordingly, we used the following equation to calculate SVD values for *A. punctipennis*, *D. platura* and *O. varia*: $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2$, where $Y$ is the predicted SVD value, $\beta_0$ is the intercept, $\beta_1$ is the coefficient for the face entropy, $X_1$ is the entropy value for the face, $\beta_2$ is the coefficient for the thorax dorsal entropy and $X_2$ is the entropy value for the thorax dorsal.

3.2.5 Estimating pollination services

We estimated the pollination services provided by each species by dividing the number of visits within each observation session by the number of flowers in each quadrat, and then multiplying...
this value by the mean SVD value for each species (Kremen et al., 2002). We focus on this pollination service measure because it is an unambiguous quantification of the contribution and insurance value of wild pollinator species. In all analyses, species were only included if they were sufficiently abundant (minimum 15 visits observed) and relatively widespread (present at three or more sites).

3.2.6 Measuring seed production

We harvested seed from each *B. rapa* field once pods were ripe (approximately 2 weeks after flowering commenced). In each field, we harvested three plants randomly from 1 m² quadrats; four quadrats were placed 5 m from the corners of the plot and one was placed in the centre (total = 15 plants per site). For each plant, we counted the total number of developed and undeveloped pods. To estimate seed production per flower for each plant we calculated pod set rate by dividing the number of developed pods by the total number of flowers (developed + undeveloped pods). We then counted the number of seeds in 15 random developed pods on each plant. From this, seed production was calculated by multiplying pod set rate by the average number of seeds across the 15 pods.

3.2.7 Agricultural intensification’s impact on the evenness of species’ contributions to pollination services

Evenness of species’ contributions to pollination services was calculated for each site using the aggregate number of pollen grains deposited per pollinator species per flower. We used a spectrum of Hill’s diversity numbers ($\alpha = 0.25, 0.5, 1, 2, 4, 8$ and $\infty$) to quantify evenness of species’ contributions to pollination services (Hill, 1973). Hill’s diversity numbers differ in their sensitivity to the presence of rare species according to parameter $\alpha$; as $\alpha$ increases, the Hill function becomes less sensitive to rare species in the community. Thus, Hill’s diversity numbers are more robust than traditional diversity indices because they provide a continuum of possible diversity measures instead of a point description of community structure.
Additionally, Hill’s diversity numbers produce an evenness value that is normalised between 0 and 1, which is simple to interpret and compare across different communities.

### 3.2.8 Statistical analyses

To determine how agricultural intensification affects aggregate pollination services provided by wild insects we used a linear mixed effect model (LMM) with the `lme` function in the “`nlme`” package (Pinheiro et al., 2017). Fixed effects were the proportion of agriculture (continuous), time of the day (categorical) and the agriculture : time interaction. The agriculture : time interaction was included to determine if changes in pollination services across the agricultural gradient varied according to time of the day. Site was included as a random effect. The summed number of pollen grains deposited per 5 min observation for all species was the response and was log-transformed (x + 1) to meet the assumptions of normality and homoscedasticity. To further improve homogeneity of residuals, we included a constant variance structure using the `varIdent` function (Cleasby & Nakagawa, 2011) in “`nlme`” package. To identify the best model for aggregate pollination services, we performed small sample corrected Akaike Information Criterion (AICC) model selection on the global model (pollen deposition ~ agriculture + time + agriculture : time) using the `dredge` function in the “`MuMIn`” package (Barton, 2016). To determine the effect of agricultural intensification on *B. rapa* seed production we used a LMM. The response was seed production per flower and the fixed effect was the proportion of agriculture (seed production ~ agriculture). Site was included as a random effect. We included a constant variance structure using the `varIdent` function in “`nlme`” package to improve homogeneity of residuals. We also assessed the effect of exotic, native and honeybee visitation on seed production (seed production ~ exotic visitation rate + native visitation rate + honeybee visitation rate + all two and three-way interactions) using a LMM and model selection as described above.

To determine if exotic species compensate for loss of pollination services provided by native pollinators due to agricultural intensification, we used a zero adjusted gamma (ZAG) hurdle
linear mixed model (GLMM) (Zuur & Ieno, 2016). We used a ZAG hurdle GLMM because our data are continuous and zero inflated. Hurdle GLMMs consist of a binary component (presence-absence) and a continuous (presence only) component; for the binary component we used a Bernoulli distribution and for the continuous component we use a gamma distribution with a log link function. To fit both components of the hurdle GLMM we used the \textit{glmer} function in the \textit{"lme4"} package (Bates et al., 2015). The summed number of pollen grains deposited by either native or exotic species per 5 min observation was the response. Fixed effects were native status (native or exotic; categorical), the proportion of agriculture (continuous), time of day (categorical) and the three way interaction between the fixed effects (summed pollen deposition [exotic/native] \sim exotic/native + agriculture + time + exotic/native : agriculture + exotic/native : time + agriculture : time + exotic/native : agriculture : time). Time was included to determine if the relative pollination service delivery from exotic vs. native species at different times of the day varied with agricultural intensification. Site was included as a random effect. To identify the best model we used AICc model selection on the global model with the \textit{dredge} function in the \textit{"MuMIn"} package.

Finally, to test the effect of agricultural intensification on the evenness of species’ contributions to pollination services, we used a linear mixed effects model (LMM). The evenness value for Hill’s number ($\alpha = 1$) for each site was the response variable and proportion agriculture was the fixed effect. Site was included as a random effect. LMMs using evenness values calculated with additional Hill’s numbers ($\alpha = 0.25, 0.5, 2, 4, 8$ and $\infty$) were performed and are provided for comparison (see Appendix B; Table B4 and Figure B4). To determine the importance of the proportion agriculture fixed effect we used a Wald chi-square test in the \textit{“car”} package (Fox et al., 2016). We validated all fitted models by examining the distribution of residuals plotted against fitted values (Crawley, 2002; Zuur et al., 2009). All data were analysed in R version 3.2.4 (R Development Core Team, 2014).
3.3 Results

In total, we analysed 7,025 visits (97.16% of all recorded wild insect visits) from 11 wild insect pollinator species for which we observed a minimum 15 visits, and were present at three or more sites. This included three bee species (one exotic, two native) and eight fly species (six exotic, two native) (see Appendix B; Table B5). Native bees from the genera *Leioproctus* and *Lasioglossum* were not identifiable to species level on the wing, so were grouped within each genus.

3.3.1 How does agricultural intensification effect aggregate pollination services provided by wild insects?

We found that aggregate pollination services (pollen grains per flower) to *Brassica rapa* from wild insects increased with agricultural intensification (Figure 1; slope ± SE = 1.33 ± 0.75). The best model for predicting summed pollen deposition included agriculture, time and the agriculture : time interaction (see Appendix B; Table B1). Overall, pollination services from wild insects increased by 94.84% from low to high agricultural intensity sites (Figure 1). However, we found that agricultural intensification had no effect on seed production (slope ± SE = -1.99 ± 2.50; \(\chi^2_1 = 0.40; P = 0.53\); Figure B4). Surprisingly, neither exotic, native or honeybee visitation rate or the two and three-way interactions between these variables had an effect on seed production (see Appendix B; Table B3).
CHAPTER 3. EXOTIC SPECIES MAINTAIN POLLINATION SERVICES

3.3.2 Do exotic species compensate for loss of pollination services provided by native pollinators with agricultural intensification?

We found that exotic and native species varied in their contribution to pollination services with agricultural intensification and this was dependent on the time of the day (see Appendix B; Table B2 and Figure 1). Exotic species over-compensated for the loss of pollination services from native species with agricultural intensification (exotic species = 242.56% increase, native species = 80.81% decrease; Figure 1). The large increase in pollination services from exotic species was predominantly driven by the exotic fly, *Eristalis tenax*, which was an effective pollinator (Figure 2) and increased its pollination service delivery by 275.03% across the agricultural intensification gradient (Figure B2).

**Figure 1.** Model estimated pollination services for exotic species, native species and the full pollinator community, for each time period, across the agricultural intensification gradient. The dashed black line is the change in pollination services averaged across all time periods.
3.3.3 Does agricultural intensification cause a temporal shift in pollination service delivery?

Agricultural intensification caused a substantial shift in temporal patterns of pollination service delivery. Overall, pollination services from wild insects increased with agricultural intensification at all time periods, except in the late evening (Figure 1). This was driven by increased pollination services from exotic species, which deposited pollen throughout the day (Figure 2). The largest aggregate change was at 14:00, where pollination services increased by 307.97% with agricultural intensification. This was because pollination services from exotic species increased by 722.96%, whereas services from natives decreased by 25.09% (Figure 1). We also found substantial increases in aggregate pollination services in the morning, again due to large increases in pollination services from exotic species during this time period (whereas pollination services from native species decreased) (Figure 2).
3.3.4 Does agricultural intensification cause a change in the evenness of species’ contributions to pollination services?

The evenness of pollination services delivery from different species had a strong negative association with agricultural intensification (slope $\pm SE = -0.32 \pm 0.087; \chi^2 = 13.50; P < 0.001$; Figure 3). This trend was ubiquitous for all other Hill’s diversity numbers that we considered (see Appendix B; Table B4). Reduced evenness of species’ functional contributions was largely driven by the exotic fly *E. tenax*, which increased its relative contribution to aggregate pollination services from 39.70% to 77.97% with agricultural intensification.

**Figure 3.** Model estimated evenness of pollination services provided by different species, as determined by Hill’s diversity number ($\alpha = 1$), across the agricultural intensification gradient. The red shaded area inside the dashed red line denotes the confidence interval ($\pm 1$ SE).
3.4 Discussion

Numerous studies worldwide have found that agriculture is a principal driver of reduced ecosystem services (Foley et al., 2011). In contrast, we found that pollination services from wild insects increased with agricultural intensification, due to over-compensation by exotic pollinator species. Further, pollination services increased substantially in the morning and afternoon, reflecting the shift in pollinator community composition to exotic fly species, which were active throughout the day. Consistent with this, the evenness of pollination service delivery from different species decreased with agricultural intensification due to increased dominance of exotic species, particularly the exotic fly *Eristalis tenax*.

We found that wild exotic pollinators compensated for the loss of pollination services from native species with agricultural intensification. In contrast, most studies show that natural habitat enhances pollination services from wild insects because it provides food and nesting resources that support more abundant and diverse pollinator communities (Garibaldi et al., 2011). Our results agree with these studies for some species (i.e., native *Leioproctus* bees), but this is masked by large increases in pollen deposition from exotic insects. Many exotic species tolerate and adapt to a broad range of biotic and abiotic conditions, so they are resistant to anthropogenic disturbances (Schlaepfer et al., 2011). Exotic pollinators are typically more generalised in resource use than natives and thus have a competitive advantage in disturbed ecosystems (Kuppler et al., 2017).

Studies of exotic pollinators primarily address their negative impacts on natural ecosystems, such as competition with native species, pollination of invasive weeds, genetic introgression, alteration of native pollination networks and impact on native pollination function (Traveset & Richardson, 2006). Studies that consider potential positive contributions of exotic pollinators are scarce, and those that do mostly assess crop pollination from a few widely managed taxa (e.g., *Apis, Bombus* and *Megachile*). It is unknown how generalisable our results are to other crops worldwide, especially for crop species that have specialised reproductive structures. However, in native ecosystems, exotic species can compensate for the loss of endemic
pollinators (Pattemore & Wilcove, 2012; Sanguinetti & Singer, 2014). In any case, most crops are cultivated outside their native range and therefore rely largely on novel pollinator species with which they share no co-evolutionary history (Garibaldi et al., 2015). Thus, our findings could be more ubiquitous than is currently recognised.

Most exotic species in our study (five out of six) are flies. In high-intensity agroecosystems, flies rather than bees are likely to play key roles in buffering pollination services against environmental disturbance. Bees are central place foragers that usually travel only short distances from their nests and therefore, are directly affected by surrounding land-use (Greenleaf et al., 2007). In contrast, the agricultural matrix is more permeable to flies because they have no central nest location and commonly use resources in intensive agricultural landscapes (Rader et al., 2016). Yet most global pollination synthesises focus on bees (e.g., Kennedy et al., 2013; Kleijn et al., 2015) and fly contribution to pollination is unknown for many crops (Rader et al., 2016).

We found that, although aggregate pollination services from wild insects increased with agricultural intensification throughout the day (except the late evening), the magnitude of change was strongly temporally dependent. This was due to changes in the relative pollination contributions of species with different temporal foraging patterns. For example, native Leioproctus bees delivered most pollen in the late-morning and midday, whereas exotic E. tenax delivered pollen throughout the day, and these species responded very differently to agricultural intensification. Differences in the temporal foraging patterns of species could indicate potential response diversity (i.e., differing, species-specific responses to environmental disturbance) to future climate change (Rader et al., 2013b), because insect temporal foraging patterns and crop stigma receptivity are strongly linked to thermal constraints (Bishop et al., 2016; Willmer, 1983). Further, we only sampled during specific weather condition (i.e., warm, sunny, little wind); thus species that are unimportant pollinators under these conditions could increase their contributions in different weather conditions (Brittain et al., 2013). Nonetheless, shifts in pollinator community composition could have
serious implications for plants that are only receptive to pollen deposition at certain times of
the day or during particular seasons (Hoehn et al., 2008; Potts et al., 2001).

Despite reduced evenness in pollination service delivery from different species, we found that
pollination increased with agricultural intensification, mostly because a single species, *E. tenax*, increased its contribution to pollination services dramatically. This implies that
pollination services are reliant on fewer species, diminishing the insurance provided by
multiple species (Yachi & Loreau, 1999). However, ecological communities universally have
strong dominance in terms of species’ abundances (McGill et al., 2007) and there is growing
evidence suggesting that fluctuations in the abundance of dominant species is more important
for driving changes in ecosystem functions than species richness or community composition
(e.g., Cariveau et al., 2013; Dangles & Malmqvist, 2004; Kleijn et al., 2015; Smith & Knapp,
2003; Winfree et al., 2015). Although dominant species are important for ecosystem services
in simplified systems (e.g., pollination to a single crop), biodiversity often becomes more
important as temporal and spatial scales increase (Cardinale et al., 2012; Hillebrand &
Matthiessen, 2009). Further, we only consider the additive effects of biodiversity on pollination
services. Loss of synergistic effects (i.e., due to loss of species interactions) may further
exacerbate vulnerability of pollination services. Therefore, diverse pollinator assemblages are
likely required to ensure pollination services are robust to future environmental change.

Although we found substantial changes in pollination service delivery, yield (seed production)
remained stable across the agricultural gradient. Further, we found no association between seed
production and visitation rate from exotic species, native species or honeybees. Unlike pollen
deposition, factors determining yield are complex and include pollination services from wild
insects, in addition to pollination from managed honeybees, soil characteristics, climatic
variables and pest pressures (Bartomeus et al., 2015). Here, we focus on pollination service
delivery because it is an unambiguous measure of pollinator contribution and the insurance
value of diverse wild species.
3.4.1 Conclusion

Enhancing the performance and stability of pollination services from wild insects requires land-use practices that meet the habitat and resource requirements of diverse taxa. This necessitates detailed understanding of the basic biology and resource requirements of pollinator species, which is currently lacking (Klein et al., 2007b). It is also key that researchers identify life history traits that cause differential responses among species to land-use change and consequently, fluxes in ecosystem service delivery and stability. This is critical for informing land management decisions to enhance the robustness of ecosystem services, such as pollination.

Nonetheless, our findings show that where intensive agriculture causes loss of pollination services from native pollinator communities, exotic species could ameliorate or even over-compensate crop pollination service delivery. This challenges the prevailing view and suggests the potential positive impacts of exotic species on ecosystem services should be considered, particularly in systems subject to strong anthropogenic disturbance (i.e., > 90% agriculture), where native species have declined. Given that control or eradication for many exotic species is not feasible, it is critical that we quantify and recognise their potential positive contributions to ecosystem services. However, the role of exotic species in providing ecosystem services should not be considered in isolation from their impacts on natural ecosystems, which are often negative and far-reaching.
Chapter 4 – Changes to plant and pollinator numerical evenness alter network structure and seed production

Abstract

Worldwide, anthropogenic change is causing biodiversity loss, disrupting many critical ecosystem functions. However, most studies investigating the relationship between biodiversity and ecosystem functioning focus on one biodiversity component, species richness, predominantly for functions related to productivity. Consequently, there is limited understanding of how other biodiversity measures, such as species evenness (the relative numbers of individuals per species), affect complex multitrophic functions such as pollination. To address this, we manipulated plant and pollinator species’ abundances within large flight cages to test how differences in numerical evenness affect network structure and consequently, seed production. We found that community-level interaction evenness was highest when plant and pollinator communities were even. At the species level, floral visitation rate and visitation rate evenness were highest when pollinator communities were dominant, but plant communities were even. Overall, seed production declined with community-level interaction evenness, but increased with species-level visitation rate evenness. Thus, we show that numerical evenness, irrespective of species richness, composition and total abundance, can alter important aspects of plant–pollinator networks and consequently, plant reproduction. Understanding how species evenness affects ecosystem functioning is crucial as anthropogenic disturbances continue to alter species’ abundances, likely disrupting ecosystem functions long before extinctions occur.

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4.1 Introduction

The accelerating rate of global biodiversity loss has prompted many experimental attempts to disentangle the relationship between biodiversity and ecosystem functioning (Cardinale et al., 2012). In numerous studies, more diverse ecological communities have greater productivity, use resources more efficiently, and are more resistant to disturbances (Hooper et al., 2005; Loreau et al., 2001). However, a comprehensive understanding of how multitrophic ecosystem functions respond to biodiversity loss remains elusive. This is because most studies focus on how species richness affects productivity-based functions (e.g., plant biomass; Balvanera et al., 2006) rather than multitrophic functions, such as pollination and seed dispersal (Schleuning et al., 2015). Furthermore, other biodiversity components important for ecosystem functioning, such as the relative abundances of species (species evenness), have received little attention (Hillebrand et al., 2017; Lewandowska et al., 2016).

Ecological communities universally have a skewed species abundance distribution where a few species are common and the rest are rare (McGill et al., 2007). The degree to which communities exhibit this skewed abundance is described as ‘species evenness’. Anthropogenic disturbances generally reduce species evenness by homogenising resources in ecosystems, favouring a subset of species that then become dominant (Hillebrand et al., 2008). Species evenness often responds more rapidly to anthropogenic disturbance than other commonly-used measures such as species richness. This is because the relative abundance of different species can change long before extinctions occur, whilst still having major consequences for ecosystem functioning (Hillebrand et al., 2017). Although extinctions eliminate interactions between species, changes in the evenness of species’ abundances may alter species interactions in ways that cannot be detected by species richness studies (Dangles & Malmqvist, 2004).

The relevance of controlled biodiversity-ecosystem function (B-EF) experiments with high evenness for real-world ecosystem functions is contentious (Díaz et al., 2003; Thompson et al., 2005; Wardle, 1999). Most B-EF experiments are designed so that all species have equal abundance, which magnifies the positive effects of complementarity (different species
performing a function in a complementary way; Loreau & Hector, 2001). However, in nature, complementarity between species is typically reduced in comparison to experimental conditions, because species’ abundances vary widely, such that rare species contribute little to interactions (Winfree et al., 2015). Furthermore, in many B-EF experiments, function is driven by the selection (or sampling) effect, where functionally dominant species have a disproportionate influence on function. The same may occur in evenness–function experiments, where increased dominance could have either a positive or negative effect on function, depending on the dominant species’ functional performance (Jiang et al., 2009). Yet, in the real world, there is no reason to expect an association between species dominance and functional performance, especially for multitrophic functions such as pollination (Larsen et al., 2005). Thus, experiments can avoid artificial (researcher-based) selection effects by using species dominance patterns that better represent real-world communities.

There is a lack of consensus on evenness-function relationships. Varying associations between species evenness and ecosystem functioning are reported for a few studies, but these mostly focus on productivity-based functions (Hillebrand et al., 2017). Plant communities in low productivity systems generally have extremely uneven species abundance distributions, whereas those with high productivity fit a lognormal curve and thus, have higher evenness (Grime, 1998; McGill et al., 2007). Similarly, empirical studies show that primary productivity increases with higher species evenness (e.g., Mattingly et al., 2007; Nijs & Roy, 2000; Stevens & Carson, 2001; Wilsey & Potvin, 2000), although this relationship strongly depends on environmental variability and temporal scale (Norberg et al., 2001). In global crop systems, fruit production increases with pollinator species richness, but only when there is also an increase in pollinator species evenness (Garibaldi et al., 2015). In contrast, decomposition rate in streams was enhanced by reduced decomposer evenness (i.e., increased dominance) (Dangles & Malmqvist, 2004). Other studies have found that fluctuations in the abundance of dominant species, rather than changes to species richness, drive ecosystem functioning. For example, in grassland systems, primary productivity increased with the removal of rare species, indicating compensation from dominant species, probably via rapid response to increased
resource availability (Smith & Knapp, 2003). Similarly, fluxes in the abundance of dominant pollinator species, rather than changes in species richness or composition, drove changes in pollination function (Winfree et al., 2015).

Several studies have experimentally tested the effects of pollinator diversity on plant reproduction, finding that increased functional diversity (Albrecht et al., 2012; Fontaine et al., 2006) and species richness (Fründ et al., 2013) enhanced seed production. No study has explicitly investigated the effects of species evenness on functioning in plant-pollinator systems. Here, we experimentally manipulated plant and pollinator evenness, independent of species richness, composition and total abundance, to investigate the effects of evenness on plant–pollination interaction networks and subsequently, plant reproduction (seed production). Within cages, we assembled plant and pollinator communities with standardised abundances, species richness and composition. We then quantified seed production for seven plant species and assessed whether changes in evenness altered plant-pollinator interactions and consequently, seed production. We focused on three key questions: (i) Does evenness affect plant-pollinator interactions; if so, (ii) how does evenness effect species-level seed production; and, (iii) how does evenness effect community-level seed production? We predicted that increased evenness would increase complementary interactions between species within plant–pollinator networks. Consequently, we expected that increased evenness would enhance species-level and community-level seed production due to greater complementarity effects.

4.2 Methods

4.2.1 Experimental design

We constructed 24 mesocosms (each 2.4 m × 3 m x 2 m = 14.4 m³) with fine netting to ensure insect pollinators could not escape or enter cages (0.58 mm; Cropsafe Protection Mesh, Cosio Industries Ltd, Auckland, New Zealand). Cages were closed when the first plants were introduced (beginning 30 November 2015) and were inspected every 2-3 days until the start of the experiment, to ensure no insect pollinators were present. The experiment commenced on
26 December 2015 when pollinators were introduced into the cages. All pollinators were removed from cages on 11 January 2016. Fruits were harvested over a four week period, from 28 January to 26 February 2016. The experiment was conducted in Hamilton, New Zealand (37°46’4”S; 175°18’45”E), in spring and summer.

The experimental design involved four treatments: high pollinator dominance and high plant dominance (DomPoll – DomPlant); high pollinator dominance and high plant evenness (DomPoll – EvenPlant); high pollinator evenness and high plant dominance (EvenPoll – DomPlant) and high pollinator evenness and high plant evenness (EvenPoll – EvenPlant) (Figure 1a and b). All pollinator and plant species were present in all cages, and total abundance for pollinators and plants was equal across cages.

We determined the number of individuals to include in our experiments for each plant and pollinator species in dominant communities using the “rpartitions” package (Locey & McGlinn, 2012). Relative species’ abundances in dominant treatment communities were calculated by simulating species abundance distributions (SADs) (Locey & White, 2013). We created uniform random partitions for dominant communities, considering the total sum of individuals (30 for pollinators and 140 for plants) and the number of species (five for pollinators and seven for plants; Figure 1a and b) in the community. We then retained simulated communities with an SAD dominance skew in the 99th percentile, so that dominant communities were representative of those with highest probable numerical dominance in nature. Even treatment communities had equal numbers of individuals for each species. Each treatment was replicated five times (i.e., four treatments × five replicates = 20 cages). We included four control cages, from which all insect pollinators were excluded. Control cages were inspected daily to ensure no pollinators were present.
4.2.2 Plant community

Seeds for the plants were purchased from commercial suppliers (Kings Seeds NZ Ltd; South Pacific Seeds NZ Ltd; Awapuni Nurseries Ltd; Egmont Seed Company Ltd). Seeds were initially sown into standard biodegradable seedling trays and individual seedlings were later planted out into standardised 1.7L pots. We used commercial grade organic soil (Top Soil and Sand Ltd, Hamilton, New Zealand). The sowing date for different plant species was staggered, based on the estimated number of days to flowering, to ensure flowering synchrony among different plant species. Once planted into pots, all plants were transferred directly into the cages. Plants were watered daily (at night) for 20 mins with an automatic watering system. We controlled unsown weedy plants by regular hand removal.

We used seven species in our plant communities that are pollinator dependent to some degree: *Brassica rapa* (Brassicaceae), *Fagopyrum esculentum* (Polygonaceae), *Coriandrum sativum* (Apiaceae), *Centaurea cyanus* (Asteraceae), *Cucurbita pepo* (Cucurbitaceae), *Lupinus hartwegii* (Fabaceae) and *Salvia farinacea* (Lamiaceae). These species represent diverse floral structures that vary in size, colour and accessibility to pollinators. *B. rapa* has a yellow, cruciferous flower that is hermaphroditic with fully exposed reproductive organs (Dixon, 2007). *F. esculentum* has white, actinomorphic flowers that are hermaphroditic with exposed reproductive organs (Cawoy et al., 2009). *Coriandrum sativum* has white/pink flowers in umbels (Diederichsen, 1996). The peripheral flowers are hermaphroditic and have exposed reproductive organs whereas the central flowers are stamine and often sterile. *Centaurea cyanus* has blue flowers aggregated in an inflorescence (Penet et al., 2012). The peripheral ray florets are sterile but the central disk florets are fertile and hermaphroditic with exposed reproductive organs. *Cucurbita pepo* has yellow monocious flowers; male flowers are produced 3-4 days before female flowers (Abu-Hammour & Wittmann, 2010). Staminate *Cucurbita pepo* flowers have three large anthers and pistillate flowers have a thick style and a large stigma, both have a showy corolla. *L. hartwegii* has a hermaphroditic papilionoid blue/purple flower with the reproductive organs enclosed within the corolla (Tucker, 2003). *S. farinacea* has a labiate purple flower with the reproductive organs partially enclosed within the corolla.
To pollinate *S. farinacea*, insects must trigger a lever type mechanism in early stage (male) flowers, which results in pollen deposition on the insect. The insect must then visit a late stage (female) flower to transfer pollen to the stigma.

We did not have data on plant species dominance in real-world ecosystems, so this was determined based on the accessibility of the floral reproductive structures. Highly accessible (generalist) species were selected as dominants, while less accessible (specialist) species were sub-dominants (Figure 1a). Plant species with specialist pollination structures are usually rarer and have greater extinction risk due to loss of their specialist pollinators (Aguilar et al., 2006; Aizen et al., 2012; Bond, 1994; Johnson & Steiner, 2000).

### 4.2.3 Pollinator community

We selected pollinator species that were representative of the local pollinator community (Figure 1c) (see Stavert et al., 2017; chapter 2). This included two fly species (*Eristalis tenax* and *Oxysarcodexia varia*), one social bee species (*Bombus terrestris*), one small solitary bee (*Megachile rotundata*), and one large native solitary bee (*Leioproctus paahaumaa*). We were unable to source adequate populations of small native bees (*Lasioglossum*), so we used *M. rotundata* as a substitute. All pollinators were introduced into cages on the same day (26 December 2015). Flies (*E. tenax* and *O. varia*) were collected from a nearby field site and transferred directly into cages. Bumblebees (*B. terrestris*) were purchased in small colonies (Zonda Beneficials Ltd, Auckland, New Zealand) and modified to match the target number of workers for each cage. Colonies included a queen and brood to encourage natural foraging behaviour. Excess *B. terrestris* workers were removed daily to ensure that the target number of workers was maintained. We translocated native bee (*L. paahaumaa*) larvae from nearby nest aggregations to artificial nest boxes 3-4 days before the experiment commenced. We only translocated larvae that were in a late developmental stage (~1 week from emergence). Since *L. paahaumaa* are ground nesting (Donovan, 2007), we provided bare soil nest sites within cages to encourage natural nesting behaviour, and we regularly observed female bees.
provisioning nests within cages. *M. rotundata* larvae were incubated at 28°C for 30 days so that adult emergence was synchronised with the predicted start date of the experiment. *M. rotundata* that hatched early were stored in dark chambers until the experiment began. Within the cages, we provided wooden branches with pre-drilled 4 mm holes as nest sites for *M. rotundata* and observed females actively provisioning nests during the experiment. The pollinator community dominance order was determined based on that for sites with strong land-use change in the surrounding landscape (i.e., > 85% agriculture; Figure 1) (Stavert et al., 2017; chapter 2).

**Figure 1.** Abundances of species in (a) even vs. dominant experimental plant communities; (b) even vs. dominant experimental pollinator communities; and (c) real-world pollinator communities with > 85% agriculture at 2,000 m radius (data from Stavert et al., 2017; chapter 2). *Megachile rotundata* was substituted for *Lasioglossum sordidum* in cages because the latter species could not be collected in sufficient numbers.

### 4.2.4 Plant–pollinator interaction rate

We conducted pollinator visitation observations for two weeks (27 December 2015 to 10 January 2016). To quantify plant–pollinator interaction rates, we recorded all visits from pollinator species to flowers on each of the seven plant species during 3 min observation sessions. This resulted in a total observation time of 21 mins per cage per observation session. At the end of each observation session we recorded the number of open flowers for each plant.
species. Visitation rate for each pollinator species to different plant species was calculated as the number of visits per flower per 3 mins. Five observation sessions were conducted per day (09:30-11:00; 11:00-13:00; 13:00-15:00; 15:00-17:00; 17:00-18:30). On any given sampling day within each cage, visitation was observed for two time periods and never at consecutive time periods. In each cage, visitation was sampled on at least three separate days for all observation sessions. We randomly assigned identification numbers to cages to ensure that observation order was independent of cage treatment and spatial arrangement. Observations were only performed when the weather was sunny or partly cloudy with wind speeds < 5 m/sec.

### 4.2.5 Seed production

To quantify ecosystem function we measured seed production per flower for each plant species, which provided an unambiguous measure of pollination success. During the experiment, we marked open flowers using fine wire strips. Fruits from marked flowers were picked once they had ripened sufficiently (28 January to 26 February 2016). Harvested fruits were dried and seeds were counted in the laboratory.

### 4.2.5 Statistical analyses

*Does evenness affect plant-pollinator interactions?* We calculated various plant-pollinator interaction metrics for each community (cage) and determined their relative importance for seed production. Firstly, *visitation rate* is the number of pollinator visits to flowers for each plant species per 3 min observation period. To test for differences in visitation rate between treatments, we fitted a *generalized linear mixed effect model* (GLMM) with a gamma distribution and a log link function, using the “lme4” package (Bates et al., 2014). In this model, visitation rate was the response variable and treatment (categorical) was the fixed effect. We included cage number and plant species as random effects.
Second, visitation rate evenness is a measure of the equitability of visits from pollinator species to a given plant species, taking into account the total number of open flowers for that plant species. To measure visitation rate evenness of the pollinator community to each plant species in each cage we calculated Pielou’s $J$ (Pielou, 1969). Visitation rate evenness is bounded by zero and one, where a value of one for a given plant species means visits from pollinator species are uniformly distributed. To test for differences in visitation rate evenness ($Pielou’s \ J$) between treatments, we fitted a GLMM with a gamma distribution and a log link function. Visitation rate evenness was the response variable and treatment (categorical) was the fixed effect. Cage number and plant species were included as random effects.

Third, floral niche complementarity describes the extent of similarity in the plant species that pollinator species visit. We calculated functional niche complementarity for each pollinator community using the $fc$ function in the “Bipartite” package (Dormann et al., 2016). Firstly, we created a matrix of interaction frequencies for cages by summing the number of visits from pollinator species to plant species across all sampling periods. Using these data we created a distance matrix (Canberra distance, to ensure pairwise distances were not unduly weighted towards species with high visitation rates; Warton et al., 2012) and subsequently, generated a dendrogram wherein pollinator species were clustered according to similarities in the plant species they visited. Branch length distances between pollinator species are equivalent to dissimilarity in the plant species that each pollinator visits. Thus, the longer branch lengths are between two pollinator species, the more dissimilar are the plant species they visit. Finally, total dendrogram branch length was calculated, which represents the degree of floral niche complementarity for each pollinator community (Dormann et al., 2009). Greater floral niche complementarity occurs when pollinator species specialise on different plants (longer branch length), whereas lower floral niche complementarity (shorter branch length) occurs when the pollinator community is more generalist. To test the effect of treatment on floral niche complementarity we used a linear mixed effect model (LMM) (total dendrogram branch length was the response variable, treatment was the fixed effect and cage number was a random effect) in the “nlme” package (Pinheiro et al., 2017).
Finally, interaction evenness is a measure of the equitability of visits from pollinator species to plant species at the community-level and thus equals one where pollinator-plant interactions are uniformly distributed between species (Tylianakis et al., 2007) as given by:

\[
I.E = \frac{\sum p_i \log_2(p_i)}{\log_2 N}
\]

where \(p_i\) is the proportion of the total number of plant-pollinator interactions \((N)\), represented by interaction \(i\). We measured interaction evenness using the networklevel function in the “Bipartite” package (Dormann et al., 2016). We summed the number of visits from pollinator species to plant species across all sampling periods, as with the floral niche complementarity analysis. To test the effect of treatment on interaction evenness we used a LMM (interaction evenness was the response variable, treatment was the fixed effect and cage number was a random effect). Finally, we compared each metric between treatments using Tukey’s HSD in the “lsmeans” package (Lenth, 2016).

To investigate how the above interaction metrics effect seed production, we fitted a GLMM with a negative binomial distribution and a log link function (Zuur & Ieno, 2016). The negative binomial error structure allowed us to account for overdispersion in the data, caused by a large number of zero seed production values. GLMMs were constructed using the “glmmADMB” package, which has two options for fitting the association between the mean and the variance: family = ‘nbinom1’, which assumes variance = \(k \times \text{mean}\), and family = ‘nbinom2’, which assumes variance = \(\text{mean}(1 + \text{mean}/k)\) (Fournier et al., 2012). We fitted models with each of these options and selected the model with the lowest Akaike information criterion (AIC) score, which most parsimoniously explained the greatest degree of variance in our data. In this model, the number of seeds produced per flower was the response variable and the plant-pollinator interaction metrics (floral niche complementarity; visitation rate; visitation evenness; interaction evenness) were fixed effects. We included cage number and plant species as random
effects. On the global model, we performed $AIC_C$ model selection using the “MuMIn” package (Barton, 2016). We then used model averaging to calculate estimates for the 95% confidence set of best-ranked models (models with cumulative Akaike weight $\geq 0.95$) (Symonds & Moussalli, 2011). We presented a visual representation of the estimates for fixed effects that were significant (Wald Z tests, $\alpha = 0.05$) following model averaging.

*How does evenness effect species-level seed production?* To test if seed production for different plant species varied between treatments, we used a GLMM with a negative binomial distribution and a log link. For this model, the number of seeds produced per flower was the response and plant species identity (categorical), treatment level (categorical) and the plant species : treatment interaction were fixed effects. We included cage number as a random effect. Further, to determine the importance of the plant species : treatment interaction, we applied type III analysis of variance (ANOVA) with a chi-square statistic in the “car” package (Fox *et al.*, 2016). Finally, we compared seed production for each plant species between treatments using Tukey’s HSD in the “lsmeans” package (Lenth, 2016). All fitted models were validated by examining the distribution of residuals plotted against fitted values (Crawley, 2002; Zuur *et al.*, 2009). All statistical analyses were conducted in R version 3.3.3 (R Development Core Team, 2014).

*How does evenness effect community-level seed production?* We quantified plant community-level function for each treatment by calculating seed production over a range of threshold levels using the “multifunc” package (Byrnes *et al.*, 2014). This approach allowed comparison of seed production for multiple plant species, simultaneously between treatments, and over a range of thresholds, providing a more comprehensive picture of how evenness affects community-level function. Threshold levels covered the full range of possible percentage values (1-99%) of maximum observed seed production for each plant species. In order to reduce the potential effect of outliers, maximum seed production was estimated by averaging the five highest seed production values per plant species. For each threshold level, linear models were fitted using the “multifunc” package (number of functions $\geq$ threshold was the response and treatment was
the predictor). We then performed Tukey’s HSD on models for each threshold level, to compare community-level seed production between each treatment, using the “lsmeans” package (Lenth, 2016). We visualised community-level seed production by plotting the estimated number of plant species in each treatment producing seed ≥ control, across all threshold levels. Finally, we computed a range of multifunctionality metrics ($T_{\text{min}}$, $T_{\text{max}}$, $T_{\text{mde}}$, $R_{\text{mde}}$, $P_{\text{mde}}$, $M_{\text{min}}$, $M_{\text{max}}$) for each treatment, to examine the relationship between evenness and community-level function (Byrnes et al., 2014). $T_{\text{min}}$ is the lowest threshold where community-level seed production for a given treatment differs significantly from another treatment. $T_{\text{max}}$ is the highest threshold where community-level seed production for a given treatment differs significantly from another treatment. $T_{\text{mde}}$ is the threshold range, where each treatment has its strongest positive effect on community-level seed production. $R_{\text{mde}}$ are the model estimates for the number of plant species producing more seed compared to the control at $T_{\text{mde}}$. $P_{\text{mde}}$ is $R_{\text{mde}}$ divided by the number of plants species in each community. $M_{\text{min}}$ is the number of functions achieving the threshold at $T_{\text{min}}$. $M_{\text{max}}$ is the number of functions achieving the threshold at $T_{\text{max}}$. $N_{\text{func}}$ is the number of functions (plant species) in each community.

4.3 Results

4.3.1 Does evenness affect plant–pollinator interactions?

In total, we recorded 32,480 plant–pollinator interactions across all treatments. At the community level, evenness had no effect on pollinator floral niche complementarity (Figures 2 and 3); pollinator species tended to visit the same suite of flower species regardless of evenness treatment. However, we found substantially lower interaction evenness (community-level interaction equitability) in cages with dominant pollinator and dominant plant communities (DomPoll – DomPlant), compared with other treatments (Figure 3). Further, cages with the DomPoll – DomPlant treatment had lower visitation rate evenness (species-level interaction equitability) and visitation rates (species-level interaction rate) compared to cages with dominant pollinator and even plant communities (DomPoll – EvenPlant).
Figure 2. Bipartite networks of plant and pollinator communities for different treatments. Thickness of links between each plant and pollinator partner represents the relative number of interactions for that treatment. Pollinator species are at the top of plots and plant species are at the bottom. Plants and pollinators are ordered according to dominance rank (for treatments with dominant communities), with the most dominant species on the left and the least dominant species on the right.
Both community-level and species-level interaction evenness had strong effects on seed production. Community-level interaction evenness had a negative effect on seed production and was present in four out of five confidence set models (model-averaged slope = -0.96; \( SE = 0.40; Z = 2.39; P = 0.017; \) Table 1; Figure 4). Species-level visitation rate evenness had a strong positive effect on seed production and was present in all 95% confidence set models (model-averaged slope = 1.00; \( SE = 0.23; Z = 4.41; P < 0.0001 \)). Visitation rate (model-averaged slope = -0.17; \( SE = 0.76; Z = 0.22; P = 0.83 \)) and functional complementarity (model-averaged slope = -0.01; \( SE = 0.01; Z = 0.87; P = 0.38 \)) had weak negative effects on seed production and were present in only two of five confidence set models.
Table 1. 95% confidence set of best ranked models (models with cumulative Akaike weight, \( w_i \), ≤ 0.95) investigating the effects of plant–pollinator interaction metrics on seed production. Values in bold are model-averaged coefficients for the 95% confidence set.

<table>
<thead>
<tr>
<th>Visitation rate</th>
<th>Visitation evenness</th>
<th>Functional niche complementarity</th>
<th>Interaction evenness</th>
<th>( df )</th>
<th>AICc</th>
<th>( \Delta i )</th>
<th>( w_i )</th>
<th>acc ( w_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>0.96</td>
<td>-0.02</td>
<td>-1.04</td>
<td>8</td>
<td>32888.4</td>
<td>0</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>0.98</td>
<td>-</td>
<td>-0.93</td>
<td>7</td>
<td>32889.2</td>
<td>0.8</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>-0.60</td>
<td>1.08</td>
<td>-0.02</td>
<td>-1.06</td>
<td>9</td>
<td>32890.2</td>
<td>1.8</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>-0.59</td>
<td>1.10</td>
<td>-</td>
<td>-0.95</td>
<td>8</td>
<td>32891.0</td>
<td>2.6</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>0.93</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>32893.0</td>
<td>4.6</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>-0.17</td>
<td>1.00</td>
<td>-0.01</td>
<td>-0.96</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Model regression plane for plant-pollinator interaction metrics that had statistically significant effects (\( \alpha = 0.05 \); determined using Wald Z tests on model-averaged parameters from the 95% confidence set) on seed production.
4.3.2 How does evenness effect species-level seed production?

We found a strong association between plant species identity and effect of evenness treatment ($\chi^2_{18} = 104.54; P < 0.0001$) indicating that, overall, seed production for different plant species varied according to treatment (Figure 5). However, we found no differences in seed production between treatments for five of the seven plant species in our experiment (all $P$ values $> 0.05$; Figure 5). The hermaphroditic open-access flower, *Fagopyrum esculentum*, had greater seed production in DomPoll – DomPlant compared with EvenPoll – EvenPlant (Figure 5). The monecious flower *Cucurbita pepo* had highest seed production in the DomPoll – DomPlant treatment (Figure 5).

![Figure 5](image)

**Figure 5.** Seeds produced per flower for each plant species in different treatments. Dots are actual seed production values and violins illustrate the data probability density. Black horizontal lines are mean seed production values. Letters above violins indicate statistical differences in seed production between treatments, as determined by Tukey’s HSD ($\alpha = 0.05$).
4.3.3 How does evenness effect community-level seed production?

EvenPoll – DomPlant had the highest community-level seed production (Figure 6). This treatment had a maximum of 5.3 plant species producing more seed than the control (at the 33-40% threshold levels; Table 2; Figure 6). EvenPoll – DomPlant had significantly more plant species with higher seed production compared with DomPoll – EvenPlant (thresholds 79-90% and 95-96%) and EvenPoll – EvenPlant (thresholds 81% and 83-86%). However, differences were relatively small (1.0 – 2.2 more plant species producing seed above the threshold).

Table 2. Multifunctionality metrics for different evenness treatments. NAs are given where a treatment did not differ significantly in seed production from other treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$T_{min}$</th>
<th>$T_{max}$</th>
<th>$T_{mde}$</th>
<th>$R_{mde}$</th>
<th>$P_{mde}$</th>
<th>$M_{min}$</th>
<th>$M_{max}$</th>
<th>$N_{func}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DomPoll - EvenPlant</td>
<td>0.79</td>
<td>0.96</td>
<td>0.33-0.37</td>
<td>5.1</td>
<td>0.73</td>
<td>1.55</td>
<td>0.4</td>
<td>7</td>
</tr>
<tr>
<td>EvenPoll - EvenPlant</td>
<td>0.81</td>
<td>0.86</td>
<td>0.22</td>
<td>4.7</td>
<td>0.67</td>
<td>1.35</td>
<td>1.15</td>
<td>7</td>
</tr>
<tr>
<td>EvenPoll - DomPlant</td>
<td>0.79</td>
<td>0.96</td>
<td>0.33-0.40</td>
<td>5.3</td>
<td>0.76</td>
<td>3.55</td>
<td>1.4</td>
<td>7</td>
</tr>
<tr>
<td>DomPoll - DomPlant</td>
<td>$NA$</td>
<td>$NA$</td>
<td>0.33-0.39</td>
<td>5.3</td>
<td>0.76</td>
<td>$NA$</td>
<td>$NA$</td>
<td>7</td>
</tr>
</tbody>
</table>
4.4 Discussion

In this first experiment investigating the effects of plant and pollinator numerical evenness on plant reproduction, differences in evenness caused important changes to plant–pollinator network structure. At the species level, visitation rate and visitation rate evenness were highest when the pollinator community was numerically dominated by a few species, but the plant community was even. However, at the community level, interaction evenness was highest where both plant and pollinator communities were even. Overall, seed production was negatively affected by community-level interaction evenness but was positively associated
with species-level visitation rate evenness. Considering this trade-off, community-level function was highest when the number of individuals per species in the pollinator community was even, but the plant community was dominant (where plant species had uneven numbers of individuals).

The majority of floral visits across treatments were provided by two pollinator species, a hoverfly (*Eristalis tenax*) and a bumble bee (*Bombus terrestris*). In particular, *B. terrestris* was a broad generalist, dominating visits to most plant species. Yet, although *E. tenax* and *B. terrestris* dominated interactions, this is not a selection effect as the pollinator species dominance order was derived from data from real-world pollinator communities. Furthermore, although *B. terrestris* displayed relatively high visitation frequency, it was not numerically dominant in any treatment. In contrast, the fly *Oxysarcodexia varia* was second in the dominance order but contributed the least visits of any pollinator species. This shows that in this case, pollinator numerical dominance was not linked to interaction rate. This contradicts the assumption that species’ interaction frequencies (Vázquez *et al.*, 2005) and/or functional contributions (i.e., the mass ratio hypothesis; Grime, 1998) are relative to their abundances. It is also important to consider that pollinator functional performance is a product of interaction rate and pollen deposition effectiveness, so the most frequent floral visitor is not necessarily the most important or effective pollinator (Kremen *et al.*, 2002; Mayfield *et al.*, 2001). There is no reason to expect an association between pollinator numerical dominance and pollen deposition effectiveness.

Although numerical dominance did not directly translate to functional dominance (visitation frequency), there were clear differences in network structure between treatments. At the community level, interaction evenness was substantially lower in cages with dominant pollinator and plant communities (DomPoll – DomPlant), compared to all other treatments. This was because *E. tenax* and *B. terrestris* strongly dominated visits to most plant species and the dominant plant species, *B. rapa*, received a disproportionate number of pollinator visits across treatments. However, evenness had no effect on community-level floral niche
complementarity. This contradicts the prediction that pollinator floral niche complementarity should be greatest when pollinator and plant communities are even (EvenPoll – EvenPlant), due to high interspecific competition for floral resources (Hillebrand et al., 2008). These results could indicate that in our experiment, interspecific competition was too similar between treatments to drive variation in pollinator floral niche differentiation (i.e., there were sufficient floral resources for pollinators in all treatments). For example, in a study where bee species richness was manipulated in cages, high species richness increased functional complementarity due to greater interspecific competition and subsequent niche separation between bee species (Fründ et al., 2013). Thus, changes in niche complementarity could be particularly sensitive to species invasions and extinctions, rather than changes in evenness (Hillebrand et al., 2008; Hooper et al., 2005).

At the species level, visitation rate and visitation rate evenness were both lowest in DomPoll – DomPlant and highest in DomPoll – EvenPlant treatments. Interestingly, the dominant plant species, B. rapa, had a much higher visitation rate in even plant treatments. This could be because the increased conspecific density of the dominant plant species reduced visitation rate, so that intraspecific competition for pollinators exceeded intraspecific facilitation (as occurs for pollinator attraction via large conspecific floral displays; Brys et al., 2008; Dauber et al., 2010; Rathcke, 1983). For a plant, the trade-off between intraspecific competition for pollinators and the benefits of having conspecific floral displays could regulate dominance in real-world plant communities. Intraspecific competition for pollinators may limit dominance by any one plant species, thus facilitating the persistence of other species in the community. Interspecific competition for pollinators may also increase with increasing plant species evenness, reducing pollination of the dominant plant species (e.g., via increased interspecific pollen transfer, pollen loss and hybridisation) (Morales & Traveset, 2009; Pauw, 2013). This could explain why there were no clear differences in seed production between treatments in our study. Intriguingly, the rare species, Lupinus hartwegii, had higher visitation rates in even plant treatments. However, instead of reduced intraspecific competition for pollinators, as with B. rapa, this was possibly due to the positive effects of larger conspecific floral displays and/or
reduced interspecific competition for pollinators with more abundant plant species. Further investigation of how species evenness affects plant competition for pollinators is clearly required for understanding the relationship between species evenness and pollination function.

Seed production was highest where there was community-level interaction asymmetry (i.e., interactions dominated by a few plant and pollinator species), but where the frequency of those interactions was relatively even between species. This was because interaction evenness had a negative effect on seed production, indicating that greater community-level interaction asymmetry increased functioning. In contrast, visitation rate evenness at the species level had a strong positive effect on seed production. Thus, lower community-level interaction evenness could enhance seed production when the average performance of the pollinator species that dominate interactions is greater than that of the other species in the community (Jiang et al., 2009). We did not measure pollinator effectiveness (single visit pollen deposition) to plant species in our study, but other studies show that, at least for *B. rapa*, *E. tenax* and *B. terrestris* are relatively effective pollinators (Howlett et al., 2011; Rader et al., 2009). Where a subset of pollinators dominate interactions, greater visitation rate evenness between those species could enhance seed production in several ways. For example, if different pollinator species preferentially visit flowers at different heights (e.g., Hoehn et al., 2008), greater visitation rate evenness between species should increase seed production. Complementary interactions could also occur at the individual flower level, where behavioural differences between pollinator species cause variation in pollen transfer, increasing seed production (e.g., Chagnon et al., 1993). In addition, pollinator species could display temporal complementarity such as visiting flowers at different times of the day (e.g., Hoehn et al., 2008) or environmental complementarity such as visiting flowers in different weather conditions (e.g., Brittain et al., 2013; Fründ et al., 2013).

At the community level, pollination function was highest in EvenPoll – DomPlant, although statistical differences were only detected between treatments under high seed production thresholds. This indicates that if plants require high seed production rates to maintain
populations, then communities with high pollinator evenness but low plant evenness are optimal. Indeed, for monoculture crops, increased pollinator evenness enhances fruit set rate (Garibaldi et al., 2015). Greater plant dominance could reduce the probability of heterospecific pollen transfer, increasing seed production (Morales & Traveset, 2009), although we did not measure this directly. Yet, despite some clear differences in community-level function between treatments, these differences were relatively small. For example, there were up to two plant species more producing seed above the threshold in EvenPoll – DomPlant compared with DomPoll – EvenPlant. Nevertheless, when translated to real-world plant communities, which are often much more species rich (see Wilson et al., 2012), this effect could be greatly magnified.

We found that two out of the seven plant species differed significantly in seed production between treatments. Firstly, the open-access *Fagopyrum esculentum* produced more seed in DomPoll – DomPlant compared to EvenPoll – EvenPlant. Reduced *F. esculentum* seed production in EvenPoll – EvenPlant was possibly because of excessively high visitation from a single pollinator, *B. terrestris*, which in an even plant community, could increase heterospecific pollen transfer (Morales & Traveset, 2008). Extremely frequent visitation can also result in removal of pollen from stigmas without pollen replacement, increased conspecific pollen deposition (geitonogamy) and damaged floral reproductive structures (Morales et al., 2017). Broadly speaking, the benefits of visitation increase asymptotically with interaction frequency, whereas the associated costs increase linearly, so a mutualistic interaction can shift to an antagonism at very high interaction frequencies (Aizen et al., 2014). Excessive visitation from a single pollinator species has been shown to reduce seed production in other plants, including raspberry (Sáez et al., 2014) and native wild flowers (Magrach et al., 2017). *Cucurbita pepo* had reduced seed production in treatments with high plant evenness, although this was possibly driven by higher conspecific density and thus increased intraspecific competition for pollinators (Ghazoul, 2006; Rathcke, 1983). Data on the effectiveness of different pollinators for plant species in our study, and the effects of excessive visitation, are required to better understand species-level variation in seed production.
4.4.1 Conclusion

Differences in species evenness can cause important changes to plant–pollinator interaction network structure, with both positive and negative effects on seed production. Contrary to predictions, greater species’ evenness did not positively affect seed production at the species or community level. Thus, the positive effects of species richness on ecosystem functioning, as reported by many studies, may not hold for complex multitrophic functions, where communities have realistic species abundance distributions. We also found that species’ abundances did not directly correspond with their functional contributions. Therefore, at least for multitrophic functions, future studies should consider evenness of interactions, rather than species numerical evenness \textit{per se}. For pollination systems, these experiments should take into account the differences in plant species’ floral abundances and pollinator visitation rates. This current study lays important groundwork for future research attempting to identify the effects of species evenness on ecosystem functioning. Improving this understanding is particularly important as anthropogenic disturbances continue to alter the dominance structure of ecological communities, likely disrupting ecosystem functions long before extinctions occur.
Chapter 5 – Hairiness: the missing link between pollinators and pollination

Abstract

Functional traits are the primary biotic component driving organism influence on ecosystem functions; in consequence traits are widely used in ecological research. However, most animal trait-based studies use easy-to-measure characteristics of species that are at best only weakly associated with functions. Animal-mediated pollination is a key ecosystem function and is likely to be influenced by pollinator traits, but to date no one has identified functional traits that are simple to measure and have good predictive power. Here, we show that a simple, easy to measure trait (hairiness) can predict pollinator effectiveness with high accuracy. We used a novel image analysis method to calculate entropy values for insect body surfaces as a measure of hairiness. We evaluated the power of our method for predicting pollinator effectiveness by regressing pollinator hairiness (entropy) against single visit pollen deposition (SVD) and pollen loads on insects. We used linear models and AICc model selection to determine which body regions were the best predictors of SVD and pollen load. We found that hairiness can be used as a robust proxy of SVD. The best models for predicting SVD for the flower species Brassica rapa and Actinidia deliciosa were hairiness on the face and thorax as predictors ($R^2 = 0.98$ and $0.91$ respectively). The best model for predicting pollen load for B. rapa was hairiness on the face ($R^2 = 0.81$). We suggest that the match between pollinator body region hairiness and plant reproductive structure morphology is a powerful predictor of pollinator effectiveness. We show that pollinator hairiness is strongly linked to pollination – an important ecosystem function, and provide a rigorous and time-efficient method for measuring hairiness. Identifying and accurately measuring key traits that drive ecosystem processes is critical as global change increasingly alters ecological communities, and subsequently, ecosystem functions worldwide.

Publications status: Published.
CHAPTER 5. HAIRINESS PREDICTS POLLEN DEPOSITION

5.1 Introduction

Trait-based approaches are now widely used in functional ecology, from the level of individual organisms to ecosystems (Cadotte et al., 2011). Functional traits are defined as the characteristics of an organism’s phenotype that determine its effect on ecosystem level processes (Naeem & Wright, 2003; Petchey & Gaston, 2006). Accordingly, functional traits are recognised as the primary biotic component by which organisms influence ecosystem functions (Gagic et al., 2015; Hillebrand & Matthiessen, 2009). Trait-based research is dominated by studies on plants and primary productivity, and little is known about key traits for animal-mediated and multi-trophic functions, particularly for terrestrial invertebrates (Didham et al., 2016; Gagic et al., 2015; Lavorel et al., 2013).

Most animal trait-based studies simply quantify easy-to-measure morphological characteristics, without a mechanistic underpinning to demonstrate these “traits” have any influence on the ecosystem function of interest (Didham et al., 2016). This results in low predictive power, particularly where trait selection lacks strong justification through explicit ecological questions (Gagic et al., 2015; Petchey & Gaston, 2006). If the ultimate goal of trait-based ecology is to identify the mechanisms that drive biodiversity impacts on ecosystem function, then traits must be quantifiable at the level of the individual organism, and be inherently linked to an ecosystem function (Bolnick et al., 2011; Pasari et al., 2013; Violle et al., 2007).

Methodology that allows collection of trait data in a rigorous yet time-efficient manner and with direct functional interpretation will greatly enhance the power of trait-based studies. Instead of subjectively selecting a large number of traits with unspecified links to ecosystem functions, it would be better to identify fewer, uncorrelated traits, that have a strong bearing on the function of interest (Carmona et al., 2016). Selecting traits that are measurable on a continuous scale, would also improve predictive power of studies (McGill et al., 2006; Violle et al., 2012). However, far greater time and effort is required to measure such traits,
exacerbating the already demanding nature of trait-based community ecology (Petchey & Gaston, 2006).

Animal-mediated pollination is a multi-trophic function, driven by the interaction between animal pollinators and plants (Kremen et al., 2007). A majority of the world’s wild plant species are pollinated by animals (Ollerton et al., 2011), and over a third of global crops are dependent on animal pollination (Klein et al., 2007b). Understanding which pollinator traits determine the effectiveness of different pollinators is critical to understanding the mechanisms of pollination processes. However, current traits used in pollination studies often have weak associations with pollination function and/or have low predictive power. For example Larsen, Williams & Kremen (2005) used body mass to explain pollen deposition by solitary bees even when the relationship was weak and non-significant. Many trait-based pollination studies have subsequently used body mass or similar size measures, despite their low predictive power. Similarly, Hoehn et al (2008) used spatial and temporal visitation preferences of bees to explain differences in plants reproductive output. They found significant relationships (i.e. low \( P \) values) between spatial and temporal visitation preferences and seed set, but with small \( R^2 \) values, suggesting these traits have weak predictive power. To advance trait-based pollination research we require traits that are good predictors of pollination success.

Observational studies suggest that insect body hairs are important for collecting pollen that is used by insects for food and larval provisioning (Holloway, 1976; Thorp, 2000). Hairs facilitate active pollen collection, e.g., many bees have specialised hair structures called scopae that are used to transport pollen to the nest for larval provisioning (Thorp, 2000). Additionally, both bees and flies have hairs distributed across their body surfaces which act to passively collect pollen for adult feeding (Holloway, 1976). Differences in the density and distribution of hairs on pollen feeding insects likely reflects their feeding behaviour, the types of flowers they visit, and whether they use pollen for adult feeding and/or larval provisioning (Thorp, 2000). However, despite anecdotal evidence that insect body hairs are important for pollen collection
and pollination, there is no proven method for measuring hairiness, nor is there evidence that hairier insects are more effective pollinators.

Here, we present a novel method based on image entropy analysis for quantifying pollinator hairiness. We define pollination effectiveness as single visit pollen deposition (SVD): the number of conspecific pollen grains deposited on a virgin stigma in a single visit (King et al., 2013; Ne'eman et al., 2010). SVD is a measure of an insects’ ability to acquire free pollen grains on the body surface and accurately deposit them on a conspecific stigma. We predict that hairiness, specifically on the body parts that contact the stigma, will have a strong association with SVD. We show that the best model for predicting pollinator SVD for pak choi *Brassica rapa* is highly predictive and includes hairiness of the face and thorax dorsal regions as predictors, and the face region alone explains more than 90% of the variation. Similarly, the best model for predicting SVD for kiwifruit *Actinidia deliciosa* includes the face and thorax ventral regions and has good predictive power. Our novel method for measuring hairiness is rigorous, time efficient and inherently linked to pollination function. Accordingly, this method could be applied in diverse trait-based pollination studies to progress understanding of the mechanisms that drive pollination processes.

### 5.2 Materials and Methods

#### 5.2.1 Imaging for hairiness analysis

We photographed pinned insect specimens using the Visionary Digital Passport portable imaging system (Figure 1). Images were taken with a Canon EOS 5D Mark II digital camera (5616 x 3744 pix). The camera colour profile was sRGB IEC61966-2.1, focal length was 65mm and F-number was 4.5. We used ventral, dorsal and frontal shots with clear illumination to minimise reflection from shiny insect body surfaces. All photographs were taken on a plain white background. Raw images were exported to Helicon Focus 6 where they were stacked and stored in .jpg file format.
Figure 1. Face of a native New Zealand solitary bee *Leioproctus paahaumaa* (a) and the corresponding entropy image (b). Warmer colours on the entropy image represent higher entropy values (shown by the scale bar on the right). Black dots on the entropy image are near-round and small objects that have been removed from the analysis by the pre-processing function.

### 5.2.2 Image processing and analysis

We produced code to quantify insect pollinator hairiness using MATLAB (MathWorks, Natick, MA, USA), and functions from the MATLAB Image Processing ToolBox. We quantified relative hairiness by creating an entropy image for each insect photograph, and computed the average entropy within user-defined regions (Gonzales *et al.*, 2004). To calculate entropy values for each image we designed three main functions. The first function allows the user to define up to four regions of interest (RoIs) within each image. The user can define regions by drawing contours as closed polygonal lines of any arbitrary number of vertexes. All information about regions (location, area and input image file name) is stored as a structure in a .mat file.

The second function executes image pre-processing. We found that some insects had pollen grains or other artefacts attached to their bodies, which would alter the entropy results. Our pre-processing function eliminates these objects from the image by running two filtering
processes. First, the function eliminates small objects with an area less than the user definable threshold (8 pixels by default). For the first task, each marked region is segmented using an optimized threshold obtained by applying a spatially dependant thresholding technique. Once each region has been segmented, a labelling process is executed for all resulting objects and those with an area smaller than the minimum value defined by the user are removed. Secondly, as pollen grains are often round in shape, the function eliminates near-circular objects. The perimeter of each object is calculated and its similarity to a circle (S) is defined as:

$$S = \frac{4\pi \cdot \text{Area}}{\text{Perimeter}^2}$$

Objects with a similarity coefficient not within the bounds defined by the user (5% by default) are also removed from the image. Perimeter calculation is carried out by finding the object’s boundary, and computing the accumulated distance from pixel centre to pixel centre across the border, rather than simply counting the number of pixels in the border. The entropy filter will not process objects that have been marked as “deleted” by the pre-processing function. This initial pre-processing provides flexibility by allowing users to define the minimum area threshold and the degree of similarity of objects to a circle. Users can also disable the image pre-processing by toggling a flag when running the entropy filter.

Once pre-processing is complete, each image is passed to the third function, which is the entropy filter calculation stage. The entropy filter produces an overall measure of randomness within each of the user defined regions on the image. In information theory, entropy (also expressed as Shannon Entropy) is an indicator of the average amount of information contained in a message (Shannon, 1948). Therefore, Shannon Entropy, \(H\), of a discrete random variable \(X\) that can take \(n\) possible values \(\{x_1, x_2, \ldots, x_n\}\), with a probability mass function \(P(X)\) is given by:

$$H(X) = -\sum_{i=1}^{n} P(x_i) \cdot \log_2(P(x_i))$$
When this definition is used in image processing, local entropy defines the degree of complexity (variability) within a given neighbourhood around a pixel. In our case, this neighbourhood (often referred to as the structuring element) is a disk with radius \( r \) (we call the radius of influence) that can be defined by the user (7 pixels by default). Thus for a given pixel in position \((i,j)\) in the input image, the entropy filter computes the histogram \( G_{ij} \) (using 256 bins) of all pixels within its radius of influence, and returns its entropy value \( H_{ij} \) as:

\[
H_{ij} = - G_{ij} \cdot \log_2(G_{ij})
\]

where \( G_{ij} \) is a vector containing the histogram results for pixel \((i,j)\) and \((\cdot)\) is the dot product operator. Using default parameters, our entropy filter employs a 7 pixel (13 x 13 neighbourhood) radius of influence, and a disk-shaped structuring element, which we determined based on the size of hairs. Therefore, in the entropy image, each pixel takes a value of entropy when considering 160 pixels around it (by default). We determined the optimal radius of influence for the entropy filter by running our entropy function with the radius of influence set as a variable parameter. We then visually compared the contrast in areas of low vs. high hairiness in the resulting entropy images (i.e., Figure 1). We found that a 7 pixel radius of influence gave the best contrast between low and high hairiness areas for our species set. Hair thickness values across species typically ranged between 3.5-4.5 pixels and therefore, the 7 pixel radius of influence is approximately two times the width of a hair.

The definition of the optimum radius of influence depends on the size of the morphological responsible for the complexity in the RoI. This is defined not only by the physical size of these features but also by the pixel-to-millimetre scaling factor (i.e., number of pixels in the sensor plane per mm in the scene plane). Thus, although 7 pixels is the optimum in our case to detect hairs, the entropy filter function takes this radius as an external parameter which can be adjusted by the user to meet their needs.
CHAPTER 5. HAIRINESS PREDICTS POLLEN DEPOSITION

The entropy filter function is a process that runs over three different entropy layers (\(E_R\), \(E_G\), \(E_B\)), one for each of the camera’s colour channels (Red, Green, and Blue), for each input image. These three images are combined into a final combined entropy image \(E_S\), where each pixel in position \((i,j)\) takes the value \(E_{S(i,j)}\):

\[
E_{S(i,j)} = E_{R(i,j)} \cdot E_{G(i,j)} \cdot E_{G(i,j)}
\]

Once entropy calculations are complete, our function computes averages and standard deviations of \(E_S\) within each of the regions previously defined by the user, and writes the results into a .csv file (one row per image). Entropy values produced by this function are consistent for different photos of the same region on the same specimen (Appendix D; Table D1). The scripts for the image pre-processing, region marking and entropy analysis functions are provided, along with a MATLAB tutorial (see Appendix D).

5.2.3 Model flower floral biology and pollinator collection

We used pak choi Brassica rapa var. chinensis (Brassicaceae) and kiwifruit Actinidia deliciosa (Actinidiaceae) as model flowers to determine if our measurement of insect hairiness is a good predictor of pollinator effectiveness.

Both B. rapa and A. deliciosa are important mass flowering global food crops (Klein et al., 2007a; Rader et al., 2009). B. rapa has an actinomorphic open pollinated yellow flower with four sepals, four petals, and six stamens (four long and two short) (Walker et al., 1999). The nectaries are located in the centre of the flower, between the stamens and the petals, forcing pollinators to introduce their head between the petals. B. rapa shows increased seed set in the presence of insect pollinators and the flowers are visited by a diverse assemblage of insects that differ in their ability to transfer pollen (Rader et al., 2013a). A. deliciosa is dioecious with individual plants producing either male or female flowers. Flowers are large (4-6 cm in diameter) and typically have 5-9 white/cream coloured petals (Devi et al., 2015). Flowers have
multiple stamens and staminodes with yellow anthers. Female flowers have a large stigma with multiple branches that form a brush-like structure. Both male and female flowers do not produce nectar but both produce pollen, which acts as a reward to visitors. Like *B. rapa*, *A. deliciosa* flowers are visited by a diverse range of insects that differ in their ability to transfer pollen, and seed set is increased in the presence of insect pollinators (Craig *et al.*, 1988).

We collected pollinating insects for image analysis during the summer of December 2014 – January 2015. Insects were chilled immediately and then killed by freezing within 1 day and stored at -18°C in individual vials. All insects were identified to species level with assistance from expert taxonomists.

**5.2.4 Image processing**

We measured the hairiness of 10 insect pollinator species (*n* = 8-10 individuals per species), across five families and two orders. This included social, semi-social and solitary bees and pollinating flies. Regions marked included: 1) face; 2) head dorsal; 3) head ventral; 4) front leg; 5) thorax dorsal; 6) thorax ventral; 7) abdomen dorsal and 8) abdomen ventral. All entropy analysis was carried out using our image processing method outlined above. For estimates of body size, we took multiple linear measurements (body length, body width, head length, head width, foreleg length and hind leg length) of each specimen using digital callipers and a dissecting microscope.

**5.2.5 Single visit pollen deposition (SVD) and pollen load**

For *B. rapa* we used SVD data for insect pollinators presented in Rader *et al.* (2009) and Howlett *et al.* (2011); a brief description of their methods follows.

Pollen deposition on stigmatic surfaces (SVD) was estimated using manipulation experiments. Virgin *B. rapa* inflorescences were bagged to exclude all pollinators. Once flowers had opened, the bag was removed, and flowers were observed until an insect visited and contacted the stigma in a single visit. The stigma was then removed and stored in gelatine-fuchsin and the
insect was captured for later identification. SVD was quantified by counting all *B. rapa* pollen grains on the stigma. Mean values of SVD for each species are used in our regression models.

To quantify the number of pollen grains carried (pollen load), *sensu* Howlett *et al.* (2011), insects were collected while foraging on *B. rapa* flowers. Insects were captured using plastic vials containing a rapid killing agent (ethyl acetate). Once dead, a cube of gelatine-fuchsin was used to remove all pollen from the insect’s body surface. Pollen collecting structures (e.g., corbiculae, scopae) were not included in analyses because pollen from these structures is not available for pollination. Slides were prepared in the field by melting the gelatine-fuchsin cubes containing pollen samples onto microscope slides. *B. rapa* pollen grains from each sample were then quantified by counting pollen grains in an equal-area subset from the sample and multiplying this by the number of equivalent sized subset areas within the total sample.

We measured SVD for *A. deliciosa* (*n* = 8-12 per pollinator species). SVD measurements were taken for insect movements from staminate to pistillate flowers, using a method that differed from *B. rapa*. Individual pistillate buds were enclosed within paper bags 2-3 days prior to opening, and were later used as test flowers to evaluate pollen deposition by flowering visiting species. Each bag was secured using a wire tie (coated in plastic) that was gently twisted to exclude pollinators from visiting the opening flowers. Following flower opening, the bag was removed and the flower pedicel abscised where it joined the vine. The test flower was then carefully positioned using forceps to hold the pedicel 1-2 cm from a staminate flower containing a foraging insect, avoiding any contacting between flowers. If the test flower was visited by an insect, we allowed it to forage with minimal disturbance until it moved from the flower on its own accord. The first stigma touched by the foraging insect was then lightly marked near its base using a fine black felt pen. We then placed the marked stigma onto a slide and applied a drop of Alexander stain (Dafni, 2007). Alexander stain was used due to its effectiveness to stain staminate and pistillate pollen differently (pistillate pollen - green-blue, staminate pollen - dark red) (Goodwin & Perry, 1992).
5.2.6 Statistical analyses

We used linear regression models and AICc (small sample corrected Akaike information criteria) model selection to determine if our measure of pollinator hairiness is a good predictor of SVD and pollen load. We constructed global models with SVD or pollen load as the response variable, body region as predictors and body length as an interaction (SVD or pollen load ~ body length * entropy face + entropy head dorsal + entropy head ventral + front leg + entropy thorax dorsal + entropy thorax ventral + entropy abdomen dorsal + entropy abdomen ventral). We included body length in our global model as a proxy for body size as it had high correlation coefficients (Pearson’s r > 0.7) with all other body size measurements. Global linear models were constructed using the \texttt{lm(stats)} function. AICc model selection was carried out on the global models using the function \texttt{glmulti()} with \texttt{fitfunction = “lm”} in the “\texttt{glmulti package}”. We examined heteroscedasticity and normality of errors of models by visually inspecting diagnostic plots using the \texttt{glmulti} package (Crawley, 2002). Variance inflation factors (VIF) of predictor variables were checked for the best models using the \texttt{vif()} function in the \texttt{“car”} package (Fox \textit{et al.}, 2016). All analyses were done in R version 3.2.4 (R Development Core Team, 2014).

5.3 Results

5.3.1 Body hairiness as a predictor of SVD

For SVD on \textit{B. rapa}, the face and thorax dorsal regions were retained in the best model selected by AICc, which had an adjusted $R^2$ value of 0.98. The subsequent top models within 10 AICc points all retained the face and thorax dorsal regions and additionally included the abdomen ventral (adjusted $R^2 = 0.98$), head dorsal (adjusted $R^2 = 0.98$), and thorax ventral (adjusted $R^2 = 0.97$) and front leg (adjusted $R^2 = 0.97$) regions respectively (Table 1; Figure 2). The model with the face region included as a single predictor had an adjusted $R^2$ value of 0.88, indicating that this region alone explained a majority of the variation in the top SVD models.
Figure 2. Relationships between mean entropy for each body region and mean single visit pollen deposition (SVD) on *Brassica rapa* for 10 different insect pollinator species. Black lines are regressions for simple linear models.

The best model for predicting SVD on *A. deliciosa* included the face and thorax ventral regions as predictors (adjusted $R^2 = 0.91$) (Table 1; Figure 3). However, the subsequent top four models were within two AICc points of the best model and therefore cannot be discounted as the potential top model. The face, thorax ventral, head ventral and abdomen ventral regions were retained in four of the five top models, which indicates that hairiness of the face and ventral regions is important for pollen deposition on *A. deliciosa*. For both *B. rapa* and *A. deliciosa*, body length and the body length interaction were not included in the top models.
Figure 3. Relationships between mean entropy for each body region and mean single visit pollen deposition (SVD) on Actinidia deliciosa for seven different insect pollinator species. Black lines are regressions for simple linear models.

5.3.2 Body hairiness as a predictor of pollen load

The best model for pollen load retained the face region only and had an adjusted $R^2$ value of 0.81 (Figure 4; Table 1). The subsequent best models retained the abdomen dorsal (adjusted $R^2$ value of 0.73), the face and head dorsal (adjusted $R^2 = 0.83$), the face and abdomen dorsal (adjusted $R^2 = 0.82$) and the abdomen dorsal and front leg (adjusted $R^2 = 0.8$) regions respectively. For pollen load, body length and the body length interaction were not included in the top models.
**Figure 4.** Relationships between mean entropy for each body region and the mean number of *Brassica rapa* pollen grains carried by nine different insect pollinator species. Black lines are regressions for simple linear models.
Table 1. Top regression models examining the effect of insect body region entropy on single visit pollen deposition (SVD) for *Brassica rapa* and *Actinidia deliciosa* and pollen load for *B. rapa*. Models are presented in ascending order based on AICc values. $\Delta i$ is the difference in the AICc value of each model compared with the AICc value for the top model. $w_i$ is the Akaike weight for each model and acc $w_i$ is the cumulative Akaike weight. Top models for each response variable are highlighted in bold.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Model</th>
<th>Adj $R^2$</th>
<th>AICc</th>
<th>$\Delta i$</th>
<th>$w_i$</th>
<th>acc $w_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SVD (B. rapa)</strong></td>
<td>Face + Thorax dorsal</td>
<td>0.98</td>
<td>88.29</td>
<td>0.00</td>
<td>0.82</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Face + Thorax dorsal + Abdomen ventral</td>
<td>0.98</td>
<td>93.09</td>
<td>4.80</td>
<td>0.07</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>Face + Head dorsal + Thorax dorsal</td>
<td>0.98</td>
<td>93.81</td>
<td>5.52</td>
<td>0.05</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Face + Thorax ventral + Thorax dorsal</td>
<td>0.97</td>
<td>96.59</td>
<td>8.29</td>
<td>0.01</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Face + Thorax dorsal + Front leg</td>
<td>0.97</td>
<td>97.02</td>
<td>8.72</td>
<td>0.01</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>Pollen load (B. rapa)</strong></td>
<td>Face</td>
<td>0.81</td>
<td>168.47</td>
<td>0.00</td>
<td>0.64</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Abdomen dorsal</td>
<td>0.73</td>
<td>171.59</td>
<td>3.12</td>
<td>0.13</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Face + Head dorsal</td>
<td>0.83</td>
<td>173.59</td>
<td>5.12</td>
<td>0.05</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Face + Abdomen dorsal</td>
<td>0.82</td>
<td>173.76</td>
<td>5.29</td>
<td>0.05</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Abdomen dorsal + Front leg</td>
<td>0.80</td>
<td>174.86</td>
<td>6.39</td>
<td>0.03</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>SVD (A. deliciosa)</strong></td>
<td>Face + Thorax ventral</td>
<td>0.91</td>
<td>74.18</td>
<td>0.00</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Abdomen dorsal</td>
<td>0.81</td>
<td>74.21</td>
<td>0.03</td>
<td>0.15</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Face</td>
<td>0.80</td>
<td>74.35</td>
<td>0.17</td>
<td>0.14</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Head ventral</td>
<td>0.79</td>
<td>74.84</td>
<td>0.66</td>
<td>0.11</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Abdomen ventral</td>
<td>0.78</td>
<td>75.08</td>
<td>0.90</td>
<td>0.10</td>
<td>0.65</td>
</tr>
</tbody>
</table>
5.4 Discussion

Here we present a rigorous and time-efficient method for quantifying hairiness, and demonstrate that this measure is an important pollinator functional trait. We show that insect pollinator hairiness is a strong predictor of SVD for the open-pollinated flower *B. rapa*. Linear models that included multiple body regions as predictors had the highest predictive power; the top model for SVD retained the face and thorax dorsal regions. However, the face region was retained in all of the top models, and when included as a single predictor, had a very strong positive association with SVD. In addition, we show that hairiness, particularly on the face and ventral regions, is a good predictor of SVD for *A. deliciosa*, which has a different floral morphology, suggesting our method could be suitable for a range of flower types. Hairiness was also a good predictor for pollen load, and the face region was again retained in the top model for *B. rapa*. The abdomen dorsal, head dorsal and front leg regions were also good predictors of pollen load and were retained in the subsequent top models. Our results validate the importance of insect body hairs for transporting and depositing pollen. Surprisingly, we did not find strong associations between SVD and body size, and top models did not contain the body length interaction. Similarly, body length was not retained in the top models for pollen load. This indicates that our measure of hairiness has far greater predictive power than body size for both SVD and pollen load.

When deciding on which body regions to measure hairiness, researchers may first need to assess additional pollinator traits, such as flower visiting behaviour. This is because the way in which insects interact with flowers influences what body parts most frequently contact the floral reproductive structures (Roubik, 2000). For some open pollinated flowers, such as *B. rapa*, facial hairs are probably the most important for pollen deposition because the face is the most likely region to contact the anthers and stigma. However, for flowers with different floral morphologies, facial hairs may not be as important because the floral reproductive structures have different positions relative to the insect’s body structures. For example, disc-shaped flowers tend to deposit their pollen on the ventral regions of pollinators, while labiate flowers deposit their pollen on the dorsal regions (Bartomeus et al., 2008). We found that hairiness on
the face and ventral regions of pollinators was most important for pollen deposition on *A. deliciosa* flowers. The reproductive parts of *A. deliciosa* form a brush shaped structure and therefore are most likely to contact the face and ventral surfaces of pollinators. Accordingly, where studies focus on a single plant species i.e. crop based studies, it is important to consider trait matching when selecting pollinator body region(s) to analyse (Butterfield & Suding, 2013; Garibaldi *et al*., 2015).

It is important to consider that pollinator performance is a function of both SVD and visitation frequency, and these two components operate independently (Kremen *et al*., 2002; Mayfield *et al*., 2001). Here, we focus on a single trait that is important for pollinator efficiency (SVD), but to calculate pollinator performance researchers need to measure both efficiency and visitation rate. Additional pollinator traits related to visitation rate, as well as other behavioural traits such as activity patterns relative to the timing of stigma receptivity (Potts *et al*., 2001) and foraging behaviour, e.g., nectar vs. pollen foraging (Herrera, 1987; Javorek *et al*., 2002; Rathcke, 1983), may be important for predicting pollination performance. In some circumstances it might also be important to consider trait differences between male and female pollinators, particularly for some bee species. Male and female bees may have different pollen deposition efficiency due to differences in their foraging behaviour and resource requirements. For example, female bees are likely to visit flowers to collect pollen for nest provisioning while males simply consume nectar and pollen during visits (Cane *et al*., 2011). For some flowers, male bees have a similar pollination efficiency compared to females (e.g., summer squash Cucurbita pepo; Cane *et al*., 2011) while for others, female bees are more effective than males (e.g., lowbush blueberry Vaccinium angustifolium; Javorek *et al*., 2002).

For community-level studies that use functional diversity approaches, our method could be used to quantify hairiness for several body regions and weighted to give better representation of trait diversity within the pollinator community. This is necessary where plant communities contain diverse floral traits i.e. open-pollinated vs. closed-tubular flowers (Fontaine *et al*., 2006). Hairs on different areas of the insect body are likely to vary in relative importance for
pollen deposition depending on trait matching (Bartomeus et al., 2016b). Our method requires hairiness to be measured at the individual-level (Figure D1), which makes it an ideal trait to use in new functional diversity frameworks that use trait probabilistic densities rather than trait averages (Carmona et al., 2016; Fontana et al., 2016). Combining predictive traits, such as pollinator hairiness, with new methods that amalgamate intraspecific trait variation with multidimensional functional diversity, will greatly improve the explanatory power of trait-based pollination studies.

One of the greatest constraints to advancing trait-based ecology is the time-demanding nature of collecting trait data. This is because ecological communities typically contain many species, which have multiple traits that need to be measured and replicated (Petchey & Gaston, 2006). To improve the predictive power of trait-based ecology and streamline the data collection process we must firstly identify traits that are strongly linked to ecosystem functions and secondly, develop rigorous and time-efficient methodologies to measure traits at the individual level. We achieve this by providing a method for quantifying a highly predictive trait at the individual-level, in a time-efficient manner. Our method also complements other recently developed predictive methods for estimating difficult-to-measure traits that are important for pollination processes i.e. bee tongue length; Cariveau et al. (2016).

5.4.1 Conclusion

Predicating the functional importance of organisms is critical in a rapidly changing environment where accelerating biodiversity loss threatens ecosystem functions (McGill et al., 2015). Our novel method for measuring pollinator hairiness could be used in any studies that require quantification of hairiness, such as understanding adhesion in insects (Bullock et al., 2008; Clemente et al., 2010) or epizoochory (Albert et al., 2015; Sorensen, 1986). It is also a much needed addition to the pollination biologist’s toolbox, and will progress the endeavour to standardise trait-based approaches in pollination research. This is a crucial step towards developing a strong mechanistic underpinning for trait-based pollination research.
Chapter 6 – General discussion

We are now living in the Anthropocene – a geological epoch defined by human modification of the biosphere (Ellis et al., 2010). Humanity’s demand on earth’s natural resources is greater than ever and continuing to increase as human populations grow (Hoekstra & Wiedmann, 2014). In consequence, we are applying unprecedented pressure on ecosystems that provide natural resources, which are essential for society (Foley et al., 2011). However, our understanding about how ecosystems respond to anthropogenic habitat modification is insufficient for predicting the consequences of these changes. Furthermore, we have limited knowledge of whether ecological communities have inbuilt resilience mechanisms that could buffer them against anthropogenic disturbances. In this study I sought to understand how pollinator communities in New Zealand respond to a key anthropogenic disturbance, agricultural intensification, and identify the consequences for associated pollination function.

6.1 Linking pollinator functional roles with their responses to land-use change

One of the key challenges in ecology is identifying how species’ functional roles in ecosystems are associated with species’ responses to environmental change (Laliberté et al., 2010; Larsen et al., 2005). This is critically important in a rapidly changing world, where human demands on earth’s natural resources are putting increasing pressure on ecological communities which provide these resources (Foley et al., 2011). Accordingly, the first aim of my PhD thesis (chapter two) was to identify the association between pollinator functional niches (species’ functional roles in ecosystems) and their environmental niches (how species respond to environmental change). First, at the pollinator community level, I found strong response diversity (differential change in species abundances with agricultural intensification) (Figure 6.1). This finding reflects studies on North American native bee communities, which also displayed strong response diversity to land-use change (Cariveau et al., 2013; Winfree & Kremen, 2009). However, I found that response diversity to agricultural intensification was driven largely by differences in exotic vs. native species responses; native species mostly
responded negatively to agriculture, whereas exotic species mostly responded positively. This raises important questions about the role of exotic species for ecosystem functions and services, which I investigate further in chapter three.

My key finding in chapter two was that although some functionally redundant species show response diversity, most do not (Figure 6.1). This implies that agricultural intensification drastically altered the composition of the pollinator community; some redundant species abundances increased massively, while others decreased or changed little. Furthermore, I showed that where functionally redundant species displayed response diversity, this did not stabilise aggregate abundance of the functional group. The relationship between functional redundancy and response diversity has previously been investigated in plant communities, using the effect-response trait framework (Laliberté et al., 2010). In this previous study, there was an association between species’ functional roles and their response traits, echoing results from other studies, in which the species that are most vulnerable to disturbance are also the most functionally important (Larsen et al., 2005; Purvis et al., 2000). In my study, I directly quantified species responses by measuring changes in their abundances along a disturbance gradient, rather than inferring response diversity from species response traits. This novel approach is an important contribution given that we know very little about which traits actually determine species’ responses to disturbances, particularly for animals (Bartomeus et al., 2017).

My findings in chapter two have important broader implications for understanding the stability of ecological communities and associated functions. Firstly, I found that the stabilising effect of response diversity was relatively minimal for functionally redundant species. Whether this holds for other communities is uncertain, although North American native bee communities show similar patterns (Cariveau et al., 2013). Thus, the direction and magnitude of the dominant species’ response within a functional group is potentially more important for driving fluxes in ecosystem functions than response diversity per se. This is consistent with a recent study in which changes in the abundance of common species were more important for ecosystem functioning than changes in species richness and composition (Winfree et al., 2015).
Accordingly, I explored the importance of species evenness/dominance for pollination function in chapters three and four.

In chapter two, I focused on species’ abundance responses to a single disturbance, over a relatively short temporal period. My approach was also limited as I assumed species within functional groups were absolutely equivalent and absolutely distinct from those in other functional groups. Thus, I may have underestimated true response diversity and overestimated functional redundancy. Accordingly, studies are required that investigate species responses to multiple disturbances, over greater temporal scales. Methods are also required that combine continuous functional redundancy measures (e.g., Carmona et al., 2016) with response diversity. Rigorous and time efficient methods for quantifying predictive functional traits (e.g., hairiness; chapter five) will improve estimations of functional redundancy.

### 6.2 Land-use change effects on pollination services

Following my findings in chapter two, I used a model crop “pak choi” (*Brassica rapa*) in chapter three, to investigate the effects of agricultural intensification on pollination service delivery from the wild pollinator community. I found that pollination services from native species declined with agricultural intensification, but surprisingly, there was a large increase in pollination from exotic species (Figure 6.1). This resulted in an overall increase in pollination services from the wild pollinator community with increased agricultural intensity. These findings are the first instance that I am aware of showing exotic species compensate for loss of ecosystem services from native species, due to agricultural intensification. Given the continuing expansion and intensification of agriculture, and the spread of exotic species globally, this finding could be more ubiquitous than is currently recognised. Chapter three challenges the prevailing view that impacts of exotics species are mostly negative (e.g., Morales et al., 2017; Traveset & Richardson, 2006). Thus, I suggest that at least in highly modified systems, exotic species could deliver important ecosystems services and provide some insurance against future species losses.
CHAPTER 6. GENERAL DISCUSSION

However, another important finding in chapter three was that agricultural intensification reduced the evenness of species’ contributions to pollination service delivery (Figure 6.1). When agriculture was highly intensive, pollination services were largely dependent on just one or a few species. This finding is particularly relevant considering the biodiversity insurance hypothesis, which states function is more stable through time and space when systems have greater biodiversity (Yachi & Loreau, 1999). Diminished populations or local extinctions of some pollinator groups, such as native solitary bees, could also reduce potential response diversity, increasing vulnerability of pollinator communities to future environmental change (Mori et al., 2013). Furthermore, I found that temporal pollen deposition patterns were altered by agricultural intensification, mostly due to increased dominance of exotic flies, which tended to deposit pollen throughout the day. This change could have important implications for plants that are receptive at particular times of the day (Bishop et al., 2016; Willmer, 1983). Therefore, although exotic species may provide compensation in highly modified environments, we should focus on conserving and restoring natural habitat within productive landscapes, to enhance the robustness of pollinator communities and associated pollination services. Effective restoration and conservation of pollinator habitat requires improved understanding of native pollinator resource requirements and basic biology, which is currently very limited.

6.3 The importance of species evenness for driving pollination function

In chapter three, I show that anthropogenic disturbance can strongly alter the evenness of pollination service delivery from the wild pollinator community. With agricultural intensification, pollination was increasingly delivered by a few common species. This was unsurprising given that the skewed species abundance distribution is one of the few universal laws in ecology (McGill et al., 2007), and that anthropogenic disturbance typically increases dominance (Hillebrand et al., 2008). However, biodiversity-ecosystem function experiments have traditionally focused on describing the relationship between species richness and ecosystem function, mostly for primary productivity based functions (Reiss et al., 2009). Only a few studies have attempted to disentangle the relationship between species evenness and function (e.g., Dangles & Malmqvist, 2004; Garibaldi et al., 2015; Mattingly et al., 2007; Nijs
& Roy, 2000; Smith & Knapp, 2003; Stevens & Carson, 2001; Wilsey & Potvin, 2000). No study has experimentally manipulated species evenness to test the effects of evenness on a multitrophic function, independent of species richness, composition and total abundance. Subsequently, in chapter four, I aimed to contribute to developing a mechanistic understanding of how species evenness affects ecosystem functioning, using plant and pollinator communities.

Despite not finding strong effects of numerical evenness on pollinator floral niche complementarity, I did detect important changes to other plant–pollinator network characteristics, particularly community-level interaction evenness and species-level visitation rate evenness. Visitation rate evenness had a strong positive effect on seed production, suggesting that some form of complementarity had enhanced function (e.g., architectural, temporal or environmental complementarity; Blüthgen & Klein, 2011; Hoehn et al., 2008). However, increased community-level interaction evenness negatively affected seed production. Thus, seed production was greatest where there was strong community-level interaction asymmetry (i.e., interactions were dominated by a few plant and pollinator species), but where those interactions occurred at similar frequencies (i.e., pollinators visited plant species at equal rates) (Figure 6.1). This finding reflects previous studies in real world landscapes that show dominant pollinator species are more important for driving pollination service delivery, than species richness and composition (Kleijn et al., 2015; Winfree et al., 2015). However, as larger spatial and temporal scales, and multiple disturbances are considered, mechanisms such as response diversity from diverse communities could become critical for the stability of pollination function (Mori et al., 2013).

In chapter four, I also found interesting effects of evenness on pollinator visitation rate for different plant species. Visitation rate to the dominant plant species, Brassica rapa, was lower in dominant plant communities compared with even plant communities, probably due to dilution of pollinator visits. Yet visitation rate to the rare plant species Lupinus hartwegii was also lower in dominant plant treatments, likely because of reduced conspecific display size.
Therefore, I suggest that dominance in plant communities could be constrained, to some degree, by the trade-off between the benefits of large conspecific displays and intraspecific competition for pollinators. Larger conspecific displays should increase pollinator visitation rate but where they become too large, visitation rate may decrease due to dilution effects, thus representing a hump shaped relationship. However, for rare plant species, pollinator visitation rate should increase with conspecific display size (i.e., as the plant community shifts from dominant to even). This finding is tentative and clearly, further work is required to understand this relationship and determine whether it occurs in natural communities.

Despite the constraints of small scale experiments, my findings in chapter four have some important implications for the real world. Firstly, increased plant and pollinator numerical evenness may not necessarily increase floral niche complementary and consequently, enhance function. This contrasts with previous studies that show increased biodiversity, specifically species richness, positively affects complementarity and increases function (e.g., Albrecht et al., 2012; Fründ et al., 2013). However, such studies are designed with high species evenness and thus, are not necessarily reflective of the real world (Hillebrand et al., 2008). We now require experimental studies that test the importance of species evenness on ecosystem functioning, particularly for complex, multitrophic functions for which we have limited understanding (Schleuning et al., 2015). This is particularly important as anthropogenic disturbance continues to increase dominance within ecological communities, likely altering ecosystem processes and functions long before extinctions occur. Using data from real-world ecosystems to inform the design of small scale experimental studies, such as that in chapter four, should facilitate development of a mechanistic understanding of how natural ecosystems function. Furthermore, studies are required that investigate how anthropogenic disturbances alter plant–pollinator interactions and consequently, plant reproduction in the real world (e.g., Magrach et al., 2017).
6.4 Moving towards more predictive trait-based pollination ecology

Trait-based approaches are now widely used in ecology, often because traits are assumed to be more strongly associated with ecosystem functions compared with conventional biodiversity measures, such as species richness and composition (Gagic et al., 2015). However, many studies use traits that lack strong mechanistic underpinning, or are weakly associated with the ecosystem function of interest (Didham et al., 2016). This is particularly true for animal-based functional trait studies. Accordingly, in chapter five, I presented a novel and rigorous method for measuring pollinator hairiness and demonstrated that this trait is strongly predictive of pollinator effectiveness (single visit pollen deposition). Specifically, I showed that the degree of hairiness on pollinators’ face and thorax body regions is strongly associated with pollination effectiveness for two flower species (pak choi, *Brassica rapa* and kiwifruit, *Actinidia deliciosa*) (Figure 6.1). This has some intriguing implications for understanding pollinator–flower trait matching, which is an emerging field in pollination ecology (Garibaldi et al., 2015). Future studies should investigate whether hairiness predicts pollinator effectiveness for other flower types (e.g., tubular flowers), and whether strong matching exists between floral structure and hairiness on different pollinator body regions.

Identifying and measuring predictive functional traits, such as hairiness, is an important first step towards transforming trait-based pollination research into a predictive discipline. Trait-based pollination research now requires effective tools for quantifying other important functional traits (e.g., pollinator behavioural characteristics). This should not be limited to effect traits (traits that are important for pollinator performance), but should also be extended to response traits (traits that determine species’ responses to environmental change). As shown in chapter two, functional traits, such as hairiness, can be used effectively for determining how environmental change impacts the functional composition and resilience of pollinator communities.

As I demonstrated in chapter five, methods for measuring functional traits must be rigorous and time efficient. Rigour is required to capture both interspecific and intraspecific trait
variation. In particular, intraspecific trait variation is likely to yield important information about the relationship between functional traits and ecosystem functioning (Violle et al., 2012). Time efficiency is important considering the large number of species in many community-level studies, for which multiple traits must be measured (Petchey & Gaston, 2006). Most importantly, the use of particular functional traits should be strongly justified through robust ecological questions. Instead of simply measuring a wide variety of traits that are not necessarily predictive of ecosystem functioning, quantifying fewer traits with strong inherit links to the ecosystem function of interest is required. The explanatory power of trait-based research will be greatly enhanced where researchers combine predicative traits, with methods that integrate intraspecific trait variation and multidimensional functional diversity.
Figure 6.1 Summary infographic of the key findings from each data chapter. Blue arrows indicate the direction of flow of ideas and findings throughout the thesis.
6.5 Concluding remarks

My study has shown that pollinator communities have inbuilt resilience mechanisms that can buffer the impacts of anthropogenic disturbance. This was illustrated by strong community-level response diversity and compensation in pollination service provision with agricultural intensification. However, positive responses and increased pollination service provision were mostly provided by a few opportunistic exotic species that were not functionally equivalent to native species. I also show that equal visitation from a few functionally dominant pollinator species may actually increase pollination functioning. Thus, increased dominance may not be detrimental to pollination function in the short-term. Nonetheless, changes to the functional structure and dominance of pollinator communities is likely to increase vulnerability to future environmental change. As spatial and temporal scales increase, biodiversity should become more important to the stability of pollination function. Therefore, we must consider the habitat and resource requirements of diverse pollinator species to maintain the stability of pollination function. This requires improved understanding of pollinator species natural history and research-guided changes to land-use management.
References


Bates, D., Maechler, M., & Bolker, B. M. (2015). lme4: Liner mixed-effects models using S4 classes.


Appendix A – supplementary information for chapter 2

Table A1. List of species that were included in functional redundancy and response diversity analyses. ‘Total collected’ is the total number of individuals collected per species across all sites. ‘No. sites present’ is the total number of sites that each species was present at.

<table>
<thead>
<tr>
<th>Species</th>
<th>Order</th>
<th>Family</th>
<th>Exotic/Native</th>
<th>Functional group</th>
<th>Total collected</th>
<th>No. sites present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthomyia punctipennis</td>
<td>Diptera</td>
<td>Anthomyiidae</td>
<td>Exotic</td>
<td>4</td>
<td>84</td>
<td>6</td>
</tr>
<tr>
<td>Bombus terrestris</td>
<td>Hymenoptera</td>
<td>Apidae</td>
<td>Exotic</td>
<td>5</td>
<td>66</td>
<td>8</td>
</tr>
<tr>
<td>Calliphora stygia</td>
<td>Diptera</td>
<td>Calliphoridae</td>
<td>Exotic</td>
<td>1</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Campylia sp</td>
<td>Diptera</td>
<td>Tachininae</td>
<td>Native</td>
<td>2</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Delia platura</td>
<td>Diptera</td>
<td>Anthomyiidae</td>
<td>Exotic</td>
<td>4</td>
<td>736</td>
<td>12</td>
</tr>
<tr>
<td>Delia sp</td>
<td>Diptera</td>
<td>Anthomyiidae</td>
<td>Exotic</td>
<td>4</td>
<td>434</td>
<td>12</td>
</tr>
<tr>
<td>Eristalis tenax</td>
<td>Diptera</td>
<td>Syrphidae</td>
<td>Exotic</td>
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<td>1,010</td>
<td>12</td>
</tr>
<tr>
<td>Helophilus hochstetteri</td>
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<td>Syrphidae</td>
<td>Native</td>
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<td>31</td>
<td>10</td>
</tr>
<tr>
<td>Hydrotaea rostrata</td>
<td>Diptera</td>
<td>Muscidae</td>
<td>Exotic</td>
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<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Inopus rubriceps</td>
<td>Diptera</td>
<td>Stratiomyiidae</td>
<td>Exotic</td>
<td>4</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Lasioglossum cognatum</td>
<td>Hymenoptera</td>
<td>Halictidae</td>
<td>Native</td>
<td>6</td>
<td>236</td>
<td>10</td>
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<tr>
<td>Lasioglossum sordidum</td>
<td>Hymenoptera</td>
<td>Halictidae</td>
<td>Native</td>
<td>6</td>
<td>99</td>
<td>9</td>
</tr>
<tr>
<td>Leioproctus boltoni</td>
<td>Hymenoptera</td>
<td>Colletidae</td>
<td>Native</td>
<td>3</td>
<td>73</td>
<td>5</td>
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<tr>
<td>Leioproctus huakiwi</td>
<td>Hymenoptera</td>
<td>Colletidae</td>
<td>Native</td>
<td>3</td>
<td>17</td>
<td>4</td>
</tr>
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<td>Leioproctus imitatus</td>
<td>Hymenoptera</td>
<td>Colletidae</td>
<td>Native</td>
<td>3</td>
<td>90</td>
<td>7</td>
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<td>Species</td>
<td>Order</td>
<td>Family</td>
<td>Status</td>
<td>Max Count</td>
<td>Min Count</td>
<td>Average</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------</td>
<td>-----------------</td>
<td>---------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------</td>
</tr>
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<td>Leioproctus kanapuu</td>
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<td>Colletidae</td>
<td>Native</td>
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<td>6</td>
</tr>
<tr>
<td>Leioproctus paahaumaa</td>
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<td>Colletidae</td>
<td>Native</td>
<td>3</td>
<td>124</td>
<td>7</td>
</tr>
<tr>
<td>Melangyna novaezelandiae</td>
<td>Diptera</td>
<td>Syrphidae</td>
<td>Native</td>
<td>2</td>
<td>46</td>
<td>10</td>
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<td>Melanostoma fasciatum</td>
<td>Diptera</td>
<td>Syrphidae</td>
<td>Native</td>
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<td>213</td>
<td>12</td>
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<td>Odontomyia sp</td>
<td>Diptera</td>
<td>Stratiomyidae</td>
<td>Native</td>
<td>1</td>
<td>19</td>
<td>3</td>
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<td>Oxysarcodexia varia</td>
<td>Diptera</td>
<td>Sarcophagidae</td>
<td>Exotic</td>
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<td>11</td>
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<td>Spilagona melas</td>
<td>Diptera</td>
<td>Muscidae</td>
<td>Exotic</td>
<td>2</td>
<td>18</td>
<td>4</td>
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</tbody>
</table>
Figure A1. The relationship between pollinator abundance and the proportion of agriculture, comparing bees vs. flies and native vs. exotic species; purple lines are exotic species and green lines are native species.
Appendix B – supplementary information for chapter 3

Table B1. Model selection statistics for community-summed pollination services to *Brassica rapa* across the agricultural intensification gradient. The model highlighted in bold is the best model, as determined by AICc model selection.

<table>
<thead>
<tr>
<th>Model</th>
<th>%Agriculture</th>
<th>Time</th>
<th>%Agriculture: Time</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>Weight</th>
<th>Cum. Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td><strong>1825.5</strong></td>
<td>0.0</td>
<td><strong>0.99</strong></td>
<td><strong>0.99</strong></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
<td>1835.8</td>
<td>10.3</td>
<td>0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>+</td>
<td></td>
<td>1837.4</td>
<td>11.9</td>
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<td>1.00</td>
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<tr>
<td>4</td>
<td>+</td>
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<td></td>
<td>2003.9</td>
<td>178.4</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Null</td>
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<td></td>
<td></td>
<td>2004.5</td>
<td>179.0</td>
<td>0.00</td>
<td>1.00</td>
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</table>
Table B2. Model selection statistics for pollination services provide by wild exotic vs. native insect species to *Brassica rapa* across the agricultural intensification gradient. The model highlighted in bold is the best model, as determined by AICc model selection.

<table>
<thead>
<tr>
<th>Model</th>
<th>Exotic/Native %Agriculture</th>
<th>Time</th>
<th>Exotic/Native: %Agriculture</th>
<th>Exotic/Native: Time</th>
<th>%Agriculture: Time</th>
<th>Exotic/Native: %Agriculture</th>
<th>Exotic/Native: Time</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>Weight</th>
<th>Cum. Wt</th>
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<tr>
<td>1</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>4625.7</td>
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<tr>
<td>2</td>
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<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Exotic/Native: %Agriculture</td>
<td>Time</td>
<td>4653.1</td>
<td>27.4</td>
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<td>1.00</td>
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<td>+</td>
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<td>5212.5</td>
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Table B3. Model selection statistics for visitation rate effects on yield (seed production) for different pollinator groups. The model highlighted in bold is the best model, as determined by AIC_c model selection.

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<td>+</td>
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<td></td>
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<td></td>
<td>+</td>
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<td>753.1</td>
<td>3.52</td>
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Table B4. Effects of agricultural intensification on the evenness of pollination service delivery from different species for a range of Hill’s diversity numbers. Importance of the proportion agriculture fixed effect was determined using Wald chi-square tests on linear mixed effects models.

<table>
<thead>
<tr>
<th>Hill's number</th>
<th>Estimate</th>
<th>SE</th>
<th>$\chi^2_{df}$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>-0.12</td>
<td>0.05</td>
<td>6.131</td>
<td>0.01</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.21</td>
<td>0.07</td>
<td>9.001</td>
<td>0.003</td>
</tr>
<tr>
<td>1</td>
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<td>0.08</td>
<td>12.811</td>
<td>&lt; 0.001</td>
</tr>
<tr>
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<td>-0.32</td>
<td>0.09</td>
<td>13.591</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>4</td>
<td>-0.26</td>
<td>0.08</td>
<td>10.191</td>
<td>0.001</td>
</tr>
<tr>
<td>8</td>
<td>-0.23</td>
<td>0.08</td>
<td>8.031</td>
<td>0.005</td>
</tr>
<tr>
<td>Infinity</td>
<td>-0.19</td>
<td>0.07</td>
<td>6.831</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Table B5. List of wild pollinator species that were included in pollination services analyses. ‘Total no. visits’ is the total number visits to open flowers from each pollinator species across all sites. ‘No. sites present’ is the total number of sites that visitations from each pollination species were recorded.

<table>
<thead>
<tr>
<th>Species</th>
<th>Order</th>
<th>Family</th>
<th>Total no. visits</th>
<th>No. sites present</th>
<th>Exotic/Native</th>
<th>SVD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anthomyia punctipennis</em></td>
<td>Diptera</td>
<td>Anthomyiidae</td>
<td>226</td>
<td>8</td>
<td>Exotic</td>
<td>16.35</td>
</tr>
<tr>
<td><em>Bombus terrestris</em></td>
<td>Hymenoptera</td>
<td>Apidae</td>
<td>134</td>
<td>7</td>
<td>Exotic</td>
<td>236.63</td>
</tr>
<tr>
<td><em>Calliphora stygia</em></td>
<td>Diptera</td>
<td>Calliphoridae</td>
<td>16</td>
<td>3</td>
<td>Exotic</td>
<td>61.00</td>
</tr>
<tr>
<td><em>Delia sp</em></td>
<td>Diptera</td>
<td>Anthomyiidae</td>
<td>1,075</td>
<td>12</td>
<td>Exotic</td>
<td>2.65</td>
</tr>
<tr>
<td><em>Eristalis tenax</em></td>
<td>Diptera</td>
<td>Syrphidae</td>
<td>3,605</td>
<td>12</td>
<td>Exotic</td>
<td>106.64</td>
</tr>
<tr>
<td><em>Helophilus hochstetteri</em></td>
<td>Diptera</td>
<td>Syrphidae</td>
<td>27</td>
<td>6</td>
<td>Native</td>
<td>99.00</td>
</tr>
<tr>
<td><em>Lasioglossum sp</em></td>
<td>Hymenoptera</td>
<td>Halictidae</td>
<td>682</td>
<td>11</td>
<td>Native</td>
<td>30.14</td>
</tr>
<tr>
<td><em>Leioproctus sp</em></td>
<td>Hymenoptera</td>
<td>Colletidae</td>
<td>437</td>
<td>11</td>
<td>Native</td>
<td>153.20</td>
</tr>
<tr>
<td><em>Melanostoma fasciatum</em></td>
<td>Diptera</td>
<td>Syrphidae</td>
<td>424</td>
<td>12</td>
<td>Native</td>
<td>6.36</td>
</tr>
<tr>
<td><em>Melangiyna novaezelandiae</em></td>
<td>Diptera</td>
<td>Syrphidae</td>
<td>36</td>
<td>7</td>
<td>Native</td>
<td>16.13</td>
</tr>
<tr>
<td><em>Oxysarcodexia varia</em></td>
<td>Diptera</td>
<td>Sarcophagidae</td>
<td>363</td>
<td>11</td>
<td>Exotic</td>
<td>32.53</td>
</tr>
</tbody>
</table>
Figure B1. Pollen deposition at different times of the day across all sites faceted by taxa (bee/fly) and exotic/native status.
Figure B2. Pollination services provided by different wild pollinator species. Line colours represent species’ native/exotic status (green lines are native species, orange lines are exotic species). Note that Y axes (pollen deposition values) for different species are on different scales.
Figure B3. Evenness of pollination services as determined by a range of Hill’s diversity numbers, across the agricultural intensification gradient. The red shaded area inside the dashed red line denotes the confidence interval (± 1 SE). Hill’s diversity number 1 is not included as this was used in the main results of the chapter.
Figure B4. Number of seeds produced per flower (seed production) for *Brassica rapa* across the agricultural intensification gradient.
Appendix C – supplementary information for chapter 4

Figure C1. Model estimated visitation rate of each pollinator species to each plant species across treatments. Coloured points are the model estimates and error bars are ± the model estimate standard error.
Figure C2. Model estimated visitation rate of all pollinator species to each plant species across treatments. Coloured points are the model estimates and error bars are ± the model estimate standard error.
**Figure C3.** Image of the experimental cage set up before plants and pollinators were introduced.

**Figure C4.** Image inside an experimental cage.
Figure C5. Images of pollinator species on flowers in experimental cages: a) *Leioproctus paahaumaa* on a *Coriandrum sativum* flower; b) *Bombus terrestris* on a *Centaurea cyanus* flower; c) *Eristalis tenax* on a *Fagopyrum esculentum* flower; d) *Oxysarcodexia varia* on a *Coriandrum sativum* flower and; e) *Megachile rotundata* on a *Fagopyrum esculentum* flower.
Appendix D – supplementary information for chapter 5

MATLAB function to perform image pre-processing for entropy analysis.

```matlab
function [RegionImage, RegionBinaryImage, RegionX, RegionY] = PreProcessRegion(RegionLoc, INPUT_IM, SmallObjThres, RoundThresh, EnableFiltering)
% This function deletes the small and the rounded objects and creates
% the images of the zone to be process by the entropy filter.
% Use:
% [RegionImage, RegionBinaryImage] = PreProcessRegion(RegionLoc, INPUT_IM, SmallObjThres, EnableFiltering)
% Where--
% RegionLoc=> Region area loaded from locations file
% INPUT_IM => Input Image variable
% SmallObjThres => Threshold to define an object as small and delete it
% RoundThresh => Coeff to define an object as round and delete it
% EnableFiltering => Flag (1 or 0) to Enable filtering small and rounded
% objects
% (c) Gustavo Liñan Cembrano
% IMSE-CNM-CSIC
% linan@imse-cnm.csic.es

Region = zeros(size(INPUT_IM,1),size(INPUT_IM,2));
Region(RegionLoc) = 1;
RegionProps = regionprops(Region,'centroid');
RegionX = RegionProps.Centroid(1);
RegionY = RegionProps.Centroid(2);
RegionBinaryImage = regionprops(Region,'Image');
RegionBinaryImage = RegionBinaryImage.Image;
RegionBBox = regionprops(Region,'BoundingBox');
RegionBBox = RegionBBox.BoundingBox;
RegionBBox = round(RegionBBox);
RegionImage = uint8(zeros(size(RegionBinaryImage,1),... size(RegionBinaryImage,2),... 3));
RegionImage(:,:,1) = INPUT_IM(RegionBBox(2):RegionBBox(2)+RegionBBox(4)-1,... RegionBBox(1):RegionBBox(1)+RegionBBox(3)-1,... 1);
RegionImage(:,:,2) = INPUT_IM(RegionBBox(2):RegionBBox(2)+RegionBBox(4)-1,... RegionBBox(1):RegionBBox(1)+RegionBBox(3)-1,... 2);
RegionImage(:,:,3) = INPUT_IM(RegionBBox(2):RegionBBox(2)+RegionBBox(4)-1,... RegionBBox(1):RegionBBox(1)+RegionBBox(3)-1,... 3);

if (EnableFiltering)
    % Removing rounded & small objects from image and substituting them by average around them
    threshold = graythresh(RegionImage);
    bw = im2bw(RegionImage, threshold);
    % Erasing small objects
    bw2 = bwareaopen(bw, SmallObjThres);
    SmallObjects = xor(bw, bw2);
    [L, NUM] = bwlabel(SmallObjects, 8);
    fprintf('Deleted %d Small Objects in Region
', NUM);
end
```

(c) Gustavo Liñan Cembrano
IMSE-CNM-CSIC
linan@imse-cnm.csic.es
RegionBinaryImage = and(RegionBinaryImage, not(SmallObjects));
% fill a gap in the pen's cap
se = strel('disk', 2);
bw = imclose(bw2, se);

% fill any holes, so that regionprops can be used to estimate
% the area enclosed by each of the boundaries
bw = imfill(bw, 'holes');
[B, L] = bwboundaries(bw, 'noholes');
stats = regionprops(L, 'Area', 'Centroid', 'PixelIdxList');

threshold = RoundThresh; % the closer to 1 the more round
% loop over the boundaries
Deleted = 0;
for k = 1:length(B)
    % obtain (X,Y) boundary coordinates corresponding to label 'k'
    boundary = B{k};

    % compute a simple estimate of the object's perimeter
    delta_sq = diff(boundary).^2;
    perimeter = sum(sqrt(sum(delta_sq, 2)));

    % obtain the area calculation corresponding to label 'k'
    area = stats(k).Area;

    % compute the roundness metric
    metric = 4*pi*area/perimeter^2;

    % mark objects above the threshold with a black circle
    if metric > threshold
        Deleted = Deleted + 1;
        RegionBinaryImage(stats(k).PixelIdxList) = 0;
    end
end
fprintf('Deleted %d round objects in Region
', Deleted);
end
end
MATLAB function "Mark_4_RegionsBees()" for defining regions on images for entropy analysis.

```matlab
function Mark_4_RegionsBees()
% Request the user to mark 4 regions in all .jpg images in an input
% directory. Regions are defined by drawing polygons in the input image.
% right-button click closes the region. Then use double click at the last
% point to validate the region and pass to the next one. In case of drawing
% error, press CTRL-C to abort the function and run the code again without
% deleting previous results. The program checks if a locations file for the
% current image exists and if so, skips it from the selecting region
% process.
% Typical Example of usage:
%       Mark_4_RegionsBees();
% (c) Gustavo Liñan Cembrano
% IMSE-CNM-CSIC
% linan@imse-cnm.csic.es

clear all;
close all;
cic;

IM_PATH=uigetdir([], 'Select Image Folder');

cd(IM_PATH)
DeletePrevious = questdlg('Delete previous regions?','Delete','No');
if(strcmp(DeletePrevious,'Yes'))
    delete('*.mat');
    delete('*.tiff')
elseif(strcmp(DeletePrevious,'Cancel'))
    return;
else
    %Do nothing
end

%Getting File name structure

FileName=dir('*.jpg');
NFiles=length(FileName);

msgstr=sprintf('%d Images Found in directory',NFiles);
h=msgbox(msgstr);
pause(1); delete(h)

for j=1:NFiles

    % Accessing file
    CURRENT_FILE=FileName(j).name;
    OutFileName=strcat(CURRENT_FILE,'_Locations.mat');

    %Only going through the loop if file with locations does not exist
    %no need to change j index
    FileExist=exist(OutFileName, 'file');
    if(FileExist~=0)
        msgstr=sprintf('%s exists in current path\nSkipping this file',OutFileName);
        h=msgbox(msgstr);
    else
```
pause(0.5); delete(h)
else
    INPUT_IM=imread(CURRENT_FILE);
    imshow(INPUT_IM)
    Nregions=question();
    if Nregions>=1
        h=msgbox('Mark region 1'); pause(0.2); close(h);
        h=impoly; wait(h);
        region1=createMask(h);
        region1=find(region1==1);
    end
    if(Nregions>=2)
        h=msgbox('Mark region 2'); pause(0.2); close(h);
        h=impoly; wait(h);
        region2=createMask(h);
        region2=find(region2==1);
    end
    if(Nregions>=3)
        h=msgbox('Mark region 3'); pause(0.2); close(h);
        h=impoly; wait(h);
        region3=createMask(h);
        region3=find(region3==1);
    end
    if(Nregions==4)
        h=msgbox('Mark region 4'); pause(0.2); close(h);
        h=impoly; wait(h);
        region4=createMask(h);
        region4=find(region4==1);
    end
end %Saving to File. Do not change OutFileName variable %as it has to agree with what's defined in the entropy calculation code
switch Nregions
    case 1
        save(OutFileName,'region1','-v7.3')
    case 2
        save(OutFileName,'region1','region2','-v7.3')
    case 3
        save(OutFileName,'region1','region2','region3','-v7.3')
    case 4
        save(OutFileName,'region1','region2','region3','region4','-v7.3')
end
end

close all;
end

function N=question(varargin)
%Preguntamos por algo
N=nargin;

switch N
    case 0,
        prompt={'¿How many regions to be marked?'};
        name='Nregions';
    case 1,
        prompt={varargin{1}};
        name='Question box';
end

numlines=1;
defaultanswer={''};
answer=inputdlg(prompt,name,numlines,defaultanswer);
options.Resize='on';
options.WindowStyle='normal';
options.Interpreter='tex';

N=str2double(answer);

end
MATLAB function “EntropyTest_4Regions ()” to quantify mean entropy values for multiple regions, as defined by the “Mark_4_RegionsBees( )” function.

```matlab
function EntropyTest_4Regions(SmallObjThres,RoundThresh,NhoodRad,Filter)
%EntropyTest_4Regions(SmallObjThres,RoundThresh,NhoodRad,Filter)
%This function process the entropy in the input *.jpg images in the selected folder
%Processing parameters can be changed inside the function
%SmallObjThres defines the pixel count of an object which defines it as small, and make it deletable.
%Default Value = 8
%RoundThresh is a simmilarity coefficient with a perfect circle object. the closer to 1, the closer to a perfect circle an object is.
%Objects marked as circles are deleted by the preprocessing function
%Default Value = 0.95
%NhoodRad defines Neighborhood size for entropy calculation 7 pixels by default----means 13x13
%Default Value = 7
%Filter is a flag which defines whether preprocessing is small and round objects are deleted or not
%Typical Example of usage:
% EntropyTest_4Regions(8,0.95,7,1);
% (c) Gustavo Liñan Cembrano
% IMSE-CNM-CSIC
% linan@imse-cnm.csic.es

% Getting input dir
IM_PATH=uigetdir([],’Select Image Folder’);
cd(IM_PATH)
DeletePrevious = questdlg(’Delete previous results?’,’Delete’,’No’);
if(strcmp(DeletePrevious,’Yes’))
    delete(’*.tiff’)
    delete(’*.csv’);
elseif(strcmp(DeletePrevious,’Cancel’))
    return;
else
    %Do nothing
end

% Getting File name structure
FileName=dir(’*.jpg’);
NFiles=length(FileName);

msgstr=sprintf(’%d Images Found in directory’,NFiles);
h=msgbox(msgstr);
pause(1); delete(h)
clc;

% Creating output variables
Region1AvgEntropy=zeros(1,NFiles);
Region2AvgEntropy=zeros(1,NFiles);
```

Region3AvgEntropy=zeros(1,NFiles);
Region4AvgEntropy=zeros(1,NFiles);

Region1StdEntropy=zeros(1,NFiles);
Region2StdEntropy=zeros(1,NFiles);
Region3StdEntropy=zeros(1,NFiles);
Region4StdEntropy=zeros(1,NFiles);

Region1Area=zeros(1,NFiles);
Region2Area=zeros(1,NFiles);
Region3Area=zeros(1,NFiles);
Region4Area=zeros(1,NFiles);

ProcTime=zeros(1,NFiles);

%Pixels count of an object which defines it as small, and make it deletable
% SmallObjThres=8;
% Simmilarity coefficient with a perfect circle object. 1-->Perfect Circle
% RoundThresh=0.95;
% Neighborhood for entropy calculation 7 pixels by default---means 13x13
% area
% NhoodRad=7;
STREL=strel('disk',NhoodRad);
NHOOD=getnhood(STREL);

%% Looping
for j=1:NFiles
    t1=tic;
    % Accessing file
    CURRENT_FILE=FileName(j).name;
    fprintf('###################################################### 
');
    fprintf('      Accessing File %s
', CURRENT_FILE);
    LocationsFile=strcat(CURRENT_FILE,'_Locations.mat');
    LocFileExist=exist(LocationsFile,'file');
    if(LocFileExist)
        load(LocationsFile)
        Nregions=length(whos('region*'));
        fprintf('Found location definition for %d regions
',Nregions);
        DoIt=1;
    else
        MSG=sprintf('Locations file for %s does not exist skipping this
file', CURRENT_FILE);
        h=msgbox(MSG);
        DoIt=0;
    end
    if(DoIt)

        INPUT_IM=imread(CURRENT_FILE);

        % Increasing contrast
        INPUT_IM=imsharpen(INPUT_IM);

        % Preprocessing regions before entropy calculation
        fprintf('Preprocessing regions and preparing images
');
        if(Nregions>=1)

            [Region1Image,Region1BinaryImage,Region1X,Region1Y]=PreProcessRegion(region
1,INPUT_IM,SmallObjThres,RoundThresh,Filter);
APPENDIX D

end
if(Nregions>=2)

[Region2Image, Region2BinaryImage, Region2X, Region2Y] = PreProcessRegion(region 2, INPUT_IM, SmallObjThres, RoundThresh, Filter);
end
if(Nregions>=3)

[Region3Image, Region3BinaryImage, Region3X, Region3Y] = PreProcessRegion(region 3, INPUT_IM, SmallObjThres, RoundThresh, Filter);
end
if(Nregions>=4)

[Region4Image, Region4BinaryImage, Region4X, Region4Y] = PreProcessRegion(region 4, INPUT_IM, SmallObjThres, RoundThresh, Filter);
end

%% Entropy Calculation
fprintf('Processing Entropy for file %s\n', CURRENT_FILE);

fprintf('***********************************************************************\n');
if(Nregions>=1)
    Region1EntropyImage = entropyfilt(Region1Image, NHOOD);

    Region1EntropyImage = Region1EntropyImage(:,:,1).*Region1EntropyImage(:,:,2).*Region1EntropyImage(:,:,3);
    Region1EntropyImage = Region1EntropyImage.*double(Region1BinaryImage);
    figure(1);
    imagesc(Region1EntropyImage); colormap jet; colorbar;
    print(gcf,'-dtiff', strcat(CURRENT_FILE, '_Region1EntropyImage.tiff'));
    Region1AvgEntropy(j) = mean(Region1EntropyImage(find(Region1BinaryImage==1))) ;
    Region1StdEntropy(j) = std(Region1EntropyImage(find(Region1BinaryImage==1)));
    Region1Area(j) = length(region1);
end
if(Nregions>=2)
    Region2EntropyImage = entropyfilt(Region2Image, NHOOD);

    Region2EntropyImage = Region2EntropyImage(:,:,1).*Region2EntropyImage(:,:,2).*Region2EntropyImage(:,:,3);
    Region2EntropyImage = Region2EntropyImage.*double(Region2BinaryImage);
    figure(2);
    imagesc(Region2EntropyImage); colormap jet; colorbar;
    print(gcf,'-dtiff', strcat(CURRENT_FILE, '_Region2EntropyImage.tiff'));
    Region2AvgEntropy(j) = mean(Region2EntropyImage(find(Region2BinaryImage==1))) ;
    Region2StdEntropy(j) = std(Region2EntropyImage(find(Region2BinaryImage==1)));
    Region2Area(j) = length(region2);
end
if(Nregions>=3)
Region3EntropyImage = entropyfilt(Region3Image, NHOOD);

Region3EntropyImage = Region3EntropyImage(:,:,1).*Region3EntropyImage(:,:,2).*Region3EntropyImage(:,:,3);

Region3EntropyImage = Region3EntropyImage.*double(Region3BinaryImage);
figure(3);
imagesc(Region3EntropyImage); colormap jet; colorbar;
print(gcf, '-dtiff', strcat(CURRENT_FILE, '_Region3EntropyImage.tiff'));

Region3AvgEntropy(j) = mean(Region3EntropyImage(find(Region3BinaryImage == 1)))
Region3StdEntropy(j) = std(Region3EntropyImage(find(Region3BinaryImage == 1)));
Region3Area(j) = length(region3);

end
if (Nregions == 4)

Region4EntropyImage = entropyfilt(Region4Image, NHOOD);

Region4EntropyImage = Region4EntropyImage(:,:,1).*Region4EntropyImage(:,:,2).*Region4EntropyImage(:,:,3);

Region4EntropyImage = Region4EntropyImage.*double(Region4BinaryImage);
figure(4);
imagesc(Region4EntropyImage); colormap jet; colorbar;
print(gcf, '-dtiff', strcat(CURRENT_FILE, '_Region4EntropyImage.tiff'));

Region4AvgEntropy(j) = mean(Region4EntropyImage(find(Region4BinaryImage == 1)))
Region4StdEntropy(j) = std(Region4EntropyImage(find(Region4BinaryImage == 1)));
Region4Area(j) = length(region4);

end

%% Displaying resulting images
pause(1); close all;

%% Calculating mean std and area for entropy regions

figure(1)

imshow(INPUT_IM);
if (Nregions >= 1)
text(Region1X, Region1Y, strcat('+ Region1Entropy=', num2str(Region1AvgEntropy(j))), 'color', [0.6 0 0]);
end
if (Nregions >= 2)
text(Region2X, Region2Y, strcat('+ Region2Entropy=', num2str(Region2AvgEntropy(j))), 'color', [0.6 0 0]);
end
if (Nregions >= 3)

end
text(Region3X, Region3Y, strcat('Region3Entropy=', num2str(Region3AvgEntropy(j))),'color',[0.6 0 0]);
end
if(Nregions==4)
    text(Region4X, Region4Y, strcat('Region4Entropy=', num2str(Region4AvgEntropy(j))),'color',[0.6 0 0]);
end
print(gcf, '-dtiff', strcat(CURRENT_FILE,'_ENTROPY_RESULTS.tiff'));
ProcTime(j)=toc(t1);
fprintf('Processing time = %1.3fs\n',ProcTime(j));
end
end

OutFile='Results.csv';
fid=fopen(OutFile,'w+');
fprintf(fid,'FileName,');
fprintf(fid,'Reg1AvgEntropy,Reg1StdEntropy,Reg1Area,');
fprintf(fid,'Reg2AvgEntropy,Reg2StdEntropy,Reg2Area,');
fprintf(fid,'Reg3AvgEntropy,Reg3StdEntropy,Reg3Area,');
fprintf(fid,'Reg4AvgEntropy,Reg4StdEntropy,Reg4Area');
fprintf(fid,'ProcTime(s)\n');
for j=1:NFiles
    fprintf(fid,'%s,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f,%2.3f%,\n',FileName(j).name,...
            Region1AvgEntropy(j),Region1StdEntropy(j),Region1Area(j),...
            Region2AvgEntropy(j),Region2StdEntropy(j),Region2Area(j),...
            Region3AvgEntropy(j),Region3StdEntropy(j),Region3Area(j),...
            Region4AvgEntropy(j),Region4StdEntropy(j),Region4Area(j),...
            ProcTime(j));
end
fclose(fid);
The following screen shots provide a tutorial on how to implement the MATLAB functions provided above for image entropy analysis.

Add folders (and subfolders) containing the script and images to the file path.

Type the mark regions command into the command window.
Select the folder containing the images to be analysed.

Define the number of regions to be marked. This can be up to four per image.
Manually mark the region of interest.
Double click over the first location
point will finalise the region location

Enter the entropy test regions command in the
command window and select the folder
containing the images and region location files
Entropy image, locations file and results file are saved in the folder containing the image file.

Analysis summary shows the number of regions processed and the number of objects deleted from the image.
Table D1. Variation in entropy values for multiple photos \((n = 5\) per region per species) for two different body regions of the same specimen.

<table>
<thead>
<tr>
<th>Species</th>
<th>Face</th>
<th>Thorax dorsal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean ((n=2))</td>
</tr>
<tr>
<td><em>Bombus terrestris</em></td>
<td>156.3</td>
<td>155.9</td>
</tr>
<tr>
<td><em>Leioproctus sp.</em></td>
<td>157.2</td>
<td>158.9</td>
</tr>
</tbody>
</table>
Figure D1. Intraspecific variation in entropy values across different body regions of insect pollinators used in our study. Boxes represent the interquartile range, horizontal lines within boxes are median values, whiskers are the range and single dots are outliers.
Figure D2. Photograph of a pak choi *Brassica rapa* flower. Labels show the key reproductive structures.

Figure D3. Photographs of a female (a) and male (b) kiwifruit *Actinidia delicosa* flower. Labels show the key reproductive structures.