

# **Honeybee declines in a changing landscape: interactive effects of honeybee declines and land-use intensification on pollinator communities**

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## Abstract

Honeybees are used as a major agricultural input around the world and their pollination services have been valued at US\$14.6 billion to the United States alone. Dramatic declines in honeybee populations around the globe, however, questioned the sustainability of this reliance on a single pollinator species. In this study, I investigated the response of wild pollinator communities to declining honeybee density and changing land use intensity to determine the potential of wild pollinators to compensate for honeybee loss in increasingly human-modified environment. I generated a gradient of declining honeybee density using increasing distances from commercial bee hives, and conducted flower observations on experimentally-grown plants across this gradient. I investigate how declining honeybee densities and intensifying land use combine to influence the composition of the pollinator community as a whole, then go on to explore individual trends in the most common pollinator species. I then analyze how this impacts the transport of viable pollen by the pollinator community and determine how these changes alter seed set in several common plant species. I then change my focus away from the composition of the pollinator community, and instead investigate how declining honeybee densities and land-use intensification influence the structuring of interactions between plants and pollinators within the community. I identify the pollen species carried by pollinators, and use this to construct a network of pollination interactions. I then use this network to analyze how changes in the way species interact influences the pollination services delivered by the pollinator community to different plant groups (weeds, native plants, and crop species).

My findings show that honeybee declines may have a large impact on community structure and interactions within pollination systems. I observed a significant shift in the wild pollinator community composition as honeybee densities declined, from a generally bee/hoverfly dominated community to one more dominated by large flies. This was associated with a significant decline in the total pollen load transported by the community, indicating that pollination services may suffer in the absence of honeybees. As honeybee densities declined, however, I also observed a shift toward greater specialisation of pollinators on abundant resources, increased pollinator constancy, and a higher viability rate of the pollen transported. These findings show that although the total amount of pollen transported by the community declined as honeybee densities decreased, the probability of this pollen transport resulting in effective pollination likely increased. Thus, I observed no decrease in seed set with honeybee declines in any of the three plant species tested, and one of these even showed a significant increase. Finally, I also demonstrated that this change differentially affected different plant types, and that the extent of changes to each plant species differed between land-use types. This reflected changes in the relative abundance of pollen types in different land uses, with greater specialisation in the absence of honeybees disproportionately benefiting common species. These findings have strong implications for several contemporary issues in pollination biology, both locally within New Zealand and on a global scale. These are discussed in the following sections.

Finally, I conclude by discussing the implications of this research on several contemporary issues in pollination biology, namely the ability for wild pollinators to compensate for honeybee declines, the impact of honeybees on natural New Zealand ecosystems, the contribution of honeybees to invasive weed pollination and finally the management of surrounding land use types to maximize the effectiveness of wild pollinators.

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## Chapter 1: Introduction

### 1.1 The importance of pollination

It is rumoured that Albert Einstein once may have claimed, “*If the bee disappeared off the surface of the globe then man would only have four years of life left. No more bees, no more pollination, no more plants, no more animals, no more man*” (Benjamin & McCallum 2009). While this view represents an extreme of current scientific views, pollination is none-the-less extremely important for plant reproduction. Nearly 90% of flowering plant species worldwide depend on insect pollination (Ollerton *et al.* 2011). Similarly, three quarters of commercial crop species depend to some extent on insect pollinators and these are critical to global food security (Klein *et al.* 2007). Although a few species of wind pollinated crops (such as grains) and pollination independent crops (such as tubers) comprise a large proportion of global food consumption (Klein *et al.* 2007), insect-pollinated crops are critical to the supply of many essential oils, proteins and vitamins people require as part of a balanced diet (Thrupp 2000; Frison *et al.* 2011) and pollination therefore directly impacts human welfare (Meffe 1998; Carpenter *et al.* 2006; Díaz *et al.* 2006). Although modern crop breeding programs are attempting to reduce the reliance of agriculture on insect pollinators through the development of seedless or self-fertile crops, global food demands are increasing at such a rate that our reliance on insect pollinators is deepening (Aizen *et al.* 2008a; Aizen & Harder 2009). In the United States alone, honeybee pollination has been valued at USD\$14.6 billion (Morse & Calderone 2000), with wild insect pollinators contributing a further USD\$3 billion to the national economy (Losey & Vaughan 2006).

### 1.2 Honeybees as pollinators

The domesticated western honeybee (*Apis mellifera*) is by far the largest contributor to pollination services globally. Honeybees, native to Europe, Asia and Africa, have been spread worldwide by humans both because of their value as pollinators (Vanengelsdorp & Meixner 2010), and due to their capacity to produce honey (Vanengelsdorp & Meixner 2010). As honeybees are easily domesticated, form large colonies (up to 50,000 bees in a single hive) and are easily transportable (hives can easily be trucked for hundreds of kilometres), they are easily manipulated on an as-required basis by farmers (Morse & Calderone 2000). Thus, millions of colonies worldwide are commercially rented to farmers, and these are often considered essential to ensure maximal crop yields (Morse 1991b; Morse & Calderone 2000). Contributing to their success as crop pollinators, honeybees have a complex communication system between workers, allowing the transfer of accurate information about floral resources (Von Frisch 1967). As a result, foragers can recruit more workers to large resource supplies, such as mass flowering crop fields, on which workers focus their foraging efforts.

### 1.3 Honeybee declines

While honeybees are undoubtedly convenient pollinators, high reliance on any single species carries inherent risk, and recent honeybee declines are making this risk increasingly obvious. In the United States, the number of managed honeybee colonies has declined by nearly 60% over the last half century (Potts *et al.* 2010b), with over 30% of overwintering colonies perishing annually in recent years (Hayes *et al.* 2008). Similar trends have been recorded globally (Potts *et al.* 2009; Potts *et al.* 2010a). While the precise causes of these declines are unknown, a wide range of factors have been implicated; including pesticides, pathogens (particularly the mite *Varroa destructor*), monocultural agricultural methods and commercial beekeeping practices (Bromenshenk *et al.* 2010; Johnson *et al.* 2010; Le Conte *et al.* 2010; Potts *et al.* 2010a). While none of these factors alone is solely responsible for the decline, these causes are likely to interact synergistically (Potts *et al.* 2010a). For example, while agricultural monocultures may provide abundant floral resources which may be beneficial to healthy beehives, a diet low in pollen diversity has been shown to impede honeybee immune systems (Alaux *et al.* 2010). This, may not necessarily detrimental in isolation, however when exposed to harmful viruses, honeybees raised on a monoculture diet can be more susceptible to virus-induced mortality (Cox-Foster *et al.* 2007). Similarly, the parasitic mite *Varroa destructor* (Figure 1.1) can be treated by beekeepers and is seldom the exclusive cause of mortality in commercial hives (Le Conte *et al.* 2010). *Varroa* mites, however, feed on the haemolymph of bees, and can be important vectors for the spread of viral diseases (Shen *et al.* 2005). Thus, this can interact synergistically with both monoculture crop methods and viral diseases to contribute to the global decline of honeybees.



Figure 1.1. A *Varroa destructor* mite on the head of a honeybee. As well as directly killing bee larvae, *Varroa* mites spread viruses between bees and can weaken bee immune systems

## 1.4 Wild pollinators

Given the current uncertainty regarding the future sustainability of our reliance on honeybees, an increasing number of studies are investigating the contribution of unmanaged pollinators to pollination (Corbet *et al.* 1991; Kremen *et al.* 2002; Kremen *et al.* 2004; Hoehn *et al.* 2008; Tylianakis 2008; Winfree *et al.* 2008; Garibaldi *et al.* 2013). Of the diversity of other animals that are capable of performing pollination, the most often recognised are wild bee species (Figure 1.2) (Bohart 1972), although a wide variety of other insect groups (Heine 1937), birds (Clout & Hay 1989), bats (Pattamore & Wilcove 2012), lizards (Olesen & Valido 2003) and even rodents (Pattamore & Wilcove 2012) can also be important pollinators. While the importance of conserving wild pollinators has been argued (Ghazoul 2005, 2007), there is increasing recognition that these may contribute greatly to pollination (Winfree *et al.* 2008; Garibaldi *et al.* 2013). Many species of wild pollinators have been shown to be equally efficient as honeybees at transporting pollen between flowers (Rader *et al.* 2009), and these may help to compensate for honeybee declines by ensuring pollination (Ricketts *et al.* 2004; Winfree *et al.* 2007; Rader *et al.* 2012). A recent global study investigating pollination in over 600 field sites of 40 different crop systems worldwide, revealed that wild pollinators are capable of effectively pollinating a wider variety of crop species than honeybees, and that these boost crop yields, independently of honeybee visits to the flower (Garibaldi *et al.* 2013). This shows that, while honeybees may provide some pollination services to farmers, wild pollinators in many cases may be equally, if not more, capable of providing this service.



Figure 1.2. A wild solitary bee *Lassioglossum mataroa* feeds from a yarrow (*Achillea millefolium*) inflorescence. Wild pollinators, such as this, are becoming increasingly recognised for their role in pollination

## 1.5 Impact of honeybees on wild pollinators

As with any species sharing similar resources, there is potential for honeybees to compete with other pollinators. Numerous studies have provided anecdotal evidence showing honeybees to affect a diverse array of wild pollinators (Badano & Vergara ; Gross & MacKay 1998; Kato *et al.* 1999; Gross 2001; Goulson *et al.* 2002; Dupont *et al.* 2004). Reviews on the subject, however, have found evidence to be inconclusive regarding widespread or long-term reductions in pollinator populations in the presence of honeybees (Goulson 2003b; Paine 2004). This is generally considered to be due to the difficulties of conducting large-scale studies capable of detecting competition and changing abundances of pollinators, rather than a lack of any underlying trends (Goulson 2003b; Paine 2004). As pollinator communities can be highly diverse, and pollinators are highly mobile and frequently generalist foragers, population-level studies on pollination have struggled to detect widespread changes in wild pollinator communities as a result of competition with exotic pollinators. Thus, whether pollinator communities change as a result of competition with honeybees, and how this affects the actual pollination of plant communities, remain unknown. The recent expansion of network-analytical approaches, however, may provide a powerful means to understand these direct and indirect competitive effects (Forup & Memmott 2005; Tylianakis 2008; Bascompte 2009). By directly analysing changes in the interactions between plants and pollinators that result in pollination, network approaches to this question also provide information about structure that relates to emergent properties such as stability (Olesen *et al.* 2002; Bascompte *et al.* 2003)

## 1.6 Land-use intensification and its effect on pollinators

Wild pollinators have shown some capacity to compensate for declining honeybee densities (Goulson 2003a; Winfree *et al.* 2007; Rader *et al.* 2012; Garibaldi *et al.* 2013), however, their ability to do so may depend on surrounding land uses (Ricketts *et al.* 2004; Klein *et al.* 2007; Kremen *et al.* 2007). Land-use intensification has been shown to negatively impact a variety of wild pollinators (Klein *et al.* 2007), both through the alteration of floral resource supplies, and the destruction of native habitat requirements, such as viable nesting sites (Kremen *et al.* 2007). Changes in floral resource supplies, for example the decreased flower diversity in agricultural systems, have been shown to reduce pollinator diversity and abundance (Kremen *et al.* 2007). Similarly, many bee species are severely limited by bare ground, hollow plant stems and other nesting requirements in agricultural systems (Greenleaf & Kremen 2006; Kim *et al.* 2006; Morandin *et al.* 2007).

Pollination services provided by wild pollinators have been shown to decrease with distance from natural habitats, and are often negatively correlated with agricultural intensity (Klein *et al.* 2003; Greenleaf & Kremen 2006; Kim *et al.* 2006). If the promotion of wild pollinators has any potential for reducing our dependence on managed honeybees and compensating for their decline, land-use management may become increasingly important as a tool to manipulate pollinator communities and manage pollination. The importance of this is clearly demonstrated in several regions of China, where intensive agriculture, particularly the excessive use of pesticides, has resulted in pollinator declines to such an extent that crop pollination routinely fails, and it is not uncommon for orchardists to employ workers to hand pollinate fruit trees (Partap *et al.* 2000).

## 1.7 Pollination in NZ

In New Zealand, pollination systems are unique in that there are no native social bee species, the butterfly fauna is highly depauperate, and dipteran flies compose an unusually high proportion of pollinators (Heine 1937). The pollinating fauna historically consisted primarily of solitary bee species (Colletidae and Halictidae), flies, and a number of moth, parasitoid wasp and beetle species (Heine 1937; Primack 1983). Native birds, and possibly short tailed bats, have also had a high historical importance in pollination (Thomson 1927; Anderson 2003; Anderson *et al.* 2011; Pattemore & Wilcove 2012), however due to predation by introduced mammals, these are now depleted across much of the mainland (Anderson 2003; Anderson *et al.* 2011; Pattemore & Wilcove 2012). In addition to these native pollinators, New Zealand has eight species of exotic bee deliberately introduced to aid in the pollination of crops (only four of these, *Apis mellifera* and three *Bombus* species, are widespread) (Howlett & Donovan 2010), and a number of other introduced insects that regularly perform pollination.

Because of this historically diverse but unspecialised pollinator guild, most native plant species in New Zealand are adapted to pollination by a large range of generalist pollinators (Heine 1937; Primack 1983), and consequently have predominantly small, white flowers (Heine 1937; Lloyd 1985). In contrast, many invasive and/or introduced plant species in New Zealand have brightly coloured, showy flowers and are adapted to attract exotic pollinator species, like introduced social bees (Goulson 2003b). While pollination provided by exotics is generally of great benefit to society, there is some concern that these may facilitate the spread of invasive weeds (Butz-Huryn 1997). Although the evidence for this has been debated (Huryn & Moller 1995; Butz-Huryn 1997), exotic pollinators have been shown to preferentially visit exotic plant species (Goulson & Hanley 2004; Webber *et al.* 2012), and in some cases these are the primary pollinators of exotic weeds (Paynter *et al.* 2010), a pattern congruent with overseas findings (Aizen *et al.* 2008b).

In New Zealand, the bee mite *Varroa destructor* has recently spread to the South Island and is currently having an enormous impact on honey bee (*Apis mellifera*) populations throughout the country (Donovan 2007). In the near future, *Varroa* is expected to almost completely eliminate feral bee populations and reduce numbers of commercial hives nationwide (Donovan 2007). Treatment is possible in commercial hives, however, this is costly and requires extensive management (Simpson 2003). Even with *Varroa* management, honey production is significantly lowered, and the commercial viability of bee keeping is reduced (Simpson 2003). This is expected to result in the removal of commercial hives from many less-productive ecosystems, such as high-country grassland, and is likely to have an enormous effect on pollination systems within the high-country ecosystem. This could potentially lead to significant impacts on both insect pollinators and plant communities in this environment.

## 1.8 Study Location

To understand the combined impacts of honeybee declines and land use on wild pollinator communities, I conducted a manipulative field experiment in the Mackenzie basin; a large subalpine

river basin in New Zealand's Southern Alps. Prior to European settlement, there is evidence that the area was at least partially covered with subalpine scrub (McGlone & Moar 1998), however upon European arrival, the basin was almost exclusively vegetated with native grasslands (Connor & Vucetich 1964). This change probably resulted from fires initiated by Polynesians (McGlone & Moar 1998). Since European settlement, the basin has undergone further land-use change. The discovery of its grazing potential in 1855 by the outlawed sheep rustler James Mackenzie, led to the establishment of many renowned high country sheep stations (Connor & Vucetich 1964). As a result of this grazing, the tussock landscape has suffered repeated burning, overgrazing, and the introduction of many exotic grass, tree and herbaceous weed species (Connor & Vucetich 1964; Norton *et al.* 2006). Despite this grazing pressure, however, the Mackenzie basin has had historically low agricultural intensity (Norton *et al.* 2006). The impacts of sheep grazing in the Mackenzie basin have been minimal compared with other regions of NZ (Norton *et al.* 2006), and the basin still harbours high native biodiversity of both plant and animal species. A survey in the basin in 1984, revealed the presence of ten native bee species (Quinn 1984), giving it among the highest bee diversity in the country. Additionally, five species of exotic bee (*Apis mellifera* and four *Bombus* species), and a diverse range of both native and exotic pollinating flies, wasps, butterflies and moths are also present, giving the basin a diverse pollinator assemblage.

In recent decades, however, there has been a rapid shift toward more intensive agriculture (Figure 1.3). Modern technology, particularly large centre-pivot irrigation systems, have led to the encroachment of intensive cropping and dairy farming into historic tussock grasslands, and this is alleged to be causing widespread environmental degradation (Lee *et al.* 2008).

This combination of high pollinator diversity and rapidly-intensifying agriculture makes the Mackenzie basin an ideal study location to investigate the interacting effects of declining honey bee densities and land use intensification. Furthermore, the open, predominantly grassland landscape is severely limited in honeybee nesting cavities, making feral honeybee colonies rare and allowing honeybee sources to be easily identified and manipulated.





Figure 1.3 A view across the western edge of the Mackenzie basin, from the Benmore Range looking toward lake Ohau (upper left) and Ben Ohau peak (upper central). Installation of centre pivot irrigators (centre), has allowed intensive agriculture to encroach into native tussock grasslands (lower).

## 1.9 Objectives and Study Outline

This research aims to determine the response of pollinator communities to the simultaneous influences of declining honeybee densities and intensifying land use. While the effects of these changes have been studied in isolation, little is currently known about simultaneous effects of these even though agricultural intensification is increasing our reliance on honey bees. The potential for these multiple drivers to interact synergistically to modify pollination interactions has been suggested in the literature; however until now this has been untested. Further, given our dependence on pollination, it is imperative to understand how changes in the way plants and pollinators interact directly impact the pollination success and seed set of plants.

To begin, I investigate in Chapter Two how declining honeybee densities and intensifying land use combine to influence the composition of the pollinator community as a whole, then go on to explore individual trends in the most common pollinator species. I then analyze how this impacts the transport of viable pollen by the pollinator community and determine how these changes alter seed set in several common plant species.

In Chapter Three, I change my focus away from the composition of the pollinator community, and instead investigate how declining honeybee densities and land-use intensification influence the structuring of interactions between plants and pollinators within the community. I identify the



pollen species carried by pollinators, and use this to construct a network of pollination interactions. I then use this network to analyze how changes in the way species interact influences the pollination services delivered by the pollinator community to different plant groups (weeds, native plants, and crop species).

Finally, in chapter four I summarize findings and discuss how these relate to the existing literature. I then discuss the implications of my findings for several major contemporary questions facing pollination biology. Finally I conclude by discussing potential gaps in our knowledge which may be lucrative avenues for further research.

## Chapter 2: Changes in pollinator community composition and resulting pollination rates

### 2.1 Introduction

Three quarters of global food crop species rely to some extent on animal pollination (Klein *et al.* 2007), and this dependence on animal pollinators is increasing (Aizen & Harder 2009). Meanwhile, pollinator populations around much of the globe are facing catastrophic declines (Holden 2006; Potts *et al.* 2010a; Burkle *et al.* 2013; Tylianakis 2013). Honeybees, in particular, are suffering in much of the world from a combination of diseases and syndromes attributed to anything from pathogens and pesticides to land-use change and electromagnetic radiation (Holden 2006; Ratnieks & Carreck 2010; Williams *et al.* 2010). As a result, the number of managed honeybee hives in the United States dropped by 59% between 1950 and 2007, with similar trends seen globally (Potts *et al.* 2010a).

While many species of wild insects frequently visit flowers and are often highly-efficient pollinators (Winfree *et al.* 2007; Rader *et al.* 2009; Rader *et al.* 2012), high managed honeybee densities are often maintained during crop flowering periods to ensure pollination should wild pollinators be absent or insufficient to ensure maximal yields (Morse 1991a; Morse & Calderone 2000). Honeybee densities can be easily manipulated and large numbers can be transported to fields of mass-flowering crops specifically to coincide with the flowering season (Morse 1991a; Howlett & Donovan 2010), thus they are frequently relied upon as a major agricultural resource (Howlett & Donovan 2010).

This high reliance on a single pollinator species is concerning for the effectiveness and resilience of pollination services (Winfree *et al.* 2007). Recent studies have shown that diversity of pollinators is more important than the overall abundance of pollinators for predicting seed set in crops (Hoehn *et al.* 2008; Brittain *et al.* 2013a), and that honeybees may be less effective pollinators than other wild pollinating insects (Winfree *et al.* 2007; Garibaldi *et al.* 2013). A large recent analysis of pollination of over 40 crop systems worldwide, showed that visits from wild pollinators across the world result in, on average, double the increase in seed set produced by the equivalent number of honeybee visits, and benefit a much wider diversity of plants (Garibaldi *et al.* 2013). Further, wild pollinators seem to act complementarily to honeybees, with wild pollinators enhancing pollination rates even in flowers frequently visited by honeybees (Garibaldi *et al.* 2013).

Many wild pollinator populations, however, have been negatively affected by anthropogenic land-use change, and the associated habitat degradation, loss of nesting habitats and insecticide use (Steffan-Dewenter 2003; Biesmeijer *et al.* 2006; Greenleaf & Kremen 2006; Ricketts *et al.* 2008). This may make wild pollinators less able to compensate for declining honeybee densities in areas of intensive land use. Although several studies have investigated the effects of either changing land use or honeybee declines on pollination (Butz-Huryn 1997; Goulson 2003b; Dupont *et al.* 2004; Kremen *et al.* 2004; Ricketts *et al.* 2008; Whittingham 2011), the interactive effects of these are unknown. Given the potential value of wild pollinators, it is imperative to understand how wild pollinator

communities will respond to current and future numbers of honeybees, particularly in an increasingly human-modified environment (Morse 1991a; Holden 2006).

In this study I address this question using a manipulative field experiment comprising experimentally-exposed plants across a gradient of honeybee density and land-use types. In particular, I focus on three main hypotheses:

- 1) The composition of the wild pollinator community will respond to changes in the local density of honeybees.
- 2) Any change in the observed pollinator community may impact the pollination effectiveness of the community, altering pollen transportation patterns, potentially affecting pollen viability, and ultimately the seed set rates of plants.
- 3) The surrounding land-use type may affect the ability of the wild pollinator community to compensate for declining honeybee density.

## 2.2 Methods

### 2.21 Study site locations and characteristics

The study was conducted using a manipulative field experiment based in the Mackenzie Basin, South Canterbury, New Zealand. The Mackenzie Basin, a large subalpine river basin in New Zealand's Southern Alps, was selected as the focus for this study both because of its high native plant and insect-pollinator diversity (Quinn 1984), and its valuable agricultural industry (Butcher 1997). Also, because of the relatively open, predominantly grassland landscape, feral honeybee colonies are likely to be severely limited by the availability of nest cavities. This, combined with the effects of the invasive mite *Varroa destructor* (Simpson 2003), means that feral honeybee colonies are rare, making honeybee source populations easy to identify and manipulate.

Table 2.1. Location, distance from bee hives and surrounding land-use type for each site

Site number	Distance from nearest bee hive (m)	Land-use type	Latitude (WGS84)	Longitude (WGS84)
1	6330	Degraded tussock	44 25'0.29"S	170 12'15.18"E
2	200	Riverbed	44 20'1.00"S	170 10'25.86"E
3	1650	Degraded tussock	44 20'24.26"S	170 8'37.36"E
4	4650	Riverbed	44 18'26.79"S	170 13'21.17"E
5	570	Riverbed	44 18'22.08"S	170 8'14.19"E
6	6790	Riverbed	44 15'23.29"S	170 11'49.71"E
7	4280	Degraded tussock	44 12'0.30"S	170 6'10.43"E

8	5180	Tussock	44 12'33.69"S	169 57'36.56"E
9	7430	Tussock	44 15'1.96"S	169 49'16.76"E
10	2810	Tussock	44 17'10.76"S	169 56'27.28"E
11	820	Riverbed	44 15'51.02"S	169 59'17.03"E
12	940	Riverbed	44 16'25.08"S	170 1'13.55"E
13	100	Crop	44 18'6.14"S	170 4'46.95"E
14	350	Crop	44 19'1.33"S	170 4'44.74"E
15	2000	Degraded tussock	44 20'3.57"S	170 5'38.36"E
16	20	Crop	44 20'14.04"S	170 3'20.19"E
17	1325	Crop	44 20'51.26"S	170 3'53.68"E
18	2760	Degraded tussock	44 22'19.98"S	170 4'48.88"E

## 2.22 Honeybee density gradient

To determine the effects of honeybees on pollinator communities and pollination success, I used a naturally-occurring gradient of honeybee density, which was generated by selecting 18 sites (Table 2.1) at increasing distances from all known managed hives. All commercial apiaries (beehive locations) around the Twizel area were mapped following communication with local bee keepers. Study sites ranged from 20m to 7800m away from the nearest commercial apiary (Table 2.1). Apiaries each contained 18-22 individual hives. Although honeybees have been recorded foraging up to 10 km from their source hive (Beekman & Ratnieks 2000), the possible foraging area scales exponentially as the distance from a hive increases, and thus bee densities would be expected to decline exponentially with increasing distance from the hive. To confirm this, two pan traps (plastic containers, 170mm x 170mm wide, 85mm deep) were placed at each site for a 3 month sampling period and honeybee trap rates were recorded. One trap was white and one yellow, to get a representative sample of the entire pollinator community because colour preference varies between species (Leong & Thorp 1999; Wilson *et al.* 2008). These were filled with one litre of liquid mix, containing two parts water to one part propylene-glycol preservative, and a drop of fragrance-free dishwashing liquid to act as a surfactant.

To determine the pattern of honeybee decline across the putative distance gradient (Figure 2.1), I modelled change in honeybee pan trap catch rates with increasing distance using a generalized linear model (GLM), with Poisson errors, a log link function, and fitted with quasi-likelihood to deal with over dispersion. This was conducted using the `glm` function in the base package of the R Statistical Software (R Development Core Team 2011).

Figure 2.1 Relationship (+/- 95% confidence interval) between observed bee density at the sites, and the distance from commercial bee hives. While an occasional bee was caught foraging at high

distances, bee densities at over 4000 m from the hive were not significantly different from zero (95% CI includes zero). A possible outlier at site 5 (4700m) may be caused by a wild bee hive in the vicinity.

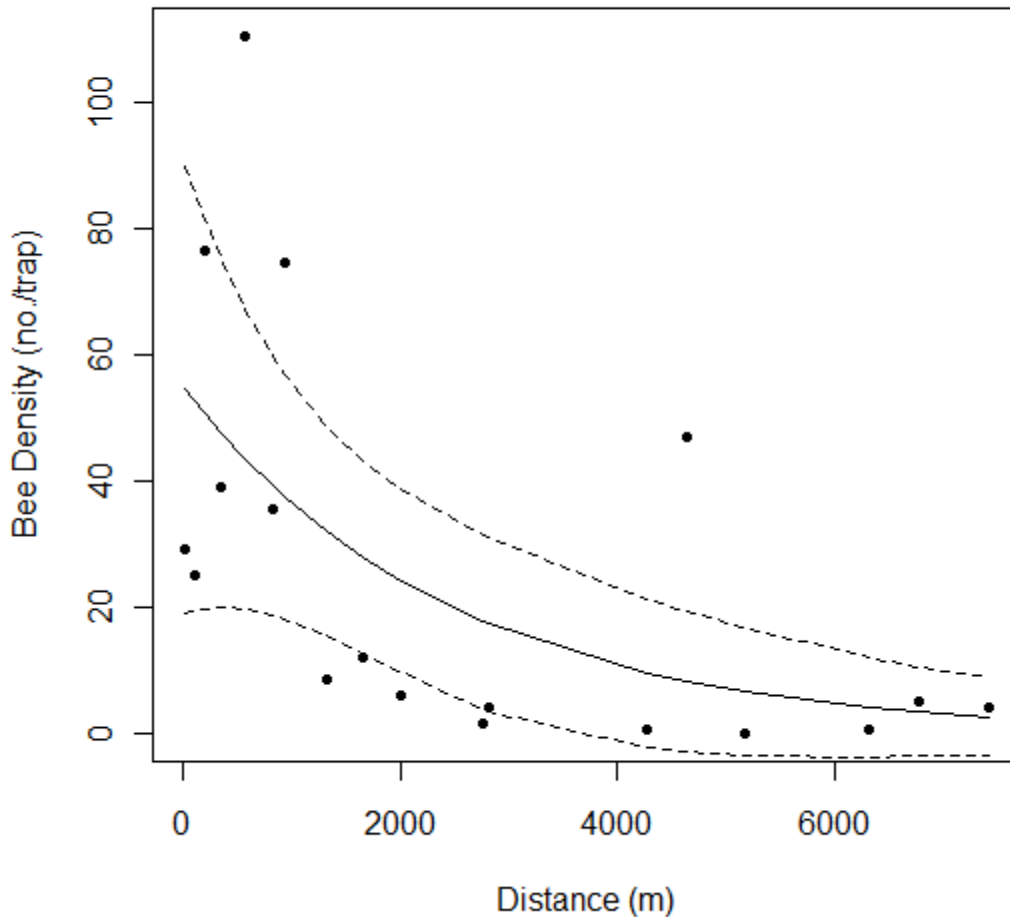


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Honeybee catch rates in pan traps declined with increasing distance from the nearest hive (Figure 2.1,  $F = 9.51$   $P = 0.007$ ), suggesting that distance from commercial hives was a good proxy for honeybee density in my study region. As distance from honeybee hives was the underlying driver behind the changing abundance of honeybees at the sites, I used this distance as an explanatory variable in analyses rather than honeybee abundance from the traps. Furthermore, as day to day honeybee foraging patterns are variable, I decided that distance from hives was more likely to correlate with long-term honeybee densities. Although an occasional bee was caught foraging at large distances, the majority of foraging occurred within 2km from the nearest hive (Figure. 2.1), and

trap rates more than 4000m from the nearest hive were not significantly different from zero (a 95% confidence interval includes zero at greater distances) (Figure 2.1).

### 2.23 Land-use categories

To determine the effects of land use on wild pollinators, I categorised the predominant land-use type within a 50m radius surrounding each site into one of four categories that reflected the predominant habitats in the region and comprised an approximate gradient of land-use intensity. These categories were: 1) Tussock Grassland - predominantly native vegetation, stock absent or at very low densities and with generally few exotic plant species. 2) Riverbed scrub – stock absent but patchy vegetation cover with a lot of bare gravel/sand. Vegetation primarily a mix of native and exotic shrubs. 3) Degraded tussock - Historically native tussock grasslands, but with high grazing intensity (>100 sheep/ha) and significant invasion of introduced pasture species and exotic weeds to give a mix of native and exotic ground vegetation. 4) Crop/pasture - High stock density, little (if any) native vegetation, often with irrigation and fertiliser input. These four land-use categories (Figure 2.2) reflected an approximate gradient of intensifying agricultural land use; with crop and pasture fields having highly intensive agriculture, degraded tussock areas having less, but still significant agricultural intensity, and riverbed and tussock areas having very little or no agriculture (although riverbed areas had other forms of human disturbance, for example vehicle damage, and were highly prone to weed invasion). As some pollinator guilds (particularly blowflies) are dependent on livestock during their larval stages, sheep density within an approximate 500m radius surrounding each site was also calculated, using stocking data obtained from farmers.



Figure 2.2 Typical land-use types in the study area, from left to right: Pasture; Degraded tussock grassland; Riverbed; Tussock grassland.

As bee hive locations were beyond my control, and tended to be placed by bee keepers in areas with abundant floral resources to maximise honey yield (Peter Bell, personal communication), intensive land uses were generally associated with close proximity to bee hives (Figure 2.3). As a result, my two predictor variables of interest (honeybee density, measured as distance from hives, and land-use intensity) were collinear, with tussock grassland tending to be more common at large distances, and more sites falling within the crop/pasture category at shorter distances (Figure 2.3). To account for this collinearity, I tested each effect after controlling for the possible effects of other variables in the model. That is, for all results presenting effects of bee density or land use, I used adjusted (Type III) sums of squares in linear models and tested partial coefficients in mixed effects models.

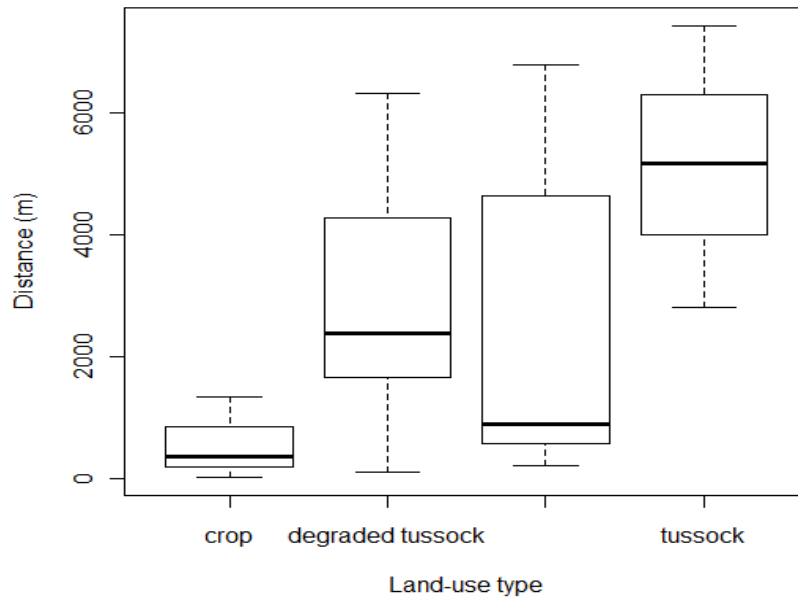


Figure 2.3 Land uses across the honeybee density gradient. There is a significant correlation between agricultural intensity and honeybee density, with beehives generally found in close proximity to crop and pasture fields.

## 2.24 Selection of plant species for pollinator observations

Common plant species were noted at each site and, excluding wind-pollinated species, these were categorised as natives, crop plants or exotic weeds. Of these, a total of eight species were selected as focal study species based on their abundance at the sites and availability. These were - Natives: *Leptospermum scoparium*, *Epilobium melanocaulon*; Crops: *Trifolium repens*, *Medicago sativa*, *Brassica napus*; Weeds: *Echium vulgare*, *Cirsium vulgare*, *Achillea millefolium*.

*Echium vulgare*, *A. millefolium*, *M. sativa* and *B. napus* were grown from seed, with *E. vulgare* and *A. millefolium* sourced from local wild populations and *M. sativa* and *B. napus* from seed donated by PGG Wrightson seeds, Christchurch, New Zealand. *Trifolium repens* was produced asexually from cuttings taken from the University of Canterbury Cass field station, and *C. vulgare* and *E. melanocaulon* were cultivated from overwintering root cultures also collected from the Cass field station. Two-year-old *L. scoparium* saplings were purchased from Wai-ora nurseries Limited, Christchurch, New Zealand. All plants were cultivated in glasshouses on the University of Canterbury campus for at least 4 months prior to flowering, and acclimatised to outdoor conditions for one month prior to transport to the Mackenzie Basin at the commencement of the study.

## 2.25 Observation of flower visitations by pollinators

To determine the pollinator community present at each site, I conducted monthly flower observations at each site using the eight plant species listed above. Standardised experimental plants were used to eliminate variation in flower attractiveness due to site-specific environmental factors; for example, changes in nectar quality due to water stress or nutrient deficiencies, which would hinder interpretation of pollinator community differences across sites. I transported three individuals of the above-named flowering species to all sites and placed them in a square array approximately 0.5 m apart. I then observed the potted flowers for a two hour period, during which I caught all insects interacting with the flowers, and noted the flower species from which each insect was collected. An interaction was defined as any contact between the flower and the insect where pollen was potentially collected from the anthers or deposited on the stigma (Engel & Irwin 2003). Temperature was recorded at the beginning of each observation period, and observations were only conducted in temperatures greater than 14°C, from 10am-6pm to cover optimal insect foraging times, and were not conducted in the rain.

Observed insects were caught and stored individually in collection jars to avoid pollen contamination until they were frozen and any pollen was removed. Insects were later identified to species level (with the assistance of taxonomists where required) or grouped into morphospecies where morphological characteristics were insufficient to differentiate species accurately. A reference collection of these was deposited at the University of Canterbury.

## 2.26 Community composition analysis

To test for compositional changes in the pollinator community (excluding honeybees, as these were experimentally manipulated) across the honeybee density and land-use gradient, I used a multivariate permutation analysis of variance (PERMANOVA) (Anderson 2001), conducted in the PERMANOVA+ add-on for the statistical program PRIMER. Community similarity was incorporated using a Bray-Curtis similarity matrix, as this integrates information on both abundance and composition of the community (Magurran 2004). Both distance from hive and surrounding land-use type (tussock grassland, non-farmed riverbed, degraded tussock, crop/pasture), and the interaction between these were included as predictor variables. As bee hive locations and surrounding land use were correlated (Figure 2.2), there was potential for distance from hive effects to be confounded by surrounding land-use variation (or vice versa) rather than any effect of honeybees. To control for this collinearity, I incorporated surrounding land-use categories as a cofactor in the statistical models, and partitioned out any variation in the pollinator communities due to land-use effects before testing distance from hive effects (and vice versa). To test whether additional site variables helped to explain any community shifts, I also incorporated surrounding sheep density and temperature during the observations as covariates in the PERMANOVA analyses. Sheep density surrounding the site was incorporated because several common fly species that appeared to be important pollinators are dependent on stock animals and their dung during larval life stages. Temperature was included because pollinator behaviour can change with increasing temperature (Roubik 1992), and temperature may have differentially affected visitation rates in different species during the observations. This maximal model was then simplified using step-wise model simplification based on minimising the Akaike Information Criterion (AIC). Temperature and sheep density both increased the AIC value when incorporated into the model, so were removed from the final model.



To understand which species were driving the overall change in community composition, I examined the separate responses of the most abundant pollinator species to declining honeybee densities (distance from hive) and local land use. I ran a GLM (with poisson errors, fitted with quasilikelihood where necessary to deal with overdispersion) on all species present at more than half (nine or more) of my sites (Table 2.1). The response variable was the abundance of a given species, and the predictors were distance from hive, surrounding land-use type, and their interaction. Stocking density and average temperature while sampling were not included as the previous permanova analysis found these to have no significant effect on community composition.

## 2.27 Pollen load and viability

To determine how changes in the pollinator community influenced pollen transportation, I analysed the pollen load being carried by insects. The total number of pollen grains being carried was counted for ten insects of every species (to give a standardised samples size per species), from every site, for each sampling round, excluding pollen carried in the Corbiculae (pollen baskets) of bees as this is unlikely to be deposited on flowers (Dafni *et al.* 2005). For this, pollen was removed from the insects using the fuchsin gel method detailed in (Dafni *et al.* 2005), whereby a sticky cube of fuchsin gel was dabbed over the surface of the insect to collect pollen, then melted onto a microscope slide. The slides were then photographed at 100x magnification using fluorescence microscopy, and the number of pollen grains in each resulting photo was counted electronically using the 'Analyse Particles' function on the image processing program Image J (Rasband 1997).

As the viability of pollen grains decreases over time (Dafni & Firmage 2000), pollen grains being transported may not be viable when they reach a stigma. To control for this, I tested the viability of pollen transported by a subset of the insects within 24 hours of collection of each insect while still in the field, as pollen viability decreases with age. Twenty pollen grains from one insect of every species, from every site, from every sampling round were tested for viability using the Diaminobenzidine (DAB) test protocol described in Dafni *et al.* (2005). For this, pollen was rinsed off the insect using 70% ethanol, and dried onto a microscope slide prior to application of the reagent. The slides were then scanned visually under a dissecting microscope and the proportion of the first 20 pollen grains to come into view that were viable was recorded.

As larger pollinators have a greater surface area exposed to pollen, and thus, should theoretically accumulate greater pollen loads, I measured the intertegular span (distance between wing bases) for every single insect as a proxy for body size. This was measured using digital callipers and was included in the analysis as a predictor of pollen load and viability.

Changes in both pollen load (Poisson errors) and proportion viability (binomial errors) across the honeybee density and land-use gradient were analysed using linear mixed effects models, conducted using the lmer function in the lme4 package (Bates *et al.* 2011) of the R Statistical Software (R Development Core Team 2011). The models used pollen load or pollen viability as response variables, and, distance from hive, surrounding land use and intertegular span as predictor variables. To determine if any potential changes were caused by changes were due to shifts in species behaviour or changes in species composition we also compared this model with and without species identity as a random effect. Site was also included as a random effect to control for the non-

independence of insects from a given site. Stock density, observed honeybee density and temperature were not included in the analysis, as these had no clear mechanism of affecting pollen transport other than through altering pollinator abundance or behaviour, and this was the subject of the previous analyses.

I calculated the dispersion parameter for glmms as the ratio of sum of squared Pearson residuals to the residual degrees of freedom, and comparison of this parameter to a Chi squared distribution provided evidence of significant overdispersion (Bolker *et al.* 2009). To deal with this, I incorporated an observation-level random effect into each model (Elston *et al.* 2001).

## 2.28 Seed set

To link the pollinator community to pollination, I intended to test how the pollination success of my eight focal plant species responded as community composition changed across the honeybee density gradient. For each sampling round, three individuals of each flowering plant species were taken to the field sites, and five freshly-opened flowers on each were marked with a small tag around their stem. The plants were left at the sites for 3 days to be exposed to pollination, and then collected. Their marked flowers were bagged with a fine mesh bag to prevent further pollination, and the plants were returned to a communal 'shade house', where they were left until the resulting seeds were mature. The use of experimental plants developing under the same conditions meant that differences in seed set across sites could be attributed to differing pollination success during the exposure period, rather than differences in plant quality or environmentally-driven fruit abortion rates across sites (Bos *et al.* 2007). Seeds were then collected and counted.

Due to rabbit browsing and harsh environmental factors in the Mackenzie Basin, many plants failed to survive the three day exposure period and many replicates were lost. Consequently, sufficient replicate numbers remained to allow analysis only for three of my original nine plant species- *C. vulgare*, *B. napus* and *A. millefolium*.

In *C. vulgare* flowers, seed set was measured as the proportion of seeds in each flower head to develop endosperm, indicating pollination success. This was not possible for all species, however, as undeveloped seeds were difficult to identify for those species with small seeds, so seed set in *B. napus* and *A. millefolium* was recorded as the number of seeds produced per flower. Seed set was tested in relation to distance from hive using a GLM with Poisson errors for *B. napus* and *A. millefolium* seed production (count data), and a binomial GLM for *C. vulgare* seed (proportion data). All generalized linear and mixed models used the canonical link function for the error distribution stated.

## 2.3 Results

A total of 3778 insects, across 79 species, were captured visiting flowers over the 3 month sampling period. These were composed primarily of solitary bees (26% - Families Colletidae and Halactidae)

and large-bodied flies (62% - mostly Syrphidae, Calliphoridae, Sarcophagidae, Stratiomyidae and Tachinidae), with the remainder of insects split among 7 orders and 20+ families.

### 2.31 Pollinator community

Less than 1% of visitors across all sites were honeybees (*Apis mellifera*), but despite their low visitation rates, PERMANOVA analysis revealed that increasing distance from beehives was correlated with a significant shift in the composition of the wild pollinator community (Pseudo-F = 1.67,  $p = 0.047$ ). Surrounding land use had no significant effect on the composition of the overall pollinator community (Pseudo-F = 1.33,  $p = 0.081$ ), but was correlated with significant changes in the abundance of some particular species (see below). Overall flower visitation frequency did not change significantly across the bee density gradient ( $F = 0.07$   $p = 0.794$ ), nor between land-use types ( $F = 0.36$   $p = 0.783$ ).

### 2.32 Individual species

Of the 12 common pollinator species examined separately, most showed no significant change in abundance across the honeybee density gradient (Appendix 1), although there were some notable exceptions. After controlling for variation due to surrounding land use, the solitary bee *Leioproctus fulvescens*, and the most common syrphid species, *Melangyna novaezelandiae*, were both significantly more abundant in close proximity to bee hives (Appendix 1). The trend in *L. fulvescens* however, was apparently driven by a strong outlier at site 16 and if this data point was removed the trend became non-significant.

In contrast, blowflies, particularly *Lucilla sericata*, and green soldier flies (*Odontomyia* spp.) became significantly more abundant as honeybee density declined (after controlling for variation due to surrounding land use) (Appendix 1). The decline in *Odontomyia* spp. with decreasing honeybee density was most strongly pronounced in areas of less intensive land use (land use x distance interaction, Appendix 1). The trend in *Lucilla sericata* was partially driven by particularly high abundances at site 8, however this point did not have high enough leverage to be considered a true statistical outlier (Cook's distance < 1), so was not removed for analysis. However, if this data point is removed the trend becomes marginally non-significant ( $p=0.065$ ).

The trends in *M. novaezelandiae*, *L. sericata* and *Odontomyia* spp. were initially highly significant, however only *L. sericata* remained significant after a Bonferroni correction to control for Type I error following the large number of individual tests (one model per species). Bonferroni corrections have been argued to often be overly conservative (Moran 2003), however, the trends observed for *M. novaezelandiae* and *Odontomyia* spp. should be interpreted with caution.

Although changing land use did not significantly affect the overall composition of the community, it is worth noting that this did have an impact on the abundance of many individual species (Appendix 1). These included the bees *Bombus terrestris*, *Lassioglossum mataroa*, *Leioproctus fulvescens*, the flies *Lucilla sericata*, *Melangyna novaezelandiae*, *Odontomyia* spp, and the butterfly *Zizina labradus*. The direction and intensity of this change across land-use types differed between species and in some species a significant interaction between intensifying land use and distance from hives was observed. The coefficients for each land-use type and interaction are presented in Table 2.

### 2.33 Pollen Load and Viability

Total pollen transport by the community decreased significantly across the gradient of declining honeybee density ( $Z = -7.03$ ,  $p < 0.0001$ ). This remained significant ( $Z = -6.88$   $p < 0.0001$ ) when controlling for species identity as a random effect in the model, showing that this effect is not simply due to changes in the species composition toward a less effective pollinator community, but rather that individual insects within each species carry less pollen at greater distances from beehives. Surrounding land use had no significant effect on total pollen load (Appendix 2). Some insect species, particularly frequent flower visitors, such as bees (e.g., *Bombus* species, *Lassioglossum* species) and hoverflies (e.g., *Eristalis tenax*, *Helophyllus hochetteri*, *Melangyna novaezelandiae*), carried significantly more pollen than other, more opportunistic flower visitors (Appendix 2), and pollen load scaled with body size (intertegular span), with larger insects carrying significantly more pollen (Appendix 2).

Pollen viability increased significantly across the gradient of declining honeybee density (Appendix 3). A few species (*Allograpta* spp, *Eristalis tenax*, *Helophyllus hochetteri*, *Lassioglossum mataroa*, *Oxysarcodexia varia* and *Apis mellifera*) carried significantly more viable pollen than others (Appendix 3) and the overall trend in viability became non-significant when a species level random effect was added to the model ( $Z = 0.20$   $p = 0.842$ ), showing that the change in viability across the honeybee density gradient is due to a shift in the species composition toward species that tend to carry more viable pollen, rather than changes in pollen viability between individuals within given species across the gradient. Pollen viability was significantly higher in degraded tussock than in other land-use types, but there was no significant interaction between distance from hive and land use (Appendix 4).

### 2.34 Seed set

For the three species tested, GLMs showed no significant change in seed set for either *Brassica napus* (Figure 5a,  $F = 1.08$   $p = 0.307$ ) or *Cirsium vulgare* (Figure 5b  $F = 0.68$   $p = 0.425$ ) as distance from hive increased. However, *Achillea millefolium* showed a strong positive relationship between seed set and distance from hive (Figure 5c,  $F = 15.79$   $p = 0.002$ ). Surrounding land use had no significant effect on any of the plant species (*B. napus*  $F = 0.11$   $p = 0.954$ ; *C. vulgare*  $F = 0.23$   $p = 0.872$ ; *A. millefolium*,  $F = 0.77$   $p = 0.558$ )

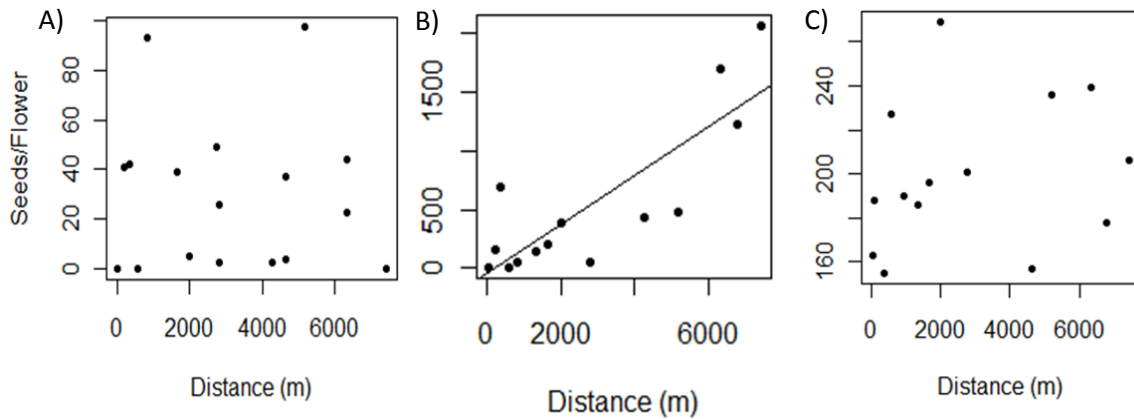


Figure 2.4 Seed set in plants exposed to pollination across the honeybee density gradient a) *Brassica napus* b) *Achillea millefolium* c) *Cirsium vulgare*.

## 2.4 Discussion

### 2.4.1 Impact of honeybees on the composition of the wild pollinator community

Supporting my first hypothesis, I observed a significant shift in the composition of the wild pollinator community as honeybee density declined. This result remained significant after controlling for surrounding land use, showing that the trend is not simply due to the correlation between agricultural intensity and honeybee density. This supports a growing bulk of literature demonstrating that the competitive effects of honeybees can influence populations of other pollinators (Gross & MacKay 1998; Kato *et al.* 1999; Goulson *et al.* 2002; Dohzono & Yokoyama 2010). While the bulk of these studies investigate interactions between only a few species (Stout & Morales 2009), several reviews on the subject have demonstrated the diversity and widespread nature of these effects (Goulson 2003b; Stout & Morales 2009). Thus, this finding came as no surprise, however, detecting this effect despite honeybees composing less than 1% of the observed visitations suggests that the impact of honeybees on wild pollinators may be strong, even at relatively low honeybee densities. Alternatively, honeybees may have been more abundant than their low visitation to my experimental plants suggests, as their communication and group foraging may mean that they visit certain abundant resources and ignore isolated plants (Visscher & Seeley 1982).

Analysis of this change in community composition at an individual species level revealed that different pollinator species responded differently to honeybee declines. Of the common pollinator species, the wild bee *Lieoproctus fulvescens* and syrphid *Melangyna novaezelandiae* were significantly more abundant in close proximity to honeybee hives, whereas the blowfly *Lucilla sericata* and soldierfly *Odontomyia spp.* were more abundant further from honeybee hives. This indicates that a general shift occurred from a bee and hoverfly dominated community close to commercial hives, to one more dominated by large bodied flies as honeybees declined. This conclusion is largely speculative as the trends in three of these species became non-significant after a Bonferroni correction, however, the vulnerability of large bodied flies in New Zealand to the

competitive effect of honeybees has been demonstrated previously in the literature (Murphy & Robertson 2000; Bennik 2009).

## 2.42 Impact of honeybees on pollen transport patterns and pollination rates

### *Pollen transport*

To link the change in the wild pollinator community to changes in pollen transportation, I analysed both the quantity and quality (viability) of pollen being carried by insects. Some insect species (such as bees and syrphid flies) carried significantly more pollen than other, more opportunistic flower visitors. Of more interest, however, is that the wild pollinator community in close proximity to bee hives carried more pollen than that at greater distances. This remained significant when controlling for species identity as a random effect in the model, showing that this effect is not simply due to changes in the species composition toward species less effective at transporting pollen as honeybee densities declined, but rather that individual insects within each species carry less pollen at greater distances from beehives. Surrounding land-use type had no effect on pollen loads, suggesting that changes in flower type or abundance were unlikely to have been a driving mechanism behind this trend. Therefore, the trend appears to be due to a change in behaviour of wild pollinators as honeybee density declined. (Brittain *et al.* 2013b) demonstrated that competition between bee species can result in such behavioural changes, resulting in increased pollen transport efficiency of particular species. While this study was in the context of wild bees influencing the behaviour and efficiency of honeybees, the inverse could potentially also occur. This mechanism would explain the trend I observed in pollen transport, and it would be interesting to test this possibility in the future. Another possible mechanism which may explain the higher pollen loads closer to beehives, is a higher frequency of flower visitation in wild pollinators at high honeybee densities. While honeybees feed on both nectar and pollen, nectar harvests are proportionally much greater than their pollen consumption (Rortais *et al.* 2005). This is partially because a large honey store is required for honeybee colonies to remain active during winter, but this is also probably exaggerated by the extraction of large quantities of honey from commercial bee hives. This possibly leads to nectar, but not pollen, becoming a limiting resource at high honeybee densities (Bennik 2009). This heavy consumption and reduction of available nectar by honeybees may result in nectar-feeding insects needing to visit more flowers to obtain a given quantity of nectar, and thus higher pollen loads per insect in the presence of honeybees. This mechanism could also partially explain the greater abundance of solitary bees and syrphid flies (both groups rely on pollen as a major protein source (Haslett 1989; Donovan 2007)) relative to other large-bodied flies (exclusively nectar feeders), at high honeybee densities, although further testing would be required to confirm this.

### *Pollen viability*

In terms of the viability of pollen carried by the wild pollinator community, the opposite pattern was observed. Pollen viability carried by the community significantly increased as distance from bee hives increased (Appendix 3). As pollen viability decreases over time (Dafni & Firmage 2000), this implies a faster pollen turnover rate in areas of low honeybee density. Such a pattern could occur via one of two scenarios. Firstly, as honeybee hive locations tended to be concentrated around areas with abundant flower supplies, pollinators may become satiated in areas close to bee hives. As

flower abundance increases, visitation rates per flower could be predicted to decline if pollinators cannot aggregate rapidly enough to utilise the abundant resource. This would result in pollen accumulating on the anthers for longer periods and thus decreasing in viability as it ages, before being collected by pollinators. Alternatively, a shift in pollinator composition or behaviour due to competition with honeybees may have led to a change in the community toward insects that more effectively collect pollen. This could result in a shorter pollen standing time and greater viability on the flowers or on the insect as new pollen is continuously collected and deposited. As pollen viability did not differ between land use types, nor did flower visitation rates did not change significantly across the bee density gradient, it seems unlikely that this result was due to increased flower abundance as predicted by the first hypothetical mechanism. In addition, my finding that the trend in viability became non-significant when a species level random effect was added to the model suggests that a shift in the species composition toward species that carry more viable pollen is possible, though further experiments would be needed to untangle these and other potential mechanisms. Nevertheless, together these results imply that, despite declines in the total pollen load being transported as honeybee densities decreased, pollen deposition and actual pollination rates may compensate by being greater than expected at low honeybee densities, and my seed set results were congruent with this hypothesis.

### *Seed set*

Honeybees are commonly viewed as being important pollinators for many crop species. If this were the case for the three plant species investigated, seed production would be expected to decline along the bee density gradient. No such pattern was observed in any of the three plant species tested. *Cirsium vulgare* and *B. napus* plants showed no significant change in seed production across the bee density gradient. These are both commonly cited as honeybee pollination dependent species (Williams *et al.* 1987; Michaux 1989; Hanley & Goulson 2003; Hayter & Cresswell 2006), so no significant decline in the absence of honeybees was an interesting result, which indicates that sufficient pollination may still occur at low honeybee densities. *Achillea millefolium* (considered to be predominantly fly pollinated, and primarily visited by native New Zealand insects(Primack 1983))interestingly showed a significant increase in seed production as honeybee densities declined. These results show that wild pollinators are capable of compensating for honeybee declines, such that pollination rates of these species can be maintained in the absence of honeybees. The different effects of the changing pollinator community on different plant species may in the future enable management of the wild pollinator community to be targeted toward pollination of particular plant species of interest.

### **2.43 Impact of surrounding land use on pollination by wild pollinators**

With the sustainability of our agricultural reliance on honeybees in question (see Chapter 1), management of wild pollinator communities is becoming increasingly important. My results show that the ability of the wild pollinator community to compensate for honeybee declines can depend on land use. In contrast to previous studies(Aguilar *et al.* 2006; Winfree 2010; Rands & Whitney 2011), I found that surrounding land use had no direct effect on the overall pollinator community, possibly because I used standardised flowers (rather than different plants in each land-use type, which would partly generate land-use effects). However, land use did significantly affect the

abundance of many individual pollinator species, and in some species a significant positive interaction between intensifying land use and distance from hives was observed (Appendix 1).

Manipulation of surrounding land-use practices could therefore be a useful tool to manipulate the wild pollinator community, as particular pollinator species responded differently to changing land use. Blowflies, for example, and particularly *Lucilla sericata*, were significantly more abundant at low honeybee densities and were frequent flower visitors. Thus, they could be increasingly economically valuable pollinators as honeybee densities decline. The abundance of these was affected by surrounding land-use type, as they rely on stock and stock dung during larval stages (Holdaway 1930).

By maintaining agricultural systems as a matrix of crop and pasture fields, thus keeping stock in close proximity to pollinator-dependent crops, it may be possible to maintain adequate pollination rates in the absence of honeybees. Similarly, it has been shown that maintaining natural areas and nesting habitats in close proximity to crops can be beneficial for maintaining diverse wild pollinator communities, and this may compensate for short- or long-term honeybee declines.



## Chapter 3: Changes in plant-pollinator interactions

### 3.1 Introduction

Human-induced global environmental change is driving species extinctions and ecosystem degradation at unprecedented rates (Sala *et al.* 2000; Tilman *et al.* 2001; Foley *et al.* 2005). This global environmental change is a complex issue driven by many different but interacting factors (Didham *et al.* 2007; Tylianakis *et al.* 2008). Among these, land-use intensification and the spread of exotic species are having particularly devastating effects on natural ecosystems (Sala *et al.* 2000), which are threatening many ecosystem services valuable to society (Chapin *et al.* 2000; Daily *et al.* 2000), such as the pollination of crops by wild insects (Schweiger *et al.* 2010). (Sala *et al.* 2000)

Three quarters of crop species around the world are dependent on animal pollination to some extent (Klein *et al.* 2007), and any reduction in this valuable service may ultimately reduce global food supplies (Garibaldi *et al.* 2011a; Tylianakis 2013). Despite our dependence on pollination, wild pollinator populations are considered by many researchers to be declining worldwide (Potts *et al.* 2010b; Cameron *et al.* 2011; Burkle *et al.* 2013) and land-use intensification has been strongly implicated in this decline (Björklund *et al.* 1999; Kremen *et al.* 2004; Klein *et al.* 2007; Kremen *et al.* 2007; Ricketts *et al.* 2008; Tylianakis *et al.* 2008)

It has been argued that commercially-managed honeybees may compensate for the loss of wild bees (Morse 1991b; Carreck & Williams 1998; Aebi *et al.* 2012), but commercial beekeeping faces a number of threats (Holden 2006; Ratnieks & Carreck 2010; Williams *et al.* 2010). Recent studies also suggest that honeybees may be less efficient pollinators of many crops than previously thought, and therefore that they may provide poor compensation for a diverse wild pollinator community (Garibaldi *et al.* 2011b; Garibaldi *et al.* 2013). Even if honeybees are effective, complementary behaviour of different pollinator species means that diverse pollinator assemblages can nevertheless be more effective than any single species (Hoehn *et al.* 2008; Tylianakis 2008; Brittain *et al.* 2013b). Given this doubt about the ability of commercial beekeeping to compensate for wild pollinator losses, it is imperative that we develop an understanding of how exotic honeybees integrate into communities of wild pollinators in order to predict how these networks might respond to declining honeybee densities and global environmental change.

While the impacts of exotic bees or land-use intensification on any particular plant or pollinator species may at times be apparent, predicting changes in pollination at the community scale has been a major challenge to scientists (Traveset & Richardson 2006). Effective pollination depends not only on the diversity and abundance of plants and pollinators within the community, but also on the ways in which species interact (Tylianakis *et al.* 2008). Therefore, to accurately determine changes in pollination across the entire community, we must analyse changes to both the species themselves and the interactions between them. The recent expansion of network-analytical approaches may provide a powerful means to achieve this goal (Bascompte 2009) (Forup & Memmott 2005; Tylianakis 2008), exposing emergent properties of the community that cannot be predicted from the pairwise interactions alone, such as the existence of different functional guilds within the community or the

stability of pollination services they provide (Bascompte *et al.* 2003; Bascompte *et al.* 2006; Bascompte 2009; Thébault & Fontaine 2010). Consequently, a surge of recent publications have examined the effects of exotic species or changing land-use on pollination networks (e.g.,(Lopezaraiza-Mikel *et al.* 2007; Aizen *et al.* 2008b; Hagen *et al.* 2012; Pocock *et al.* 2012; Traveset *et al.* 2013).

One consistent pattern to emerge from the network literature is that exotic species are often extreme generalists, and by interacting with many other species they increase the overall connectivity of pollination networks (Aizen *et al.* 2008b; Traveset *et al.* 2013). For example, the indiscriminant foraging behaviour of honeybees makes them an extremely generalist exotic (in much of their global range) species (Butz-Huryn 1997), which consequently affects the structure of pollination networks in which they are present (Aizen *et al.* 2008b). Therefore, if honeybees decline in their range or abundance, we may expect pollination networks to become less connected, unless generalist wild pollinators move in to fill their role. The ability of generalist wild pollinators to fill this role, however, is unknown. As changing land use has been shown to have strong effects on wild pollinator communities (Björklund *et al.* 1999; Kremen *et al.* 2002; Kremen *et al.* 2004; Klein *et al.* 2007; Kremen *et al.* 2007; Ricketts *et al.* 2008; Tylianakis *et al.* 2008), the response of wild pollinators to declining honeybee densities will likely differ between land-use types (Kremen *et al.* 2002). While no studies have specifically investigated the interactive effects of exotic honeybees and land-use change, Didham *et al.* (2007) discuss two broad types of interaction effects between exotic species invasion and land-use change that may occur: interaction chain effects, whereby one driver alters the propensity of another driver to occur, but does not change its effect; and interaction modification effects, where one driver changes the per-unit or per-capita effect of the other driver. Both these mechanisms are likely to occur in pollination systems. For example, competition for pollinators by the exotic weed *Impatiens glandulifera* has been shown to alter interactions between the native plant communities and their pollinators (Lopezaraiza-Mikel *et al.* 2007). As weed invasion is frequently facilitated by land-use intensification(Tsai *et al.* 2012; Peltzer & MacLeod 2013) and human disturbance, interaction chain effects are also likely.

In this study, I use a pollination-network approach to investigate the interactive impacts on communities of the declining abundance of a key exotic pollinator, the European honeybee (*Apis mellifera*), and land-use intensification. I investigate changes in network structure to determine the role of honeybees in the wider pollination network, and to determine how this changes with altered land use. I then determine how changes in community structure impact the pollination services carried out by species in the pollination network. Specifically, I test for changes in the pollen transport patterns of native, crop and weed species in these networks, and discuss their implications for pollination services.

## 3.2 Methods

### 3.21 Insect collection

This study used pollen transport networks constructed from samples collected from the Mackenzie Basin, South Canterbury, New Zealand (See Chapter 2 methods). Flower-visiting insects were collected at 18 field sites in the Mackenzie basin, across gradients of declining honeybee density (manipulated by selecting sites at increasing distances from commercial hives) and changing land use (classified into one of four levels, based on predominant land uses in the landscape - tussock

grassland, riverbed scrub, degraded tussock grassland or pasture/crop fields) (See Chapter 2 methods). These insects were collected during flower observations on arrays of experimentally grown plants at the sites from December 2011 to February 2012. (See “flower observations” Chapter 2 Methods). After capture, insects were stored individually to prevent pollen contamination between insects until pollen was later removed, quantified and identified. The insects were then identified to species level with the help of taxonomists where possible, or grouped to morphospecies where morphological characteristics were insufficient to differentiate species accurately. A reference collection of these was deposited at the University of Canterbury.

### 3.22 Pollen counts and identification

To determine the presence of interactions between the pollinator and plant communities, I analysed the pollen load carried by each insect (excluding pollen carried in the scopae or corbiculae of bees, as this is unlikely to be deposited on flowers). I removed pollen samples from each insect and mounted this on microscope slides using the fuchsin gel method detailed in (Dafni *et al.* 2005). From these, pollen samples were identified for a standardised number of pollinator individuals of every species (4 individuals from each sampling round at every site). These pollen samples were systematically examined and the first twenty pollen grains on each slide were identified to species, based on a reference collection of pollen grains collected from the observed flowers and surrounding vegetation at each site.

### 3.23 Network construction

To analyse the impact of honeybee declines and changing land use on pollination interactions at the community level, I constructed a network of pollen transport interactions (Lopezaraiza-Mikel *et al.* 2007). I constructed binary pollen transport networks for each site using the identities of pollen grains carried by a standardised number of insects from every species (see “Pollen counts and Identification” above). An interaction (i.e. a network ‘link’) was deemed to occur between a plant and pollinator species if pollen of this plant species was identified on any members of an insect species. However, the amount of pollen carried by a pollinator may not be representative of the importance of that species for the plant’s reproduction. In fact, the quantity of pollen transported by pollinators was highly variable, differing by over three orders of magnitude between insects. As high pollen loads can result from a single visitation, and thus depend highly on pollinator morphology and behaviour as well as visitation rates, I decided that pollen load was not directly comparable between pollinator species as a measure of pollination effectiveness. Therefore, I opted to construct more conservative binary networks, whereby interactions are present or absent depending on the presence or absence of pollen transport by pollinators, rather than quantitative links weighted by the amount of pollen carried. However, because morphology and behaviour should be more consistent within species, and because one objective of this study was to examine how the pollen preferences of pollinator species change, when calculating metrics at the species level to make within-species comparisons, I incorporated quantitative link weights based on the proportion of each pollen species being transported. Individual networks were compiled for each of the 18 sites, and properties of these (Connectance and Nestedness) were calculated using the “bipartite” package (Dormann *et al.* 2008) within R Statistical Software (R Development Core Team 2011).

Connectance and nestedness were selected as metrics of community structure because these closely relate to underlying biological properties. Connectance, the proportion of the total possible links

between species in the network that are realised, is a measure of species specialisation (Jordano 1987). Specialist pollinators visit only a few of the possible flower species, thus leading to networks with low connectance, whereas generalist pollinators visit a wide range of flowers and therefore generate high connectance. I hypothesised that the generalist pollination strategy of honeybees would cause a decline in overall network connectedness when they are absent from the network. Nestedness measures the extent to which specialist species (those with few links) interact with species that also interact with generalists (Bascompte *et al.* 2003). It is known to be important for the stability of mutualist networks (Bascompte *et al.* 2003; Thébault & Fontaine 2010) and has been shown to increase with the presence of exotic generalists (Traveset *et al.* 2013). Nestedness was calculated using the algorithm “BINMATNEST” (Rodríguez-Gironés & Santamaría 2006) in R. Both of these metrics were calculated using binary interactions, i.e. I did not incorporate any measure of link strengths between plant and pollinator species.

### 3.24 Analysis Methods

To determine how the overall network structure changed with declining honeybee density and changing land use, I analysed the response connectance and nestedness to each gradient. These were tested using a general linear model (GLM) for each metric (the response variable), and honeybee density, surrounding land-use type and the interaction between these as predictors. This analysis was conducted using the `lm()` function in the base package of R, and the maximal model was simplified to the best-fitting model based on minimising the value of the Akaike Information Criterion (AIC). The residuals of these models were checked to be normally distributed and to conform to the assumption of homoscedasticity, and the response variable was transformed appropriately where these assumptions were not met.

As honeybees are an integral part of the pollinator community, I included honeybees in the networks, despite manipulating their densities using the distance to the nearest hive. However, to differentiate between changes in network structure simply due to the absence of honeybees, as opposed to changes in the interactions between other species throughout the wider community induced by the presence of honeybees, I re-ran these analyses with honeybees removed from the network and compared these models. To control for the known relationship between connectance and species diversity (as diversity increases, there are more possible links between species and a lower probability of these being realised)(Dunne *et al.* 2002), I incorporated the number of possible links (plant diversity x pollinator diversity) at each site as a covariate in the model testing for changes in connectance. Connectance between species within a network can also be influenced by sample size (abundance of individuals), as increasing sample size increases the probability of observing rare interactions (Dunne *et al.* 2002). To control for this, I also added the sample size (number of

pollinator individuals) at each site as a covariate in the model.

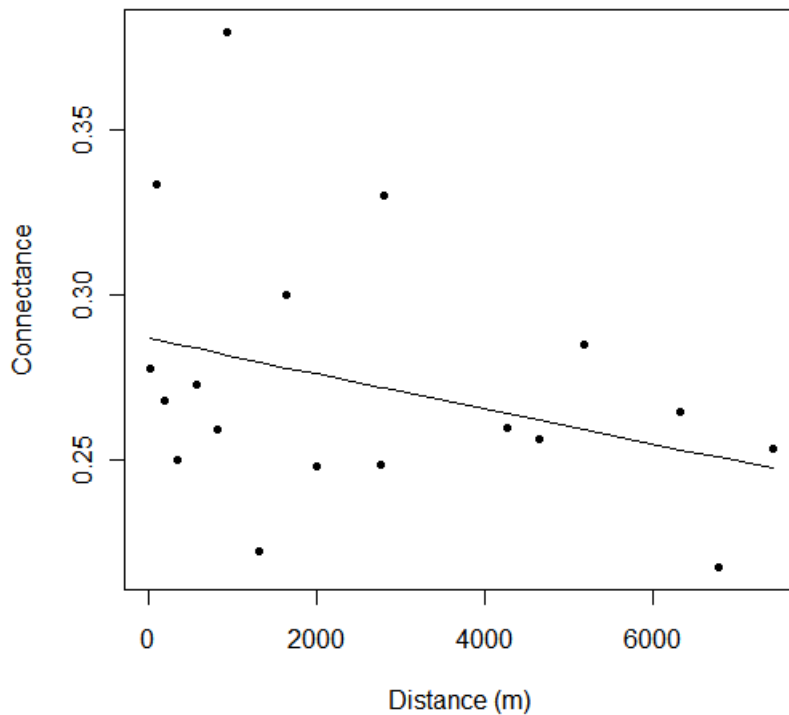


Figure 3.1 Change in network connectance at increasing distances from commercial bee hives.

To untangle the mechanisms driving any changes in network structure, I analysed connectivity at the species level. If community-level connectedness were to change according to any of my predictor variables, this could be caused by a shift in species composition toward a more generalist community, or alternatively by individual species becoming more generalist. To distinguish between these possibilities I analysed changes in the connectedness of each species (measured as normalised species degree) with changing land use and honeybee density. Normalised species degree (the number of links per species, scaled by the number of possible links) was calculated for every pollinator species at each site (i.e. each network) using the “species level” function of the bipartite package in R. This was tested against the distance and land-use gradients using a linear mixed effects model, conducted using the lmer function of the lme4 package (Bates *et al.* 2011) in R. For this model, normalised species degree was the response variable, land use and distance from bee hives (and their interaction) were fixed predictors. Species identity was included as a random factor to test for within-species changes in connectedness across sites (i.e. to test whether each species was behaviourally specialising more on fewer plant species, rather than the community changing to include species that differed in their inherent specialisation). Site was also included as a random factor to account for the non-independence of multiple species within a network (site). This maximal model was simplified to the best-fitting model based on minimising the value of the Akaike Information Criterion (AIC). Because the calculation of error degrees of freedom in mixed effects models is the subject of debate, I followed (Bolker *et al.* 2009) by testing hypotheses within this model using Monte Carlo Markov Chain (MCMC) resampling, which was conducted using the pvals.fnc() function in the languageR package (Baayen 2008) in R. If any of my predictors remained

significant, it would indicate that the generality of each species were changing, rather than a community shift towards more generalist species.

To determine the effect of any changes in connectivity on the pollination services carried out by the community, I analysed how the transport of different pollen types (crop vs. native or weed pollen) changed with species-level connectivity of pollinators in the network. Crops were defined as exotic plant species of which the spread or cultivation is intentionally facilitated by farmers, whereas weeds were defined as exotic plants not meeting this criterion. Pollen transport was analysed using a linear mixed effects model with a binomial distribution, with the proportion of each pollen type carried by each species as a response variable, and the type of pollen (factor with three levels: crop, native or weed pollen) and the normalised degree for each species (including their interaction term) as predictors. Species identity and site were also included as random factors to control for the non-independence of different pollen types from a given bee species, and of different species from a given site. Obviously bees with lower connectivity by definition carry fewer species of pollen, however, this model tested whether any behavioural specialisation (reduced connectivity of a species) favoured a particular type of pollen.

### 3.3 Results

Connectance of the pollen transport network decreased significantly as honeybee densities declined ( $F = 8.79$   $p = 0.011$ ). Land-use intensification and the land use x honeybee density interaction had no significant effect on connectance, and were not included in the best-fitting model. Similarly, the number of possible links between species and the sample size both had no significant effect on connectance and were removed from the best-fitting model. The observed change in connectance remained significant when data for honeybees were removed from the network ( $F = 9.84$   $p = 0.0079$ ), showing that this change was at least partially due to changes in the interactions between other species in the community. Despite the change in connectance, there was no significant change in nestedness across either gradient, and this was best predicted by a null model with only the intercept and not incorporating either honeybee density or changing land use.

To untangle the mechanisms driving this change in connectance, I analysed connectivity at the species level using the normalised degree (the number of observed links, scaled by the number of possible links per species) of each species. As with connectance at the network level, normalised species degree decreased significantly as honeybee densities declined ( $p_{\text{MCMC}} = 0.022$ ), but did not change significantly between land-use types or with the distance x land use interaction, which were both removed from the best-fitting model. However, this honeybee density effect on individual-level connectedness became non-significant when species identity was removed from the model as a random factor ( $p_{\text{MCMC}} = 0.253$ ), indicating that changes in the behavioural specialisation of individual species drove the change in connectedness, rather than a community shift towards generalist species. Despite changes in connectivity, the diversity of plant species carried by the community showed no significant change ( $F = 0.9892$   $p = 0.3347$ ) across the honeybee density gradient as connectivity decreased, indicating that pollinators did not simply become more specialised because there were fewer plants on which to feed.

The proportion of the different pollen types (crop, weed or native pollen) carried by insects changed across the different land uses, which would be expected based on differing availability of plant species. For example, in crop/pasture sites, significantly more weed pollen was carried than any other pollen type ( $Z = 5.27$ ,  $p < 0.001$ ), and the proportions of each pollen did not differ significantly from this in degraded tussock. In contrast, this ratio changed significantly in riverbeds, where the proportion of crop pollen increased ( $Z = 3.07$ ,  $p = 0.002$ ) and both native (land use (riverbed) x native pollen interaction:  $Z = -2.18$ ,  $p = 0.029$ ) and weed (land use (riverbed) x weed pollen interaction:  $Z = -3.80$ ,  $p = 0.0001$ ) pollen proportionately decreased.

However, changing connectedness (normalised degree) of species in the network was associated with dramatic changes in the proportion of each pollen type they carried, and how this changed with land use. There was a highly significant 3-way interaction between normalised degree, pollen type transported, and land use (Appendix 5). Specifically, normalised degree was significantly negatively associated with the proportion of weed pollen carried in pasture sites (normalised degree x weed pollen interaction:  $Z = -3.02$ ,  $p = 0.003$ ), but this effect was reversed in all other land-use types (land use types x normalised degree x weed pollen interactions:  $Z > 2.32$ ,  $p < 0.020$  in all cases). The non-crop/pasture habitats were also associated with an increased positive association between normalised degree and the proportion of native pollen carried (land use x normalised degree x native pollen interactions:  $Z > 2.68$ ,  $p < 0.007$  in all cases). Normalised degree was not associated with changes in the proportion of crop pollen in crop/pasture sites, but it was negatively related with the proportion of crop pollen carried in all other land uses (land use x normalised degree interactions:  $Z < -2.46$ ,  $p < 0.014$  in all cases).

### 3.4 Discussion

In pollination networks, network structure can reveal emergent properties of the system that are not apparent by investigating pairwise interactions alone (Bascompte 2009). I investigated the change in several measures of network structure across gradients of declining honeybee density and changing land use, and my results show that network structure changed as honeybee density declined. Specifically, I observed a significant decrease in connectance at both network and species level across the pollen transport networks as honeybee densities decreased.

Obviously, as honeybees are a highly generalist pollinator, their removal from the community would be expected to result in a decrease in network connectance. Honeybee visits, however, composed less than 1% of the total number of visitations and the observed change in connectance remained significant when honeybees were removed from the network. This change also remained significant after controlling for the number of possible links between species, showing that this was not due to an increase in species diversity as honeybee densities declined, but rather due to a shift in the foraging behaviour of pollinator species toward a higher degree of specialisation on certain plant species.

As honeybee densities declined, other pollinators tended to visit a narrower selection of flowering plants. Theoretically, this change could be caused by a shift in community composition from a predominantly generalist community toward a guild of more specialist pollinator species. However,



my mixed model with species included as a random factor indicated a change in the behaviour of the species themselves, as individuals within a given species tended to visit a narrower diversity of plant species. Interestingly, the diversity of plant species carried by the entire community showed no significant change across the bee density gradient as connectivity decreased. This implies that foraging preferences between pollinator species allowed diversity of pollen transport to be maintained throughout the community, yet each pollinator species had a higher degree of fidelity to particular plant species in the absence of honeybees.

This increasing specialisation would theoretically result in more efficient pollination, as transported pollen has a higher probability of being deposited on a flower of the same species (Waser 1986). Therefore, the reduced connectivity of other species with declining honey bee density would theoretically result in more efficient pollination of the plant species that are visited. Other recent studies have shown that honeybees can be less efficient pollinators than wild pollinators, and may provide poor compensation for declining wild pollinators (Winfree *et al.* 2007; Hoehn *et al.* 2008; Garibaldi *et al.* 2013). My result expands on this, showing an association between high honeybee density and generalist visitation behaviour by other species in the network. This indicates that rather than simply being less efficient pollinators themselves, honeybees may also reduce the pollination efficiency of wild pollinators in the surrounding landscape.

Although my experimental design was not designed to test the mechanisms causing this change in network connectance, it is possible that increased competition for floral resources at high honeybee densities drives wild pollinators to forage more opportunistically. In an optimal, competition-free world, pollinator species might be expected to specialize to a high degree on the flower species that give them the greatest reward (Abrol 2006). However, as competition for floral resources increases, the maximal rewards from any single species would decrease and these specialists might be forced to become more opportunistic, scavenging resources from a wider array of flower species. As honeybees are highly efficient foragers, partially due to their complex communication systems (Visscher & Seeley 1982), they might be better able to dominate optimal resource supplies than wild pollinators, and thereby induce greater generalism in competing insect species (Roubik & Villanueva-Gutierrez 2009). This displacement of wild pollinators by honeybees has been frequently demonstrated to occur on an individual species basis (e.g. (Roubik 1980; Roubik *et al.* 1996; Stout *et al.* 2002a; Dupont *et al.* 2004), but to my knowledge this has never been shown at a community wide scale.

The decreasing network connectance observed with declining honeybee abundance had consequences for the transport of pollen throughout the network. I found a significant three-way interaction between a species' connectivity, the proportion of different pollen types it carried, and surrounding land use. Significantly more weed pollen than native or crop pollen was carried by pollinators across all of my sites, and the proportion of weed pollen was higher in crop and pasture than all the other land-use types. However, the proportion of weed pollen relative to native or crop pollen changed with decreasing connectedness. Reduced connectedness was associated with an increase in the proportion of weed pollen carried by the community in crop and pasture areas where weeds were abundant, but a decrease in the proportion of weed pollen in all less intensive land uses where weed pollen was significantly less abundant



As the proportion of weed pollen carried was significantly higher in crop/pasture than other land use types, this may be explained by changes in weed abundance in these landscapes, combined with the greater specialisation of pollinators on more abundant resources in the absence of honeybees. In land-use types where weeds were less common, specialisation appears to be toward the more abundant native and crop species, resulting in reduced transport of weed pollen. Where weeds were more abundant, however, this greater specialisation was associated with higher levels of weed pollen transport. This is consistent with my above hypothesis; suggesting that that, as competition between pollinators increases, pollinators are forced to forage on less-rewarding flower species. I hypothesise that in areas where flowers of a particular type are abundant, wild pollinators are able to specialise on these in the absence of honeybees. As honeybee densities increase, wild pollinators are out-competed on these species and forced to switch to foraging on marginal species.

Combined with my findings of greater pollen-species constancy in the absence of honeybees, this has significant implications for the impact of honeybees on pollination systems. Exotic pollinators, particularly social bees, have often been reported to favour exotic flower species over natives (Goulson & Hanley 2004; Hingston 2005; Aizen *et al.* 2008b; Webber *et al.* 2012). As a result, exotic pollinators have been implicated in spread of many weed species (Huryn & Moller 1995; Simberloff & Von Holle 1999; Barthell *et al.* 2001; Goulson 2003b; Hanley & Goulson 2003; Simpson *et al.* 2005; Gross *et al.* 2010). My results show that this effect not only applies to honeybees themselves, but that they may also indirectly influence weed pollination through their influence on the connectedness of other pollinator species in the network.

Finally, this study emphasises the importance of investigating the many drivers of global environmental change simultaneously. While land use had no direct effect on network structure in this pollination system, it affected pollination services delivered by the community when faced with declines in the abundance of honeybees and resulting changes in the pollination network structure. This change would not be detectable when investigating either driver in isolation, demonstrating the importance of testing these simultaneously.

## Chapter 4: Discussion

### 4.1 Effects of honeybee declines in changing landscapes

Pollination, as an ecosystem service, is directly linked to global food security and human welfare (Meffe 1998; Carpenter *et al.* 2006; Díaz *et al.* 2006; Klein *et al.* 2007), and much of this service is provided through the globalisation of the western honeybee, *Apis mellifera* (Morse & Calderone 2000). However, a broad suite of inflictions are currently having adverse effects on honeybee colonies around the globe, and the future sustainability of beekeeping has been questioned (Johnson *et al.* 2010; Le Conte *et al.* 2010; Potts *et al.* 2010a). Simultaneously, global population growth has increased human food demands, resulted in rapidly-increasing agricultural intensity in many areas worldwide (Tilman *et al.* 2011), increased demand for pollination services (Aizen *et al.* 2008a; Aizen & Harder 2009), but these changes potentially threaten the ability of ecosystems to provide these services (Kremen *et al.* 2002; Greenleaf & Kremen 2006; Kremen *et al.* 2007).

In this study, I investigated the combined impacts of honeybee declines and land-use intensification on pollination services, showing that honeybee declines may have a large impact on community structure and interactions within pollination systems. I observed a significant shift in the wild pollinator community composition as honeybee densities declined, from a generally bee/hoverfly dominated community to one more dominated by large flies (Chapter Two). This was associated with a significant decline in the total pollen load transported by the community (Chapter Two), indicating that pollination services may suffer in the absence of honeybees. As honeybee densities declined, however, I also observed a shift toward greater specialisation of pollinators on abundant resources (Chapter Three), increased pollinator constancy (the tendency of pollinators to show high fidelity to a single species (Waser 1986), Chapter Three), and a higher viability rate of the pollen transported (Chapter Two). These findings show that although the total amount of pollen transported by the community declined as honeybee densities decreased, the probability of this pollen transport resulting in effective pollination likely increased. Thus, I observed no decrease in seed set with honeybee declines in any of the three plant species tested, and one of these even showed a significant increase. Finally, I also demonstrated that this change differentially affected different plant types, and that the extent of changes to each plant species differed between land-use types. This reflected changes in the relative abundance of pollen types in different land uses, with greater specialisation in the absence of honeybees disproportionately benefiting common species. These findings have strong implications for several contemporary issues in pollination biology, both locally within New Zealand and on a global scale. These are discussed in the following sections.

### 4.2 Compensation of wild pollinators for honeybee declines

Given current uncertainty regarding the sustainability of our reliance on honeybees, understanding the ability of wild pollinators to compensate for honeybee declines may become crucial to ensuring global food security (Klein *et al.* 2007; Winfree *et al.* 2007; Garibaldi *et al.* 2011a). Recent studies have shown that wild pollinators may be more important than once thought for crop pollination

(Winfree *et al.* 2008; Garibaldi *et al.* 2013), and my findings support this claim. I found that wild pollinators were responsible for 99% of all pollen transport in the area studied; however, this trend may have been exaggerated by my experimental design. Due to the communication systems of honeybees, they tend to recruit to large resource supplies and forage primarily on a few high-quality flower patches (Visscher & Seeley 1982). My pollinator observations were conducted on small, temporary, experimental flower patches, and therefore may have under represented the importance of honeybees in areas close to hives.

Nevertheless, the Mackenzie basin is known for its high native bee diversity (Quinn 1984) and semi-natural landscape, therefore wild pollinators are likely to be more abundant than national (and perhaps global) averages. Consequently, I observed high visitation rates and no decline in the seed set of a range of plant species at large distances from bee hives, suggesting that wild pollinators do indeed compensate for the loss of honeybees. Furthermore, I observed increases in pollinator constancy and pollen viability in the absence of honeybees, which suggests that the pollination efficiency of wild pollinator species may even increase as honeybee abundances decline. Therefore, the ability of wild pollinators to compensate for pollination loss in the absence of honeybees may be greater than predicted based on their abundance alone or their contribution to pollination in the presence of honeybees.

### 4.3 Impact of honeybee declines on NZ ecosystems

While wild bees are frequently the focus of studies and conservation efforts (e.g. (Banaszak 1992; Goulson *et al.* 2008; Brown & Paxton 2009), fly species can be equally important pollinators (Chapter 2)(Rader *et al.* 2009; Rader *et al.* 2011; Rader *et al.* 2012; Rader *et al.* 2013). In New Zealand, pollination systems are unique in that there are no native social bee species, and that dipteran flies comprise an unusually high proportion of pollinators (Heine 1937; Primack 1983). In fact, a high proportion of New Zealand native plants have small, colourless and often scented flowers, which is considered to be an adaptation to pollination by the diverse fly fauna (Heine 1937; Primack 1983; Lloyd 1985). However, my findings that large-bodied flies increased most in the absence of honeybees suggests that they may be particularly vulnerable to competition from honeybees (Chapter 2), and this has implications for the management of honeybees near native ecosystems. As honeybee densities declined, I observed a shift in the pollinator community from a generally bee- and hoverfly-dominated community, to one more composed of large flies (particularly blowflies (Calliphoridae) and soldierflies (Stratomyiidae), Chapter 2). Greater flower visitation rates by flies in the absence of honeybee competitors would be expected to result in greater pollination rates and seed set in plants adapted to fly pollination, and indeed I observed increased seed set in a common fly-adapted plant (*Achillea millefolium*). Although this was an introduced plant, its floral characteristics are similar in many respects to many native, fly-pollinated species and it has been shown to be primarily visited by native insects in New Zealand (Primack 1983). This indicates that high honeybee densities may possibly alter interactions between native plants and their fly pollinators, potentially reducing seed set.

While the results of Chapter 2 provide evidence for some benefits associated with honeybee declines in indigenous ecosystems, this conclusion is based only on data from a single (exotic) fly-pollinated plant, and further study would be required to extrapolate these results to other species.

Finally, although a large proportion of native plants are apparently adapted to fly pollination, birds have historically also been important pollinators, and a number of indigenous plant species have specialised ornithophilous (bird-adapted) flowers (Anderson *et al.* 2011). Pollinating birds however, have suffered drastic declines on the New Zealand mainland, as a result of habitat loss and predation by introduced mammals (Anderson 2003; Anderson *et al.* 2011), and several ornithophilous flower species are now severely pollen limited (Montgomery *et al.* 2001; Anderson 2003; Anderson *et al.* 2011). While some native insects do visit these flower species, their deposition of pollen is highly ineffective due to their small size (Robertson *et al.* 2005). Given the much larger body size of exotic bees, and their introduction into New Zealand coinciding with bird declines, it is possible that these may be important to the remaining pollination of some ornithophilous flowers and partially compensate for the functional loss of pollinating birds. Therefore, the impact of honeybees on native ecosystems is a complex issue, and further study is required before the impacts of honeybee declines on pollination of New Zealand's native plant species can be fully understood.

#### 4.4 Weed invasion

It has been widely speculated that exotic bees may facilitate the invasion of exotic weeds (Huryn & Moller 1995; Goulson 2003b; Aizen *et al.* 2008b; Howlett & Donovan 2010). While many studies have shown that exotic pollinators often preferentially visit exotic plant species (Goulson & Hanley 2004; Webber *et al.* 2012), and that many invasive weed species are highly dependent on exotic pollinators (Stout *et al.* 2002b; Gross *et al.* 2010; Paynter *et al.* 2010; Beavon & Kelly 2012), other studies have concluded that facilitation of exotic weeds by honeybees is unlikely (Huryn & Moller 1995). In addition to my above findings of increased pollination of a weed (*A. millefolium*) in the absence of honeybees, Chapter 3 investigated the effects of changing pollination interactions on the transport of weed pollen by the pollinator community as honeybee densities declined. I found that, as honeybee densities declined, there was a shift in the network structure of the pollinator community toward decreased connectance. This reduced connectivity of the network was associated with an increase in the proportion of weed pollen carried by the community in crop and pasture areas where weeds were abundant, but a decrease in the proportion of weed pollen in all less intensive land uses where weed pollen was significantly less abundant. This shows that weed facilitation by pollinators may be a complex issue. Firstly this shows that facilitation may occur both directly and indirectly, with honeybees not only pollinating weeds themselves, but also altering the propensity of wild pollinators to visit weeds. Secondly, the propensity of pollinators to pollinate weeds depended on the surrounding land-use, possibly as a result of increased behavioural specialisation on available flowers. Wild pollinators became more specialised (i.e. they interacted with fewer plant species) as honeybee densities declined. In land-use types where weeds were less common (e.g. native tussock grasslands), this specialisation appears to have been toward the more abundant native and crop species, resulting in reduced transport of weed pollen. Where weeds were more abundant (e.g. in crops and pastures), however, this greater specialisation was associated with higher levels of weed pollen transport. Overall, this suggests that honeybees may facilitate the initial invasion of invasive weeds, increasing their weed reproductive rates when they are rare, however once weed populations reach a critical density this benefit to weed pollination is lost. Conversely, if honeybees decline, weed pollination may benefit, particularly in areas where weeds are common.

## 4.5 Land-use management as a tool to manage pollination

Wild pollinators have shown some capacity to compensate for declining honeybee densities, both in the present study and in others (Goulson 2003a; Winfree *et al.* 2007; Rader *et al.* 2012; Garibaldi *et al.* 2013), however their ability to do so may depend on surrounding land uses (Klein *et al.* 2007; Kremen *et al.* 2007; Cariveau *et al.* 2013; Scheper *et al.* 2013). Land-use intensification has been shown to negatively impact a variety of wild pollinators (Klein *et al.* 2007; Cariveau *et al.* 2013), both through the alteration of floral resource supplies, and the destruction of native habitat requirements, such as viable nesting sites (Kremen *et al.* 2007).

My results show that, although land-use type did not have a direct impact on the pollinator community composition, it did influence several aspects of pollination. A major change in the pollinator community as honeybees declined was the importance of blowflies as pollinators. During their larval life stages, these blowflies are highly dependent on stock and animal dung (Holdaway 1930). While I did not detect significant changes in the abundance of these with changes in the surrounding land use or stocking rates, this is likely a result of the high mobility of these adult flies and their dispersal from source populations (Smith & Wall 1998). Therefore, it may be less important which land use is sampled, provided there is stock grazing somewhere in the landscape. Land-use intensification in the Mackenzie basin is leading to a complex mosaic of natural, semi natural and intensive agricultural systems in close proximity to one another (Figure 1.3). The increasing value of these blowflies as pollinators when honeybees are absent demonstrates the value of this habitat complexity for pollination. By maintaining high stock densities within dispersal range of crop or natural landscapes, the spill over of dung-dependent pollinators may allow greater pollination success in adjacent habitats. Thus, maintaining high stock densities in close proximity to crop fields during flowering periods may allow farmers to compensate for honeybee declines and ensure pollination, and the practical application of this possibility warrants further exploration.

## 4.6 Future research

While this research contributes to our understanding of how pollinator communities may respond to honeybee declines in increasingly human-modified landscapes, my findings also open several avenues which may prove lucrative for future research on both local and global scales. The first of these is on the management of honeybees in New Zealand's conservation estate. Commercial beekeeping is widely permitted in close proximity to many protected natural areas (Huryn & Moller 1995), primarily due to the high value of honey produced from these hives. This study indicates that high honeybee densities might influence wild pollinators, potentially adversely affecting the pollination of the many fly-pollinated native plants. This result, however, is highly speculative and based on extrapolation from a limited portion of our data. In contrast to this, it is also possible that honeybees may partially compensate for declines in bird pollinators on the New Zealand mainland, providing valuable pollination to species dependent on this. Further research into the relative costs or benefits of honeybees to both fly- and bird-pollinated plants in New Zealand will be required before advice can be given on the management of bee keeping in natural areas.

Secondly, my findings show that different pollinator species respond differently to both honey bee declines and changing landscapes. It is known that different insect guilds have visitation preferences toward different flower characteristics. Therefore, further research into the differential responses of pollinators to management solutions, such as changing land-use practices at landscape scales, may allow promotion of specific pollinator taxa, providing targeted delivery of pollination services to particular plant species of interest while reducing the reproductive success of others.

Finally, this study clearly demonstrates the potential value of wild pollinators to society, particularly when faced with potential declines in the viability of honeybee pollination. While the benefits of promoting wild pollinator species are becoming increasingly obvious, little is currently known on how best to maximise this at landscape scales. My findings indicate that simple modifications of land-use practices, such as maintaining a matrix of diverse land use types in close proximity, may provide a tool for land managers to sustain wild pollinators. Further research into the resources provided to pollinators by differing land-use practices, and the complementarity between these, may allow for greater utilisation of these findings, thereby maximising the potential of wild pollinators to compensate for honeybee declines in our changing world.

## Appendix

Appendix 1. Poisson GLM results for effect of distance from hive site and land use type, for all species present at more than half of the sites. Where there is a significant land use effect or interaction, co-efficients are given for individual land use types

Species		$\beta$	F	P
Allograpta spp.	<b>Land use</b>		2.60	0.11
	<b>Distance</b>	0.00	0.80	0.39
	<b>Interaction</b>		0.18	0.91
Phasia Campbelli	<b>Land use</b>		1.64	0.24
	<b>Distance</b>	0.00	2.06	0.18
	<b>Interaction</b>		0.71	0.57
Bombus terrestris	<b>Land use</b>		43.22	<b>0.00</b>
	Crop	-0.16		
	Degraded tussock	-1.09		
	Riverbed	1.39		
	Tussock	4.06		
	<b>Distance</b>	0.00	1.53	0.22
	<b>Interaction</b>		7.63	<b>0.00</b>
	Crop x Distance	-0.16		
	Degraded tussock x Distance	-1.09		
	Riverbed x Distance	-0.16		
	Tussock x Distance	-1.09		
Eristalis tenax	<b>Land use</b>	1.39	0.70	0.57
	<b>Distance</b>	4.06	0.02	0.89
	<b>Interaction</b>		0.30	0.83
Lassioglossum mataroa	<b>Land use</b>	0.00	3.81	<b>0.05</b>
	Crop	2.30		
	Degraded tussock	-0.23		
	Riverbed	4.62		
	Tussock	3.30		
	<b>Distance</b>	0.00	0.12	0.74
Lassioglossum sordidum	<b>Interaction</b>		0.86	0.49
	<b>Land use</b>		2.02	0.17
	<b>Distance</b>	0.00	3.68	0.08
Leioproctus fulvescens	<b>Interaction</b>		1.44	0.29
	<b>Land use</b>		30.07	<b>0.00</b>
	Crop	-25.05		
	Degraded tussock	2.52		
	Riverbed	-0.04		
	Tussock	-58.11		

	<b>Distance</b>	0.02	30.07	<b>0.00</b>
	<b>Interaction</b>		12.27	<b>0.00</b>
	Crop x Distance	-25.03		
	Degraded tussock x Distance	2.52		
	Riverbed x Distance	-0.04		
	Tussock x Distance	-58.10		
<i>Lucilla sericata</i>	<b>Land use</b>		6.36	<b>0.01</b>
	Crop	2.56		
	Degraded tussock	1.47		
	Riverbed	0.20		
	Tussock	3.48		
	<b>Distance</b>	0.00	19.25	<b>0.00</b>
	<b>Interaction</b>		1.64	0.24
<i>Melangyna novaezelandiae</i>	<b>Land use</b>		5.37	<b>0.02</b>
	Crop	2.90		
	Degraded tussock	3.75		
	Riverbed	2.14		
	Tussock	2.47		
	<b>Distance</b>	0.00	6.74	<b>0.03</b>
	<b>Interaction</b>		1.86	0.20
<i>Odontomyia spp.</i>	<b>Land use</b>		5.77	0.01
	Crop	2.95		
	Degraded tussock	1.90		
	Riverbed	3.32		
	Tussock	7.67		
	<b>Distance</b>	0.00	7.83	<b>0.02</b>
	<b>Interaction</b>		4.59	<b>0.03</b>
	Crop x Distance	2.95		
	Degraded tussock x Distance	1.90		
	Riverbed x Distance	3.32		
	Tussock x Distance	7.67		
<i>Oxysarcodexia varia</i>	<b>Land use</b>		1.75	0.22
	<b>Distance</b>	0.00	0.21	0.66
	<b>Interaction</b>		0.09	0.97
<i>Platycheirus spp.</i>	<b>Land use</b>		1.75	0.22
	<b>Distance</b>	0.00	0.05	0.83
	<b>Interaction</b>		1.12	0.39
<i>Zizina labradus</i>	<b>Land use</b>		4.23	0.04
	Crop	3.19		
	Degraded tussock	2.06		
	Riverbed	1.00		
	Tussock	3.40		
	<b>Distance</b>	0.00	0.826	0.384
	<b>Interaction</b>		3.547	0.055



Appendix 2. Coefficients and P values for the analysis of pollen load across all species total pollen load across all species

	Estimate	Std. Error	z value	P
(Intercept)	2.624	0.249	10.548	< 2e-16
<i>Adalia bipunctata</i>	-2.560	1.261	-2.031	<b>0.042</b>
<i>Allograpta</i> spp.	0.025	0.275	0.091	0.927
<i>Apis mellifera</i>	0.560	0.408	1.372	0.170
Bethylidae	-0.129	1.194	-0.108	0.914
Birconidae	0.237	1.668	0.142	0.887
<i>Bombus hortorum</i>	1.697	0.482	3.521	<b>0.000</b>
<i>Bombus ruderatus</i>	1.882	0.507	3.713	<b>0.000</b>
<i>Bombus terrestris</i>	1.810	0.331	5.461	<b>0.000</b>
<i>Bruchidius villosus</i>	1.461	1.657	0.882	0.378
<i>Calcigeria incidens</i>	1.921	1.653	1.162	0.245
<i>Calliphora vicina</i>	0.075	0.640	0.117	0.907
Calliphoridae spp.	-0.696	0.782	-0.890	0.373
<i>Campylia</i> spp.	0.454	0.980	0.463	0.643
<i>Chaetophthalmus bicolor</i>	-0.572	1.690	-0.339	0.735
Chalcidae	0.080	1.672	0.048	0.962
<i>Coccinella leonina</i>	0.096	1.666	0.058	0.954
<i>Coccinella undecimpunctata</i>	0.139	1.195	0.116	0.907
<i>Dilophus nigostigma</i>	-1.097	1.031	-1.064	0.287
<i>Eristalis tenax</i>	2.292	0.444	5.166	<b>0.000</b>
<i>Helophilus antipodes</i>	0.285	1.280	0.223	0.824
<i>Helophilus hochstetteri</i>	2.143	0.564	3.799	<b>0.000</b>
<i>Heteria appendiculata</i>	0.336	0.889	0.378	0.706

Heteria_plebia	-0.860	1.702	-0.505	0.613
Heteria_punctigera	-0.917	0.819	-1.120	0.263
Heteria_sp_1	1.084	1.659	0.653	0.514
Hylaeus_asperithorax	-2.795	1.947	-1.435	0.151
Hylaeus_capitosus	-0.795	0.521	-1.525	0.127
Hylaeus_relegatus	-2.101	1.802	-1.166	0.244
Lassioglossum_mataroa	0.876	0.245	3.575	<b>0.000</b>
Lassioglossum_sordidum	1.247	0.251	4.963	<b>0.000</b>
Leioproctus_fulvescens	2.163	0.421	5.140	<b>0.000</b>
Leioproctus_maritimus	0.593	1.660	0.357	0.721
Lucilla_sericata	-0.104	0.275	-0.380	0.704
Lycaena_boulderanum	-0.606	0.469	-1.291	0.197
Lycaena_salustius	0.269	0.488	0.550	0.583
Melangyna_novaezelandiae	0.623	0.254	2.451	<b>0.014</b>
Muscidae_sp._1	-0.129	0.881	-0.147	0.883
Muscidae_sp._2	-0.099	0.481	-0.206	0.837
Muscidae_sp._3	-1.603	0.969	-1.655	0.098
Muscidae_sp._6	-2.635	1.955	-1.348	0.178
Muscidae_sp._7	-2.066	1.817	-1.137	0.255
Muscidae_sp._8	-3.134	2.114	-1.482	0.138
Muscidae_sp_2	-3.292	2.052	-1.604	0.109
Odontomyia_spp.	0.597	0.254	2.355	<b>0.019</b>
Orocrambus_flexuosellus	1.202	1.657	0.725	0.468
Oxysarcodexia_varia	0.371	0.244	1.522	0.128
Pales_sp_1	-3.123	2.092	-1.493	0.135
Pales_sp_3	-0.046	0.988	-0.046	0.963

Pales_sp_4	-0.197	0.731	-0.270	0.787
Pales_sp_6	0.306	1.185	0.258	0.796
Peremptor_spp	0.562	0.979	0.574	0.566
Phasia_campbelli	0.144	0.307	0.468	0.640
Pieris_rapae	1.350	1.182	1.142	0.254
Plagiomyia_spp.	-0.402	1.211	-0.332	0.740
Platycheirus_spp.	-0.045	0.365	-0.123	0.902
Poecilohetaerella_bilineata	0.084	0.578	0.145	0.885
Pollenia_spp.	0.529	0.672	0.788	0.431
Proscissio_sp_3	1.713	1.654	1.035	0.301
Psilota_decessum	-0.014	0.545	-0.027	0.979
Tachinidae	0.166	0.357	0.464	0.643
Xanthocnemis_zealandica	-1.343	1.728	-0.777	0.437
Zizina_labradus	0.539	0.272	1.979	<b>0.048</b>
Land.usedegraded tussock	0.004	0.184	0.019	0.984
Land.useriverbed	0.125	0.184	0.679	0.497
Land.usetussock	-0.095	0.234	-0.407	0.684
Distance..m.	0.000	0.000	-5.518	<b>0.000</b>

Appendix 3. Pollen viability coefficients and P values across all species, and across land-use and bee density gradients

	Estimate	Std. Error	z value	P
(Intercept)	0.927	0.405	2.292	0.022
Adalia_bipunctata	0.033	1.567	0.021	0.983
Allograpta_spp.	1.222	0.619	1.974	<b>0.048</b>
Apis_mellifera	1.247	0.634	1.966	<b>0.049</b>
Beris_spp.	-0.993	1.873	-0.530	0.596

Bethylidae	15.190	4139.000	0.004	0.997
Birconidae	14.870	3569.000	0.004	0.997
Bombus_hortorum	1.342	0.824	1.629	0.103
Bombus_ruderatus	1.537	1.199	1.281	0.200
Bombus_terrestris	0.300	0.512	0.585	0.559
Bruchidius_villosus	-2.170	1.460	-1.486	0.137
Calliphora_vicina	-0.256	1.092	-0.234	0.815
Calliphoridae_spp.	-0.664	1.471	-0.451	0.652
Campylia_spp.	1.817	1.408	1.290	0.197
Chaetophthalmus_bicolor	16.740	3671.000	0.005	0.996
Chalcidae	-0.344	1.523	-0.226	0.821
Coccinella_leonina	14.470	4596.000	0.003	0.997
Coccinella_undecimpunctata	-0.892	1.583	-0.564	0.573
Dilophus_nigostigma	0.310	0.967	0.321	0.748
Eristalis_tenax	1.870	0.710	2.633	<b>0.008</b>
Helophilus_antipodes	-1.412	1.747	-0.809	0.419
Helophilus_hochstetteri	2.855	0.953	2.994	<b>0.003</b>
Heteria_appendiculata	-1.013	1.324	-0.765	0.445
Heteria_punctigera	-0.297	0.963	-0.308	0.758
Heteria_sp._2	-0.676	1.809	-0.374	0.709
Heteria_sp_1	16.700	3584.000	0.005	0.996
Hylaeus_asperithorax	17.460	3506.000	0.005	0.996
Hylaeus_capitosus	-0.721	0.962	-0.750	0.453
Hylaeus_relegatus	15.400	4149.000	0.004	0.997
Lassioglossum_mataroa	1.434	0.559	2.564	<b>0.010</b>
Lassioglossum_sordidum	0.738	0.559	1.319	0.187

Leioproctus_fulvescens	0.827	0.713	1.161	0.246
Leioproctus_hudsonii	1.891	1.981	0.955	0.340
Leioproctus_imitatus	0.648	1.810	0.358	0.720
Leioproctus_maritimus	0.652	2.035	0.320	0.749
Lucilla_sericata	0.595	0.533	1.117	0.264
Lycaena_boulderanum	0.265	0.982	0.270	0.787
Lycaena_salustius	0.646	1.100	0.587	0.557
Melangyna_novaezelandiae	0.628	0.448	1.400	0.161
Muscidae_sp._1	16.970	2849.000	0.006	0.995
Muscidae_sp._2	1.350	0.925	1.460	0.144
Muscidae_sp._3	0.247	1.439	0.172	0.864
Muscidae_sp._6	16.260	3061.000	0.005	0.996
Muscidae_sp._7	16.720	2845.000	0.006	0.995
Muscidae_sp._8	15.250	4224.000	0.004	0.997
Muscidae_sp_2	0.390	1.312	0.297	0.766
Neoitamus_melanopogon	1.607	1.438	1.118	0.264
Odontomyia_spp.	0.499	0.457	1.092	0.275
Orocrambus_flexuosellus	16.540	3208.000	0.005	0.996
Oxysarcodexia_varia	2.109	0.589	3.583	<b>0.000</b>
Pales_sp_1	-0.318	1.278	-0.249	0.804
Pales_sp_3	-0.162	1.273	-0.127	0.899
Pales_sp_4	2.042	1.614	1.265	0.206
Phasia_campbelli	0.216	0.625	0.345	0.730
Pieris_rapae	16.770	2098.000	0.008	0.994
Plagiomyia_spp.	15.160	6651.000	0.002	0.998
Platycheirus_spp.	0.668	0.719	0.930	0.352

Poecilohetaerella_bilineata	15.970	1695.000	0.009	0.992
Pollenia_spp.	0.473	0.939	0.504	0.614
Protohystricia_sp_1	0.106	1.844	0.057	0.954
Protohystricia_sp_2	0.458	1.777	0.258	0.797
Psilota_decessum	0.264	0.778	0.339	0.734
Pteromalidae	15.960	4304.000	0.004	0.997
Pyronota_festiva	-0.953	1.771	-0.538	0.590
Sapropogon_sp.	-0.747	1.755	-0.425	0.671
Tachinidae	0.418	0.515	0.813	0.416
Therevidae	-0.594	1.741	-0.341	0.733
Xanthocnemis_zealandica	1.945	1.984	0.980	0.327
Zizina_labradus	2.295	0.838	2.738	<b>0.006</b>
Land.usedegraded tussock	1.015	0.388	2.612	<b>0.009</b>
Land.useriverbed	0.115	0.391	0.294	0.769
Land.usetussock	0.385	0.523	0.736	0.462
Distance..m.	0.000	0.000	3.430	<b>0.001</b>

Appendix 4. Coefficients showing overall effects of declining bee density (distance) and land use types on the pollen load pollen load carried by pollinators

	Estimate	Std. Error	z value	P
(Intercept)	2.72	0.21	12.90	< 2e-16
Intertegular distance	0.29	0.06	4.59	0.000
Land use: degraded tussock	-0.15	0.20	-0.77	0.442
Land use: riverbed	-0.08	0.20	-0.40	0.689
Land use: tussock	-0.14	0.25	-0.56	0.574
Distance	0.00	0.00	-3.95	0.000

Appendix 5. Table of coefficients and P values showing the relationship between connectedness, pollen type and land use as predictors of the proportion of pollen carried by insects.

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	0.40034	0.55981	0.715	0.474524
normalised degree	-1.1225	1.12676	-0.996	0.319142
Pollen typeNative	0.09069	0.67089	0.135	0.892474
Pollen typeWeed	3.00698	0.57072	5.269	1.37E-07
land usedegraded tussock	0.06954	0.76288	0.091	0.927365
land useriverbed	2.12234	0.69162	3.069	0.00215
land usetussock	0.6096	0.68639	0.888	0.374479
normalised degree x Pollen type Native	-0.23633	1.36114	-0.174	0.862158
normalised degree x Pollen type Weed	-3.70761	1.22646	-3.023	0.002503
normalised degree x land use Degraded tussock	-3.70789	1.5017	-2.469	0.013544
normalised degree x land use Riverbed	-6.8807	1.42654	-4.823	1.41E-06
normalised degree x land use Tussock	-3.57713	1.42983	-2.502	0.012357
Pollen typeNative x land use Degraded tussock	-0.83107	0.89073	-0.933	0.350811
Pollen typeWeed x land use Degraded tussock	0.33881	0.799	0.424	0.671537
Pollen typeNative x land use Riverbed	-1.77027	0.81303	-2.177	0.029452
Pollen typeWeed x land use Riverbed	-2.71193	0.71462	-3.795	0.000148
Pollen typeNative x land use Tussock	-1.92956	0.85926	-2.246	0.024729
Pollen typeWeed x land use Tussock	-0.57899	0.73096	-0.792	0.428299
normalised degree x Pollen type Native x land use Degraded tussock	5.0447	1.75102	2.881	0.003964
normalised degree x Pollen typeWeed x land usedegraded tussock	3.77018	1.62154	2.325	0.020069
normalised degree x Pollen typeNative x land useriverbed	4.51404	1.68321	2.682	0.007323
normalised degree x Pollen typeWeed x land useriverbed	8.79414	1.54087	5.707	1.15E-08
normalised degree x Pollen typeNative x land usetussock	5.55052	1.75934	3.155	0.001606
normalised degree x Pollen typeWeed x land usetussock	3.98826	1.58872	2.51	0.012061

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