Pollinators and pollination in changing agricultural landscapes; investigating the impacts of bioenergy crops

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Dara Anne Stanley

Department of Botany
School of Natural Sciences
Trinity College Dublin

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and is entirely my own work except where duly indicated and clearly acknowledged in the text.

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Dara A. Stanley

Summary

Pollinators, and the pollination services they provide, are essential for the reproduction of the majority of angiosperms, but also for a wide variety of crops. However pollinators are increasingly threatened by many human activities including land use change and agricultural intensification. A major shift in agricultural land use is beginning with the widespread promotion and cultivation of bioenergy crops as an alternative fuel source to combat climate change, with potentially major implications for biodiversity. The aim of this thesis was to examine the impacts of changes in agricultural regions, specifically the growth of bioenergy crops, on pollinators and pollination services in Ireland.

Using a large network of commercial fields, I investigated the impacts of growing two model bioenergy crops (oilseed rape *Brassica napus* L. and *Miscanthus X giganteus*) on pollinator diversity, abundance and community composition in comparison to the crops they replace in the landscape. I found varying effects of bioenergy production on different pollinator taxa, with the solitary bees the group most affected. Higher abundances and species richness of solitary bees were found in bioenergy crops than in conventional ones, and community composition of this group differed between bioenergy crops also. This may be due to increased floral resources in the bioenergy crops, although perennial Miscanthus did not provide more nesting resources for bumblebees than annual crops. Overall, field margins and hedgerows were the most important habitats for pollinators. This indicates that bioenergy production at the field scale in agricultural areas does not have negative implications for pollinators and may even have positive benefits for some taxa, and that a variety of crop types within the landscape can be beneficial for solitary bees.

In the same fields, I used plant-pollinator networks to examine effects of mass flowering oilseed rape during and after flowering, different bioenergy crops, and the composition of the landscape surrounding the fields on the interactions between flower visiting insects and plants. Networks were robust to changes in a mass flowering resource, but replacing arable land with bioenergy crops caused changes in network structure. However, landscape context also affected network properties, suggesting that both local and landscape effects should be considered when studying species interactions and resulting pollination services.

The most common visitors to mass flowering oilseed rape in Ireland are a cryptic bumblebee complex, but little is known about the proportions or requirements of the different cryptic species. I investigated, using molecular methods, what the proportions and colony densities of the different species were in spring oilseed rape fields, in comparison to the second most common bumblebee visitor. I found different proportions and colony densities of the different cryptic species, and that the different species responded differently to the composition of landscape surrounding the fields suggesting different ecological requirements. Interestingly, large numbers of bumblebee colonies (up to 880) were found to use individual spring oilseed rape fields as a resource.

Although oilseed rape provides forage resources for pollinators when the crop is in flower, pollinators are often associated with semi-natural habitats and field margins. I investigated whether pollinators use wild plants as a forage resource in addition to oilseed rape when the crop is in flower, and whether this has implications for pollination services to these wild species. I found that oilseed rape shares pollinator taxa and individuals with a variety of wild plants in the margins and hedgerows, and that the majority of insects foraging on oilseed rape also carried wild plant pollen. However, little crop pollen becomes deposited on wild plant stigmas suggesting this is not a mechanism for interference with pollination services to wild plants.

Lastly, in a final study I examined the pollinators and pollination of oilseed rape in Ireland. Oilseed rape is visited by a wide range of insect species, with bumblebees the most abundant pollinators followed by honeybees. Winter oilseed rape produces more seed with insect pollination, showing that insects can improve crop yields, and therefore market value, of the crop in Ireland.

Finally, I conclude with a synthesis of results and some methodological considerations. I propose some suggestions for both pollinator conservation in farmland, and bioenergy policy to mitigate impacts on biodiversity. I also highlight some potential areas for further research.

To my family

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Chapter 1

General Introduction

> "Nature's economy shall be the base for our own,
> for it is immutable, ours is secondary" *Linneus, 1763*

1 General Introduction

1.1 Biodiversity and Ecosystem services

Biodiversity, the variety of life on earth, contributes both directly and indirectly to human welfare and existence on the planet by the provision of vital goods and services (e.g. Costanza *et al.* 1997; Daily *et al.* 1997; Kearns *et al.* 1998; Palmer *et al.* 2004). These can be termed "ecosystem services" and can be described as "the benefits to human welfare provided by organisms interacting in ecosystems" (Hooper *et al.* 2005; Klein *et al.* 2007), or the "economic benefits that nature provides to people" (MEA 2005). In more recent times, both biodiversity and the ecosystem services it provides are under increasing pressure from human activities (Daily *et al.* 1997; Vitousek *et al.* 1997; Hooper *et al.* 2005; Cardinale *et al.* 2012). However, humans and biodiversity are not separate entities, and the maintenance of biodiversity and ecosystem services globally is increasingly dependent on maintaining biodiversity in landscapes dominated by humans (Fahrig *et al.* 2011).

1.2 Pollination and its importance as an ecosystem service

Pollination is an essential supporting ecosystem service required by the majority of flowering plants; it has been estimated that 87.5% of angiosperms require biotic pollination (Ollerton *et al.* 2011b), and that 62% of these flowering species are limited in reproduction by the amount of pollen they receive (Burd 1994). Pollination is the result of pollen being transferred from the anther (male part) to the stigma (female part) of another flower. Although this can happen by abiotic means (via transport in water or by wind) the majority takes place through transport on the bodies of flower visiting animals. A wide variety of organisms can act as pollinators including birds, bats, other mammals and insects (Willmer 2011), with insects being the most common. Of the insects, bees are the most important pollinators both in natural and agricultural systems (Winfree *et al.* 2008; Potts *et al.* 2010; Plate 1), primarily as they feed on floral products for the whole of their lifecycle.

Pollinators are not only responsible for the reproduction of wild plant species, but also for pollination of a large number of food crops for humans (Corbet *et al.* 1991). Thirty nine of

57 leading world food crops have higher production with pollination, with 35% of the worlds food supply coming from insect pollinated crops (Klein *et al.* 2007). Pollinator dependent crops also provide many essential nutrients required for a balanced human diet (Eilers *et al.* 2011), and are increasing in production (Aizen *et al.* 2008a) and price (Lautenbach *et al.* 2012) globally. It has been estimated that the value of pollination in agriculture to the world economy is €153 billion per year (Gallai *et al.* 2009). Although crop pollination is often attributed to the honeybee as the main pollinator, wild pollinators often carry out the majority of crop pollination (Williams *et al.* 1991; Breeze *et al.* 2011; Ollerton *et al.* 2011a; Rader *et al.* 2012). In fact, 37 invertebrate and seven vertebrate genera have been documented as crop visitors (Kremen 2008), and wild bees alone were found to provide the majority of pollination services in farms in USA (Winfree *et al.* 2007; Winfree *et al.* 2008).

Plate 1. A range of solitary bees, and the honeybee, found in Irish farmland. From top left to bottom right: *Andrena* spp., *Nomada* spp., *Andrena* spp., *Andrena* spp., *Andrena cineraria* and *Apis mellifera* (photos: DS)

1.3 Pollinator declines

Although less well publicised, declines in insects have been recorded as even greater than declines of some other vertebrates and plants (e.g. in the UK, Thomas *et al.* 1994) and there is now unequivocal evidence that pollinator declines have been recorded worldwide, in every continent except Antarctica (Allen-Wardell *et al.* 1998; Kearns *et al.* 1998; but see Ghazoul 2005). This has consequences for the persistence of both crop yields, but also for the reproduction of wild plant species and maintenance of global biodiversity and ecosystem functioning. Declines have been recorded in most pollinator groups, especially in Europe and North America, including the bumblebees (Fitzpatrick *et al.* 2007; Grixti *et al.* 2009; Bommarco *et al.* 2011; Cameron *et al.* 2011; Dupont *et al.* 2011), butterflies (Warren *et al.* 2001; Thomas *et al.* 2004; van Swaay *et al.* 2010) and honeybees (Vanengelsdorp *et al.* 2008). Declines in other groups are also apparent; across 11 European countries, 27.4% of the bee fauna is listed in Red data books (Steffan-Dewenter *et al.* 2005). Equally, insect pollinated plants are also in decline (Biesmeijer *et al.* 2006), and bumblebee forage plants have been shown to have declined in the size of their range relative to other plant species (Carvell *et al.* 2006a). The importance of pollinators, their recent declines, and the potential impacts on pollination services, are increasingly getting scientific, media and political attention (Buchmann & Nabham 1996; POST 2010; Mayer *et al.* 2011; Plate 2).

Plate 2. A "biodiversity beermat" illustrating the importance of bees and pollination in a public awareness campaign (www.biodiversityinourlives.com).

1.3.1 Drivers of pollinator decline

Pollinators are threatened by a number of different factors, and there is much debate as to what is causing the observed declines in many pollinator species. Habitat loss and fragmentation, use of pesticides and agrochemicals, pathogens, alien species and climate change have all been suggested as major drivers (Kearns *et al.* 1998; Brown & Paxton 2009; Potts *et al.* 2010). Invasive species can compete with native pollinators for resources or cause movement of pathogens and disease (Stout & Morales 2009; Goulson 2010); declines of some pollinator populations have been due to parasites or pathogens (Cameron *et al.* 2011; Evison *et al.* 2012; Szabo *et al.* 2012); and climate change can affect distribution and phenology of insects and plants, and even plant chemistry, with consequences for decline (Memmott *et al.* 2007; Hegland *et al.* 2009; Hoover *et al.* 2012).

1.3.2 Pollinators and agriculture

However, the majority of threats to, and causes of, pollinator decline are those associated directly with agricultural practice. Land used for agriculture covers approximately 37% of global terrestrial land, and agriculture is the predominant land use in Western Europe (e.g. 62% of Ireland is used for agriculture, CSO 2010). Agriculture has intensified significantly since the second World War, with reductions in crop diversity and increased reliance on fewer high-yielding varieties, increased mechanisation, use of agrochemicals, destruction of hedgerows and decreases in landscape diversity (Robinson & Sutherland 2002). This has led to associated declines in biodiversity across many taxa (e.g. birds, Benton *et al.* 2002; carabid beetles and biocontrol, Geiger *et al.* 2010; and large vertebrates and invertebrates, Medan *et al.* 2011), including pollinators (Kremen *et al.* 2002), which in turn can threaten the productivity of agriculture (Zhang *et al.* 2007; Power 2010).

There are a number of ways in which changes in agricultural practice can influence pollinators and pollination. Firstly, agricultural intensification often leads to increased use of pesticides which has negative impacts on pollinators (Thompson 2001; Brittain *et al.* 2010b; Otieno *et al.* 2011; Henry *et al.* 2012; Krupke *et al.* 2012; Whitehorn *et al.* 2012), and organic farming which traditionally uses less agrochemicals than conventional farming has been shown to be beneficial for pollinators (Morandin & Winston 2005; Holzschuh *et al.* 2008; Rundlof *et al.* 2008b), insect pollinated plants (Gabriel & Tscharntke 2007; Power *et al.* 2012) and pollination services to both crops and wild species (Power & Stout 2011; Andersson *et al.* 2012; Klein *et al.* 2012). Secondly, agricultural intensification can cause increased habitat loss and fragmentation as fields are enlarged and field margins and hedgerows removed, or as more marginal land is intensified. Habitat loss has been extensively studied in the pollination literature but independent effects of habitat fragmentation are less well documented (Hadley & Betts 2012). Declines in pollinator diversity and abundance (Hendrickx *et al.* 2007; Kohler *et al.* 2008), and pollination services (Ricketts *et al.* 2008; Carvalheiro *et al.* 2010; Klein *et al.* 2012), are found in farmland surrounded with less, or further from, semi-natural habitat fragments, and the proportion of semi-natural land in landscapes can have positive impacts on pollinators and pollination (Morandin & Winston 2006; Klein *et al.* 2012). Thirdly, changes in agriculture can lead to homogenisation of crop types and habitats across landscapes which can have negative consequences for pollinators. Heterogeneity of habitats within farmland can be beneficial for biodiversity (Benton *et al.* 2003), and more heterogeneous landscapes provide benefits for both butterflies (Weibull *et al.* 2000; Oliver *et al.* 2010) and bumblebees (Rundlof *et al.* 2008b). Intensification of agriculture can lead to a homogenization of pollinator faunas (Carre *et al.* 2009; Ekroos *et al.* 2010), with a loss of habitat specialists and poor dispersers leaving only common taxa. Lastly, the introduction of new crop types and high yielding varieties, such as GM crops, can also have impacts on pollinators (e.g. Haughton *et al.* 2003; Bohan *et al.* 2005). Therefore, any changes in agricultural regions that cause changes in land use, spatial configuration or intensity of management have the potential to have knock-on impacts on pollinators and pollination services (Osborne *et al.* 1991).

Recent discussion has led to two ways of maintaining biodiversity in agricultural regions. The "land sparing" concept suggests that agricultural areas should be farmed as intensively as possible with maximum yields, and that specific areas should be set aside or "spared" for conservation purposes (Ewers *et al.* 2009; Fischer *et al.* 2011). The "land sharing" concept suggests that agriculture and conservation should go hand in hand, with agricultural areas being made more biodiversity–friendly (Phalan *et al.* 2011). In the case of pollinator conservation, it seems that conservation of pollinators within agricultural regions is needed for a variety of reasons. Firstly, as previously discussed, many important crops require pollination by wild pollinators; without wild pollinators in intensive agricultural regions many crops could not be grown, or managed pollinators would have to be introduced to achieve maximum yields (Lye *et al.* 2011). Secondly, many wild plant species in agricultural regions that are found in hedgerows and field margins (and provide additional ecosystem services to agriculture) also benefit from pollination (Gibbs 2001; Jacobs *et al.* 2009; Jacobs *et al.* 2010), and for their persistence pollination services are needed. Thirdly, agriculture covers a large land area worldwide. As bees and other pollinators are mobile organisms they need large land areas to sustain them. Therefore, for effective conservation across the majority of the earths' surface, agricultural regions must be included. Lastly, often agricultural areas provide the best habitat for pollinators. For example, the dominant natural vegetation type across much of Western Europe is deciduous forest which may not be an ideal habitat for many pollinator species. In fact, some of the best habitats for pollinators include flower rich semi-natural grasslands (Steffan-Dewenter & Tscharntke 2000); these habitats are themselves products of extensive agricultural practice and some agricultural management is needed to sustain them. Therefore, maintaining of pollinators in agricultural regions is fundamental to their conservation, and to the persistence of the service of pollination.

1.4 Bioenergy crops

1.4.1 Bioenergy production

Recently, shifts in agricultural land-use have arisen from the adoption of the Kyoto protocol which stimulated a need to find alternatives to fossil fuels to combat climate change, but also to increase fuel security and satisfy increasing energy demands worldwide. One proposed alternative is bioenergy, derived from the growth of first and second generation bioenergy crops, which is now increasing in production worldwide and is predicted to increase further in the future (Rounsevell *et al.* 2006; Sims *et al.* 2006). This is resulting in large scale conversion of conventional agricultural land towards growing crops for bioenergy (International Energy Agency 2006), stimulated largely by policy. For example, an EU target of having 20% of energy and 10% of transport fuel from renewable energy by 2020 (Directive 2009/28/EC), and a requirement of 36 billion gallons of renewable fuel in US transportation fuel by 2022 (EISA 2007), is causing a rapid expansion of the bioenergy sector in Europe and the USA. In 2007, over 4 million hectares of non-food crops were sown in the EU, with the majority in Germany, France and the UK (DAFF 2009).

However, the rapid expansion of the bioenergy sector has resulted in concerns in a number of areas. Although use of bioenergy crops has the potential to reduce the use of fossil fuels and combat climate change, there are also implications for water use, food security and biodiversity (Field *et al.* 2008). Bioenergy crops are often grown on existing agricultural land (Donnelly *et al.* 2011), where they can compete with conventional food production (Pimentel *et al.* 2008; Tilman *et al.* 2009; Valentine *et al.* 2012); for example the grain required to fill the tank of an SUV with ethanol can feed a person for a year (Scharlemann 2008). Although bioenergy crops in current circumstances usually replace existing farmland, future projections suggest that to meet bioenergy targets semi-natural areas or forests would need to be converted to bioenergy production which would have hugely negative consequences for both carbon emissions, biodiversity and ecosystem services

(Fargione *et al.* 2008). Biofuels are designed to be carbon neutral, or even carbon negative (Tilman *et al.* 2006; Zimmermann *et al.* 2011), but concerns over whether bioenergy crops actually reduce carbon emissions when the whole life cycle is taken into account have also been discussed (Scharlemann & Laurance 2008; Searchinger *et al.* 2008), and can depend on the amount of agrochemical inputs (Stephenson *et al.* 2008) or the type of land replaced (Fargione *et al.* 2008).

There are a wide variety of crops than can be grown for bioenergy. First generation bioenergy crops are those that produce fuel from starch, sugar and vegetable oil. These include crops grown for starch and sugar such as maize (*Zea mays* L.), wheat (*Triticum aestivum* L.) or sugar cane (*Saccharum officinarum* L), or those grown for oil such as palm (*Elaeis guineensis* Jacq.) or oilseed rape (*Brassica napus* L.) (Valentine *et al.* 2012). These crops are often also used as food, and so their use for energy can compete directly with food production (Fargione *et al.* 2008; Searchinger *et al.* 2008; Valentine *et al.* 2012). Although some first generation crops can be used as solid biofuels, the majority are converted to liquid biofuels for use in transport, including ethanol for use in petrol engines, or pure plant oil or biodiesel for use in diesel engines (Valentine *et al.* 2012). Second generation bioenergy crops are lignocellulosic biomass or woody crops including grass, switchgrass (*Panicum virgatum* L.), reed canary grass (*Phalaris arundinacea* L.), Miscanthus (*Miscanthus X giganteus*), poplar (*Populus* sp.) or willow (*Salix* sp.) (Rowe *et al.* 2009). It has been suggested that these are a better option than first generation bioenergy crops as they do not compete directly for use as food, they require less agricultural inputs, and they do not lead to the destruction of natural forests as they can often be grown on existing marginal land (Valentine *et al.* 2012). These crops are commonly used as solid biofuels for heat and electricity generation, but can also be converted into liquid biofuel for transport (Rowe *et al.* 2009).

1.4.2 Bioenergy crops, biodiversity and pollinators

One of the major concerns over bioenergy crop production is the potential impact on biodiversity, which have been highlighted since the beginnings of the industry (Cook *et al.* 1991; Ranney & Mann 1994). Although landscapes dominated by arable agriculture are always changing due to changes in crop choice or crop rotations, the extent and the rapidity of change towards bioenergy is different (Haughton *et al.* 2009), and some bioenergy crops may differ substantially to conventional crops in their agronomy (Bellamy *et al.* 2009). Bioenergy crops may therefore have positive (enhanced habitat, erosion prevention, less pesticides) and/or negative (taking over of semi-natural habitats, increased intensification etc) impacts on the biodiversity and the environment (Börjesson 1999; Groom *et al.* 2008; Scharlemann 2008). Furthermore, effects of bioenergy production could be at the field scale, but also at larger landscape scales due to changes in landscape diversity or impacts on climate change (Firbank 2008).

Some second generation bioenergy crops such as Miscanthus and Willow have been suggested as beneficial for biodiversity as they are less intensively managed and, as some are perennial crops, are less disturbed (Boehmel *et al.* 2008; Haughton *et al.* 2009; Rowe *et al.* 2009; Dauber *et al.* 2010; Kleijn *et al.* 2011). Other bioenergy crops are more intensively managed with high inputs of agrochemicals and short annual rotations such as corn and oilseed rape (Boehmel *et al.* 2008). The growth of bioenergy crops on a small field scale may increase heterogeneity of agricultural landscapes increasing potential habitats and niches for different species, but on large farm or landscape scale may produce more uniform monocultures (Firbank 2008; Landis & Werling 2010), which can have negative effects on biodiversity (Engel *et al.* in press). A review of 47 studies found a predominantly positive effect of the growth of biomass crops on biodiversity, with birds the taxon studied most comprehensively (Dauber *et al.* 2010). However, given the predicted extent of bioenergy production globally, the potential impacts of this growing sector on biodiversity are not well known (Rowe *et al.* 2009; Dauber *et al.* 2010; Landis & Werling 2010).

As pollinators are threatened by changes in agricultural practice in many ways, the introduction of large tracts of new crops in agricultural areas has the potential to have large impacts on this group and the pollination services they provide. However, very little is known about the impacts of bioenergy production on pollinators or other insects (but see Landis & Werling 2010), and in addition a large number of crops used to produce biodiesel require pollination services (Vaknin 2012). Bioenergy production could result in pollinator loss through i) loss of habitat heterogeneity and increased monocultures (e.g. Brodschneider & Crailsheim 2010; Oliver *et al.* 2010); ii) increased use of agrochemicals (e.g. Henry *et al.* 2012; Whitehorn *et al.* 2012); iii) conversion of marginal land and loss of semi-natural habitats (e.g. Ricketts *et al.* 2008); and iv) changes in the types of crops grown, with a reliance on a handful of high yielding varieties and the arrival of novel crop types (e.g. Haughton *et al.* 2003). Although pollinators have been extensively studied in mass flowering crops such as oilseed rape (see section 1.8.2), and other biofuel crops have been shown to provide habitat for bees (Gardiner *et al.* 2010), the impacts of growing bioenergy crops on pollinators are largely unknown. Those studies which have looked at the impacts of bioenergy crops on pollinators, and on biodiversity, have tended not to compare bioenergy crops with any other type of land use and were carried out on experimental plots with low numbers of replicates (Dauber *et al.* 2010). In a review by Dauber *et al.* (2010), which incorporated both peer reviewed scientific literature and other reports, only four studies were found investigating butterflies in biomass crops, and four investigating other canopy invertebrates.

1.5 Resources for pollinators in agricultural environments

The growth of bioenergy crops within the landscape has the potential to have both direct and indirect effects on pollinators. Direct effects could include provision of floral resources by the crop itself, application of pesticides or changing the shading or wind conditions in an area. Indirect effects could include changes in quantity and quality of floral and nesting resources, which in turn can cause changes in pollinator abundances and community composition.

1.5.1 Floral resources

Primarily, pollinators need flowers to feed on. Although most pollinators feed on floral products for some, or all, of their lifecycle there are differences between pollinator taxa in their floral preferences, and different flowers may have adapted to attract different pollinators (Fenster *et al.* 2004). Bees feed exclusively on nectar and pollen, and so need continuous provision of flowers throughout their lifecycle; therefore floral communities can be important in structuring bee communities (Potts *et al.* 2003). Bumblebees have different lengths of tongue which can determine which flowers they visit (Fussell & Corbet 1991; Goulson & Darvill 2004; Goulson *et al.* 2005), and it has been suggested that dietary breadth can explain why some bumblebees remain common and others are declining (but see Williams 2005; Williams & Osborne 2009). Long tongued bumblebees often have strong associations with perennial flowers (Dramstad & Fry 1995; Carvell *et al.* 2006b) or flowers from the Fabaceae (Goulson *et al.* 2005), which are often associated with unimproved grasslands. Species that have declined have been shown to have had narrower diets in the past, while commoner species have benefitted from increases of some of their chosen food plants (Kleijn & Raemakers 2008). Many solitary bee species are polylectic, visiting a variety of flowers, but some are monolectic and specialise on particular plant species (Strickler 1979); for example *Colletes floralis*, although polylectic, shows a preference for flowers from the Apiaceae family (Davis *et al.* 2012). Syrphids are not floral specialists for their whole lifecycle, but different foraging guilds of hoverflies have been identified (Branquart & Hemptinne 2000). The growth of bioenergy crops could change the floral composition of field margins and hedgerows, or weeds within crops, which could have consequent impacts on both abundance and community composition of pollinators. Although mass flowering bioenergy crops can enhance pollinator densities at a landscape scale (Westphal *et al.* 2003), it is not known whether insects exclusively visit crop flowers, or whether they also require alternative forage from wild plant species.

1.5.2 Nesting resources

However, many flower visiting groups also need other resources such as nesting sites, overwintering sites and larval host plants or substrates (Gathmann & Tscharntke 2002; Kremen *et al.* 2007). The availability of potential nest sites can also be a determinant of bee density and community structure (Eltz *et al.* 2002; Potts *et al.* 2005; Steffan-Dewenter & Schiele 2008), and in some cases can be more limiting than the availability of forage resource (Samejima *et al.* 2004). The location of bee nests as either above or below ground can affect species responses to habitat loss and intensification (Williams *et al.* 2010). However, different bumblebees have different nesting preferences: *Bombus terrestris* and *B. lapidarius* prefer nesting in open terrain, while *B. lucorum* and *B. pascuorum* prefer forest boundaries (Svensson *et al.* 2000), and a strong association has been found between the subterraneous nesters *B. terrestris*, *B. lapidarius* and *B. lucorum* and banks, and *B. pascuorum* and *B. hortorum* and tussock-type vegetation (Kells & Goulson 2003). Solitary bees can be divided into two main groups based on their nesting requirements, cavity nesters and ground nesters, but there is great variety in the requirements of different species (Krombein 1967; Cane *et al.* 2007). Butterflies often require specific host plants on which to lay their eggs (Bond & Gittings 2008), and hoverflies have a variety of different larval habitats including water or animal dung (Speight 2008). The growth of bioenergy crops could alter the availability of nesting and reproductive sites both within the crop and in field margins and hedgerows, which could also cause changes in pollinator abundance and community composition.

1.5.3 Field margins and hedgerows

In agricultural regions, as most intensive farming involves annual crop rotations and high disturbance, field margins and hedgerows are often the only remaining semi-natural habitat (Marshall & Moonen 2002), and are likely to be important areas in the provision of resources for pollinators (Plate 3). Field margins usually consist of a number of different elements that can vary quite considerably in their flora and fauna; the boundary which is usually a hedgerow, fence or wall, the margin strip which occurs between the crop and the boundary and may be there for access, wildlife, recreational or agronomic reasons, and the edge of the crop itself (Marshall & Moonen 2002). Field margins and hedgerows can provide both forage and nesting resources for pollinators (Hannon & Sisk 2009; Lye *et al.* 2009), with more bumblebees found in naturally regenerated field margins than conventional cereal field margins (Pywell *et al.* 2005) and more pollinators in field margins than centres of grazed dairy pastures (Power & Stout 2011). In agricultural regions, field margins and hedgerows are important for bumblebee nesting (Svensson *et al.* 2000; Kells & Goulson 2003; Osborne *et al.* 2008b; Lye *et al.* 2009), and may be the only nest sites left. The width of margins can also be important, with wider margins supporting higher densities of bumblebees (Backman & Tiainen 2002). Hedgerows can also have effects on both bumblebee movement and pollination services (Cranmer *et al.* 2012). Recent agri-environmental objectives have involved augmenting the quality of field margins for pollinating insects by sowing flower rich mixtures, which can increase pollinator abundance and species richness (Pywell *et al.* 2005; Carvell *et al.* 2007), especially in more intensively farmed areas (Haenke *et al.* 2009; Carvell *et al.* 2011). However, crop management can affect the plant diversity of field margins (Kleijn & Snoeijing 1997), and new crops may cause changes in the size and quality of field margins. In northern Ireland it has been suggested that a decrease in the intensity of hedgerow management and the inclusion of a wider margin before hedgerows would help to maintain species diversity (Hegarty & Cooper 1994). Therefore, the growth of bioenergy crops may affect the quality of field margins for pollinators; annual crops may result in intensive margin management and hedgerow maintenance, while perennial crops may result in larger, less managed field margins and less frequent hedgerow management. Field margins may also be important in the provision of alternative forage resources for pollinators in mass flowering crops, both during and after crop flowering.

Plate 3. Field margins and associated hedgerows on Irish farms (photos: DS)

1.6 Pollination services

If bioenergy crops cause changes in the abundance and diversity of pollinators, this is likely to have knock-on impacts on the provision of pollination services to both wild plant species and to crops. For example, some wild plants show an increase in fruit set when grown beside mass flowering oilseed rape, while others show a decrease or no effect (Cussans *et al.* 2010; Diekotter *et al.* 2010; Holzschuh *et al.* 2011). These wild plants are important for pollinators in agricultural regions as they provide essential forage resources. At the same time, many wild species found in agricultural systems benefit in reproduction with pollinators (Jacobs *et al.* 2009; Power & Stout 2011), and changes in their pollination interactions could have implications for their persistence (Gibson *et al.* 2006) and have knock on impacts along the food chain (e.g. on production of berries for birds, Jacobs *et al.* 2009).

However, it is not only the abundance of pollinators that is important for the delivery of pollination services; a diversity of pollinators is also important. For example, increased pollinator diversity can increase seed set in pumpkin (Hoehn *et al.* 2008), and diversity of bees was also important for pollination of watermelon (Kremen *et al.* 2002). This is also important in maintaining wild plant communities; a study by Fontaine et al. (2006) found that functional diversity of pollinators increases functional diversity of plants. Diversity may benefit pollination for a number of reasons (Klein *et al.* 2008); 1) a higher diversity of pollinators means there is a better chance of the right pollinator existing for the right plant, 2) a higher diversity of pollinators means there is a higher chance of all flowers being pollinated over an extended blooming season and 3) a higher diversity of pollinators means that pollinators may interact with each other increasing their efficiency (e.g. Greenleaf & Kremen 2006). This highlights the importance of conservation of both pollinator abundance and diversity to maintain pollination services to both crops and wild species (Allen-Wardell *et al.* 1998; Pauw 2007; Potts *et al.* 2010).

Although conservation of pollinators is gaining general support as being important, it is becoming increasingly apparent that the conservation of species interactions is also critical (Kearns *et al.* 1998). Recently, the interactions between pollinators and the flowers they visit have been studied using a food web approach (Memmott 1999). This allows the study of the interactions between flower-visiting insects and plants that provide the service of pollination, and how stable or vulnerable these interactions might be. The structure of networks can be quantified, and may be affected independently of changes in just species richness and abundance (Tylianakis *et al.* 2007; Tylianakis *et al.* 2010). The growth of bioenergy crops could cause changes in network structure or stability, which could in turn affect the ecosystems service of pollination.

Mass flowering bioenergy crops may also cause changes in pollination services through direct competition for pollination resources between crop and wild plant species. Plants can affect the pollination of co-flowering species in two ways; by affecting visitation rates, or by affecting how pollen is transferred from one individual to another, including movement of pollen between the different species (interspecific pollen transfer, Morales & Traveset 2008). Visitation rates may be either enhanced via facilitation, decreased as a result of competition or not change in the presence of a co-flowering species (Rathcke 1983), with examples of all three outcomes found in different studies (Chittka & Schurkens

2001; Johnson *et al.* 2003; Moragues & Traveset 2005; Larson *et al.* 2006; Duffy & Stout 2008; Duffy & Stout 2011). Changes in pollen dynamics and interspecific pollen transfer can have consequences for both male and female fitness. Pollen can be lost during movements between heterospecific flowers, through grooming or through deposition on non-reproductive floral parts of flowers (especially when visiting flowers with different floral morphology to the donor flower as this can cause pollen to "scrape off" on non-reproductive structures, Murcia & Feinsinger 1996). This results in less pollen available for transfer to con-specific flowers, reducing male fitness (termed con-specific pollen loss, Morales & Traveset 2008; Muchhala & Thomson 2012). Alternatively, heterospecific pollen can become deposited on stigmas with consequences for conspecific pollen receipt and seed set, and thus potentially reducing female fitness (Brown & Mitchell 2001; termed heterospecific pollen deposition, Morales & Traveset 2008). The influence of one plant species on the pollination of another is also affected by the floral density of the species involved. At lower floral densities, a particular species may have a facilitative effect on pollination of another, whereas at higher floral densities this interaction may become competitive (Rathcke 1983; Dietzsch *et al.* 2011). As mass flowering species by definition occur at high density, mass flowering crops could have particular impacts on the pollination of co-flowering wild species. However, pollen transfer between mass flowering crops and wild plant species has not been investigated previously.

1.7 Landscape scale

It is likely that changes in pollinators and pollination services due to bioenergy crop cultivation at the local field scale could also be affected by processes acting at larger landscape scales. Landscape structures can influence the temporal and spatial availability of all types of resources needed by pollinators, and although resources may occupy the same locality, they can also be dispersed across landscapes (Steffan-Dewenter *et al.* 2002; Kremen *et al.* 2007). Therefore, pollination can be seen as a “mobile agent-based ecosystem service” that is affected not only at a local scale within habitats where the services are delivered, but also at a landscape scale that reflects the spatial distribution of resources such as nesting sites and alternative forage between habitats (Kremen *et al.* 2007). The effects of agricultural change can operate at a landscape level (Hendrickx *et al.* 2007); therefore bioenergy crop growth may cause changes in resources at a local scale, but these changes may be buffered or affected by what else is in the landscape surrounding the bioenergy fields.

Pollinators are able to exploit resources spatially separated within the landscape as they are mobile organisms with relatively large foraging ranges. Bees are central place foragers, and therefore consistently return to their nest (Zurbuchen *et al.* 2010a). Extensive work on the foraging ranges of bumblebees, using molecular, observational and tracking methods, has shown that bumblebees can fly long distances, up to a number of kilometres from their nests, and that there are differences between species in terms of how far they forage (Osborne *et al.* 1999; Goulson & Stout 2001; Darvill *et al.* 2004; Knight *et al.* 2005; Wolf & Moritz 2008; Charman *et al.* 2010; Hagen *et al.* 2011). Honeybees also can fly large distances (Beekman & Ratnieks 2000) but as solitary bees are generally smaller, although still mobile, they tend to forage over smaller distances (Gathmann & Tscharntke 2002; Greenleaf *et al.* 2007; Zurbuchen *et al.* 2010b), and so may be affected by landscape at smaller spatial scales. Both bumblebee and solitary bee foraging distance is affected by the scale of available forage, and bees often travel shorter distances during foraging bouts in more resource rich environments (Gathmann & Tscharntke 2002; Westphal *et al.* 2006b; Osborne *et al.* 2008a; Carvell *et al.* 2012), and long range foraging can have negative impacts on solitary bee reproduction (Zurbuchen *et al.* 2010a).

Although not central place foragers, butterflies and hoverflies are also very mobile and likely to be affected at the landscape scale. Some butterfly species migrate thousands of kilometres, but more sessile species also move throughout the landscape (Cant *et al.* 2005; Ouin *et al.* 2008; Ovaskainen *et al.* 2008). Less is known about foraging ranges of hoverflies and how they disperse, but some species can also migrate over long distances (Gatter & Schmid 1990), and hoverfly movement in agricultural regions can be affected by barriers such as hedgerows (Wratten *et al.* 2003).

There is lots of evidence to show that landscapes can affect ecological processes (Tscharntke *et al.* 2012), and pollinators are affected by both landscape composition and structure (Hadley & Betts 2012) at different spatial scales (Steffan-Dewenter *et al.* 2002; Westphal *et al.* 2006a). Local patterns are sometimes better explained at the landscape than the local level (Schweiger *et al.* 2005; Brittain *et al.* 2010a; but see Kovács-Hostyánszki *et al.* 2011), which demonstrates the importance of studies of both local and landscape effects. Different pollinator groups can respond differently to landscape (Sjödin *et al.* 2008). Landscape composition has different impacts on different bee taxa (Carre *et al.* 2009), and landscape context can also influence bumblebee nest survival (Goulson *et al.* 2010). Landscapes with more organic faming can support higher pollinator diversity (Holzschuh *et al.* 2008), and landscapes with high availability of mass flowering oilseed rape have been found to have higher densities of bumblebees (Westphal *et al.* 2003), but not more colonies of *B. pascuorum* (Herrmann *et al.* 2007). Landscape heterogeneity can also affect bees: species richness and abundance of bumblebees is positively related to organic farming and landscape heterogeneity in Sweden (Rundlof *et al.* 2008b), and bumblebee colonies produce larger workers in complex landscapes (Persson & Smith 2011). Elements in the wider landscape have also been shown to have implications for rarer bee species such as *B. muscuorum* (Diekotter *et al.* 2006). Different landscape elements, such as forests, can act as barriers within the landscape (Kreyer *et al.* 2004).

Butterflies and hoverflies are also affected by landscape composition, and landscape heterogeneity effects stability and diversity of butterfly communities (Weibull *et al.* 2000; Oliver *et al.* 2010). Agri-environmental schemes, such as enhancement of floral resources in field margins, can be more effective for pollinators in simple rather than complex landscapes (Heard *et al.* 2007; Holzschuh *et al.* 2007; Rundlof *et al.* 2008b; Haenke *et al.* 2009; Batary *et al.* 2011). Insect pollinated plants also respond to landscape (Power *et al.* 2012), and landscape level elements can affect pollination services. For example, the number of flower visiting bees and seed predators of *Centaurea jacea* increased with landscape complexity, but no influence was found on seed set (Steffan-Dewenter *et al.* 2001). In a review of 23 studies, Ricketts et al. (2008) found an overall negative effect of isolation from natural habitat on crop pollination services. Therefore the growth of bioenergy crops at the field scale may also be affected by the landscape context surrounding the fields.

1.8 Bioenergy production in Ireland

Ireland currently imports all of its transport fuel requirements, and a large proportion of electricity comes from non-renewable peat fired power stations. Therefore, there is huge potential for the use of bioenergy crops to produce both solid and liquid biofuels. This has been recognised by the Irish Government, and although less than 0.2% of agricultural land in Ireland is under non-food crops (declared under the Energy crops scheme in 2008), the Bioenergy Action plant for Ireland (Department of Communications Marine and Natural Resources 2007) has established targets, over and above the EU directive, of 33% of electricity from renewable resources by 2010, and that electricity production from peat-fired power stations would be co-fired with 30% biomass crops by 2015.

Approximately 64% of Irelands' land area is used for agriculture; 91 % of agricultural area is used for grass and rough grazing and approximately 9% for crops which occur mainly in the East and South East of the country (CSO 2010, Figure 1.1). Although intensifying, Irish agricultural areas are typically less intensive than some of their European counterparts. For example, 14.3 % of area on Irish pastoral farms has been identified as semi-natural habitat (Sheridan *et al.* 2011). The main bioenergy crops that can be grown in Ireland include the annual crops oilseed rape, cereals and hemp (Department of Communications Marine and Natural Resources 2007), and the perennial crops willow, Miscanthus and reed canary grass (Styles *et al.* 2008), and these crops are predominantly replacing conventional agricultural land (Donnelly *et al.* 2011). To incentivise bioenergy crop growth in Ireland, a bioenergy scheme was established in 2007 (DAFF 2010), providing farmers with 50% establishment costs of some bioenergy crops such as Miscanthus and Willow, which has resulted in increases in area of both these crops. Currently, oilseed rape is the most widely grown bioenergy crop in Ireland covering 6500 hectares in 2009, followed by Miscanthus which covered 2100 hectares by the same year (CSO 2009; McDonagh 2010). Oilseed rape is predominantly grown in the East and South East (Dublin, Meath, Kildare, Wicklow, Waterford, Carlow, Kilkenny, Wexford and South Tipperary) (CSO 2011b). Miscanthus is also being grown in similar areas including Tipperary (337ha), Limerick (332ha), Cork (308ha), Kilkenny (200ha) and Wexford (188ha) (McDonagh 2010). Both crops are continuing to increase; oilseed rape production in Ireland increased 76% between 2006-2007, and 99% between 2010 and 2011, and it now occupies about 4% of arable land in Ireland (CSO 2011a), while the area planted with Miscanthus increased 15% between 2007 and 2009 (McDonagh 2010). Baseline studies of biodiversity in agricultural grasslands in Ireland have been carried out (Purvis *et al.* 2009; Power 2011), but little is known about biodiversity in Irish arable areas (O'Brien *et al.* 2008). Therefore it is important that the potential of bioenergy crops to affect pollinators and pollination is investigated in the infancy of their production in Ireland, and they provide a useful model to study these impacts at an early stage.

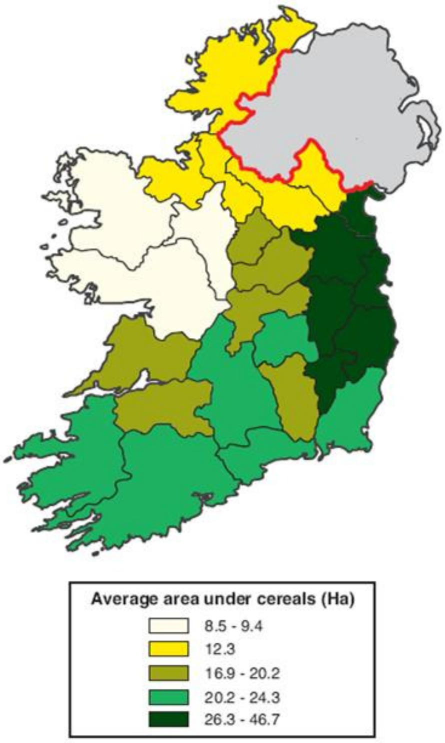

Figure 1.1. Average area under cereals (total area of cereals in county/number of farms growing cereals in county, Ha) in Ireland. Reproduced from Census of Agriculture 2010 (CSO 2010)

1.8.1 Miscanthus as a bioenergy crop and previous work on pollinators

Miscanthus (*Miscanthus X giganteus,* Plate 4) is a perennial rhizomatous grass, originally from Asia, which is grown as a second generation bioenergy crop. It has been grown in Europe under trial conditions since the 1980s (Lewandowski *et al.* 2000) and is now grown commercially in both Europe and the US (Heaton *et al.* 2004). It has been grown commercially in Ireland since the adoption of the bioenergy scheme in 2006. Once established, it is harvested annually between January and March, grows rapidly (up to 3m in one growing season, Caslin *et al.* 2010), remains in the ground for approximately 15 years and requires very low inputs of agrochemicals (Lewandowski *et al.* 2000; Clifton-Brown *et al.* 2007; Christian *et al.* 2008; Caslin *et al.* 2010). To date, positive impacts in comparison to conventional crops have been shown for birds and small mammals (Clapham & Slater 2008; Bellamy *et al.* 2009). However, little is known about the impacts of growing Miscanthus on pollinators and pollination. As a perennial crop Miscanthus may provide more stable nesting resource or perennial forage plants for pollinators. In the UK, Semere and Slater (2007b) found that Miscanthus was a better habitat for bees and butterflies than Reed canary grass, but did not compare it to any conventional crops. Haughton et al. (2009) found total abundance of butterflies was higher in Miscanthus than other arable crops in the UK (although the latter were sampled in different years), and attributed this difference to the low-input management of Miscanthus causing less disturbance and perhaps a wider variety of weedy forage plants within fields.

Plate 4. Oilseed rape (top) and Miscanthus (bottom) crops grown in Ireland (photos: DS)

1.8.2 Oilseed rape as a mass flowering bioenergy crop, and previous work on pollinators

Oilseed rape (or canola, *Brassica napus L.* Plate 4) is grown as both a food and a first generation bioenergy crop largely in Europe but also in US, Canada, India, Australia. China and Brazil (International Energy Agency 2006). The seeds are used to produce oils for human consumption, or liquid biofuel in the form of pure plant oil, and can be converted to biodiesel through a process of esterification (Rowe *et al.* 2009). The residue of the pressing process is often used as protein cake for animal feed, and the stems of the crop can be used as straw. Oilseed rape was first cultivated for oil in Europe in the 13th century (Snowdon *et al.* 2007) and has been grown since then, but new bioenergy incentives are changing the distribution and uses of the crop (Frondel & Peters 2005), and its production in Europe is increasing (EEB 2011; Eurostat 2011). However, it is an intensively managed annual crop with high inputs of agrochemicals (Becka *et al.* 2004; Boehmel *et al.* 2008), and reduced use of fertiliser on the crop in the UK has been shown to increase savings in global warming potential (Stephenson *et al.* 2008).

Although much less common in Irish agricultural landscapes than in UK and elsewhere in Europe (covering only 4% of arable land in Ireland), oilseed rape is the most widely planted mass flowering crop in Ireland followed by peas and beans (CSO 2011a). It is typically grown in a 1 in 4 rotation with other arable crops such as wheat and barley (Teagasc 2009). Although there are many different varieties of the crop which change in popularity from year to year, there are two main forms; winter oilseed rape is planted in August or September, flowers the following spring between April and June, and is harvested in July or August (Teagasc 2009), while spring oilseed rape is planted in March or April, flowers in June or July and is harvested in August or September (Hayter & Cresswell 2006). Winter oilseed rape typically has higher yields but also higher inputs, and the relative abundance of the two forms varies from year to year, largely depending on the weather conditions for sowing in August/September. Pollination efficiency is higher in spring crops (Hayter & Cresswell 2006), probably due to different abundances of insects in later compared to earlier flowering forms. Winter oilseed rape is likely to provide resources for bumblebee queens at the beginning of the colony cycle, while spring oilseed rape probably provides resources for a wider diversity and abundance of insects later on in the season. However, due to the short flowering period of both forms of the crop (approximately 4 weeks), oilseed rape crops alone are unlikely to provide sufficient forage for pollinators through their whole life cycle.

Mass flowering crops have many implications for pollinators and pollination, and most work on mass flowering crops has focussed on oilseed rape. Although it is self-fertile crop and can produce seed via wind-pollination, oilseed rape benefits from biotic pollination (Free 1993; Morandin & Winston 2005; Sabbahi *et al.* 2005; Hayter & Cresswell 2006; Oz *et al.* 2008; Bommarco *et al.* 2012; although this contribution has not been evaluated in Ireland previously), and is pollinated by wild bees (including both bumblebees and solitary bees; Banaszak 1992; Abel *et al.* 2003; Cresswell & Osborne 2004; Morandin & Winston 2005; Bommarco *et al.* 2012), hoverflies (Jauker & Wolters 2008; Bommarco *et al.* 2012; Jauker *et al.* 2012a), honeybees (Sabbahi *et al.* 2005), and is also visited by butterflies (Haughton *et al.* 2003). Therefore it can provide an important and nutritious nectar and pollen resource for wild insects (Cook *et al.* 2003; Tasei & Aupinel 2008; Jauker *et al.* 2012b). A large amount of oilseed rape planted in many countries around the world is now genetically modified (GM) and GM genes can be found in wild oilseed rape relatives (Snow *et al.* 2003), even including those from different genera to the oilseed rape (Chevre *et al.* 1997). Much work has focussed on the pollination of the crop, and the efficiencies and behaviour of pollinators, particularly in terms of geneflow from GM varieties (Cresswell *et al.* 2002; Cresswell & Osborne 2004; Hayter & Cresswell 2006; Chifflet *et al.* 2011; Jauker *et al.* 2012a). However, more recent work has investigated the impacts of oilseed rape from the pollinators' perspective and has suggested that oilseed rape may provide a beneficial resource for insects within agricultural areas (Westphal *et al.* 2003). Higher relative densities of bees and butterflies have been associated with oilseed rape compared with non-flowering arable crops in the UK (Haughton *et al.* 2003), and higher densities of bumblebees were found in landscapes with increasing proportions of oilseed rape (Westphal *et al.* 2003), possibly due to larger colony sizes (Herrmann *et al.* 2007). Although oilseed rape can have positive impacts on bumblebee numbers, this may not necessarily translate into reproductive success; bumblebee colonies gained more weight in areas with more oilseed rape, but did not produce any more reproductives at the end of the cycle (Westphal *et al.* 2009).

Less work has focussed on solitary bee species, but higher solitary bee species richness and abundance has been found in margins of flowering oilseed rape fields compared to other crop types, but more rare species were found in other crop types (Le Féon *et al.* in press). In contrast, work by Burger (2004) found no difference in the species richness of trap-nesting bees and wasps between fallows and oilseed rape fields. Solitary bees with early phenology that corresponds with the flowering of winter oilseed rape can derive reproductive benefits (Jauker *et al.* 2012b). Hoverflies are also efficient pollinators of oilseed rape (Jauker *et al.* 2012a), and more hoverflies were found in spring oilseed rape fields in more complex landscapes (Bommarco *et al.* 2012) although the density of hoverflies in oilseed rape fields was not related to the amount of woody vegetation in the surrounding landscape in Australia (Arthur *et al.* 2010). However, studies so far have been at the population level (Herrmann *et al.* 2007), focussed on specific taxonomic groups (Westphal *et al.* 2003), or focussed on species richness and diversity measures only (Haughton *et al.* 2003). Little is known about how oilseed rape affects pollinator communities, the structure of plant-pollinator networks, or pollination services to wild plant species.

1.9 Pollinators in Ireland

As a peripheral island, Ireland has a smaller insect fauna than the UK and most European countries (Regan *et al.* 2010a). The main pollinator groups found are the butterflies (Lepidoptera, Plate 5), hoverflies (Diptera; Syrphidae, Plate 6) and bees (Hymenoptera; Apoidea, Plate 7).

Ireland has 33 resident and migrant butterfly species (Nash *et al.* 2012). In a regional IUCN red list produced in 2010, 21 species are of least concern (LC), five near threatened (NT), three vulnerable (VU), three endangered (EN) and one regionally extinct (RE) (Regan *et al.* 2010b). Butterflies are threatened by a number of factors, with some of the biggest threats in Europe highlighted as agricultural intensification, followed by land abandonment and climate change (van Swaay *et al.* 2010). Declines have been noted in species associated with species rich grassland, but other less sensitive, more mobile species who are able to live in more eutrophic habitats have increased in some areas (Kuussaari *et al.* 2007).

Plate 5. A range of butterflies found in Irish farmland. From top left to bottom right: Green Veined White (*Pieris napis*), Painted Lady (*Vanessa cardui*), Small Tortoiseshell (*Agalis urticae*), Ringlet (*Aphantopus hyperantus*), Comma (*Polygonia c-album*), Speckled Wood (*Pararge aegeria*), White (*Pieris* sp.), Red Admiral (*Vanessa atalanta*) (photos: DS).

Currently, 183 hoverfly species are known from Ireland (Speight 2008). However, the known fauna is still expanding, with approximately one species added per year (Speight 2008). Approximately 50 species could be recognised as threatened, and are mostly associated with Irelands declining native woodlands (Speight 2008). The majority of hoverflies found in farmland have larvae that feed on aphids (Frank 1999; Haenke *et al.* 2009), and so this group can also provide bio-control services along with pollination. Although adult hoverflies consume both nectar and pollen, adults found on farmland are often polyphagous which may be a reason for their ability to exploit open habitats (Branquart & Hemptinne 2000).

Plate 6. Some Irish hoverflies. (from left to right: *Eristalis* spp., *Episyrphus balteatus*, *Helophilus* spp.) (photos: DS)

Ireland currently has 102 bee species, including the honeybee *Apis mellifera*, 20 species of bumblebee and 81 species of solitary bee (Fitzpatrick *et al.* 2006). According to IUCN regional classification, 38 species are of least concern (LC), 12 near threatened (NT), 14 vulnerable (VU), 10 endangered (EN), 6 critically endangered (CR), and 3 regionally extinct (RE, although the tawny mining bee, *Andrena fulva*, was recorded this year, 2012, for the first time in 87 years, National Biodiversity Data Centre 2012) (Fitzpatrick *et al.* 2006). However, 16 species were highlighted as data deficient, and 3 were not evaluated. The majority of species classified as data deficient were solitary bees, but two bumblebee species were also included in this category; *Bombus cryptarum* and *B. magnus*. *B. cryptarum* was only officially recognised in Ireland in 2006 following molecular studies (Murray *et al.* 2008). This species belongs to the *B. sensu stricto* group which comprises of five species in Europe (Williams *et al.* 2012), four of which are found in Ireland *(B.*

cryptarum, *B. lucorum, B. magnus* and *B. terrestris*, Murray *et al.* 2008). However, these species are notoriously difficult to tell apart morphologically, especially as workers (Wolf *et al.* 2010; Carolan *et al.* 2012), and although *B. lucorum* and *B. terrestris* are though to be of least concern (LC) in Ireland, data on the distribution and ecology of *B. cryptarum* and *B. magnus*, both in Ireland and throughout their range, is lacking. In fact, taxonomy and associated species specific information were highlighted as one of the key areas for future research in pollination ecology (Mayer *et al.* 2011).

Similar bumblebees are declining in Ireland as in the UK, including species such as *Bombus distinguendus*, *B. sylvarum* and *B. ruderarius*, and these later nesting rare species are now mainly found in the west of Ireland (Fitzpatrick *et al.* 2007). This may be attributed to a reduction in hay making from flower rich meadows and an increase in silage monocultures (Fitzpatrick *et al.* 2007), or due to range constrictions or later nesting behaviour (Williams & Osborne 2009). A survey of 28 Irish grassland farms found only 10 bumblebee species out of a potential 20 (Santorum & Breen 2005), with 50% reduction in abundance and diversity found in intensively farmed areas compared to current populations in the Burren region (Purvis *et al.* 2009). Work in intensive dairy farms found only five bumblebee and three solitary bee species (Power & Stout 2011). Therefore, although a limited pollinator fauna may already be found in agricultural regions in Ireland, there are a number of gaps in our knowledge, even in the basic taxonomy and ecological requirements of some of the most common bumblebee species. The value of pollination to the Irish economy has been valued at €53 million per year (Bullock *et al.* 2008), and this is expected to rise if production of oilseed rape as a bioenergy crop increases. Therefore, the maintenance and protection of existing pollinators and the services they provide in agricultural regions is essential. The growth of new crops for bioenergy has the potential to disrupt these organisms in the Irish landscape, and so deserves full investigation.

Plate 7. Common Irish bumblebees found in Irish farmland (rows 1 and 2, left to right: *B. lapidarius, B. pascuorum, B. pratorum, B. hortorum, B. sensu stricto*, *B. sensu stricto*) and rarer Irish bumblebees (row 3, left to right: *B. muscuorum, B. distinguendus* and *B. ruderarius*) (photos: DS).

1.10 Research objectives

This thesis aims to increase understanding of how changes in agricultural practice, specifically the growth of bioenergy crops, can affect pollinators and pollination services in agricultural areas, paying specific reference to landscape context. I pay special interest to the interactions between insects and flowers via plant-pollinator networks, and the use of alternative forage by pollinators in a mass flowering crop. I also examine a taxonomic issue by investigating patterns in one of the main bumblebee groups in agricultural areas, the *Bombus sensu stricto* complex.

As bioenergy crops are relatively new in the Irish landscape, I first investigated how the growth of bioenergy crops affects the abundance, diversity and community composition of pollinating insects, in comparison to the crops they replace, on a large scale using a network of commercial farms. This could be due to changes in forage resources or availability of nesting sites; therefore I also quantified impacts on floral resources and the availability of nest sites for bumblebees (Chapter 2).

Bioenergy production may not only influence pollinator abundance and diversity, but it could also have implications for the interactions between insects and flowers and the subsequent provision of pollination services. Chapter 3 investigates the impacts of mass flowering oilseed rape and bioenergy crop growth on the interactions between pollinators and plants using plant-pollinator networks. However, patterns can be driven by both changes in crop type at the local scale, but also by what is in the landscape surrounding the fields. Therefore I also investigate the relative importance of local and landscape effects.

As a mass flowering bioenergy crop, oilseed rape has particular implications for pollinators. The main flower visitors in oilseed rape fields are a cryptic complex of bumblebees, but little is known about proportions and ecological requirements of these individual species and so information on their conservation status is lacking. In Chapter 4, I investigate the proportions and colony densities of the species of the cryptic *Bombus sensu stricto* group in oilseed rape fields using molecular methods, and examine how the landscape composition around a field can influence the identity and colony densities of cryptic bees within it.

It is not yet known if pollinators exclusively visit mass flowering crops or whether they concurrently visit wild field margin and hedgerow species. If pollinators are shared between the two, this could also have implications for pollination services to these wild plants. In Chapter 5, I investigate whether the same insect taxa and individuals visit both oilseed rape and wild plants in the adjacent field margins and hedgerows, and whether oilseed rape pollen becomes deposited on wild plant stigmas.

Finally, the pollination requirements of oilseed rape have been studied in many countries, but not yet in Ireland. In Chapter 6 I investigate what insects are pollinators of winter oilseed rape crops in an Irish context, and whether oilseed rape benefits from insect pollination.

I finish with a synthesis of the overall findings, methodological considerations, recommendations for pollinator conservation in farmland and bioenergy policy, and directions for further research.

Chapter 2

Quantifying the impacts of bioenergy crops on pollinating insect abundance and diversity: a field scale evaluation reveals taxon-specific responses

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Plate 8. The five focal crop species used in Chapters 2 & 3. Left hand column: oilseed rape (top), Miscanthus on former arable land (middle) and wheat (bottom). Right hand column: Miscanthus on former grassland (top) and grass silage (bottom) (photos: DS)

2 Quantifying the impacts of bioenergy crops on pollinating insect abundance and diversity: a field scale evaluation reveals taxon-specific responses

2.1 Abstract

Global declines in pollinating insects have been linked with agricultural intensification and land use change. Increased production of novel crops for bioenergy is causing changes in agricultural practice, but the effects on different pollinating taxa have not yet been quantified. However, the major pollinating groups (social bees, solitary bees, hoverflies and butterflies) are likely to respond differently to changes in land use and shifts in crop cultivation patterns. I assessed the impacts of two bioenergy crops, oilseed rape (*Brassica napus* L.) and Miscanthus (*Miscanthus X giganteus*) on a) the diversity and abundance of four taxonomic groups of pollinating insects, b) insect community composition, c) floral resources and d) nesting sites for bumblebees, by comparing bioenergy crops with conventional arable and grass crops in a large network of commercial fields. I found that conventional crops and bioenergy crops did not differ greatly in either the abundance or richness of the most common pollinator groups (bumblebees and hoverflies) in individual fields, but there were differences in the abundance and richness of solitary bees and floral resources. In addition, impacts varied according to which crops are being replaced. Bumblebee nest-searching did not differ among crop types and was confined to field margins, but more trap nesting bees and wasps were found in Miscanthus than oilseed rape. Novel assemblages of solitary bee species were found in Miscanthus compared to oilseed rape suggesting a diversity of crop types benefits this group. Flowering plant assemblages also varied between crops, with perennial species more prevalent in Miscanthus. Whilst relatively isolated field-scale changes in crop type may not affect bumblebees and hoverflies, they may have a positive impact on solitary bees that have different floral and nesting resource requirements and mobility. To optimise habitat for pollinators in agricultural areas, our data suggest that management towards a diversity of crop types at the field scale may have positive impacts, and supports the appropriate management of field margins to maintain floral and nesting resources for pollinators.

2.2 Introduction

Declines in pollinating insects have been reported worldwide for a range of taxonomic groups (e.g. butterflies; Thomas *et al.* 2004; hoverflies; Biesmeijer *et al.* 2006; bees; Cameron *et al.* 2011). These declines, and their potential impact on pollination services, have recently received substantial scientific, political and media attention (e.g. POST 2010; Cameron *et al.* 2011). Although many human activities can directly or indirectly drive pollinator decline, many authors cite the primary drivers as agricultural intensification and land use change (Kearns *et al.* 1998; Kremen *et al.* 2002). Stimulated largely by policy, the growth of biofuel crops as alternatives to fossil fuels to combat climate change is resulting in large scale conversion of conventional agricultural land to growing crops for bioenergy (International Energy Agency 2006). This has implications for food production (Tilman *et al.* 2009; Valentine *et al.* 2012), but will also cause changes in both agricultural land use and intensification, and thus impact on pollinator biodiversity and the delivery of pollination services.

Some bioenergy crops, such as the lignocellulosic and woody biomass crops Miscanthus and willow, have been suggested as beneficial for insects in comparison to conventional ones as they have longer rotation periods, low inputs of agrochemicals, fewer disturbances during the growing season than other crops, are harvested in winter and provide a greater richness of spatial structures (Haughton *et al.* 2009; Rowe *et al.* 2009; Dauber *et al.* 2010). In addition, perennial bioenergy crops may support a diverse within-field plant community that can be beneficial for bees (Gardiner *et al.* 2010). Crops such as willow and oilseed rape can provide nectar and pollen resources for pollinators within the landscape (e.g. Bommarco *et al.* 2012; Chapter 6), although oilseed rape may be more intensively managed. However, given the degree of expected bioenergy production, and the extent to which pollinators rely on the agricultural environment, the potential effects of bioenergy crop production on pollinators have not been well studied (Semere & Slater 2007b; Rowe *et al.* 2009; Dauber *et al.* 2010; Gardiner *et al.* 2010).

It is likely that different bioenergy crops could provide different key resources to pollinating insects. Pollinating insects do not only require flowering plants to forage from, but also need nesting, mating, ovipositing, larval, and overwintering sites (Potts *et al.* 2005), and there is variation among pollinating taxa in relation to their requirements. For this study, I examined two contrasting bioenergy crops that are currently grown commercially worldwide. Firstly, oilseed rape (*Brassica napus* L.) is a mass flowering crop, and produces large amounts of nectar and pollen and so has the potential to be beneficial for pollinators as a forage resource. Previous work has found higher relative densities of bees and butterflies associated with oilseed rape compared with non-flowering arable crops in the UK (Haughton *et al.* 2003), and the abundance of worker bumblebees was higher with increasing proportions of oilseed rape in the landscape in Germany (Westphal, Steffan-Dewenter & Tscharntke 2003). Less work has focussed on solitary bee and hoverfly species, but Le Féon et al. (in press) found more solitary bees in margins of oilseed rape fields than other crop types. However, as an annually disturbed crop oilseed rape is unlikely to provide stable nesting resources for pollinators. Secondly, Miscanthus (*Miscanthus X giganteus*), due to its perennial nature and increased litter layer, may provide resources through vegetation growing within the crop or stable nesting habitat for ground and stem-nesting bees (Landis & Werling 2010). However, butterflies are the only pollinator taxa previously investigated in this crop and were found to be more abundant in Miscanthus compared with reed canary grass (Semere & Slater 2007b), and compared with arable field margins (Haughton *et al.* 2009).

Using a large network of 50 commercial fields, I compared the pollinating insects in fields of two model bioenergy crops (Miscanthus and oilseed rape) with the conventional crops they replace. I used abundance and species richness measures, but also examined community structure, floral resources and the availability of bumblebee nesting sites in the different crop types. Specifically, I answered the following questions:

1) Which pollinating insects are using bioenergy crops as a habitat?
2) Are there differences in abundance and species richness of different pollinator groups in bioenergy crops, in comparison to the conventional crops they replace? Are there differences between the margins and centres of fields?
3) Is the community structure of pollinator groups different in bioenergy crops, and do they contain novel communities of pollinators?
4) Does Miscanthus, as a perennial crop, provide better nesting opportunities for bumblebees than conventional crops?

2.3 Methods

2.3.1 Field sites

Data were collected in 2009 and 2010 in South–East Ireland, where arable, beef and dairy farming are interspersed and government grants have resulted in relatively small scale (field scale) commercial planting of Miscanthus on both former arable land and grassland since 2006 (Donnelly *et al.* 2011). Oilseed rape has been grown for much longer, but its cultivation has increased in recent years due to its use as a bioenergy crop. I compared each bioenergy crop to a conventional crop that it commonly replaces on farmland: Miscanthus planted on former arable land (MA) and winter oilseed rape (OS) were compared to a common arable crop (winter wheat, WW), and Miscanthus planted on former grass land (MG) to a common grass crop (grass silage, GS).

Ten fields of each crop type (MA, OS, WW, MG and GS) were selected in the study region. The Miscanthus fields were planted in 2006 or 2007 and were reaching maturity during our study. All fields were selected to be as similar as possible in terms of management, size (median field size 4.5 hectares), number of hedgerows, and altitude, and were at least 1km apart from each other and from any other mass flowering crops. Only one field was selected per farm to allow for independence of management, and fields of different crop types were interspersed geographically.

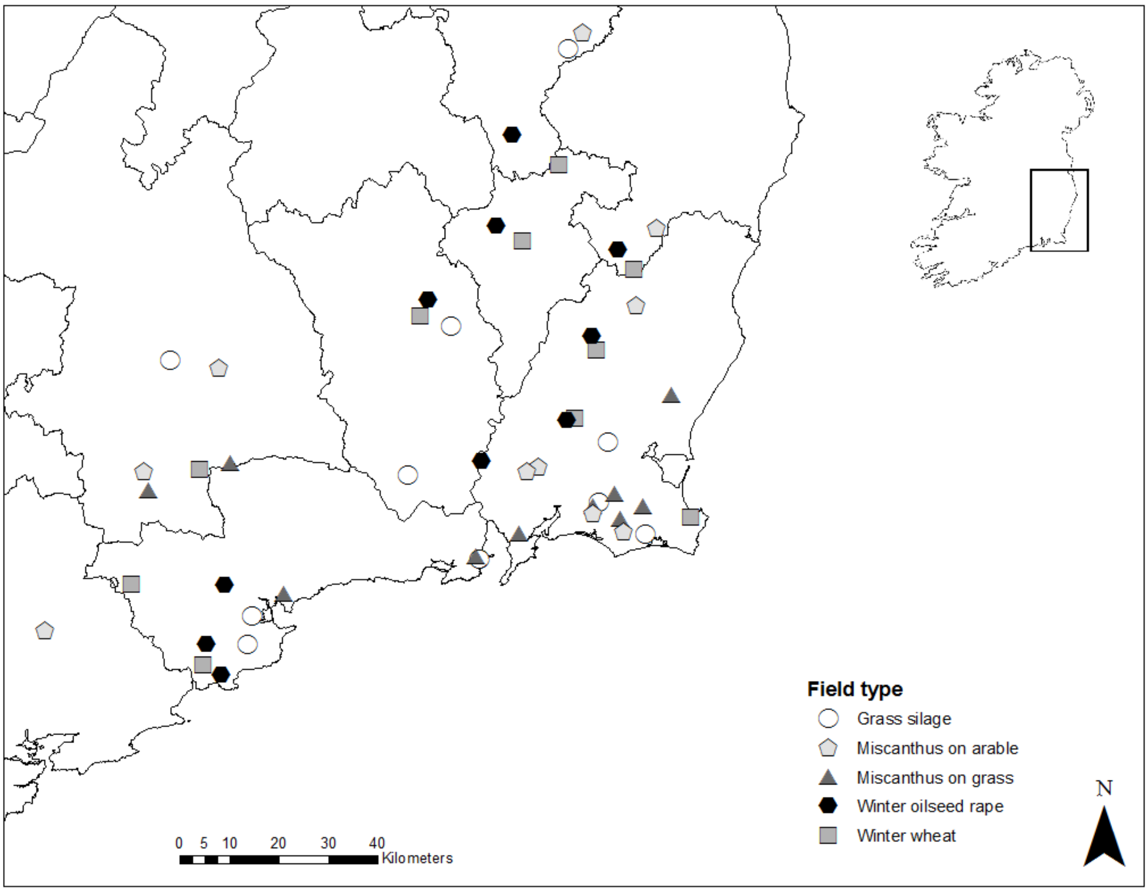

Figure 2.1. The location of the 50 sites used for pan trap sampling around South-East Ireland in 2009.

2.3.2 Pollinator sampling

To sample all pollinator groups as comprehensively as possible in each crop type, I used a number of recommended methods (Westphal 2008; Nielsen *et al.* 2011). Firstly, pan traps were used as this method allows simultaneous sampling of multiple locations, coverage of a large number of sites, laboratory identification of specimens, and is the most efficient method of sampling bees (Westphal 2008). All 50 fields were sampled once during each of two sampling periods (beginning of June when oilseed rape was in flower, and mid-July) in 2009. To maximise comparability during each sampling period, all fields were sampled in two sequential four day periods. Traps were polypropylene plastic bowls painted with white, yellow and blue UV paint (LeBuhn & Droege 2003). One bowl of each colour was attached to a post using a metal clamp and the rim of the bowl adjusted to vegetation height (Plate 11). Three posts were deployed along the margin (20m apart by a south-facing hedge, where possible) and three posts in the centre (20m apart, 30m from the margin) of each field for 48 hours. The number of floral units (the unit of a flower that a medium sized bee has to fly rather than walk between, and was either an individual flower or compact inflorescence (Dicks *et al.* 2002)) of non-crop flowering plants were also estimated in a 20x60m quadrat encompassing the three trap posts. All bees and syrphids were identified to species level in the laboratory (using Coe 1953; Prys-Jones & Corbet 1987; Stubbs & Falk 2002). Total abundance or species richness of insects in traps, and floral units, were pooled to give one value for the field margin and one for the centre of each field in each sampling period.

Secondly, transect sampling was also used as this method efficiently samples butterflies (Pollard & Yates 1993) and bumblebees (Fussell & Corbet 1992). Due to the intensive nature of this method, a subset of five fields of each crop type were sampled (**Appendix 1**). The same fields were visited three times between May and August 2009 by a single observer (D.S.), and four 100x2m standard belt transects (Pollard & Yates 1993) were walked on each visit, two in the margins along hedgerows, and two in the centre (>30m from margins, minimum 20m from each other) of each field at a slow, steady pace (0.07-0.44ms^{-1}). Oilseed rape was in flowerin during the first survey period, at the end of flowering during the second period and finished during the third period. Surveys took place in dry, bright conditions and were limited to obligate flower-visiting taxa commonly associated with pollination: bees (Apidae), butterflies (Lepidoptera) and hoverflies (Diptera: Syrphidae). Bumblebees (*Bombus* spp.) and butterflies were identified to species level (except for the butterflies Pieris napi, P. rapae and P. brassicae which were only recorded to genus, and bumblebee workers of the Bombus sensu stricto group which can be indistinguishable morphologically: Wolf *et al.* 2010), while solitary bees and syrphids were identified to genus. Taxa identified to genus were not used in analyses of species richness. Insects were mostly identified in the field, and where this was not possible were captured and identified in the laboratory along with the pan trap specimens. The number of floral units of non-crop flowering plants were also recorded at 10m intervals along each transect. For analyses, bumblebee, butterfly, hoverfly and floral unit abundance and species richness were pooled for the margins and centres of each field in each sampling period, and calculated as the number or species richness of individuals recorded per minute (insect data only) in 100m. The numbers of other bees were too low for further analysis.

To detect additional species that may not be sampled using previous methods (Westphal 2008), trap nests were deployed in the same subset of five fields used for the transects but of the arable crops only (MA, OS & WW). Six trap nests were placed in each field in early May 2009. Traps nests were made of 20x10cm sections of plastic drainpipe stuffed with 20cm lengths of common reed *Phragmites australis* (Gathmann *et al.* 1994), and eight bamboo internodes (at least one diameter > 8mm). Two nests were attached to a single post using metal clamps and one post put in the centre of each of three margins with hedgerows, 30cm from and perpendicular to the hedgerow to allow colonisation in both ends (Plate 12). At the end of August, one nest from each post was left in the hedgerow (to prevent nests acting as a population sink for solitary bees and wasps) and one collected, vernalised for 12 weeks at 5°C and then transferred to emergence boxes at room temperature. All emerging individuals were identified to species level. The abundance of trap nesting bees and wasps was calculated as the total number of emerged individuals and was analysed per trap.

To sample the availability of nesting sites for bumblebees, nest searching bumblebee queens were used as a proxy for the availability of bumblebee nest sites in a habitat (Svensson *et al.* 2000; Kells & Goulson 2003). Observations of nest searching bumblebees were carried out the following spring in April 2010. The annual crops are grown in rotation and so were in different fields; therefore six fields of each of the arable crops were sampled on the same farms as the previous year as close as possible to the original field (Miscanthus fields stayed in the same location, **Appendix 2**). The same fields were sampled twice, using similar methodology to the transect walks. In each field, five 100x2m transects were walked, three along field margins (avoiding North facing margins) and two in the centre of the field (30m from any margin), recording nest searching bumblebee queens. Nest searching behaviour was defined as a low, zig-zag flight pattern along the ground, and bees were also recorded if they emerged from a potential nest site. The total number of nest searching bees recorded was analysed per transect.

Type specimens of insect species were verified by experts (see Acknowledgements), and deposited in Trinity College Dublin.

2.3.3 Data analyses

To assess the impact of the different crop types on pollinators, linear mixed effects models were used. For each group (bumblebees, syrphids, butterflies, solitary bees and floral units) and sampling method (pan traps, transect walks, trap nests and nest-searching observations) a separate model was created. Response variables were insect or floral unit abundance or species richness, and were either log (log (x + 1)) or square root transformed when the distribution of model residuals was not normal, as this gave a better fit than using Poisson distribution. For the pan trap and transect data, fixed effects were crop type and position in field (margin vs. centre), and for the trap nest and nest searching data only crop type was used. To account for issues of spatial and temporal independence, random terms were specified: field (1-50) and sampling period were used as random terms in transect and pan trap models, while trap and field were used for trap nest data. Nest searching data did not meet parametric assumptions and so abundance of nest searching bees per site was compared among crop types using a non-parametric Kruskall-Wallace test. All models were simplified by backwards selection, first removing non-significant interactions and then non-significant main effects. Models were validated by plotting standardised residuals vs. fitted values, normal qq-plots and histograms of residuals. Analyses were carried out using the lme function in the nlme package (Pinheiro *et al.* 2012) in R (R Development Core Team 2008). If p-values were significant, post-hoc Tukey all-pair comparisons were performed using the multcomp package (Hothorn *et al.* 2008).

Differences in community composition among crop types were examined using non-parametric multivariate analyses in Primer-E (Clarke & Gorley 2006). Pan trap data for each pollinator group were used, as this data set had the highest replication at the field level and was the most taxonomically resolved. Data were pooled at the field level and any fields with zero observations were removed, and were square-root transformed to down-weight the contributions of dominant species. A Bray Curtis similarity matrix was then constructed, and data were ordinated using non-metric multidimensional scaling (NMDS, Clarke 1993). Differences in community composition between crop types were tested using pair-wise permutational multivariate analysis of variance (PERMANOVA) tests, with crop type as a factor using Type III sums of squares based on 9999 permutations of residuals. The species driving the differences between crop types were determined using a SIMPER (Similarity Percentage) analysis (Clarke 1993).

2.4 Results

2610 insects were identified from the pan traps and 3512 from the transects, comprising of 11 butterfly, 43 hoverfly, 8 bumblebee and 23 solitary bee species (see **Appendix 3** & **Appendix 4**). Over 90 flowering plant species were also recorded during both sampling methods (**Appendix 5**). A total of 502 individuals emerged from the trap nests, of two bee (*Megachile versicolor* and *Hylaeus communis*, 10 individuals in total) and seven wasp species (492 individuals in total). The majority of wasps were from the sub-family Euminae, particularly *Symmorphus bifasciatus* and *Ancistrocerus trifasciatus* which commonly prey on Lepidopteran and beetle larvae (Yeo & Corbet 1983). During nest-searching observations, 156 nest searching bumblebees of seven species were recorded. The most commonly observed were *B. terrestris* and *B. lucorum agg.* which are distinguishable as queens. Numbers of honeybees observed in all methods were too low for further analyses.

When comparing Miscanthus on arable and oilseed rape with conventional wheat that they replace, the pan trap data showed that solitary bees were more abundant and species rich in both bioenergy crops than wheat, while abundance and species richness of hoverflies was higher in oilseed rape than Miscanthus (Table 2.1, Figure 2.2). Using the transect data, bumblebees were found to be more abundant in oilseed rape than in either Miscanthus or wheat (Table 2.2, Figure 2.2). There were no differences between crop types for butterflies (Table 2.2, Figure 2.2). Data collected during both types of sampling showed higher abundance and species richness of non-crop floral units in oilseed rape than wheat and, during pan trap sampling, in Miscanthus than wheat (Figure 2.2). There were no differences in the number or species richness of nest searching bees between energy crops and wheat (Kruskall Wallace test, df = 2: Abundance χ^2 =3.7, p =0.16, Species Richness χ^2=3.04, p=0.219, Figure 2.3). However, the abundance and species richness of trap nesting bees and wasps was significantly higher in Miscanthus than in oilseed rape (lme: abundance $F_{12,30} = 4.24$, p = 0.04, species richness $F_{12,30} = 3.64$, p = 0.051, Figure 2.3).

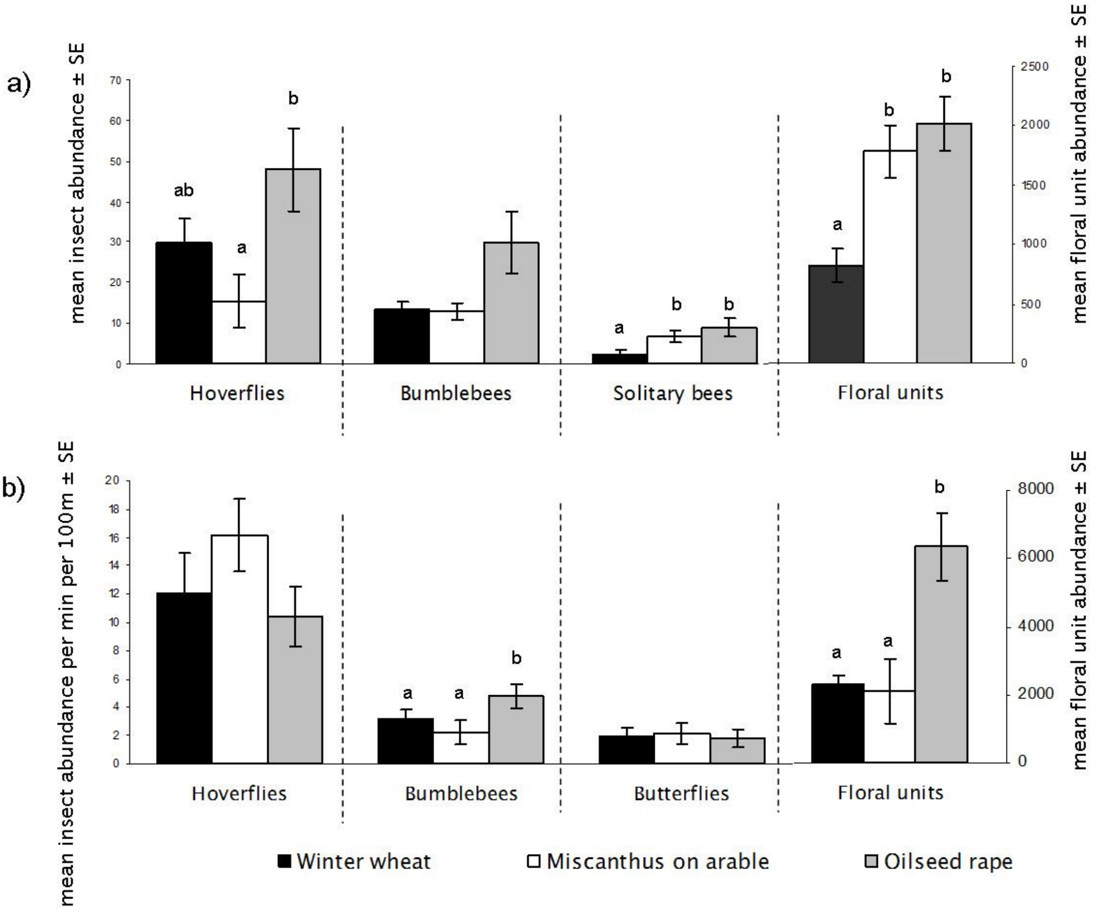

Figure 2.2. Mean abundance (± standard error) of the different pollinator groups, and non-crop floral units, per total sampling area in each field, sampled with pan trap (a, top) and transect (b, bottom) methods in the three arable crop types. Data were analysed separately for each taxon. Significant differences (p<0.05) are indicated by letters

Table 2.1. F-values of the linear mixed effects models applied on the pan trap data. (arable crops: oilseed rape, Miscanthus on arable and wheat; grass crops: Miscanthus on grass and grass silage). Number of fields per crop type = 10. Significant results are represented as follows: *p <0.05; **p < 0.01; ***p < 0.001. Non-significant first order interactions are not shown.

	Bumblebees	**Hoverflies**	**Solitary Bees**	**Floral units**
ARABLE				
Abundance				
Crop type	2.87	3.89*	5.29**	18.81***
Margin vs. Centre	6.26*	9.24**	5.94*	141.01***
Species Richness				
Crop type	2.56	3.89*	6.01*	8.58***
Margin vs. Centre	13.23**	9.24**	12.03*	66.03***
Crop type * Margin vs. Centre	-----	-----	-----	4.42*
GRASS				
Abundance				
Crop type	0.00	2.89	3.19	8.21**
Margin vs. Centre	8.27**	20.16**	4.39*	60.89***
Species Richness				
Crop type	1.75	1.16	2.73	9.08**
Margin vs. Centre	0.14	25.86***	4.31*	58.51***
Crop type * Margin vs. Centre	6.28*	-----	-----	-----

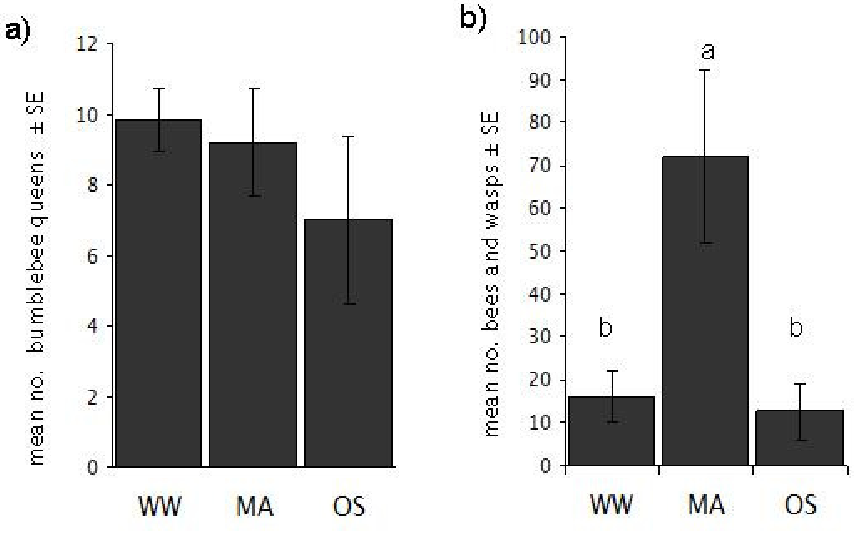

Figure 2.3. Mean (± standard error) abundance of nest searching bumblebee queens (a, left) and emerged trap nesting bees and wasps (b, right) per total sampling area in each field in the three arable crop types. Significant differences (p<0.05) are indicated by letters. WW = wheat, MA = Miscanthus on arable, OS = oilseed rape.

Table 2.2. F-values of the linear mixed effects models applied on the transect data (arable crops: oilseed rape, Miscanthus on arable and wheat; grass crops: Miscanthus on grass and grass silage). Number of fields per crop type = 5. Significant results are represented as follows: *p <0.05; **p < 0.01; ***p < 0.001. Non-significant first order interactions are not shown

	Bumblebees	**Hoverflies**	**Butterflies**	**Floral units**
ARABLE				
Abundance				
Crop type	4.58*	1.75	0.48	16.20***
Margin vs. Centre	17.81**	94.13***	36.49***	63.73***
Crop type * Margin vs. Centre	-----	-----	-----	11.30**
Species Richness				
Crop type	4.60	NA	0.42	11.01**
Margin vs. Centre	24.44***	NA	6.37*	87.23***
Crop type * Margin vs. Centre	-----	NA	3.79*	9.34**
GRASS				
Abundance				
Crop type	0.02	2.03	4.68*	1.07
Margin vs. Centre	5.29*	27.53***	27.26***	21.79**
Species Richness				
Crop type	0.02	NA	4.80*	4.01
Margin vs. Centre	9.47*	NA	18.00**	107.56***

PERMANOVA showed differences in community composition between oilseed rape, Miscanthus on arable and wheat for all taxa studied. Bumblebee and solitary bee communities differed between oilseed rape and Miscanthus, while hoverfly communities differed between all crop comparisons except Miscanthus and wheat (Table 2.3). Floral communities differed between all crop types (Table 2.3). SIMPER analyses showed that for bumblebees and hoverflies the differences in communities between crop types were not driven by the absence or presence of different taxa but different proportions of shared taxa (Figure 2.5 & **Appendix 4**). For the solitary bees, different species contributed to the dissimilarities between crop types, and only one species *Andrena bicolor* contributed to the similarities of all three arable crop types (Table 2.5). For the flowering plants, differences in communities between crop types were also driven by different species. Excluding woody hedgerow species, annuals were important in terms of defining similarities of plant communities within oilseed rape and wheat crops, whereas similarities between Miscanthus on arable fields were driven only by perennials (**Appendix 5**).

Table 2.3. T-values of PERMANOVA pair-wise tests for differences in communities among crop types. Arable crops: oilseed rape (OS), Miscanthus on arable (MA) and wheat (WW); Grass crops: Miscanthus on grass (MG) and grass silage (GS). Significant results are represented as follows: *p <0.05; **p < 0.01.

	Bumblebees	**Hoverflies**	**Solitary Bees**	**Floral units**
ARABLE				
MA – WW	0.87	0.99	1.05	2.13**
OS – WW	1.34	1.59**	0.87	2.64**
OS – MA	1.61*	1.67**	1.76**	2.95**
GRASS				
MG-GS	1.26	1.72**	0.66	1.59**

Table 2.4. Total abundances of all bumblebee species collected during pan trap sampling in each of the crop types, and results of SIMPER analysis. Species counts highlighted in grey represent those contributing to overall similarity within crop type. Arable crops: oilseed rape (OS), Miscanthus on arable (MA) and wheat (WW), Grass crops: Miscanthus on grass (MG) and grass silage (GS).

Bumblebee species	**WW**	**MA**	**OS**	**GS**	**MG**
Bombus bohemicus		3	1		
Bombus hortorum	26	25	103	28	45
Bombus jonellus	4		5		
Bombus lapidarius	7	3	30	2	3
Bombus muscuorum	1		1	1	
Bombus pascuorum	19	16	28	16	12
Bombus pratorum	23	20	39	16	31
Bombus sensu stricto	56	62	101	79	46
within group similarity (%)	69.3	61.3	63.36	58.95	65.63
between group dissimilarity (%)					
	WW-MA	34.74		GS-MG	38.47
	WW-OS	35.73			
	MA-OS	41.06			

Table 2.5. Total abundances of solitary bee species collected by crop type, and results of SIMPER analysis. Species counts highlighted in grey represent those contributing to overall similarity within crop type. Arable crops: oilseed rape (OS), Miscanthus on arable (MA) and wheat (WW); Grass crops: Miscanthus on grass (MG) and grass silage (GS).

	WW	MA	OS	GS	MG
Andrena angustior	**3**	20	4	6	11
Andrena bicolor	**5**	7	26	1	2
Andrena cineraria	1	5	8		
Andrena coitana		1	1	2	4
Andrena fucata	2	4	8	4	9
Andrena haemorrhoa	1	2	9	2	3
Andrena minutula		1			
Andrena nigroaenea	3		1		
Andrena scotica	1	2	3		1
Andrena semilaevis				1	
Andrena subopaca		1			
Colletes similis		1			
Halictus rubicundus	3	2	16	1	2
Hylaeus confusus	1	1	2	2	1
Lasioglossum albipes	1	7			5
Lasioglossum fratellum		1			
Lasioglossum leucopus		12			
Lasioglossum punctatissimum		1			
Lasioglossum villosulum			1	1	
Megachile versicolor	1				
Nomada fabriciana			1		
Nomada marshamella	**2**		7		1
Nomada ruficornis			1		
within group similarity (%)	4.97	28.92	25.68	7.37	14.1
between group dissimilarity (%)					
	WW-MA	83.61		GS-MG	83.81
	WW-OS	82.35			
	MA-OS	80.73			

Comparing Miscanthus planted on former grassland with conventional grass fields, butterflies were more abundant and species rich in Miscanthus fields than grass silage fields when sampled using the transect data, but there were no detectable patterns for any of the other insect taxa studied in either sampling method (Table 2.2, Figure 2.4). The abundance of floral units was also higher in Miscanthus than grass using data from the pan trap sampling (Figure 2.4). PERMANOVA showed few differences in community composition of insect groups between Miscanthus on grass and grass fields (Table 2.3); hoverflies were the only insect group found to differ between the two but as in the arable crop types the main differences were driven by different proportions of the same taxa

(**Appendix 4**). However, plant communities differed between the crops (Table 2.3), but in this case both crop types shared more perennials, with differences in crop types driven largely by *Lotus corniculatus* in the Miscanthus fields followed by different proportions of shared species (**Appendix 5**).

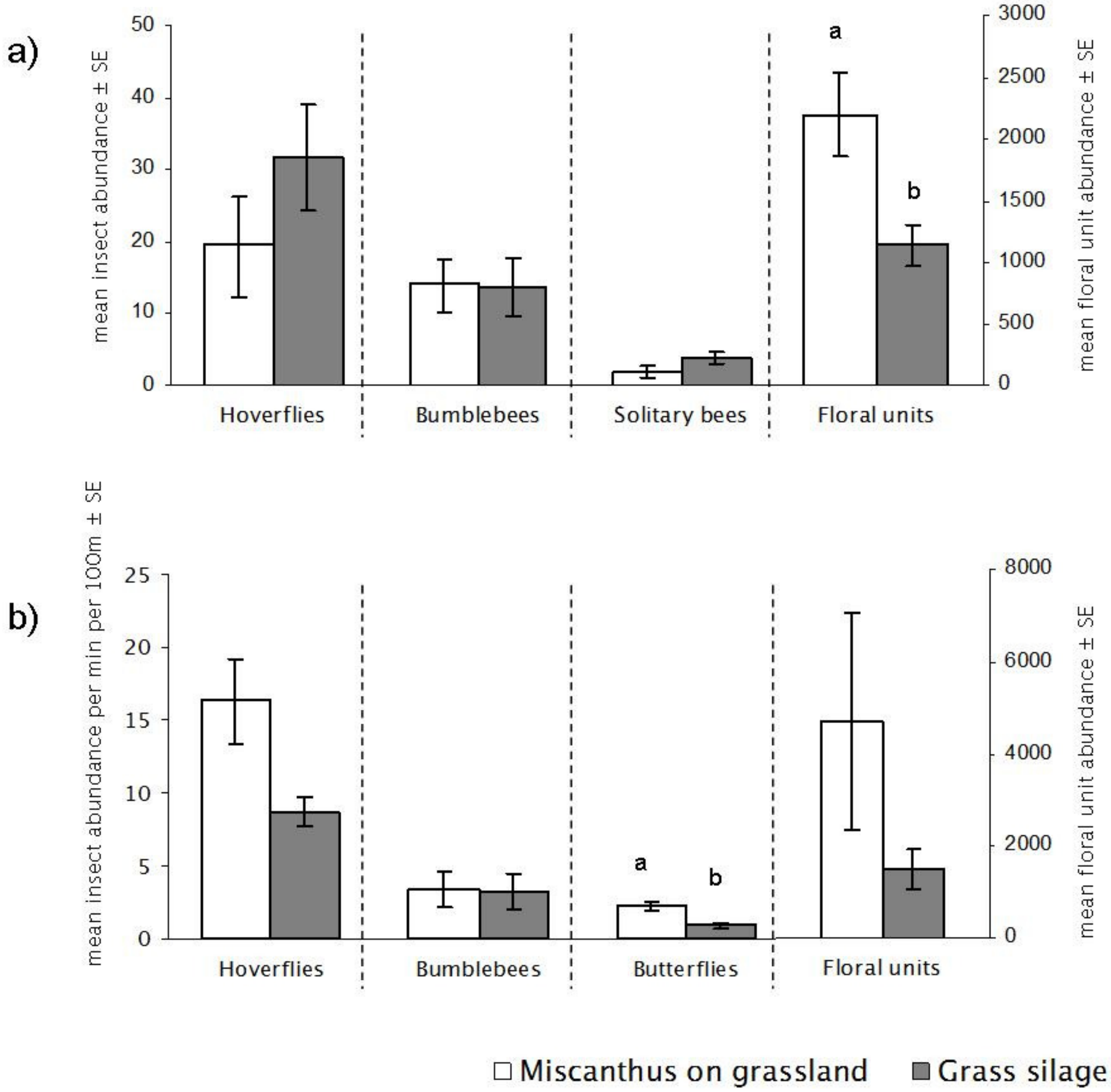

Figure 2.4. Mean abundance (± standard error) of the different pollinator groups per total sampling area in each field sampled with pan trap (a, top) and transect (b, bottom) methods in the two grass crop types. Data were analysed separately for each pollinator group. Significant differences ($p<0.05$) are indicated by letters.

There was consistently higher species richness and abundance of all insect groups studied in the field margins compared to the field centres (Table 2.1, Table 2.2, Figure 2.5) across all crop types. Species richness and abundance of non-crop floral units were also significantly higher in field margins compared to field centres, with a significant interaction between crop type and abundance in terms of floral units in arable fields using the pan trap data (Table 2.1), and abundance and species richness in arable fields using the transect data (Table 2.2). Differences between field margins and centres were particularly important for nest searching bumblebees (Figure 2.5), with 96% nest searching bees observed in field margins and not in the field centres.

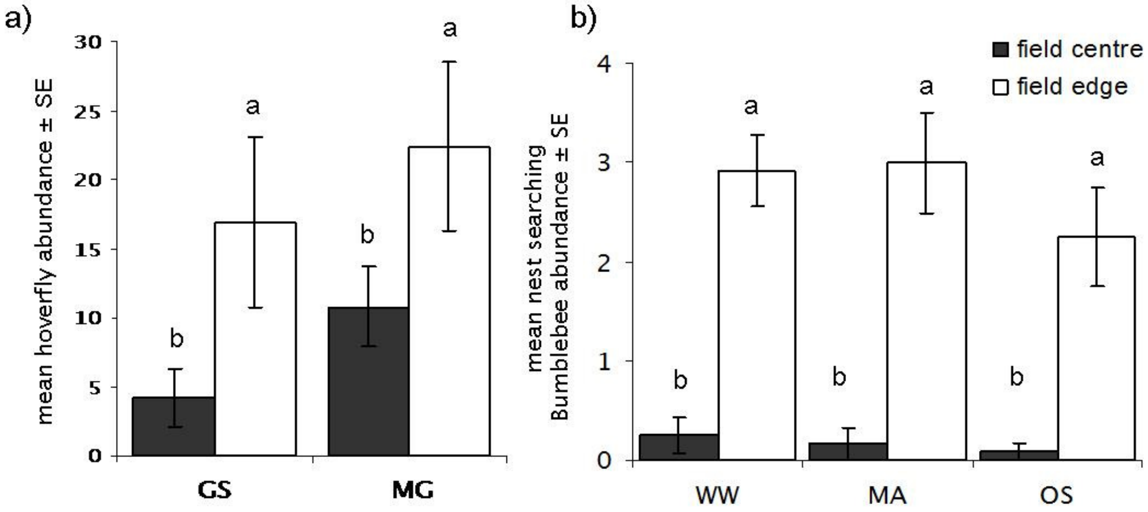

Figure 2.5. Mean abundance per total sampling area of a) of hoverflies using pan trap data and b) nest searching bumblebee queens in the edges and centres of the different crop fields. Significant differences ($p<0.05$) are indicated by letters. GS = grass silage, MG = Miscanthus on grass, WW = wheat, MA = Miscanthus on arable, OS = oilseed rape.

2.5 Discussion

Our findings suggest that in landscapes already dominated by agriculture, land use change from conventional to bioenergy crops at the field scale may not reduce pollinating insects in individual fields, but can result in increases in some pollinator groups using the fields, especially solitary bees. However, responses of pollinator communities varied according to the previous land use that was replaced (i.e. arable or grassland).

Replacing wheat with oilseed rape has implications for bees; the abundance and taxonomic richness of bumblebees and solitary bees were higher in oilseed rape than wheat. This could be explained by abundant nectar and pollen production by this crop, on which both groups of bees forage (Morandin & Winston 2005; Holzschuh *et al.* 2011), and by the non-crop floral resources which were also more abundant in oilseed rape fields. This also supports the increasing body of literature that mass flowering crops can have positive effects on bumblebees (e.g. Westphal *et al.* 2003; Morandin & Winston 2005), as they provide a short term pollen and nectar resource within the landscape early in the season. Positive impacts of mass flowering oilseed rape on solitary bees have been less well documented (Jauker *et al.* 2012b; Le Féon *et al.*) but are confirmed here. Both hoverflies and butterflies were frequently observed visiting oilseed rape, but neither group differed in species richness and abundance between oilseed rape and conventional wheat. Differential effects of land use change on hoverflies and butterflies compared with bees have also been shown in previous studies (Biesmeijer *et al.* 2006; Alanen *et al.* 2011; Power & Stout 2011), and may occur as neither group rely on floral resources during their larval phase. Therefore butterflies and hoverflies may be less influenced by a mass flowering resource and more so by other factors such as larval brood sites or host plants. However, oilseed rape has intensive annual production with high inputs of agrochemicals (**Appendix 6**), especially pesticides, and so has the potential to have knock-on harmful effects on insect pollinators and the other resources they need. Due to negative effects of seed coating pesticides observed for honeybees and bumblebees (Henry *et al.* 2012; Whitehorn *et al.* 2012) and impacts of oilseed rape on pollination services to wild plant species (Diekotter *et al.* 2010; Holzschuh *et al.* 2011), large-scale (farm or landscape) production of this crop could have indirect effects on pollinators and the resources they need, especially over longer timescales. However, appropriate management of pesticide application, alternative forage and nesting resources for pollinators could augment the positive impacts of oilseed rape.

Replacing wheat with Miscanthus could have implications for solitary bees and wasps; I found more solitary bees in Miscanthus fields than in wheat, and more trap nesting bees and wasps in Miscanthus than either oilseed rape or wheat. This may be linked to higher floral abundance in Miscanthus fields compared with wheat, or possibly because Miscanthus provides better nesting opportunities due to its low-disturbance, perennial nature and potential nest sites offered by dead stems left in the field after harvesting. Since many of the wasps found colonising the trap nests were larval predators, Miscanthus could perhaps be a reservoir for biocontrol agents. Solitary bees have shorter dispersal distances (Gathmann & Tscharntke 2002) and are thus more likely to be affected by changes at the field scale than the other more mobile groups, like the bumblebees. Since perennial flowering plants have been found to be important for supporting bumblebees (Fussell & Corbet 1992; Carvell *et al.* 2007), it is perhaps surprising that bumblebees were not more abundant in Miscanthus. I also found no differences in the number of nest-searching bumblebees between crop types, suggesting Miscanthus does not provide better nesting resources for bumblebees than annual crops. As bumblebees are highly mobile organisms with large foraging ranges (Knight *et al.* 2005), field-scale production of Miscanthus may be unlikely to affect them. In a similar way, hoverflies have been found to respond to landscape factors over local ones (Werling *et al.* 2011). As the bioenergy sector expands, and Miscanthus is planted more frequently in the wider landscape, there may be different landscape scale impacts (Westphal *et al.* 2003; Dauber *et al.* 2010), particularly on species with larger foraging ranges (Steffan-Dewenter *et al.* 2002).

Replacing conventional grassland with Miscanthus seems to have fewer implications for flower visiting insects. Both crops supported communities dominated by perennial plant species and so perhaps both provide similar levels of forage and nesting resources for pollinators. Butterflies were the only group to differ in abundance and species richness between these two crop types, which could be driven by differences in larval host plants.

Although the abundance and species richness of bumblebees was higher in oilseed rape than wheat, novel communities were not found. Only eight of the 20 Irish bumblebee species were recorded, including five common and widespread species (Fitzpatrick *et al.*

2006). Conversely, solitary bee communities were composed of different species in the different crop types, with six species found only in Miscanthus. Le Féon et al. (in press) also found more rare species in non-oilseed rape fields. Together, our studies suggest that a variety of crops within the agricultural landscape could help maintain diversity of solitary bee assemblages. Hoverfly assemblages reflected the same pattern as bumblebees: the same common species were present in different proportions in the different crop types, similar to previous studies in organic and conventional grasslands (Power 2011). As hoverflies do not rely on floral resources for their whole lifecycle, all of the crops studied and their associated margins may provide sufficient resources for the same common species.

The importance of field margins for pollinators, and biodiversity has been well documented (Marshall & Moonen 2002; Power & Stout 2011), and in our study all pollinator groups and floral resources were consistently more abundant and species rich in the field margins (with adjacent hedgerows) than in the centres. Field margins in Ireland seem to be exceptionally rich in terms of floral resources in comparison to field margins in the UK (Carvell *et al.* 2007), and high quality hedgerows may also buffer some of the effects of changes in crop type within a landscape. Although floral characteristics can be used as predictors for bee community structure, the diversity and availability of nesting sites is also important (Potts *et al.* 2005). I confirm that field margins are also important nest sites for bumblebees and possibly the only ones left in intensive farmland (Svensson *et al.* 2000), as the majority of queen bumblebees were searching in the margins. Field margins thus provide not only forage for pollinators, but also nesting and larval habitats, and out results support agri-environment schemes that conserve or supplement field margins (Carvell *et al.* 2007).

Finally, for monitoring future impacts of bioenergy crops, the choice of sampling method for pollinating insects is important as different methods can yield contrasting results (Westphal 2008; Nielsen *et al.* 2011). As in previous studies (Westphal 2008; Nielsen *et al.* 2011), we confirm that pan traps are more useful for sampling solitary bees and transect walks are useful for butterflies and solitary bees (Chapter 7, **Appendix 3**). However, it is important to note that pan trapping is a passive sampling method which, although it allows simultaneous coverage of a large amount of sites and laboratory identification of specimens, may be influenced by the surrounding floral communities with traps in florally sparse areas more attractive to insects. In addition, nest searching observations are a proxy for the availability of nesting resources in an area (Svensson *et al.* 2000; Kells & Goulson 2003); although more bees are seen to search for nests in an area this may not mean more eventually nest there, although physically locating bumblebee nests is not practical (Waters *et al.* 2011a)

2.5.1 Conclusion

Second generation bioenergy crops such as Miscanthus have been suggested as one of the most promising biofuels as they are high yielding, do not compete directly for use with food, require low inputs in terms of annual cultivation and agrochemicals and don't involve the destruction of native forests (Valentine *et al.* 2012). Here I also show no effect of growing either Miscanthus or oilseed rape on some pollinator groups studied in comparison to conventional crops, and in some cases positive impacts on less mobile solitary bee species, bumblebees, butterflies and trap-nesting bees and wasps. These results support others (e.g. Gardiner *et al.* 2010; Gevers *et al.* 2011) which showed differential responses among taxa to the growth of bioenergy crops, and I show that the effects on species vary according to the type of crop the bioenergy crops replace. I confirm the importance of field margins as floral and nesting habitat for pollinator species, and can suggest conservation of these features. Since the largest effects of field scale growth of bioenergy crops in the landscape were on the less mobile solitary bee species, occasional replacement of arable fields with bioenergy crops may enhance the numbers of solitary bee species in the landscape, driven by the increased floral resources, different floral communities and/or better nesting resources. In this study, I assumed that bioenergy crops would replace existing agricultural crops, as this is the most usual case in the study area, but it is important to note that impacts of conversion of natural, semi-natural or marginal habitats may not be the same (Rowe *et al.* 2009). Wider scale replacement of existing crop types in the future may have unpredictable consequences for both pollinator populations and consequent delivery of pollination services.

Chapter 3

Plant-pollinator network structure is not affected by a mass flowering event, but by changes in crop type and composition of surrounding landscape

To be submitted as: Stanley, D.A., Bourke, D. & Stout, J.C. Plant-pollinator network structure is not affected by a mass flowering event, but by changes in crop type and composition of surrounding landscape. *Oikos*

3 Plant-pollinator network structure is not affected by a mass flowering event, but by changes in crop type and composition of surrounding landscape

The landscape data used in this Chapter were collected by David Bourke and Evelyn Flynn.

3.1 Abstract

Plant-pollinator systems are largely generalised, with most pollinators visiting a variety of plants and most plants being visited by a variety of pollinators. Plant-pollinator interaction networks can be used to study these complex interactions that deliver the service of pollination. However, little is known about how networks respond to a mass flowering resource throughout the season, or how they are affected by landscape context. Here, I used a network of 25 sites of bioenergy and conventional crops to investigate the effects of i) mass flowering oilseed rape on network structure during and after flowering, ii) bioenergy crop production on networks and iii) both local and landscape context on networks. Although there were large differences in availability of flowers during and after oilseed rape flowering, little effect is seen on network structure suggesting re-wiring and generalised adaptive foragers buffer the impacts of this crop in agricultural regions. Replacement of arable land with bioenergy crops had greater impact on network structure than replacement of grassland, with interaction evenness and connectance being affected. However, overall landscape context (particularly hedgerow length and diversity of habitats) also affected properties of a network including number of interactions, generality and interaction evenness. As both bioenergy production and landscape context can affect network structure, they should be taken into account in the conservation of species interactions and the service of pollination.

3.2 Introduction

Biotic pollination is a key ecosystem process essential for sexual reproduction in the majority of angiosperms, as well as for the production of a third of the world's food crops (Klein *et al.* 2007; Ollerton *et al.* 2011b). Pollinators rarely visit a single plant species exclusively, and the majority of plant-pollinator interactions appear to be generalised (Waser *et al.* 1996). Efforts to conserve a single group of species, such as pollinating bees, ignores the fact that these species interact with other species, and that their persistence may depend on the occurrence of other taxa (Tylianakis *et al.* 2010). Therefore the conservation of these interactions, as well as their component species, is being increasingly recognised as important (Kearns *et al.* 1998), especially as they result in fundamental ecosystem services such as pollination. Recent studies have used food web approaches to look at plant-pollinator interactions, allowing ecological investigation at a community scale using network analyses (Jordano 1987; Memmott 1999), and it has been shown that interactions are not random but structured, and networks are more than the sum of their component parts (Tylianakis *et al.* 2010).

Plant-pollinator networks have become widely studied and some common themes are emerging. New indices to describe these networks have been developed (e.g. Bersier *et al.* 2002; Almeida-Neto *et al.* 2008); common properties have been identified, including the tendency for nestedness (Bascompte *et al.* 2003), low connectance (Olesen & Jordano 2002) and asymmetry (Vazquez & Aizen 2004); extinctions have been simulated at a community level (Memmott *et al.* 2004; Kaiser-Bunbury *et al.* 2010); invasive species can become well integrated (e.g. Olesen *et al.* 2002; Lopezaraiza-Mikel *et al.* 2007; Vila *et al.* 2009), without necessarily causing changes in network structure (Vila *et al.* 2009); networks have been used to examine success of restoration projects (Forup *et al.* 2008; Kaiser-Bunbury *et al.* 2009); and changes in network structure have been found with habitat modification when no differences in conventional species measures are seen (Tylianakis *et al.* 2007).

However, pollinators are organisms with relatively large foraging and dispersal distances (e.g. Gathmann & Tscharntke 2002; Knight *et al.* 2005). Therefore, in addition to responding to local factors such as the availability of floral and nesting resources and response to local field scale changes, they also respond to changes at larger landscape levels (Westphal *et al.* 2003; Rundlof *et al.* 2008a; Gabriel *et al.* 2010) due to their ability to move freely over wider areas. Effects of landscape composition have been widely studied in pollination literature, but effects of landscape configuration have not been extensively investigated (Hadley & Betts 2012). Previous work has shown that different pollinator taxa respond differently to changes in landscape composition (Sjödin *et al.* 2008; Jauker *et al.* 2009; Alanen *et al.* 2011) at different spatial scales (Steffan-Dewenter *et al.* 2002; probably due to differences in foraging ranges); bee and hoverfly diversity in agri-environmental schemes can be affected by surrounding landscape composition (Holzschuh *et al.* 2007; Rundlof *et al.* 2008b; Haenke *et al.* 2009); heterogeneous landscapes can benefit pollinator populations (Rundlof *et al.* 2008b; Oliver *et al.* 2010) and pollination services decrease with increasing distance from semi-natural habitat (Ricketts *et al.* 2008). Since pollinator richness and abundance, and pollination services, change with land use at a landscape scale, plant-pollinator networks may also be affected, but this has not yet been studied (Burkle & Alarcon 2011; but see Kaartinen & Roslin 2011; Ferreira *et al.* in press).

Networks often require intensive sampling (Hegland *et al.* 2010; Chacoff *et al.* 2012), and therefore there is often a trade off between network quality and the number of networks studied for robust statistical comparison (Kaiser-Bunbury *et al.* 2010). Thus, the majority of studies have been descriptive or have compared small numbers of networks (e.g. Olesen *et al.* 2002; Dupont *et al.* 2003; Forup *et al.* 2008; Kaiser-Bunbury *et al.* 2009). Fewer studies have looked at the impacts of habitat modification or land use change on plant-pollinator networks as larger numbers of networks are required for such a scale (but see Tylianakis *et al.* 2007; Forup *et al.* 2008; Hagen & Kraemer 2010; Power & Stout 2011), and fewer again have looked at the effects of landscape composition on networks. For example, more complex landscapes may provide more habitats for pollinators and plants; therefore generality and vulnerability network parameters may change, or connectance may decrease, as there is more choice for pollinators and plants within the landscape in terms of their mutualistic partners. However, the impacts of landscape composition and configuration on ecological processes, and more specifically plant-pollinator networks, are not well understood (Hadley & Betts 2012; Tscharntke *et al.* 2012).

As well as being affected at the landscape scale, pollinators are also influenced by local variation in land use at the field scale, such as occurs with the cultivation of mass flowering crops, which provide large numbers of flowers for pollinators to forage on. Although mass flowering invasive species have been shown to become integrated into native plant-pollinator networks (e.g. Vila *et al.* 2009) this is not yet known for mass flowering crops, and changes in network structure within a season have not been well studied (Burkle & Alarcon 2011). Oilseed rape (*Brassica napus* L.) is a mass flowering crop extensively studied in its impacts on pollinators (e.g. Westphal *et al.* 2003), and on pollination services to native species (Cussans *et al.* 2010; Diekotter *et al.* 2010). Crops are planted in agricultural areas which are typically less species rich than semi-natural ones, which have been the focus of invasive species studies, and networks from agricultural regions tend to be small (Power & Stout 2011); therefore impacts may not be the same. Oilseed rape flowers early on in the season providing a short pulse of resources for pollinating insects. This also raises the question as to what happens to networks after the flowering of the crop. After the flowering of a mass flowering resource you may expect measures such as generality (the mean number of plants per pollinator), vulnerability (mean number of pollinators per plant) or measures of specialisation (e.g. H2) to change, as pollinators switch from a super-abundant resource to a wider variety of different, less common species. Changes in number of interactions or insect abundance may also be expected as insects move away in search for alternative forage.

Oilseed rape has been grown in Europe for centuries but its frequency and distribution are increasing due to its use as an oil crop for bioenergy and recent bioenergy incentives. Another bioenergy crop, Miscanthus (a low input, perennial, non-biotically pollinated crop Clifton-Brown *et al.* 2001; Boehmel *et al.* 2008), has also increased in area planted in Ireland over the last 6 years. These bioenergy crops can cause changes in species richness and abundance of different pollinator groups in fields (Chapter 2) and could have knock-on impacts on interaction networks which may have been previously overlooked; and these field scale effects may be dependent on surrounding landscape context. I constructed plant-pollinator networks from mass flowering oilseed rape fields, but also from a variety of different crop types representing both bioenergy crops and the conventional crops they replace in the landscape. I used networks constructed from 25 agricultural fields to investigate the local effects of crop type in the field, and the composition of the surrounding landscape (1km^2) to examine the importance of landscape context. Specifically I asked the following questions:

1) As a mass flowering crop, are there differences in network structure when oilseed rape is flowering compared to when it is finished? Does oilseed rape become well integrated into networks?
2) Are there differences in plant-pollinator network indices at the local scale in fields of bioenergy crops Miscanthus and oilseed rape compared to previously planted crop types?
3) Are plant-pollinator networks affected at the local (by crop type) or landscape level (by the composition of the landscape surrounding the fields)?

Plate 9. Oilseed rape during (left), end (middle) and after (right) flowering (photos: DS)

3.3 Methods

3.3.1 *Field site selection*

This study was conducted in 2009 in South-East Ireland, where arable crops are interspersed with both beef and dairy farming, resulting in a mosaic of different land use types driven by a gradient in the amount of arable land to the amount of grassland in the surrounding landscape. I sampled a total of 25 fields, comprising five replicates of five crop types randomly interspersed within the study area (Figure 3.1). These crops were the bioenergy crops winter oilseed rape (OS), Miscanthus planted on former arable (MA), Miscanthus planted on former grassland (MG); and the conventional crops winter wheat (WW) and grass planted for silage (GS). Winter wheat was chosen as a control for conventional land use as it is commonly used in rotation with winter oilseed rape and is a common arable crop also replaced by Miscanthus. As a control for Miscanthus on grass I chose grass silage, as intensively grazed dairy farms are unlikely to convert to Miscanthus production. Miscanthus has been planted commercially in Ireland since 2006, and so all fields chosen were 2-3 years old and reaching maturity. Based on the current knowledge of bumblebee foraging ranges (Knight *et al.* 2005), all study fields were a minimum of 1km apart and 1km from any other mass flowering crop to prevent pollinator sharing between fields. Fields were chosen to minimise within crop type variation where possible, with similar sizes, number of hedgerows and altitude, and only one field was selected per farm to ensure independence of management.

3.3.2 *Transect sampling*

To construct plant-pollinator networks each field was sampled three times throughout the season, once during each of three sampling periods (6 – 29 May, 19 June – 7 July and 28 July – 19 August). On each visit four 100 x 2m standard belt transects were walked by a single observer at a slow steady pace (0.07-0.44ms^{-1}) (Pollard & Yates 1993). Two transects were walked in the margins along hedgerows, and two in the centre of each field (>30m from margins). Surveys were limited to taxa associated with pollination, specifically bees (Apidae), butterflies (Lepidoptera) and hoverflies (Diptera: Syrphidae), and took place between 09:00 – 18:50h in dry, bright, warm conditions (mean temperature 18˚C). All interactions between insects and flowers were recorded on each transect, as well as total abundances of insects (interacting and non-interacting). Insects and flowers were mostly identified in the field, or collected and identified in the lab. Flowers, bumblebees (*Bombus* spp.) and butterflies were identified to species level (except for the butterflies from the genus Pieris that were grouped, and bumblebees from the Bombus sensu stricto group which are indistinguishable morphologically as workers: Wolf *et al.* 2010; Carolan *et al.* 2012), while solitary bees and syrphids were identified to genus (the syrphid genera *Platycheirus* and *Melanostoma* were grouped for field identification as they are very similar when seen visiting flowers). Oilseed rape was in full flowering in the first sampling period, ending flowering in the second and over by the third. Total abundance and species richness of flowers (defined as floral units; either individual flowers or compact umbels that a medium sized bee could walk between, Dicks *et al.* 2002) were also recorded on each visit to each field by counting and identifying all floral units in 1m x 1m quadrats placed along the transect. Quadrats were placed on both sides of the transect every 10m, resulting in a total of 20 quadrats per transect.

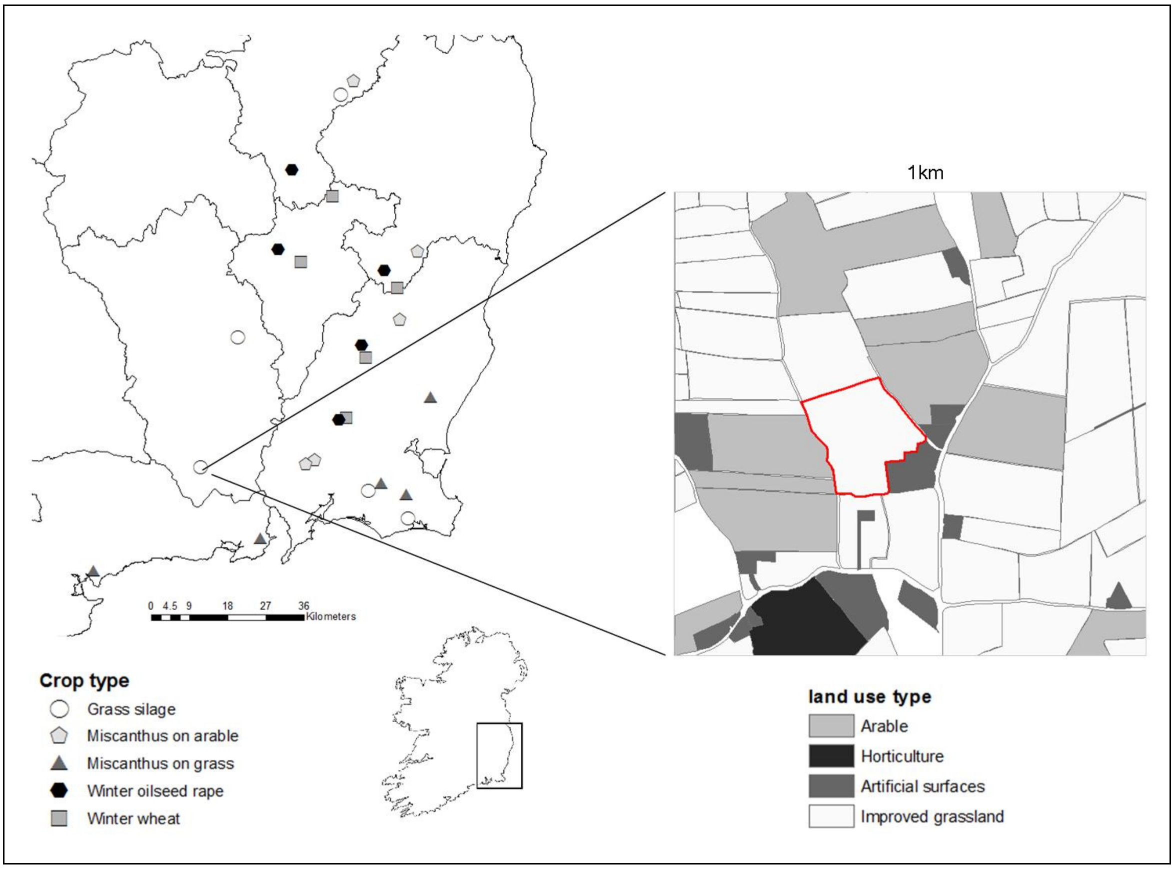

Figure 3.1. Locations of the 25 fields studied, and an example of a landscape that was mapped around each in a 1km x 1km grid. The study field is highlighted in red.

3.3.3 Landscape characterisation

Land use surrounding each field was quantified in a 1km grid with the survey field at the centre (Figure 3.1). All land cover types within this area were categorised by interviewing farmers and ground truthing, and the area and proportion of each calculated. Land cover types were classified according to the classification of habitats in Ireland (Fossitt 2000), and grouped into main habitat types including arable land, intensive grassland, semi-natural land, artificial surfaces and woodland (including a variety of different woodland types including plantations and more natural areas, Table 3.1). Hedgerow length was also measured, and Shannon diversity of all habitats calculated (using all habitat types classified to Fossitt level). All landscape analyses were carried out in ESRI ArcGIS 9.3 or using the ArcGIS extension Patch Analyst v4.3. If any landscape variables were highly correlated, only one was included in subsequent analyses and all variance inflation factor (VIF) values were under 4 (Zuur *et al.* 2010). Therefore landscape variables included in analyses were as follows: proportion of arable land (which correlated negatively with proportion of grass land), proportion of woodland, proportion of artificial land, diversity of habitats (correlated positively with proportion of semi-natural habitats) and length of hedgerows (Table 3.1).

Table 3.1. Summary of landscapes variables calculated surrounding the 25 fields

	Mean percentage ± SE (min – max)
Intensive grassland^	40 ± 8 (6-86)
Arable land	45 ± 9 (0-83)
Woodland#	3 ± 1 (0-15)
Semi-natural land*	3.5 ± 1 (0-16)
Artificial surfaces$	4 ± 1 (1-9)
Diversity of habitats (Shannon)	1.03 ± 0.06 (0.52-1.65)
	Mean length (km) ± SE (min – max)
Hedgerow length	9.5 ± 2 (3-18)

^ included both improved and semi-improved grassland categories

included mixed broadleaved woodland, mixed broadleaved/conifer woodland, mixed conifer woodland and conifer plantation

*semi-natural land included areas likely to provide good semi-natural habitat for pollinators; agri-environmental LINNET, wet grassland, scrub, marsh, and small areas of oak-ash hazel woodland and wet willow-alder-ash woodland that included extensive flower rich margins

$ artificial surfaces include farmyards, houses, gardens and urban areas

3.3.4 Data analyses

A quantitative plant-pollinator interaction matrix was produced for each field with the total number of visits observed for each plant-pollinator interaction in all sampling periods pooled, resulting in five matrices per crop type. To investigate differences in network structure during and after oilseed rape flowering, matrices were also produced for each oilseed rape field in each sampling period. For each matrix I then created a bipartite interaction network. As quantitative metrics can reveal patterns not seen in their qualitative counterparts (Bersier *et al.* 2002; Tylianakis *et al.* 2007) and as some network metrics are more sensitive to sampling artefacts than others (Dormann *et al.* 2009; Rivera-Hutinel *et al.* 2012), I calculated a variety of indices including the qualitative indices number of plant species, number of pollinator species, network size, connectance and nestedness and also the quantitative indices total number of interactions, generality, H2, interaction evenness, linkage density, species asymmetry, vulnerability and web asymmetry (Appendix 7, Dormann *et al.* 2008; for definitions of the different indices and calculations used in bipartite see Dormann *et al.* 2009). Network indices, and total insect and flower abundance and species richness (including all interacting and non-interacting individuals), were then modelled as individual response variables.

Analyses took 3 steps; firstly I investigated differences in the structure of networks in oilseed rape fields during and after flowering of the crop by comparing response variables between the three sampling periods. I accounted for autocorrelation between sampling periods within fields by using general least squares (GLS) models with time period as a random factor in the nlme package in R (Pinheiro *et al.* 2012). Secondly, I tested for differences in network structure between bioenergy crop fields and the conventional fields they replace; oilseed rape and Miscanthus on arable fields were compared with conventional wheat (arable crops) and Miscanthus and grass fields were compared with grass silage (grass crops). Lastly, I investigated the influences of local (crop type) and landscape variables on network structure in all 25 sites. For the bioenergy and conventional crop comparisons, and the local and landscape models, general linear models (GLMs) were used and fitted with Poisson error structure for any response variables that were counts, correcting for overdispersion if necessary. To account for differences in network structure found only due to network dimensions (Bengtsson 1994; Dormann *et al.* 2009), network size was included as a covariate for all network indices (except for total number of insects and flowers). Models were simplified by using backwards selection, removing non-significant effects until all variables in the model were significant. All variables were first checked for outliers, normality and homogeneity of variance, and models were validated by plotting standardised residuals against fitted values. Total plant abundance was log transformed. All analyses were conducted in R (R Development Core Team 2008), and networks were constructed and indices calculated using the bipartite package (Dormann *et al.* 2008).

3.4 Results

A total of 1700 interactions were observed, between 64 flowering plants and 23 flower visitor species groups. The flower visitors recorded included bumblebees (*Bombus* sp. 5 species, 464 interactions), hoverflies (Syrphidae, 9 genera, 1094 interactions), the honeybee (*Apis mellifera*, 73 interactions), butterflies (Lepidoptera, 7 species, 48 interactions) and solitary bees (other Apidae, 21 interactions). The most commonly visited plant species across all crop types was *Rubus fruticosus*, followed by *Senecio vulgaris*, *Heracleum sphondyllium*, *Ranunculus repens*, *Cirsium vulgare*, *Cirsium arvense* and *Brassica napus*. As the networks were constructed in a temperate agricultural zone (and as some insects were only recorded to genus) the individual networks were small with 7-30 species in each, and 15-189 interactions. This is similar in size to other temperate networks (e.g. Aizen *et al.* 2008b; Power & Stout 2011), but much smaller than highly resolved tropical webs (e.g. Kaiser-Bunbury *et al.* 2009)

3.4.1 Mass flowering oilseed rape

Oilseed rape, as a mass flowering crop, became well integrated into the networks (Figure 3.2). It was the most visited plant species in the oilseed rape fields resulting in 113 interactions (27%), followed by *Rubus fruticosus* which was involved in 68 interactions (17%). Oilseed rape was visited by 12 of the 24 pollinator groups (50%), including all bumblebee species (59 interactions; in descending order *Bombus sensu stricto*, *B. lapidarius*, *B. pratorum*, *B. pascuorum*, *B. hortorum*), honeybees (31 interactions), solitary bees (4 interactions), hoverflies (18 interactions) and one butterfly species (*Vanessa carduii,* 1 interaction). However, no insects were found to be specialists on oilseed rape only. When in flower early in the season, oilseed rape was very dominant in the networks comprising 70% of interactions in round 1, and 25% in round 2, but when flowering finished many insects switched to other plant species such as *Rubus fruticosus*. Although there were large differences in the number and species richness of available flowers between sampling periods (largely due to the flowering of mass flowering oilseed rape in the first period, Figure 3.3), there was not a significant change in insect abundance or species richness. Despite the radical changes in floral abundance between all periods, there were no significant changes in the majority of network indices (Table 3.2). Connectance was negatively, and generality and linkage density positively, related to network size (Table 3.2).

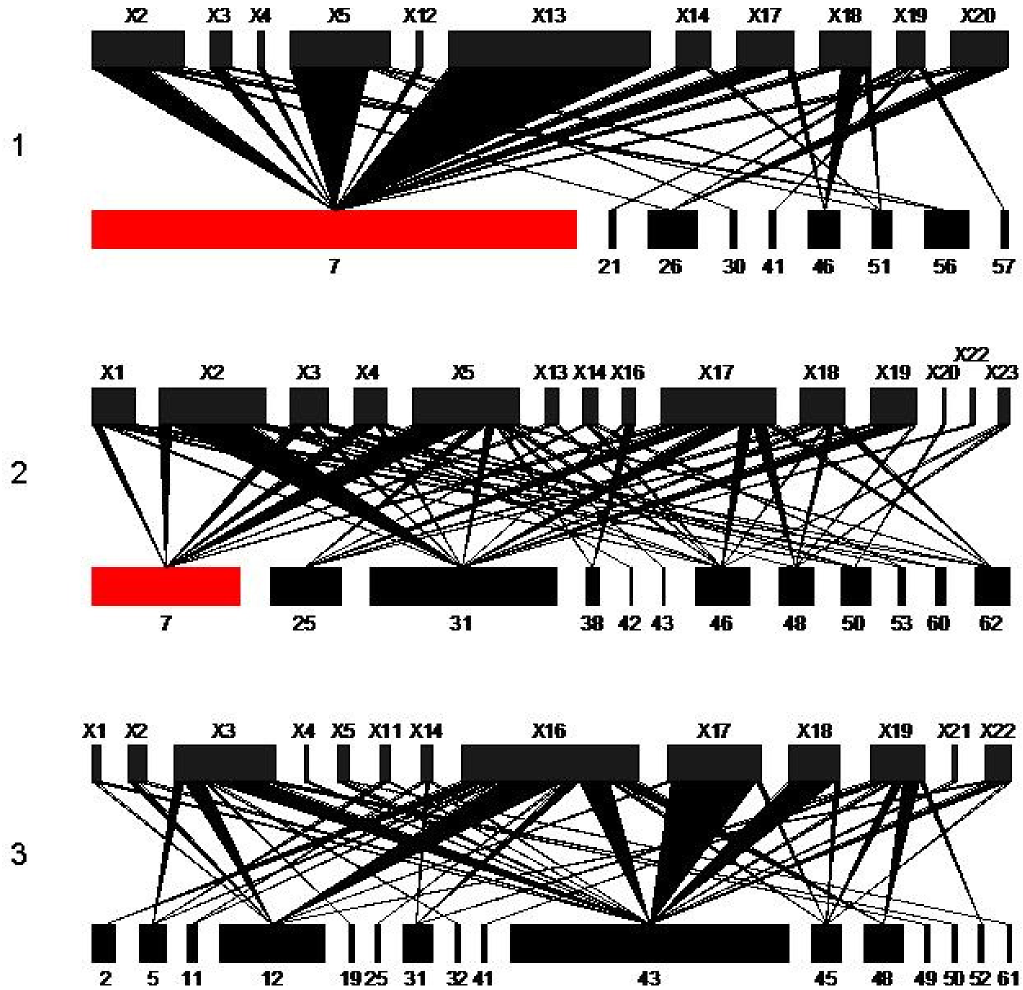

Figure 3.2. Quantitative plant-pollinator networks from the area sampled in oilseed rape fields, in each of three sampling periods (period 1 = 6 – 29 May, period 2= 19 June – 7 July, period 3 = 28 July – 19 August). Oilseed rape was in full flower in period 1, end flowering in period 2 and finished flowering in period 3, and is highlighted in red. To summarise, networks show data pooled across all five replicate fields for each sampling period, although analyses were carried out on a per field basis. Species codes are given in **Appendix 8**.

Table 3.2. F-values from comparison of species richness, abundance and network measures between three sampling periods using linear models, 1) when oilseed rape was in flower, 2) at the end of flowering and 3) after flowering in five fields. Network size was used as a covariate and is included where it has a significant effect. Significant results are represented as follows: *p <0.05; **p < 0.01; ***p < 0.001

	period	**network size**
Insect abundance (total)	ns	---
Insect species richness (total)	ns	---
Floral abundance (total)	9.35**	---
Plant species richness (total)	4.15*	---
Network size	ns	---
Connectance	ns	18.18**
Generality	ns	5.52*
H2	ns	ns
Interaction evenness	ns	ns
linkage density	ns	4.87*
Nestedness	ns	ns
Number of interactions	ns	ns
Specialisation asymmetry	ns	ns
Vulnerability	ns	ns
Web asymmetry	ns	ns

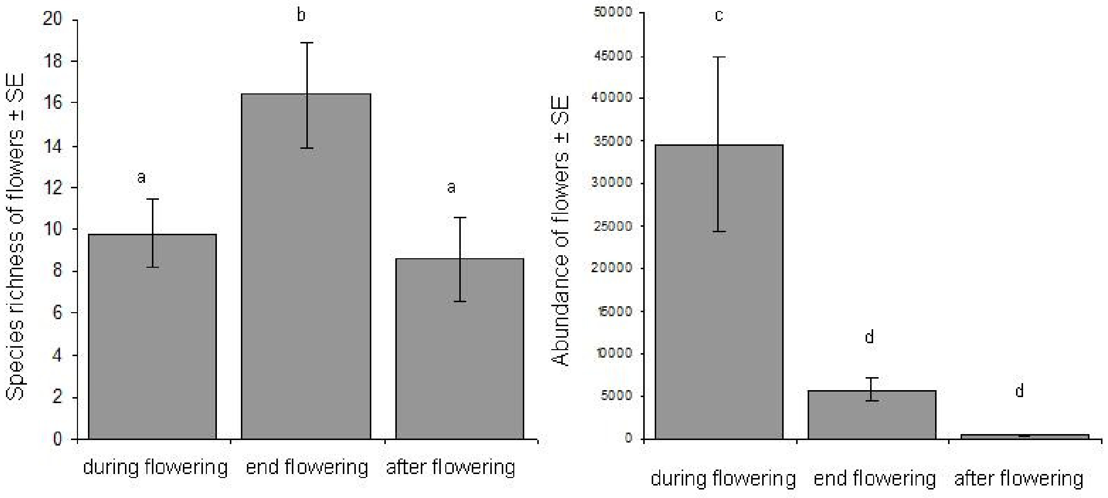

Figure 3.3. Mean species richness and abundance of floral units (measured in two 100x2 m transects per field) in oilseed rape fields during, at the end of, and after oilseed rape flowering. Significant differences (p<0.05) are represented by letters.

3.4.2 Local impacts (crop type)

Comparing the bioenergy crops oilseed rape and Miscanthus on former arable land with the conventional wheat they replace, there was higher floral abundance in oilseed rape than either wheat or Miscanthus, and more plant species and bigger networks in both energy crops than in conventional wheat (Table 3.3, **Appendix 9**). Interaction evenness was significantly higher in oilseed rape than wheat, while connectance was higher in wheat than Miscanthus (Figure 3.4). There were no differences in any of the other network indices between crop types. Number of interactions and generality were positively, and nestedness negatively, related to network size.

Comparing Miscanthus on former grassland with the conventional grass it replaces, there were no differences in any network indices between crop types, but there was a non-significant trend towards higher floral abundance in Miscanthus than grass fields. Generality and number of interactions were positively, and connectance and web asymmetry negatively, related to network size (Table 3.3).

Table 3.3. F-values from separate general linear models comparing network characters among arable crop types (Miscanthus on arable land, oilseed rape and wheat, 15 fields) and grass crop types (Miscanthus on grassland and grass silage, 10 fields), with network size as a co-variate. Significant results are represented as follows: *p<0.05; **p< 0.01; ***p< 0.001

	crop type		**network size**	
	arable	grass	arable	grass
Insect abundance (total)	-	-		
Insect species richness (total)	-	-		
Number of flowering plants (in network)	8.57**	-		
Number of pollinators (in network)	-	-		
Floral abundance (total)	38.34***	-		
network size	7.40**	-		
Connectance	9.95**	-	-	5.22*
Generality	-	-	7.72*	15.60**
H2	-	-	-	-
Interaction evenness	9.51**	-	-	-
Linkage density	-	-	-	-
Nestedness	-	-	6.15*	-
Number of interactions	-	-	15.78**	14.25**
Specialisation asymmetry	-	-	-	-
Vulnerability	-	-	-	-
Web asymmetry	-	-	-	-

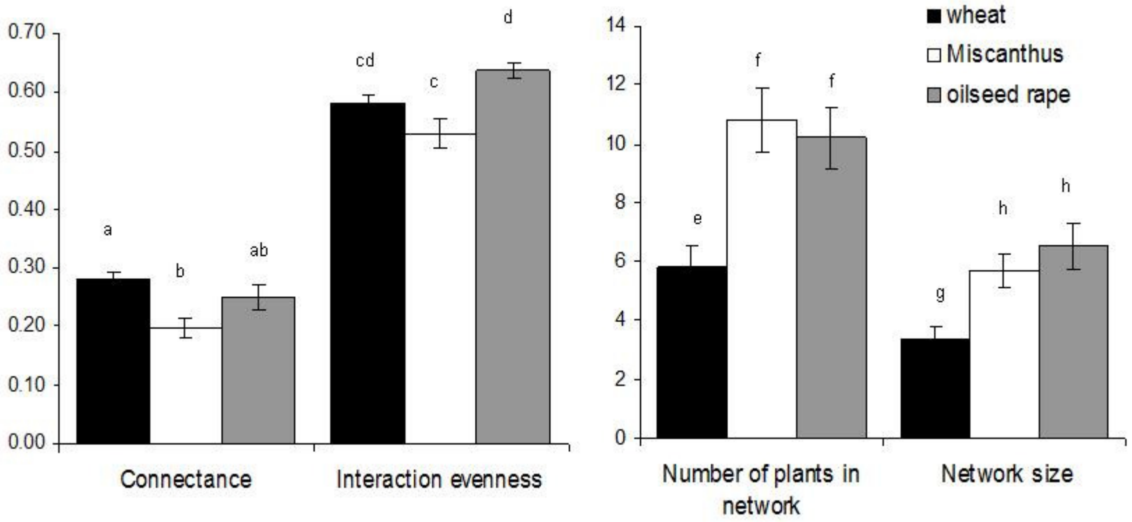

Figure 3.4. Mean (± standard error) connectance, interaction evenness, number of plants in network and network size across the five fields of each of the arable crop types. Black bars are wheat, white bars Miscanthus and grey bars oilseed rape. Significant differences ($p<0.05$) are represented by letters.

3.4.3 Local and landscape impacts

Network indices varied in their responses to local and landscape effects (Table 3.4). Floral abundance and species richness was significantly affected by local crop type only, with more flowers in oilseed rape than in all other crop types and higher species richness of flowers in oilseed rape than wheat. Total insect abundance and number of interactions were affected by crop type with more insects in energy crops than in conventional wheat or grass and more interactions in grass, oilseed rape and wheat when network size was kept constant, but also by landscape effects; both were negatively affected by hedgerow length while number of interactions was also related to the diversity of habitats and network size (Figure 3.5). Interaction evenness and generality were all related to landscape level measures only; generality was associated with diversity of habitats negatively and network size positively, and interaction evenness was positively associated with hedgerow length and negatively associated with diversity of habitats (Figure 3.6). Connectance, linkage density and web asymmetry were related only to network size.

Table 3.4. F-values from simplified models of network characters related to both local and landscape effects in all 25 fields. Non-significant factors were removed by stepwise backwards selection from the full model until all parameters were significant.

	Explanatory variables	**t**	**F**	**p**
Insect abundance (total)	crop type		3.28	0.033
	hedgerow length	-2.15	4.55	0.046
Floral abundance (total)	crop type		9.96	0.000
Insect species richness (total)	crop type		2.86	0.050
Floral abundance (minus oilseed rape)	crop type		2.74	0.057
Plant species richness (total)	crop type		3.12	0.038
Number of pollinators (in network)	-			
Number of flowering plants (in network)	-			
Network size	-			
Connectance	network size	-3.23	10.42	0.004
Generality	diversity of habitats	-2.29	5.25	0.032
	network size	5.97	35.60	0.000
H2	-			
Interaction evenness	hedgerow length	2.17	4.72	0.041
	diversity of habitats	-2.35	5.52	0.028
Linkage density	network size	1.94	3.76	0.065
Nestedness	-			
Number of interactions	network size	9.73	53.56	0.000
	crop type		3.15	0.041
	hedgerow length	-4.26	18.23	0.001
	diversity of habitats	4.05	16.82	0.001
Specialisation asymmetry	-			
Vulnerability	-			
Web asymmetry	network size	-2.848	8.11	0.009

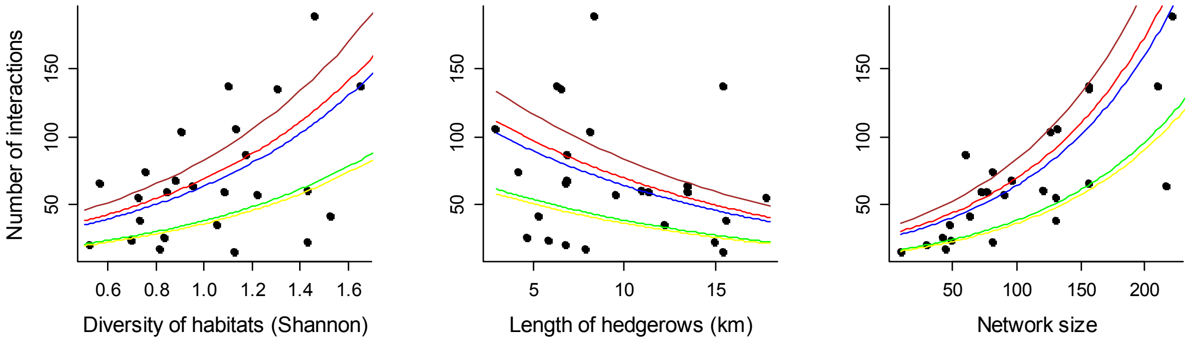

Figure 3.5. Main effects of landscape characters on the number of interactions from the best model. Points indicate measured values, and lines are model predictions when other model parameters are set to a mean value. As there was a significant crop type effect in the model, each crop type is represented as follows: Grass silage = brown, winter oilseed rape = red, winter wheat = blue, Miscanthus on arable = green and Miscanthus on grass = yellow.

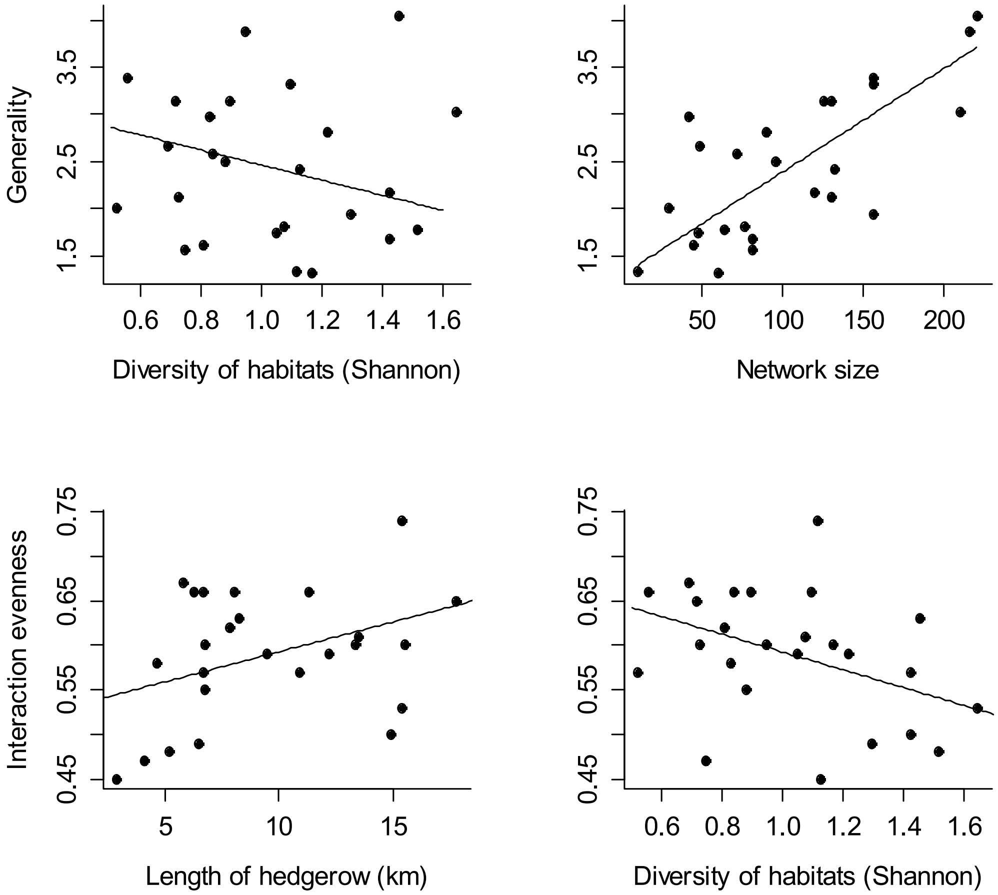

Figure 3.6. Main effects of landscape characters (from the best model) on generality (top) and interaction evenness (bottom). Points indicate measured values, and lines are model predictions when other model parameters are set to a mean value.

3.5 Discussion

In this study I investigated the effects of local and landscape elements on plant-pollinator networks, as well as the effect of an early mass flowering resource throughout the season. Here I find that in agricultural areas, where flora and fauna are more limited than in semi-natural areas (e.g. only 8 out of a total fauna of 20 species of bumblebees were recorded in these sites, and only 5 were found interacting with flowers during sampling), a mass flowering crop does not have a large detectable impact on network structure throughout the season. Although the crop was very well integrated into the network (being visited by 50% of the insect taxa who also visited other plant species) and there were large differences in flower abundances during and after flowering, I found little difference in insect abundances or network structure. This suggests that although mass flowering resources provide resources for large proportions of insect taxa, networks are reasonably robust to changes in the resource; similarly invasive species become well integrated into networks, visited by 43 and 31% of the pollinator taxa (Bartomeus *et al.* 2008b), but also may not cause changes in network structure (Vila *et al.* 2009). It has been suggested that networks can be robust to changes due to their asymmetrical structure and nestedness (Memmott *et al.* 2004; Bascompte *et al.* 2006). This supports the concept of re-wiring where species switch interaction partners resulting in little change to overall network properties (Kaiser-Bunbury *et al.* 2010), especially when strong interactors are removed. With a fauna and flora already much smaller than semi-natural areas, we might expect most of the species in agricultural areas to be generalised, adaptive foragers as they are able to cope with disturbed environments; adaptive foragers have been found to result in more stable networks (Kondoh 2003).

With a field scale replacement of conventional crops with bioenergy crops, I find varying responses in network structure depending on the crop type and the crop it is replacing. When Miscanthus replaces grass, this leads to no detectable change in network structure or the diversity of insects or plants. As both these crops are perennial, perhaps similar resources are provided in both and replacing one with the other is again buffered by adaptive foragers and re-wiring. However, when conventional wheat is replaced with Miscanthus or oilseed rape I find more flowering plants (and therefore bigger networks) in both the bioenergy crops than wheat, and also changes in both connectance and interaction evenness. For example, as there are fewer plants available for pollinators to forage on in wheat fields they have less choice; therefore insects may visit a higher proportion of all flowers in this crop type leading to higher connectance than in Miscanthus fields where insects can have more of a preference. If the goal of conservation is the preservation of more species, bioenergy production has a positive impact in comparison to conventional agriculture, but if more highly connected networks are desirable (which they may or may not be, for discussion see: Tylianakis *et al.* 2010; Heleno *et al.* 2012) then bioenergy production has a negative impact. However, although replacement of conventional agricultural land with crops for bioenergy can have impacts on network structure (predominantly in arable systems), under certain scenarios bioenergy production may not only replace existing agricultural land but may also begin to replace semi-natural habitats (Tilman *et al.* 2009; Dauber *et al.* 2010). Networks have been shown to persist for higher levels of habitat loss than random communities, but they eventually reach a tipping point (Fortuna & Bascompte 2006) at a certain level. Previous work has found no relationships between specialisation and disturbance in Argentinian forests (Vazquez & Simberloff 2002), but again these networks may not have reached the tipping point mentioned above and higher levels of disturbance could potentially have different effects. Under these alternative scenarios for bioenergy production where semi-natural land is replaced, this tipping point could be reached resulting in more dramatic changes in network properties.

Network structure can also be affected by changes at the landscape level, which has not been shown for plant-pollinator networks previously, and our data show that these landscape effects are sometimes stronger than just local effects of crop type. For example, I find differences in interaction evenness between the arable crop types, but this local effect disappears when the composition of the wider landscape is considered, where hedgerow length and diversity of habitats are the more important drivers. This reinforces that a landscape level approach is fundamental in the conservation of interactions, as well as their constituent species (Tylianakis *et al.* 2007).

Generality was negatively related to the diversity of habitats in the surrounding landscape. With a higher diversity of habitats, pollinators probably have more choice of areas to forage in and so are not strictly forced to visit more plants per pollinator at the field scale. When hedgerow length is kept constant, there are more insects in bioenergy crops than in conventional crops, but with longer hedgerows there are fewer insects. Hedgerows provide habitat for insects (Hannon & Sisk 2009), and more hedgerows means more linear features to disperse along (Cranmer *et al.* 2012) which may dilute insect abundance in the landscape. Similarly, hedgerow length was also part of the best model to describe the number of interactions most likely for similar reasons. The number of interactions was also influenced positively by the diversity of habitats; with more habitats in the landscape there can be more flowers (Power *et al.* 2012) and pollinators (Weibull *et al.* 2000) available. Therefore although generality is lower, perhaps there are more flowers and pollinators available overall which increases the number of interactions. Interaction evenness was affected by hedgerow length and diversity of habitats; when hedgerow length is kept constant there are less even interactions with increasing diversity of habitats while when diversity of habitats doesn't change, hedgerow length is positively related to interaction evenness,

Some indices were found to be related to network dimensions only, while others still had an influence when variation due to network size was accounted for. Linkage density was positively related to network size only, while connectance was negatively so. Similarly differences in network sizes but not other parameters were found between invaded and restored heathlands in Mauritius (Kaiser-Bunbury *et al.* 2009). Number of interactions and generality were positively related to network size in the simpler models, but both were also affected by wider landscape elements when network size was held constant. Similar effects of network dimensions on network indices have been reported previously (Bengtsson 1994; Dormann *et al.* 2009) but many studies do not take them into account. Network indices related only to network size may not give any extra information on interaction patterns other than abundance and species richness of interacting organisms, and therefore the interactions themselves can be considered as random (Dormann *et al.* 2009). However, if these dimensions are taken into account during a modelling process, effects over and above just dimensional changes can be detected. Connectance is often used as a measure of robustness or stability of a community (Dunne *et al.* 2002; Gilbert 2009); if a species from one trophic level is removed it is unlikely to result in the total removal of a species from the other tropic level because if that species is well connected it also interacts with other species. However if connectance is related only to network size then connectance is only a desirable trait when comparing two networks of the same size, and it should be interpreted with caution (Heleno *et al.* 2012).

Indices may also be related to network size due to incompleteness of sampling a network. Discussion occurs in the literature as to how much sampling and what methods are needed to gain a useful understanding of a network, with varying conclusions (Hegland *et al.* 2010; Gibson *et al.* 2011; Chacoff *et al.* 2012; Rivera-Hutinel *et al.* 2012). Networks also vary from year to year (Alarcon *et al.* 2008), with higher species turnover between years suggesting that networks sampled in one season may over-estimate real levels of specialisation (Petanidou *et al.* 2008), but concurrently it has also been shown that not all flower visitors carry pollen and so pollination networks can be more specialised than the flower-visitor networks commonly studied and presented here (Alarcón 2010). For many studies investigating integral properties of networks, a full description of a network is needed. However, here I was interested in comparisons between networks in different time periods or crop types. All networks across different crop types were sampled with the same intensity, and although some insect species were grouped and not identified to species level, this was consistent among sites and crop types also. Therefore effects of sampling intensity and taxonomic resolution are likely to be consistent among sites, which makes their effect on comparisons negligible.

3.5.1 Conclusion

This is one of the first studies to compare plant-pollinator networks across a season, during and after the flowering of a mass flowering crop (but see Olesen *et al.* 2008). Although I find little difference in network structure during and after the flowering of a mass flowering crop this area deserves more attention; this study was carried out in agricultural areas, and effects on semi-natural habitats could be different. Also, I studied winter oilseed rape which flowers early on in the season, but spring oilseed rape which flowers later may have different effects. Bioenergy production has a greater effect on network structure when planted on arable land than grassland, but this depends on crop type and what networks properties are desirable to conserve. I have also shown, for the first time, that the composition of the landscape surrounding the network sampling location can also affect the properties of the network, with diversity of habitats and hedgerow length being especially important. Both plants (e.g. Power *et al.* 2012) and pollinators (e.g. Steffan-Dewenter *et al.* 2002) respond to landscape composition, but often at different spatial scales and so further work on the scale at which whole networks respond could further the field. With regards to objectives for conservation, the diversity of habitats and length of hedgerows affected different networks indices differently. There is still discussion as to which network indices should be promoted over others for conservation objectives (Tylianakis *et al.* 2010), and which are desirable and which not in terms of stability or otherwise. It seems that length of hedgerows and diversity of habitats are especially important in agricultural regions, with hedgerows in Ireland already being quite species rich (Murray & Foulkes 2006), and I can suggest conservation and maintenance of these features to conserve plant-pollinator interactions. However, more work needs to be done on which network properties should be the basis for practical conservation objectives.

Chapter 4

Ecological variation in response to mass flowering oilseed rape and surrounding landscape composition by members of a cryptic bumblebee complex

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Plate 10. Oilseed rape fields planted at low density in the Irish landscape. Insets are of the bumblebee species groups used in Chapter 3: *Bombus sensu stricto* group (left) and *Bombus lapidarius* (right) (photos: DS)

4 Ecological variation in response to mass flowering oilseed rape and surrounding landscape composition by members of a cryptic bumblebee complex

4.1 Abstract

The *Bombus sensu stricto* species complex is a group of widespread cryptic bumblebee species which are important pollinators of many crops and wild plants. These cryptic species have, until now, largely been grouped together in ecological studies, and so little is known about their individual colony densities, foraging ranges or habitat requirements, which as highly mobile organisms can be influenced by land use at a landscape scale. Here, I used mass flowering oilseed rape fields as locations to sample bees of the cryptic complex, as well as the second most common visitor to oilseed rape *B. lapidarius*, and molecular RFLP methods to distinguish between the cryptic species. I then used microsatellite genotyping to identify sister pairs and estimate colony densities, and related both proportions and colony densities of the different species to the composition of the landscape surrounding the fields. I found *B. lucorum* was the most common member of the cryptic complex present in oilseed rape fields followed by *B. terrestris*, but *B. cryptarum* was also present in all but one site. High numbers of bumblebee colonies were estimated to be using oilseed rape fields as a forage resource, with *B. lucorum* colonies the most abundant. I also found that the cryptic species responded to surrounding landscape composition; relative proportions of *B. cryptarum* individuals in samples were positively associated with grassland and negatively associated with variables representing intensification. I also estimated more *B. terrestris* colonies with more soil types, and more *B. lapidarius* colonies further from woodland and urban areas. Colony densities of *B. lucorum* were affected by a combination of landscape variables. This suggests that the cryptic species have different ecological requirements, and that oilseed rape can be an important forage resource for many colonies of bumblebees. Given this I recommend sustainable management of this crop to benefit bumblebees.

4.2 Introduction

One of the most common bumblebees in North West Europe, responsible for both crop and wild plant pollination (Corbet *et al.* 1991), is the *Bombus sensu stricto* group, a cryptic complex of five species: *B. cryptarum, B. lucorum, B. magnus, B. terrestris* and *B. sporadicus* (Williams *et al.* 2012). Although advances in the taxonomy of this group have been made (Williams *et al.* 2012), most ecological studies of bumblebees and the pollination services they deliver have considered these species as a single group (e.g. Goulson *et al.* 2005; Bommarco *et al.* 2011) since the workers are morphologically indistinguishable in the field (Bertsch *et al.* 2005; Wolf *et al.* 2010; Carolan *et al.* 2012). However, this means that ecological differences between the species may have been overlooked (but see Murray *et al.* 2008; Waters *et al.* 2011b) and the pollination services delivered by, and conservation status of the species belonging to, the cryptic complex are impossible to assess (Fitzpatrick *et al.* 2006).

Over the past few decades, declines in both range and abundance have been documented for several bumblebee species in both North America and Europe (e.g. Fitzpatrick *et al.* 2007; Bommarco *et al.* 2011; Cameron *et al.* 2011), whilst other species have shown no decline, and in some cases have spread and become more abundant (Goulson *et al.* 2005; Bommarco *et al.* 2011). It is thought that the longer tongued bumblebee species, those with later starting colony development cycles, and those at range edges and with small climatic ranges are at most risk of decline (Goulson *et al.* 2005; Williams & Osborne 2009), driven largely by agricultural intensification (Goulson *et al.* 2008; Grixti *et al.* 2009; Whitehorn *et al.* 2012). Members of the *B. s. str.* cryptic complex of species are all relatively short tongued, have earlier starting colony cycles and are assumed to be ecological generalists (Goulson *et al.* 2005; Bommarco *et al.* 2011) and therefore may not be at the same risk of decline as some other species. The *B. s. str.* group in Ireland contains both species which are classified according to the IUCN criteria as of Least Concern (*B. lucorum* and *B. terrestris*) but also species which could not be assigned to a threat category and were deemed Data Deficient (*B. cryptarum* and *B. magnus*) (Fitzpatrick *et al.* 2006). However, due to the cryptic nature of these species, it is quite possible that these classifications are meaningless as the relative proportions of these cryptic species in both semi-natural and agricultural sites are not well known.

Individuals of the *Bombus sensu stricto* group (henceforth *B. s. str.)* are the most commonly observed bumblebees visiting mass flowering oilseed rape in Ireland (followed by *B. lapidarius*;) and as the crop benefits from insect pollination (Chapter 6, Bommarco *et al.* 2012), are likely to be important pollinators. Although it has been grown in Europe for centuries, the distribution of oilseed rape is changing and it is becoming more common largely due to its use as an oil crop for bioenergy (Frondel & Peters 2005). Since bumblebees, including the *B. s. str*. group, have large foraging ranges (for a summary see Charman *et al.* 2010) in comparison to other bee species (Gathmann & Tscharntke 2002), and are influenced by the composition of habitats and features within landscapes at both smaller (Murray *et al.* 2012) and larger spatial scales (Westphal *et al.* 2006a), they may be sensitive to changes in cultivation patterns of mass flowering crops such as oilseed rape. Bumblebee foraging distances can vary with the proportion of forage habitats in the landscape (Carvell *et al.* 2012), and landscape scale factors can also influence nest survival (Goulson *et al.* 2010). Several field surveys have demonstrated that the abundance of bumblebees can be influenced by landscape features. For example, more bees of the *B. s. str*. group (as well as *B. lapidarius* and *B. pascuorum*) were observed when surrounding landscapes (up to 3km from the sampling site) contained a high availability of mass flowering oilseed rape in Germany (Westphal *et al.* 2003), while oilseed rape fields had more bumblebees of all species when there was more pasture in the surrounding landscape (at an 800m radius) in Canada (Morandin *et al.* 2007). Conversely, other studies have shown no relationship between the abundance of bumblebees and landscape features (Steffan-Dewenter *et al.* 2002; Bommarco *et al.* 2012). However, many studies so far have grouped all bumblebee species together when looking at how bees are influenced by the surrounding landscape, when species-specific responses are likely, and have primarily focussed on the abundance and richness of bumblebees (Westphal *et al.* 2003; Morandin *et al.* 2007).

As bumblebees are colonial organisms, the reproductive unit is the whole colony. Therefore work on the effect of the surrounding landscape at the reproductive level (on colony densities) is important to understand impacts on populations (which may differ from impacts on abundances of individuals) and landscape scale effects on colony densities of some distinguishable species have been investigated (Knight *et al.* 2009; Goulson *et al.* 2010; Carvell *et al.* 2012). Since it is notoriously difficult to find and quantify colony densities using observational methods (Osborne *et al.* 2008b), molecular techniques have been developed allowing estimations of colony densities based on the relationships of bumblebee workers or sisters to each other (Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2005). This has allowed estimation of nest density and foraging distances, which differ remarkably between different bumblebee species (for a review see: Charman *et al.* 2010). As the most distinguishable of the *B. s. str*. complex, *B. terrestris* has been extensively studied (although lighter coloured individuals may have been overlooked: Wolf *et al.* 2010) but colony densities, foraging distances, landscape scale effects and even distributions of the other species in the complex are not well known. This ecological information is essential to understand how to manage, protect and conserve these important pollinator species.

The aim of this study was to investigate ecological differences between species within the *B. s. str.* complex by estimating the relative abundance and colony density of each species and then relating those data to landscape factors along an agricultural landscape gradient. For comparison another common non-cryptic short tongued species, *B. lapidarius*, was also included in the study. I used mass flowering oilseed rape fields as a sampling unit as they are commonly visited by *B. s. str*. and it is likely to attract in bumblebee colonies from the surrounding agricultural matrix. Specifically I set out to quantify and compare proportions and colony density estimates for members of the *B s.str* group, with *B. lapidarius* for further comparison, in an agricultural landscape, and ask whether they responded differently to landscape uses and effects. I also wanted to compare our colony density estimates from a mass flowering crop with previous estimates from non-mass flowering agricultural fields.

4.3 Methods

4.3.1 Site selection

Fourteen spring oilseed rape (*Brassica napus* L.) fields were selected for study in an area of 114km x 62 km in South East of Ireland in 2010 (Fig. 1), where arable farming is interspersed with beef and dairy, and oilseed rape is relatively rare. Fields were selected along a landscape gradient of arable to pasture dominated landscapes based on CORINE landcover data (EPA 2006). Fields were on average 15.28km apart (range 2.9km -

48.2km). Due to the current knowledge on average foraging ranges of our focal bumblebee species (B. lapidarius 260m, B. terrestris 663m Walther-Hellwig & Frankl 2000; B. terrestris median 620 - 2800m Chapman *et al.* 2003; B. lapidarius 450m, B. terrestris 758m Knight *et al.* 2005; B. terrestris 1500m Osborne *et al.* 2008a; B. cryptarum, B. lucorum and B. magnus unknown), it was assumed that given our average inter-site distance, the number of sites sharing bees from the same colony would be negligible overall.

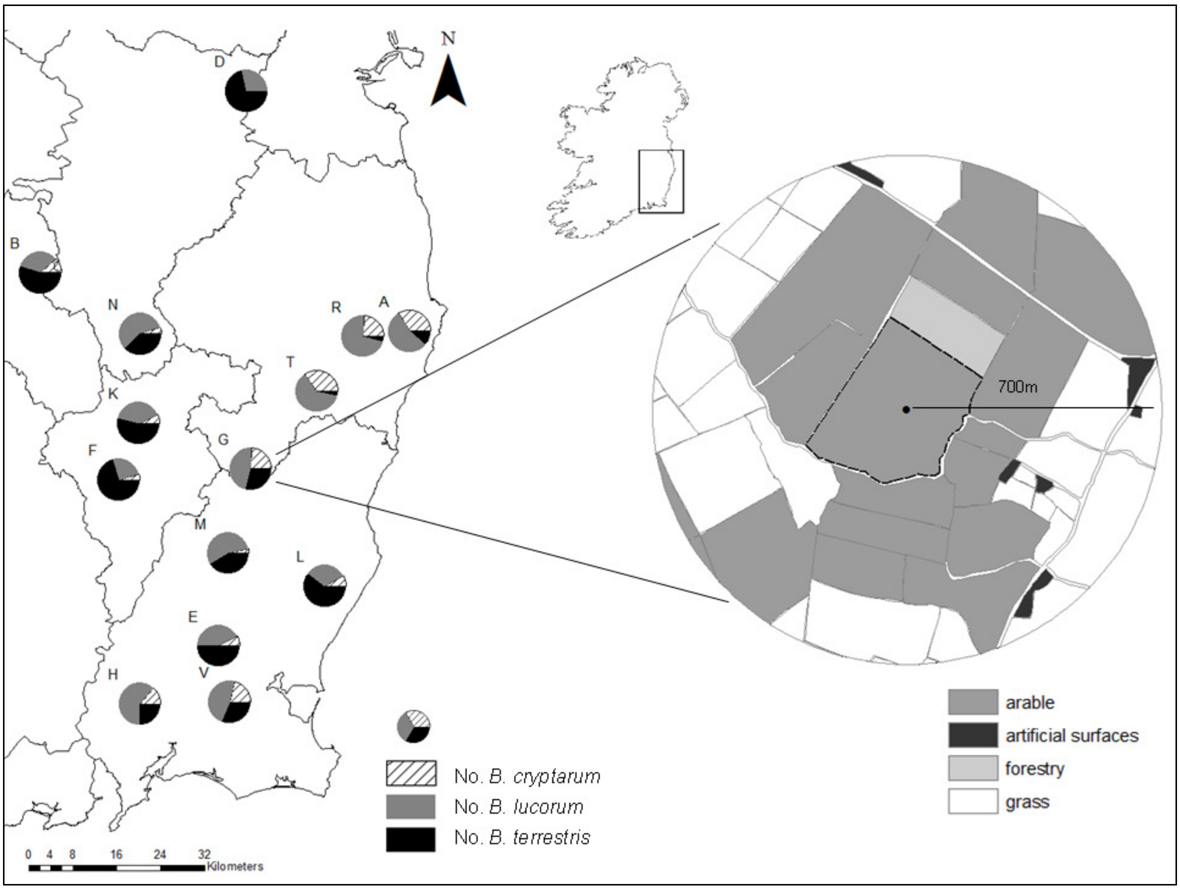


Figure 4.1. Locations of the 14 spring oilseed rape fields in South-East Ireland, proportions of the cryptic species in each field, and an example of the landscape mapped at a 700m radius around each field. The focal oilseed rape field is highlighted with a dotted line.

4.3.2 Sample collection

Fields were visited once between 13th June and 12th July 2010 during the peak flowering of spring oilseed rape. Firstly, two 100m transects were walked at each site to identify the main bumblebee groups present. Then, individuals of the *B. s .str*. group were sampled qualitatively in each field by walking around the perimeter of the field on the outermost tramline, approximately 20m into the crop, and catching individuals until a target of 60 were caught. A non-lethal sample of tarsal segment was taken (Holehouse *et al.* 2003) from the mid-leg and stored in 75% ethanol for later DNA analysis. In the seven sites where *B. lapidarius* occurred in the transects, a target of 50 *B. lapidarius* individuals were also sampled in the same way. Sampled individuals were marked using a non-toxic marker pen to avoid re-capture of the same individuals. A similar amount of time was also spent catching bees around the perimeter of a non-mass flowering field directly adjacent to the oilseed rape field to investigate patterns of worker distribution from colonies, but due to low numbers of sister pairs in both oilseed rape and adjacent fields separately, insufficient colonies were identified in both fields to answer this question. It was only possible to sample *B. s. str.* workers in nine adjacent fields as there were no suitable sampling areas (flowering vegetation) at the other five fields. All *B. lapidarius* individuals appear to be attracted to oilseed rape fields; individuals were rarely present in adjacent fields and so numbers were too low to include in further analyses. A total of 1362 individuals from the *B. s. str.* group and 330 *B. lapidarius* individuals were sampled overall.

4.3.3 Landscape characterisation

A detailed map of the landscape surrounding each oilseed rape field was also produced, up to a 700m radius from the centroid of each field (Figure 4.1). This radius was chosen based on the estimated foraging distance of the focal bee species (see above). Land cover was divided into the following categories (Table 4.1): mass flowering crops (79% oilseed rape, 14% potatoes, 7% field beans), non mass flowering arable land, grass land, forestry and extensively modified human surfaces (including buildings, yards and gardens). Land cover types were ground-truthed for each landscape as accurate distinction using aerial photographs was not possible. Linear features were also quantified using ortho-photographs and Ordinance Survey maps including length of field boundaries, roads, and rivers. Soil characteristics can also be important predictors of habitat types (Sullivan *et al.* 2011), and the number of soil types in each landscape was also quantified using the national soils database (Teagasc 2008). Distances to the nearest urban area, protected area, forest patch, peatland and sea were also calculated for each field using the CORINE landcover data (EPA 2006). All landscape analyses were carried out in ESRI ArcGIS 9.3.

Table 4.1. Summary of the landscape variables calculated surrounding the 15 fields

	Mean percentage ± SE (min – max)
Intensive grassland	46 ± 5 (17-74)
Arable land	42 ± 6 (8-78)
Forestry	6 ± 2 (0-22)
Mass flowering crops	12 ± 2 (3-34)
Artificial surfaces	6 ± 1 (1-21)
Surrounding field size (ha)	6 ± 1 (2-15)
Elevation (m)	80 ± 9 (37-169)

	Mean length (km) ± SE (min – max)
Field boundary length	22.6 ± 2 (15.2-33.4)
Distance to protected area	2.3 ± 0.5 (0.2-6.2)
Distance to peatland	11.6 ± 2 (3-25)
Distance to forest	0.5 ± 0.1 (0.08-1.4)
Distance to urban area	3.6 ± 0.7 (0.1-9)
Distance to sea	25.4 ± 5 (69-6.3)

4.3.4 Species differentiation and microsatellite genotyping

DNA was extracted by pulverising tarsal segments after cooling in liquid nitrogen, and adding 300 µL 10% solution of Chelex 100 heated to 80˚C to each sample. Samples were then heated to 100˚C for 15 minutes before centrifuging and finally cooling to 4˚C. I used a Restriction Fragment Length Polymorphism (RFLP) method developed by Murray et al. (2008) to definitively assign each sample to one of the cryptic species, before performing sibship reconstruction. Samples were amplified using a polymerase chain reaction (PCR), digested using specific restriction enzymes (EcoNI and HinFI) and then visualised using electrophoresis in 2% agarose gels (Murray *et al.* 2008), where each cryptic species has a unique banding pattern (**Appendix 10**). Sixteen individuals (including a mixture of ambiguous and confirmed banding patterns) were also sequenced at partial mitochondrial COI gene (Tanaka *et al.* 2001) to confirm RFLP identities.

Samples from all four species were subsequently genotyped at 14 microsatellite loci in two multiplex reactions (all: B10, B11, B96, B100, B118, B124, B126, B132, BT08, BT11, BL02, BL06, BTERN01, *B. terrestris* and *B. lucorum*: BL03, *B. lapidarius* and *B. cryptarum*: BL11, Estoup *et al.* 1995; Estoup *et al.* 1996; Funk *et al.* 2006, Table 4.1). PCR products were visualised on an ABI 3730xl automated sequencer (Applied Biosystems) using a GeneScan 500 LIZ size standard, and alleles were sized using GENEMAPPER software (Applied Biosystems). Where a sample failed to amplify at any locus on the first attempt, or where there was any case of scoring ambiguity, a new PCR was run and all loci were re-amplified. This also allowed calculation of scoring and allelic drop-out error rates for loci that were amplified twice. After two attempts, B100 still failed to amplify sufficiently for accurate scoring in both *B. terrestris* and *B. lucorum*, and B10, B96 and BT08 in *B. lapidarius,* and so these loci were omitted from any further analyses.

4.3.5 Data analyses

For analyses, I included any samples with a minimum of 7 of the 13 loci scored for *B. terrestris* and *B. lucorum,* and a minimum of six loci for *B. cryptarum* and *B. lapidarius*. An initial analysis comparing genetic distance (F_{st} values) between sites showed that there was a significant relationship with geographic distance between sites using Mantel tests for all species expect *B. lapidarius* (data not shown). Therefore, each site was treated as a discrete population and all data were subsequently analysed on a per site basis. Genotypes were checked for typographic error and null alleles using MICRO-CHECKER (Van Oosterhout *et al.* 2004). I then used the program COLONY (Wang 2004) to identify sister pairs for each site using allelic drop out and scoring error rates calculated from re-scoring, which implements a maximum likelihood sibship reconstruction method (Jones & Wang 2010) and has been shown to give the most accurate sibship reconstruction when compared with other methods (Lepais *et al.* 2010). Due to the assumptions of COLONY, GENEPOP 4.1 (Raymond & Rousset 1995) was used to test for deviations from Hardy Weinberg equilibrium (HWE) of individual loci by population using a probability test, and linkage disequilibrium between loci across all populations, using Bonferroni corrections for multiple comparisons of loci per individual. For GENEPOP analyses only one individual per colony was retained, as inclusion of closely related family members will inevitably lead to inflated homozygosity estimates and can lead to false evidence of population structure (Darvill *et al.* 2004; Anderson & Dunham 2008).

However, sampling was not exhaustive, and colony estimates are based only on workers successfully sampled from each site. Therefore, to account for the number of colonies we missed, I also estimated the total number of colonies present in each site for each species. To do this I used the Two Innate Rate Model (TIRM) in the CAPWIRE programme (Miller *et al.* 2005). This is a mark-recapture software and method recommended for estimating number of bumblebee nests (Goulson *et al.* 2010) instead of the more conservative Poisson distribution method (e.g. Darvill *et al.* 2004), as it assumes unequal capture rates of different nests. I carried out colony density estimates in two ways: firstly I used sister pairs identified in the oilseed rape field only to get an estimate of colonies using that resource. Secondly, since very low number of sister pairs were identified within the oilseed rape fields for both *B. cryptarum* and *B. lucorum*, I pooled these data with those from the adjacent field for each species to increase sample sizes and number of sister pairs, and therefore get a more accurate estimate of the numbers of colonies foraging in the area. CAPWIRE models were run in 0.1 increments with capturability ratios of minimum 1, maximum 20; 95% confidence intervals for the estimate on population size based on 1000 bootstrap replicates; a largest population size of 750 for dimensioning.

I also calculated the number of colonies per km^2 for the two species where foraging distance is known (*B. terrestris* and *B. lapidarius*), based on methods and foraging distances estimated from Knight et al. (2005) (*B. terrestris* 758m, *B. lapidarius* 450m).. These densities (*B. terrestris* from 12 fields and *B. lapidarius* from 7 fields) were compared to those from other studies (*B. terrestris* previous estimates data from a number of sources (5 data points), summarised in Charman *et al.* 2010; *B. lapidarius* previous estimates data (12 data points) from Goulson *et al.* 2010 and Knight et al., 2005) using non.parametric Kruskal-Wallis tests.

Total colony number estimates from CAPWIRE (using TIRM) were used in landscape analyses. Colony density estimates of each species were initially investigated for relationships with the area sampled, the number of individuals sampled, and geographic location (using ITM x and y co-ordinates) using Spearmans rank correlations. The subsquent landscape analyses consisted of two steps. Firstly I investigated relationships between proportions of cryptic species or colony densities in each site with individual landscape variables using Generalised Linear Models (GLMs). Secondly, the same response variables were modelled against all calculated landscape variables to test for additive effects. Landscape variables were first normalised ((variable-mean)/standard deviation) as they were measured in different units. After removing variables that were highly co-linear, the following landscape metrics were used in the full model for the proportions of the different species: area of arable land, artificial surfaces, mass flowering crops, forestry, length of field boundaries, surrounding field size, elevation, and distances to nearest protected area, peatland, forest, urban area and sea (Table 4.1). As sample sizes for the colony estimations were smaller, landscape variables tested for additive effects were again checked for co-linearity before analysis and some additional variables were removed where necessary. Models were simplified using stepwise backwards selection, removing non-significant terms singly until all terms in the model were significant. For proportions of the cryptic species, binomial GLMs were used to account for proportional data, and corrected for overdispersion using quasi-binomial GLMs if necessary. For the colony density estimates of the different species, Poisson GLMs were used and standard errors corrected for overdispersion using a quasi-GLM. All models were validated by plotting residuals vs fitted values and all explanatory variables. For colony densities, only sites where an estimate was obtained were used in analyses (i.e. sites where no sisters were found were not used as no accurate estimate could be calculated, perhaps excluding those with the highest colony densities). *B. cryptarum* colonies had one outlier (site T) that had a much higher estimate than all other sites, and so analyses were carried out both including and excluding this site. All analyses were carried out in R (R Development Core Team 2008).

4.4 Results

4.4.1 Proportions of cryptic species

Three of the four species of the *B. s. str.* group found in Ireland were found foraging in oilseed rape fields – *B. cryptarum*, *B. lucorum* and *B. terrestris*. No *B. magnus* individuals were found in any of the fields studied. The most abundant species was *B. lucorum*, followed by *B. terrestris*. *B. cryptarum* was also present in all but one of the fields, but in lower numbers that the other two species (Figure 4.2). Proportions of all three species varied among fields (Figure 4.2). The proportion of *B. cryptarum* was positively associated with *B. lucorum* (Spearman rank correlation: Rho = 0.53, S =2.4, p=0.05), and negatively associated with *B. terrestris* (Spearman rank correlation: Rho = -0.81, S = 822, p<0.001). There was a trend towards proportions of *B. cryptarum* being positively related to latitude (Spearmans rank correlation: S=216, p=0.057, Rho =0.53); proportions were higher in the eastern part of the study area (Figure 4.1).

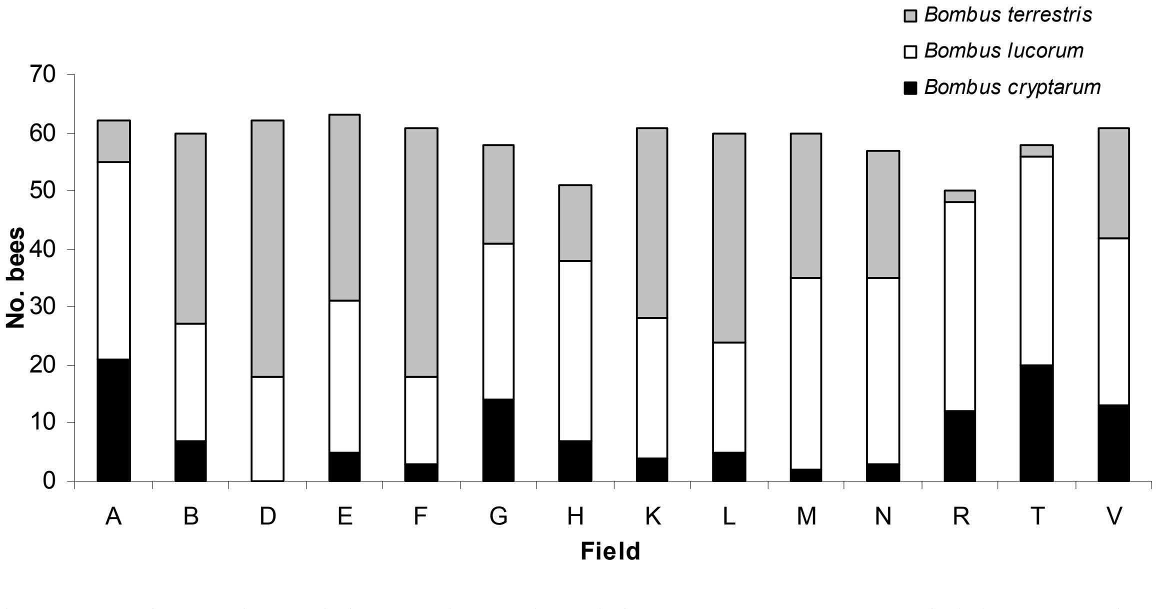


Figure 4.2. The numbers of the cryptic species of the *B. s. str.* group recorded from a random sample of ~60 individuals sampled from around the outside tramline of each oilseed rape field.

4.4.2 Microsatellite genotyping

Thirteen loci were scored for *B. lucorum* and *B. terrestris*, 14 for *B. cryptarum* and 11 for *B. lapidarius*. Generally the number of alleles found were lower for *B. lapidarius* than for the members of the *B. s. str.* group (Table 4.2).

Table 4.2. Characteristics of the microsatellite loci used in each species. References a = Estoup *et al.* (1995), b = Estoup *et al.* (1996) c = Funk *et al.* (2006).

Locus	**ref**	***B. lucorum*** **no alleles**	**Fis** ± SE	***B. terrestris*** **no alleles**	**Fis** ± SE	***B. cryptarum*** **no alleles**	**Fis** ± SE	***B. lapidarius*** **no alleles**	**Fis** ± SE
B118	b	6	0.072 ± 0.037	8	0.104 ± 0.037	7	0.148 ± 0.046	5	-0.016 ± 0.049
B100	b	n/a	-	n/a	-	10	0.082 ± 0.066	5	-0.002 ± 0.039
B132	b	25	0.018±0.016	13	0.063 ± 0.027	15	0.005 ± 0.026	5	0.036 ± 0.035
B10	a	18	-0.022±0.017	18	0.064 ± 0.024	22	0.032 ± 0.032	n/a	-
BL06	c	24	0.067 ± 0.01	15	0.045 ± 0.029	25	-0.003 ± 0.0.31	9	-0.038 ± 0.03
B124	a	8	0.012 ± 0.024	16	0.094 ± 0.019	8	0.013 ± 0.04	6	-0.05 ± 0.029
BL03	c	20	-0.019 ± 0.016	17	0.055 ± 0.027	n/a		n/a	-
BT08	c	10	-0.008 ± 0.018	19	0.082 ± 0.032	14	0.146 ± 0.048	n/a	**-**
BT11	c	10	0.066 ± 0.022	13	0.114 ± 0.03	12	-0.006 ± 0.09	4	**0.594** ± 0.11
BL02	c	22	0.01 ± 0.008	12	0.021 ± 0.029	22	0.037 ± 0.024	6	0.004 ± 0.024
B11	a	7	0.021 ± 0.035	9	0.122 ± 0.044	8	0.105 ± 0.055	6	-0.085 ± 0.037
B96	b	7	0.018 ± 0.027	8	0.07 ± 0.049	5	0.24 ± 0.069	n/a	-
BTERN01	c	11	-0.017 ± 0.023	14	0.063 ± 0.018	13	0.015 ± 0.044	4	0.012 ± 0.05
B126	a	18	0.008 ± 0.018	18	0.077 ± 0.034	19	0.11 ± 0.036	9	0.027 ± 0.068
BL11	c	n/a	-	n/a	-	24	0.05 ± 0.049	8	0.014 ± 0.033

For *B. lucorum* and *B. cryptarum* a global test showed no overall deviations of any loci from HWE (global Fishers test: *B lucorum* χ^2 =373.24, df = 364, p = 0.36, *B. cryptarum* χ^2 = 271.16, df = 316, p = 0.97). There was no significant linkage disequilibrium detected between loci for *B. cryptarum*. Although a number of loci showed significant linkage disequilibrium using a global test across all populations (sites) for *B. lucorum,* on further investigation of each case this was driven by linkage found in one population only. Due to the small number of populations affected (1 out of 14) all loci were retained in further analyses.

For *B. lapidarius*, BT11 showed significant deviation from HWE in 3 of the 6 populations, possibly due to a deficit of heterozygotes suggesting the presence of a null allele. Therefore this locus was removed from analysis and colony sibships re-run without it. There were no deviations from HWE for any of the remaining loci (global Fishers test: χ^2 = 101.18, df = 140, p = 0.99), and no significant linkage disequilibrium between any loci.

For *B. terrestris*, a global Fishers test showed significant deviation from HWE (χ^2 = infinity, df= 310, p<0.001). However, on further investigation this was caused by a small number of loci in 4 populations only: BT08 in site A, B132 in site B, B126 in site F and BT11 and BT08 in site M. The following loci showed significant linkage disequilibrium but again in one population only: B132 and B124 in site B, BL03 and BL06 in site D and B126 and B118 in site E. Due to the small number of populations affected (1 or 2 out of 14 in all cases) all loci were retained in further analyses.

4.4.3 Colony estimation

Low numbers of sister pairs of all species were found within each field, suggesting that high numbers of colonies were using this mass flowering resource (**Appendix 12**). Most colonies were represented by a single worker, and the maximum numbers of sisters from any one colony was four (from an average of 31 individuals per site). To examine the estimated numbers of colonies using mass flowering oilseed rape fields as a resource, I estimated colony densities using bees caught only in the oilseed rape. CAPWIRE estimations of total colony densities were not possible in fields where no sister pairs were identified; therefore estimations of total colony densities were possible in 12 fields for *B. terrestris* (mean 145, range 21-320 colonies) but only one field for *B. cryptarum* (46 colonies), and four fields for *B. lucorum* (mean 227, range 107-320 colonies) due to the small numbers of sister pairs, despite similar sample sizes to previous studies (e.g. Charman *et al.* 2010; Goulson *et al.* 2010). *B. lapidarius* was only found in sufficient numbers in oilseed rape fields and colony estimates ranged from 41 - 105 colonies per field (Table 4.3).

Using data from both the oilseed rape field and adjacent field (henceforth "site"), sample sizes were larger and number of sister pairs higher allowing estimates of total colony density for a larger number of sites (**Appendix 12**). Using an average across all sites where estimations were possible, the highest number of colonies found were of *B. lucorum* (Figure 4.3, 11 sites, mean 406, range 103-726), then *B. terrestris* (mean 163, 12 sites, range 37 – 332) and then *B. cryptarum* (6 sites, mean 131, range 0-710, Table 4.3). Colony densities of all species were not significantly related to the size of the fields sampled. Colony densities of *B. lucorum* and *B. lapidarius* (using only sites where colonies were sampled) were not significantly related to the number of individuals sampled, but colony densities of *B. cryptarum* (Spearmans rank correlation: Rho = 0.84, S = 5.8, p=0.04) and *B. terrestris* (Spearmans rank correlation: Rho = 0.82, S=50.18, p<0.001) were. Sites where no sisters were found were not included in subsequent analyses as no estimates of numbers of colonies were possible.

Table 4.3. Sample sizes (A) and Capwire point estimates of number of colonies (B) of the different bumblebee species in the different sites (Site names in bold are those with a total of the oilseed rape and adjacent field together). NA = no estimation possible due to lack of sister pairs. S = area of focal oilseed rape field. D = distance to nearest focal oilseed rape field (site)

Site	**Location**	**S (ha)**	**D (km)**	***B. cryptarum***		***B. terrestris***		***B. lucorum***		***B. lapidarius***	
				A	B	A	B	A	B	A	B
A	**Rathdrum, Co. Wicklow**	5.14	2.90	29	53	27	61	59	580	0	0
B	**Stradbelly, Co. Laois**	31.52	21.27	10	46	73	332	32	502	0	0
D	Celbridge, Co. Kildare	6.87	48.17	0	0	43	229	18	NA	44	78
E	**Adamstown, Co. Wexford**	3.59	10.46	10	30	55	116	54	726	0	0
F	**Bagnelstown, Co. Carlow**	6.94	10.95	8	NA	63	329	31	103	51	48
G	Carnew, Co. Wicklow	12.65	15.87	10	NA	17	37	27	NA	43	41
H	**New Ross, Co. Wexford**	2.49	16.32	5	10	32	81	44	NA	0	0
K	**Carlow, Co. Carlow**	5.55	10.95	9	NA	53	150	36	320	52	25
L	**Kilmuckridge, Co. Wexford**	5.76	18.59	15	NA	60	174	45	200	0	0
M	**Ballycarney, Co. Wexford**	4.61	15.87	5	NA	47	152	62	428	45	13
N	Castledermot, Co, Kildare	8.68	16.26	3	NA	21	213	36	213	44	21
R	Rathdrum, Co. Wicklow	4.29	2.90	12	NA	2	NA	33	269	0	0
T	**Aughrim, Co. Wicklow**	3.93	13.06	38	710	3	NA	79	522	40	48
V	**Taghmon, Co. Wexford**	8.59	10.46	21	70	35	81	60	599	0	0
	mean	**7.9**	**15**	**13**	**147**	**38**	**163**	**44**	**406**	**46***	**61***

* mean of sites with species present only.

Using colony densities calculated per km^2, I found the density of colonies of *B. terrestris* to be significantly higher than previously published estimates (Kruskal-Wallis χ^2=8.72, df=1, p=0.0031, Figure 4.3), while colonies of *B. lapidarius* were not (Kruskal-Wallis χ^2 = 1.03, df=1, p=0.3096, Figure 4.3). Comparisons for *B. cryptarum* and *B. lucorum* were not possible as, to our knowledge, there are no previously published estimates of colony densities for these species.

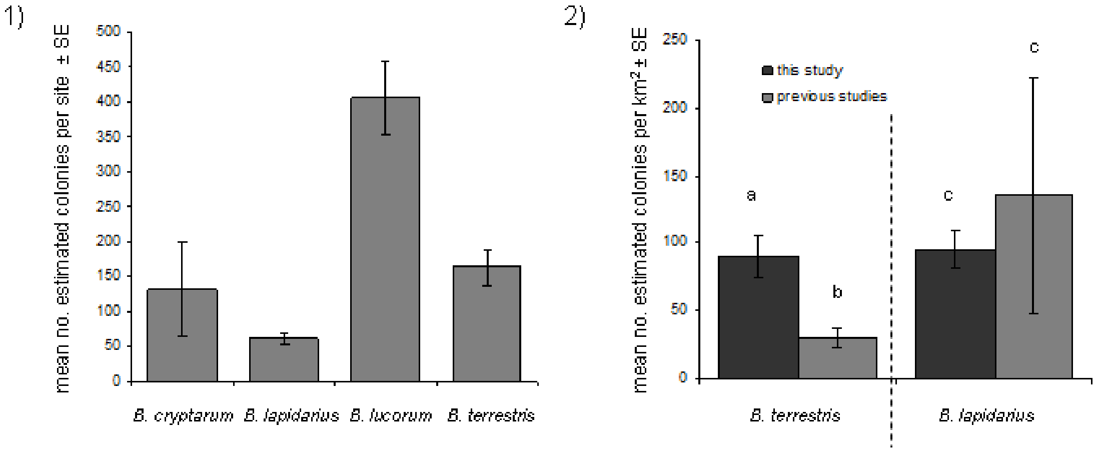


Figure 4.3. 1) mean number (± standard error) of nests estimated foraging per field for each of the species, and 2) Nest density per km^2 of *B. terrestris* and *B. lapidarius* in comparison to previous estimates (*B. lapidarius* previous estimates data from Goulson *et al.* 2010 and Knight *et al.* 2005. *B. terrestris* previous estimates data from a number of sources, summarised in Charman *et al.,* 2010). Letters indicate significant differences (p<0.05) determined using Kruskal-Wallis tests and

4.4.4 Landscape analyses

Relating each landscape variable separately to the proportions of cryptic bumblebees found in each field, the proportion of *B. cryptarum* was positively related to the amount of grassland in a 700m radius (Deviance = 1.22, F=6.21, p=0.028), and negatively related distance to the nearest forest patch (Deviance = 1.224, F=6.29, p=0.027). Proportions of *B. cryptarum* were best explained with a model containing negative relationships with arable land, artificial land, mass flowering crops and distance to urban area (Figure 4.4, Table 4.4). Proportions of *B. lucorum* and *B. terrestris* were related to the field size of surrounding fields, negatively and positively respectively, and both these proportions were best explained by a model including this variable only (Table 4.4).

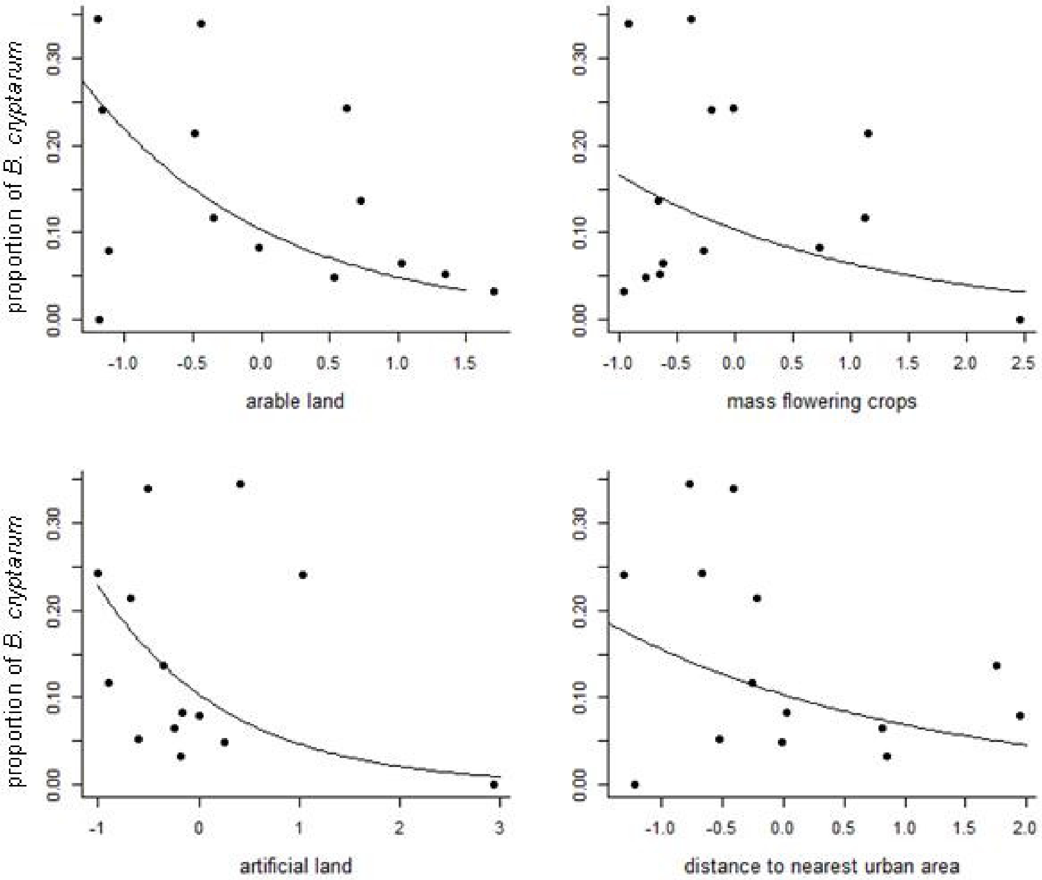


Figure 4.4. Main effects of normalised landscape components on proportions of *B. cryptarum* from the best generalized linear model. Points show normalised measured values, and lines show model predictions when other landscape variables are kept constant

Table 4.4. Final quasi-binomial generalized linear models describing the effects of landscape variables on proportions of the three cryptic species. Non-significant factors ($p>0.05$) were removed by stepwise backward selection from the full model which included: area of arable land, artificial surfaces, mass flowering crops, forestry, length of field boundaries, surrounding field size, elevation, and distances to nearest protected area, peatland, forest, urban area and sea

	Deviance	**F value**	**p value**
B. cryptarum			
residual	15.889		
arable	53.246	21.16	0.001
artificial surfaces	45.136	16.56	0.003
mass flowering crops	33.891	10.2	0.011
distance to urban	32.939	9.66	0.013
B. lucorum			
residual	41.663		
field size surrounding	60.854	5.5273	0.037
B. terrestris			
residual	135.87		
field size surrounding	185.1	4.3287	0.059

Colony density estimates of *B. terrestris* were positively related to the distance to the sea (deviance = 633.59, F=10.04, p=0.01), and best explained by a model containing a positive relationship with the number of soil types only (Table 4.5). Colony density estimates of *B. lucorum* were not related to any landscape variables individually, but were best explained by a model containing negative relationships with area of arable land (which was positively correlated with distance to forestry), forestry, number of soil types and elevation (Figure 4.5, Table 4.5). Colony estimates of *B. cryptarum* were positively related to the length of field boundary (which was correlated with amount of forestry) with the full data set (deviance = 1976.06, F=10.96, p=0.02), and area of artificial surfaces when outlier was removed (Table 4.5). Colony estimates of *B. lapidarius* were positively related to distance to forest (deviance = 637.5, F=10.314, p=0.007) and were best explained with a model containing distance to forestry and distance to urban (Figure 4.5, Table 4.5).

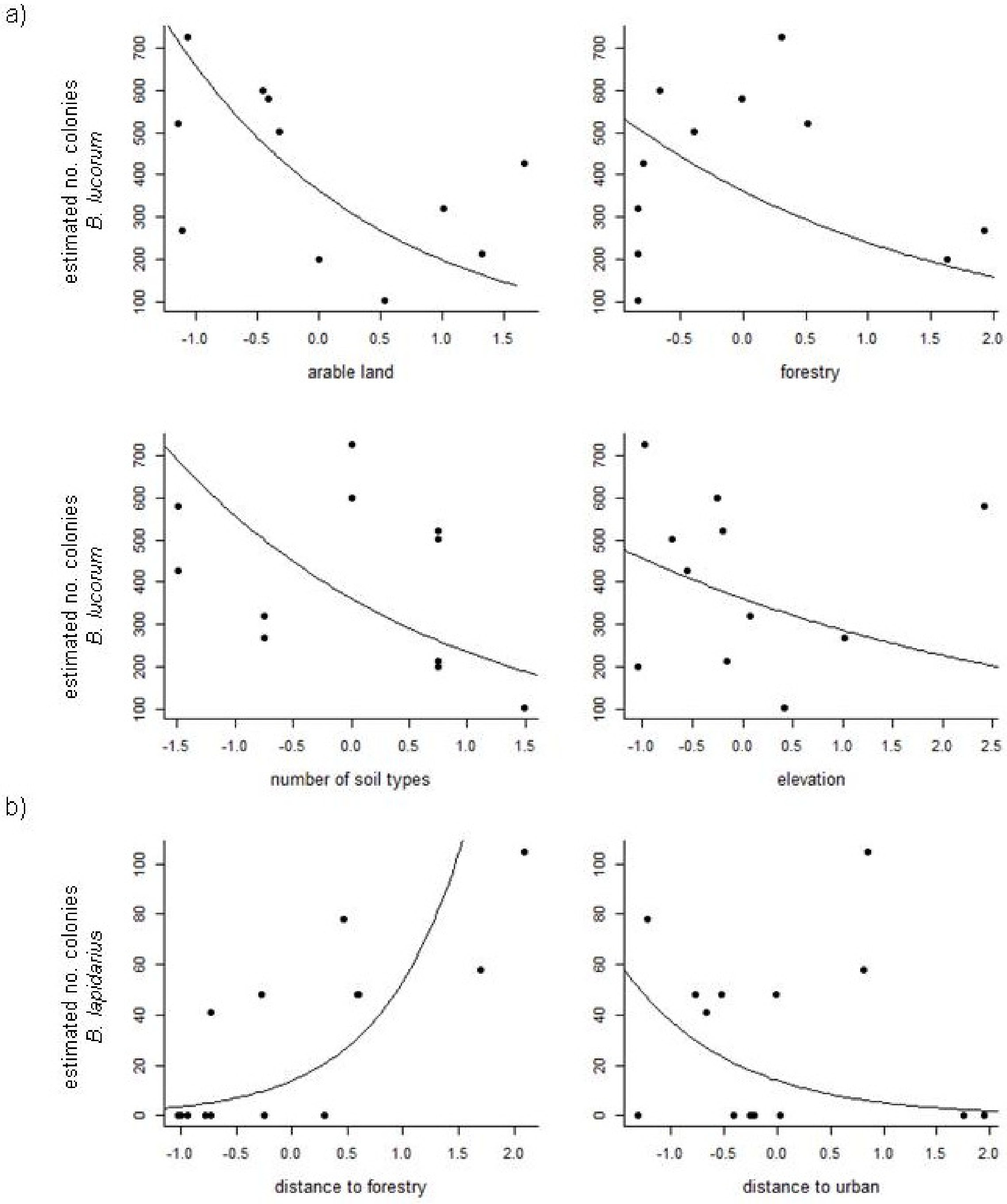


Figure 4.5. Main effects of normalised landscape components on estimated total number of colonies of a) *B. lucorum* and b) *B. lapidarius* from the best generalized linear model. Points show normalised measured values, and lines show model predictions when other landscape variables are kept constant.

Table 4.5. Final quasi-poisson generalized linear models describing the effects of landscape variables on colony density estimates of the cryptic species and *B. lapidarius*. Non-significant factors (p>0.05) were removed by stepwise backward selection from the full model.

	Deviance	F value	p value
B. cryptarum			
residual	43.82		
artificial surfaces*	133.86	8.22	0.007
B. lapidarius			
residual	216.45		
distance to urban	342.84	6.42	0.03
distance to forest	631.22	21.1	0.0008
B. lucorum			
residual	120.02		
arable	862.09	37.1	0.0009
forestry	456.58	16.8	0.006
number soil types	535.3	20.8	0.004
elevation	292.46	8.6	0.03
B. terrestris			
residual	406.03		
number soil types	633.59	5.6	0.04

* with outlier removed

4.5 Discussion

In accordance with two previous studies (one unpublished), I found that *B. lucorum* is relatively more abundant than other members of the *B. s. str.* species complex, across a broad range of habitat types in Ireland (Murray *et al.* 2008; Byrne 2011), but this is the first study to confirm this pattern in agricultural habitats. In addition, our data add evidence to suggest that *B. cryptarum* is widespread in Ireland, and certainly under-recorded due to its morphological similarity to other cryptic species. Furthermore, this is the first study to estimate colony densities of the *B. s. str.* group using molecular methods. I found that in agricultural areas, there were fewer foraging individuals and colonies of *B. cryptarum* than there were of *B. lucorum* and *B. terrestris*, but that the numbers of colonies using oilseed rape as a forage resource were high. Lastly, I found that species showed different responses to landscape composition, suggesting differences in ecological requirements.

4.5.1 Proportions of cryptic species in samples

Only three of the four species in the *B. s.str*. group were found in oilseed rape fields; *B. magnus* was not found in any of the sites studied. Previous work has suggested that *B. magnus* is an upland species associated with heathlands and Ericaceous species (Goulson *et al.* 2005; Waters *et al.* 2011b), and that *B. cryptarum* is more widespread in the UK (Waters *et al.* 2011b). As the nearest peatland areas were on average 11.6km from the fields used in this study, it is perhaps unsurprising that *B. magnus* was not present. A previous study in Ireland also found that *B. lucorum* was the most abundant of the *B. lucorum/B. cryptarum/B. magnus* complex at altitudes lower than 200m (our highest site was 169m) and found *B. magnus* in all the non-urban sites sampled (Murray *et al.* 2008), while another study also found *B. magnus* in forested sites in the Wicklow mountains (Byrne 2011). However, these studies sampled a variety of different habitat types while I was more limited to sampling more intensive farming areas. These farmed areas only have a limited bumblebee fauna; of a possible twenty species, only ten are generally found (Santorum & Breen 2005). Therefore perhaps it is not surprising that *B. magnus* is also not found there. However, this does show that *B. cryptarum* is a common and widespread bumblebee species, perhaps more so that previously thought. We also found higher proportions in the north-eastern part of the study area where the land is more mountainous and possibly less productive; although we found no association with elevation (possibly due to small elevational range), previous work has found more *B. cryptarum* at higher altitudes (Murray *et al.* 2008).

4.5.2 Colony density estimates

This is one of the first studies to look above abundance and richness estimates, and to investigate the numbers of colonies of bumblebees using a mass flowering resource. In a study of nest density in agricultural fields in the UK, Goulson et al. (2010) estimated 413 colonies of *B. lapidarius* in one site which they considered an outlier attributed to an adjacent mass flowering clover ley. Although our estimates of colony densities are likely to be conservative (see below), I estimated that there were between 694-880 colonies (total of all species per site where there was an estimate for each species) of the four most common bumblebee species using an individual oilseed rape (and in some cases adjacent) field. This indicates that mass flowering crops can provide forage resources for large numbers of bumblebee colonies, particularly when they flower at the peak of the colony cycle (as is the case with spring oilseed rape sampled in our study). This may be particularly important in intensive agricultural areas with little alternative forage. Assuming all nests are located within the 700m landscape radius measured, a nest would be located on average every 19-30m of field boundary (although some colonies may be coming from further afield). However, oilseed rape fields are usually treated with pesticides which are applied both as a seed treatment and sprayed on the crop (DAFF 2004, Appendix 6). These pesticides can then be found in the nectar and pollen of not only the crop, but also in plants growing nearby (e.g. Krupke *et al.* 2012). Although lethal levels of most pesticides for bees are now avoided, recent work has shown that neo-nicotinoid pesticides used as seed treatments on oilseed rape can have sub-lethal effects on colony growth and queen production in bumblebees (Whitehorn *et al.* 2012). Thus, given the large number of colonies found foraging in these fields and potentially exposed to these pesticides, more research into pesticide effects and a more sustainable management of oilseed rape may be beneficial.

Interestingly, I found no difference between our estimates of colony density of *B. lapidarius* and previous estimates from other studies. *B. lapidarius* was only found foraging in oilseed rape at most sites, and not in the surrounding landscape. Therefore, it seems that most *B. lapidarius* individuals within the vicinity of a mass flowering resource will use that resource exclusively. I estimated significantly more colonies of *B. terrestris* per km^2 than in previous work. This could be for two reasons; either there is a higher background number of *B. terrestris* colonies in Ireland than in the UK and Germany where previous studies have been carried out, or that *B. terrestris* will fly longer distances than it would normally to exploit a mass flowering crop, therefore inflating the colony density estimates. *B. terrestris* has been found to be able to fly large distances on occasion (Goulson & Stout 2001) and to quickly complete its colony cycle when growing next to a mass flowering crop (Goulson *et al.* 2002). In addition, it is known that bees can change their foraging distance according to floral availability (Carvell *et al.* 2012); this all suggests that perhaps *B. terrestris* could fly further to make use of the forage available in a mass flowering resource.

Although I based our sample sizes on those used in previous studies, low numbers of sister pairs were found in all landscapes studied. In some sites, no sister pairs were identified, preventing any total colony density estimations from CAPWIRE. Therefore, the estimates of colony densities have large confidence intervals (**Appendix 12**), due to the uncertainty caused by the low number of sister pairs found in relatively large populations (Miller *et al.* 2005). However, although estimates using the TIRM method can be 1.4 times higher than previous methods (Goulson *et al.* 2010) I think it is most likely that our colony density estimations are conservative for two reasons: 1) as I had to exclude sites with no sisters from further analyses, I most likely excluded sites with larger numbers of colonies that I was not able to detect and 2) estimations of colonies were based on bees found in an oilseed rape field and in most cases an adjacent field also. Therefore, some sisters pairs were found outside the oilseed rape field itself and so estimates of colony densities using a mass flowering resource may be lower than I would expect. Larger sample sizes may have allowed detection of more sister pairs and reduced the wide confidence intervals of estimations; however sample sizes were chosen based on the results of previous studies (e.g. Knight *et al.* 2009; Charman *et al.* 2010; Goulson *et al.* 2010)

4.5.3 Influence of surrounding landscape

The cryptic species were affected differently by elements in the surrounding landscape, which suggests they may have different ecological requirements. The relative proportions of *B. lucorum* and *B. terrestris* individuals in samples were related only to the size of adjacent fields, and not any other landscape characters. Interestingly, proportions of these species were also related to each other but not to *B. cryptarum*; this possibly suggests an interaction between these species, or competition for similar resources. I found higher estimated numbers of *B. terrestris* colonies further from the sea, and in landscapes with a higher number of soil types, while colony density estimates of *B. lucorum* were related to area of arable land (or forestry), distance to forestry, number of soil types and elevation. Both *B. lucorum* and *B. terrestris* are ground nesters and so it is not surprising that the number of colonies is affected by number of soil types; more soil types may mean a wider variety of different nesting places, or may mean an increase in a particular soil type that is good for nesting. Previous work has also shown *B. lucorum* to be less common at higher altitudes (Murray *et al.* 2008). It is likely that many studies have not been able to distinguish between all individuals of *B. terrestris* and *B. lucorum* and the rest of the *B. s str*. group due to unreliability of colour characteristics (Wolf *et al.* 2010), and so therefore subtle habitat associations have yet to be teased out. However, the colony density estimates of *B. terrestris* and *B. cryptarum* in this study were correlated with the number of individuals sampled; this suggests that adding more individuals to the sample would increase estimates, and therefore landscape associations with this species in particular should be interpreted with caution.

In comparison, proportions of *B. cryptarum* were positively associated with the amount of grassland in the surrounding landscape (which in this region was mainly pasture with some silage cutting), and were best explained by a model including the amount of arable land, artificial surfaces, mass flowering crops and distance to nearest urban area. Colony densities of *B. cryptarum*, in a similar way to the proportional data, were related to the amount of artificial surfaces in the surrounding landscape. Not only does this suggest that *B. cryptarum* responds differently to the other two species, but that it is associated with grassland which is probably less intensively managed in the study area, and that it is less common intensive arable land and areas with more human habitation and interference.

B. terrestris colony density estimates did not respond to the composition of the surrounding landscape; therefore it is possible that this species may respond to some landscape factors at a larger spatial scale than measured here. Although I based our landscape radius selection on estimated average foraging ranges, *B. terrestris* has also been shown to be able to return home after being released 9.8 km from its nest, and to fly back out to forage at a site 4.3km away (Goulson & Stout 2001). This suggests that, at least in these habitats in Ireland, this species has a larger foraging range than both *B. lucorum* and *B cryptarum* (both of which responded to measures of landscape composition at the radius measured), or that *B. terrestris* at least flies further distances than these species to access a mass flowering resource.

The distribution of *B. lapidarius* in agricultural areas in Ireland appears to be patchy, with no individuals found in some fields sampled and very large numbers being found in others. This species is classified as Near Threatened (NT) in the Irish red list (Fitzpatrick *et al.* 2006), although in this study I find it locally abundant. I found higher numbers of *B. lapidarius* colonies further from woodlands and nearer urban areas. Urban areas and gardens have been found to provide important resources for bumblebees (Goulson *et al.* 2002). Previous work has also shown woodland to affect colony densities of *B. lapidarius* (Goulson *et al.* 2010), with negative effects on colony survival and colony densities later in the season, suggesting that perhaps woodland acts as a barrier to *B. lapidarius* in the landscape. *B. lapidarius* queens have also been found to prefer nest searching in open habitats and not along forest boundaries (Svensson *et al.* 2000), suggesting that woodlands and their boundaries do not provide nesting resources for this species.

4.5.4 Conclusion

I found differences in proportions and colony densities of the *B. s. str.* group using oilseed rape as a resource, and differential impacts of landscape on these species and *B. lapidarius,* suggesting that they have different ecological requirements. For example, *B. cryptarum* appeared to be less common in areas with more arable land, mass flowering crops and human interference. This knowledge may help conservation efforts targeted to conserve this species, or may help to predict the distributions of the cryptic species which are not well known. I also found large numbers of bumblebee colonies using oilseed rape fields as a resource. This suggests that mass flowering crops provide important forage for pollinators within agricultural areas, but it also highlights the possible severity of any negative effects of pesticides on bumblebee populations. A more sustainable management of this crop should be considered, with less pesticide input and appropriate spraying times, until more is known about pesticide effects on bumblebees.

Chapter 5

Pollinator sharing between a mass flowering bioenergy crop and co-flowering wild plants; implications for pollination services

To be submitted as: Stanley, D.A. & Stout, J.C. Pollinator sharing between a mass flowering bioenergy crop and co-flowering wild plants; implications for pollination services. *GCB Bioenergy*, special issue on bioenergy and biodiversity.

5 Pollinator sharing between a mass flowering bioenergy crop and co-flowering wild plants; implications for pollination services

5.1 Abstract

Oilseed rape production in Europe is increasing due to demands for biofuels to meet current bioenergy targets. However, crop yields and market value are improved with insect pollination. Pollinators are often associated with semi-natural habitats in farmland, including field margins and hedgerows, although little is known as to whether insects concurrently use mass flowering crops and wild plants as forage and whether this has implications for pollination services and fecundity of wild species. I wanted to investigate 1) whether oilseed rape and wild plants in field margins and hedgerows share the same pollinators, 2) whether the same individuals visit both the crop and the wild plants and 3) whether oilseed rape pollen becomes deposited on the stigmas of the wild plants. To do this, I carried out focal observations of visitors to flowers of winter oilseed rape and co-flowering wild plants, collected insects for analysis of pollen carried on their bodies, and examined pollen deposited on wild plant stigmas. I found that all insect taxa (bumblebees, hoverflies, solitary bees and other flies) visited oilseed rape and wild plants, and the majority of individuals carried both crop and wild species pollen on their bodies, indicating they visited both whilst foraging. More than half of the insects visiting oilseed rape also carried pollen from wild species, and on average insects carried more than two different pollen types. However, very little oilseed rape pollen was deposited on wild plant stigmas. This shows that 1) field margins and hedgerows are important sources of alternative forage for pollinating insects even when a mass flowering crop is in flower in spring and 2) that limited crop pollen deposition on wild plant stigmas is unlikely to cause reductions in seed set of wild plants. Therefore I can suggest the maintenance and augmentation of field margins and hedgerows to provide alternative forage for pollinators to continue provision of pollination services to entomophilous mass flowering bioenergy crops.

5.2 Introduction

Current demands for bioenergy are resulting in increased production of oilseed rape (or canola, *Brassica napus* L.) in Europe and worldwide (CSO 2011a; EEB 2011). Although conventionally grown for vegetable oil or animal feed, oilseed rape is now being increasingly produced for use as a liquid biofuel, either directly as pure plant oil or through conversion to biodiesel (Frondel & Peters 2005; Rowe *et al.* 2009). Although partially wind pollinated, oilseed rape increases in yield and market value with insect pollination (Bommarco *et al.* 2012). However, global declines in many pollinator groups have led to concerns over the stability of crop yields faced with pollinator decline (Allen-Wardell *et al.* 1998) which provides an economic incentive to conserve pollinators in agricultural regions (Gallai *et al.* 2009).

In agricultural areas, beneficial insects are often associated with field margins and their associated hedgerows (Croxton *et al.* 2002; Pollard & Holland 2006; Hannon & Sisk 2009; Morandin *et al.* 2011), and these areas are often some of the only remaining suitable forage and nesting habitats for pollinators left in farmland (Lye *et al.* 2009). Although mass flowering resources differ from cereal crops in that they provide forage resource for pollinators, pollinating insects are often found more abundantly in field edges than field centres of both oilseed rape fields (Chapter 2), and other mass flowering crops such as almonds (Klein *et al.* 2012). As a result, stability of flower visitor richness, visitation rate and fruit set in mass flowering crops can all decrease with increasing distance from natural areas (Garibaldi *et al.* 2011). Pollinators may be more abundant in field edges due to their proximity to alternative forage resources in the margins and hedgerows. Previous studies have investigated pollination services within mass flowering crop fields (Hayter & Cresswell 2006; Rader *et al.* 2009), but whether pollinators forage exclusively on mass flowering crops or supplement their diets with alternative pollen sources from the margins and hedgerows is not yet known. This has implications for the persistence of pollinators in agricultural regions, but also for the sustained provision of pollination services to mass flowering crops.

If pollinators visit both mass flowering crops and wild plant species in the field margins and hedgerows, there is also the potential for the crop to interfere with pollination services to the wild plants (Morales & Traveset 2008; Cussans *et al.* 2010). As the majority of flowering plant species are limited in reproduction by the amount of pollen they receive (Burd 1994), decreases in pollination services and the resulting effects on plant fecundity can be an early step in the decrease of plant populations (Aizen *et al.* 2002), which can have knock-on negative feedback on pollinators. Plants can affect the pollination of co-flowering species in two ways; by affecting visitation rates, or by affecting how pollen is transferred from one individual to another. Pollen can be lost as a result of pollen transfer between species (interspecific pollen transfer, Morales & Traveset 2008); this can be due to loss of pollen through movement on or between flowers of different species, or due to deposition of pollen on heterospecific flowers (Murcia & Feinsinger 1996; Brown & Mitchell 2001; Muchhala & Thomson 2012), with consequences for both male and female fitness. Therefore interference by a mass flowering crop in wild plant pollination can have implications for the persistence of wild plant species in agricultural areas where they provide a number of other ecosystem services (Jacobs *et al.* 2009; Isbell *et al.* 2011; Quijas *et al.* 2012), including the provision of alternative forage resources for pollinating insects.

Previous work investigating the impacts of mass flowering species (mostly invasive aliens) on pollination services to less abundant species have found predominantly negative effects (Bjerknes *et al.* 2007; Morales & Traveset 2009). Previous studies on the impacts of mass flowering crops on pollination of co-flowering species has been limited to effects on seed set of wild plants. One wild hedgerow species, *Lotus corniculatus* was shown to have increased seed set when grown beside oilseed rape in the UK, while there was no effect on *Glechoma hederacea* (Cussans *et al.* 2010). *Primula veris* was also shown to have reduced seed set when oilseed rape occurred in low densities in the surrounding landscape (Holzschuh *et al.* 2011), while no effect of oilseed rape was found on seed set of *Trifolium pratense* (Diekotter *et al.* 2010). However, the mechanisms behind these species specific results are not clear. Although pollen transfer between flowers within mass flowering crops has been investigated (e.g. Hayter & Cresswell 2006; Rader *et al.* 2009), pollen transfer dynamics between mass flowering crops and wild plants have not been investigated previously and it is not known if mass flowering crop pollen can become deposited on wild plant stigmas. As there are differences in pollinator efficiencies and the amounts of pollen carried by different pollinator species (Rader *et al.* 2009; Rader *et al.* 2011), different pollinator groups may have different potentials to be involved in the transfer of pollen between crops and wild species.

The aim of this study was to investigate whether pollinators in mass flowering crops also use resources in adjacent margins and hedgerows, and whether this has implications for the pollination services to these wild plants. Specifically I tested i) whether mass flowering oilseed rape crops share flower visitor taxa with co-flowering wild plants, ii) whether the same individual insects visited both the crop and wild species and iii) whether oilseed rape pollen becomes deposited on wild plant stigmas. I also investigated whether there were differences in floral preference and pollen loads of different flower visiting insect taxa.

5.3 Methods

5.3.1 Site selection

In summer 2010, two winter oilseed rape fields were selected in South-East Ireland, an area where arable, beef and dairy farming are interspersed, in Ballyhamilton, Co. Wexford (field A) and Ballymurphy, Co. Carlow (field B, **Appendix 11**). Only two fields were selected due to the intensive nature of sampling, but more than one was chosen to account for some between field variation. Fields were not close to any other type of mass flowering crop (e.g. field beans or peas), and were not close to each other (17km apart). In each field, a south facing focal hedgerow and field margin was selected with the highest number of wild species co-flowering with oilseed rape; *Stellaria holostea*, *Lamium purpureum*, *Ulex europeus* and *Fumaria* spp. in field A, and *Ranunculus repens, Stellaria holostea*, *Veronica chamaedrys* and *Vicia sepium* in field B (Figure 5.1).

Figure 5.1. The focal species. From top left to bottom right: *Fumaria* spp., *Ulex europeus*, *Vicia sepium*, *Lamium purpureum*, oilseed rape (*Brassica napus)*, *Veronica chamaedrys*, *Ranunculus repens* and *Stellaria holostea*.

5.3.2 Focal observations

To examine whether the same insect taxa foraged on the crop and on wild plants in the margins, each field was visited seven times between 8th May and 14th June, spanning the period when oilseed rape was in flower. On each visit to each field, six approximately 30 x 30 cm sized patches of each focal species and oilseed rape itself were observed, within a 150m transect along the south-facing field margin with adjacent hedgerow. The number of flowers of the focal species in each patch was counted before each observation period. Each patch was observed for 5 minutes and any visiting insects recorded (no a priori decision was made as to which insects were pollinators, and all flower visitors were recorded except for pollen beetles (*Meligethes aeneus*) who were not observed frequently moving between flowers), resulting in a total observation time of 210 minutes for each focal species. Observations were carried out between 10am and 5pm on dry, bright days (average temperature 18.7˚C, average wind 2 on Beaufort scale)

Bumblebees and butterflies were identified to species level (except for members of the Bombus sensu stricto group which are impossible to reliably distinguish morphologically, Wolf *et al.* 2010; Carolan *et al.* 2012), hoverflies and solitary bees were identified to genus (*Eristalis* including: *E. abusivus, E. arbustorum, E. horticola, E. interruptus, E. pertinax,* *E. tenax* and *Eristalinus sepulchralis*; *Helophilus* including: *H. hybridus* and *H. pendulus*; *Platycheirus/Melanostoma* including: *P. albimanus*, *P. scutatus* and *P. granditarsus*; and *Cheilosia* including: *C. albitarsus*, *C. antiqua* and *C. pagana*) and other flies were grouped to morpho-groups.

5.3.3 Insect pollen loads

On each visit to each field, insects were captured for analysis of pollen loads to identify i) if the same individuals visited both oilseed rape and wild species and ii) to quantify the abundance and number of types of pollen they were carrying. For every insect group (bumblebees and honeybee to species, solitary bee and hoverfly to genus) observed in the focal observations visiting a particular plant species, I aimed to catch five individuals performing that same interaction for pollen analyses. Insects were caught straight into clean plastic vials and were cooled immediately and frozen the same day. In the lab, each individual was systematically swabbed with a 2.5mm^3 cube of fuchsin-stained gel (Dafni 1992), avoiding pollen storage areas (i.e. corbiculae) on bees, as pollen stored here is unlikely to be available for pollination. Gel was melted onto a slide, covered with a cover slip and sealed using nail varnish. All pollen grains were then identified and counted on each slide under x400 magnification, using both a reference collection collected from each field and other resources (Sawyer 1981; Moore *et al.* 1999; Chandler & Rennison 2005). Although this method did not give a count of the total pollen insects were carrying, it gave a measure of relative pollen density. As it is not possible to identify many pollen types to species level, I named pollen grains according to what species were present in the reference collection from the surrounding area, or to broader groupings (e.g. genus or family) when a number of similar species were present. Therefore it is possible that pollen identified to species level may have come from a closely related species not in the reference collection, and so pollen identifications are referred to as “types” rather than “species”. Insects may pick up heterospecific pollen in the environment without actually visiting that species; therefore I only counted species with more than 5 pollen grains present on a particular insect as being visited (this level was set in accordance with work with a monolectic species; Bosch *et al.* 2009). All swabbed insects were identified (bees and hoverflies to species level, other flies to morpho-species).

5.3.4 Stigmatic pollen loads

To investigate whether oilseed rape pollen becomes deposited on wild plant stigmas, stigmas were also collected from each focal plant species in each field during two visits at peak flowering of oilseed rape. Stigmas were collected from along the whole transect at the end of the day to ensure that flowers had been open for at least a full day to allow time for visitation. Twenty stigmas were collected from each focal species (ten on each visit). In the laboratory, stigmas were stained using 0.5% safranin in 50% alcohol and squashed onto a microscope slide under a cover slip. Pollen grains on the stigmas were then identified as self pollen, heterospecific pollen or oilseed rape, and abundance counted (counts may not have been comprehensive as some grains may have been obscured under bits of stigmatic tissue). *Stellaria holostea* has 3 stigmas, and pollen grains were counted on all three. *Ranunculus repens* has many carpels and two were examined per individual flower for pollen.

5.3.5 Data analyses

To visualise pollinator sharing, bipartite interaction networks were plotted for both the visitation data and pollen load data from both fields using the Bipartite package (Dormann *et al.* 2008) in R (R Development Core Team 2008). Visitation networks were created from quantitative matrices containing total counts of the numbers of visits of each observed interaction between pollinator group and plant species, and pollen transport networks were constructed from matrices including total counts of pollen grains of each plant species on each pollinator group. The “network level” command was used to calculate network indices of connectance (the realised proportion of links in a network) and links per species (mean number of species a species is connected with) to compare between visitation and pollen networks, and the “species level” command was used to calculate species degree (the sum of interactions per species), strength (the sum of dependencies of each species, Bascompte *et al.* 2006), partner diversity (Shannon diversity of the interactions of each species) and number of effective partners of oilseed rape and the different focal plant species in the network (Dormann *et al.* 2008).

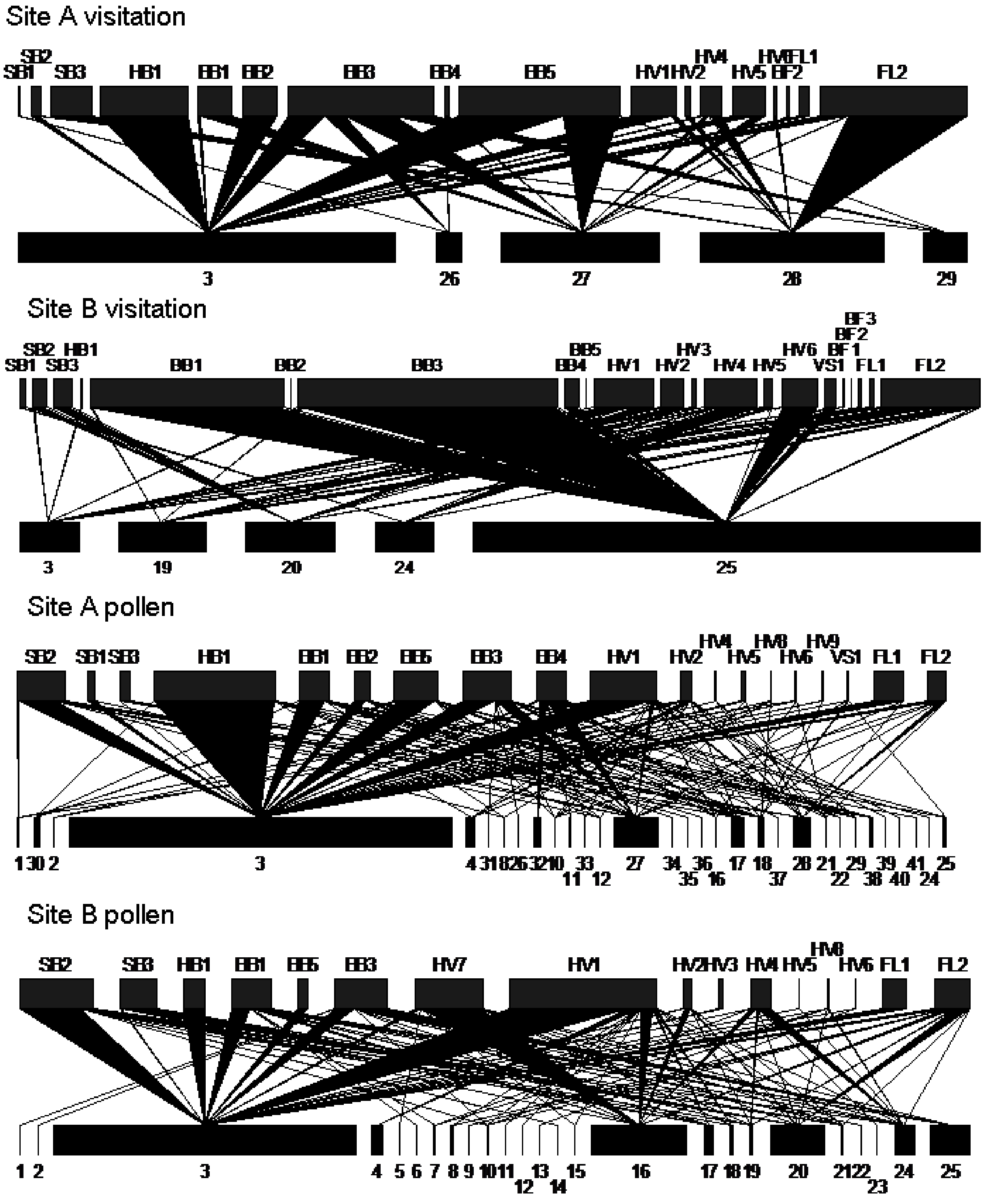
Differences in number of pollen types carried, the total abundance of pollen grains carried and the abundance of oilseed rape pollen carried were also investigated between insect taxa (bumblebees, solitary bees, honeybee, hoverflies and other flies) and between insects caught foraging on the oilseed rape and on wild species using general linear models (GLMs). Total numbers of pollen grains and total amount of oilseed rape pollen were log transformed in both fields to achieve assumptions of normality and homogeneity of variance. A poisson distribution was specified for models of the number of pollen types. Models were validated by visual inspection of residuals. All analyses were carried out in R, and post-hoc comparisons between insect groups were made using the multi-comp package (Hothorn *et al.* 2008). Permutational multivariate analysis of variance (PERMANOVA) was also used to test for differences in pollen load composition between the insect taxa in each field using the programme PRIMER (Clarke & Gorley 2006), with type III sums of squares and based on 9999 permutations of residuals. Data were square root transformed to down-weight the contributions of dominant species, and a Bray Curtis similarity matrix was constructed. SIMPER analysis was used to assess which pollen types contributed to the similarity within groups (Clarke 1993).

5.4 Results

Across both fields, a total of 1135 flower visits were recorded (212 to oilseed rape and 923 to wild plant species) in 52.5 hours of focal observations. For pollen analyses, 236 insects were examined (223 of which carried more than 5 pollen grains) and approximately 296,000 pollen grains counted and identified of 31 pollen types in field A and 25 pollen types in field B (Figure 5.2). Pollen networks were bigger in size than visitation networks with a higher number of links per species but lower connectance (Figure 5.2, Table 5.1).

5.4.1 Pollinator sharing

In both fields, all insect taxa were observed visiting oilseed rape (bumblebees, honeybee, solitary bees, hoverflies and other flies; only three butterflies were observed in total and so this group is not discussed further), and all but the honeybee also visited at least two of the wild focal plant species in the margins, showing a large degree of pollinator sharing between oilseed rape and wild plant species (Figure 5.2, Figure 5.3). Pollen data showed that 59% of all insect individuals caught foraging on oilseed rape carried also carried pollen from wild plants (including *Lamium purpureum*, *Aesculus hippocastanum,* Rosaceae sp. and *Salix),* which on average made up 8% (range 0-83%) of pollen loads, while 73% of insects caught foraging on wild plants carried oilseed rape pollen. In general insects did not specialise on a single flower type, and carried a mean of 2.6 ± 0.12 (standard error) different pollen types. Although social bees can pick up small amounts of pollen collected by other individuals in the nest (Paalhaar *et al.* 2008), I assumed this to be negligible. Therefore visitation and pollen data confirmed that oilseed rape shares pollinators with all the wild plant species studied; and that the same insect individuals visit both the crop and wild flowers.


Site A visitation

Site B visitation

Site A pollen

Site B pollen

Figure 5.2. Visitation (top) and pollen transfer (bottom) networks from the area sampled in Site A and Site B. See legend below. Plant species no. 3 = oilseed rape.

	Code	Insect species	Code	Plant species/pollen types
Bumblebees	BB1	*Bombus hortorum*	1	*Acer/Prunus* sp.
	BB2	*Bombus lapidarius*	2	Asteraceae (excluding *Taraxacum)*
	BB3	*Bombus pascuorum*	3	Oilseed rape *(Brassica napus)*
	BB4	*Bombus pratorum*	4	*Capasella bursa-pastoris*
	BB5	*Bombus sensu stricto*	5	*Chenopodium*
Butterflies	BF1	*Parage aegeria*	6	*Cerastium fontanum*
	BF2	*Pieris brassicae*	7	*Digitalis purpurea*
	BF3	*Pieris napis*	8	*Filipendula ulmaria*
Flies	FL1	*Fly morpho-type A*	9	Poaceae
	FL2	*Other flies*	10	*Heracleum sphondyllium*
	HB1	*Apis mellifera*	11	*Hippophae rhamnoides*
Hoverflies	HV1	*Eristalis* sp.	12	*Ilex aquifolium*
	HV2	*Helophilus* sp.	13	*Ligustrium vulgare*
	HV3	*Other hoverfly*	14	*Lilium* sp.
	HV4	*Platycheirus/Melanostoma spp.*	15	*Quercus* sp.
	HV5	*Rhingia campestris*	16	*Ranuculus repens*
	HV6	*Syrphus ribesii*	17	Rosaceae (often *Craetagus* type)
	HV7	*Cheilosia* sp.	18	*Salix* sp.
	HV8	*Sphengia clunipes*	19	*Ranunculus* sp. 2
	HV9	*Volucella* spp.	20	*Stellaria holostea*
Solitary bees	SB1	*Andrena cineraria*	21	*Taraxacum* sp.
	SB2	*Andrena* sp.	22	*Trifolium repens*
	SB3	*Nomada marshamella*	23	*Brassica* sp. 2
Wasps	VS1	*Vespidae*	24	*Veronica chamaedrys*
			25	*Vicia sepium*
			26	*Fumaria* spp.
			27	*Lamium purpureum*
			28	*Stellaria holostea*
			29	*Ulex europeus*
			30	*Aesculus hippocastanum*
			31	*Cardamine pratensis*
			32	*Geum urbanum*
			33	*Hyacinthoides non-scripta*
			34	*Lonicera periclymenum*
			35	*Pinus* sp.
			36	*Primula vulgaris*
			37	*Succisa pratensis*
			38	morpho-species 1
			39	morpho-species 2
			40	morpho-species 3
			41	morpho-species 4

Of all the focal flowering species, oilseed rape had the highest partner diversity and number of effective partners in pollen and visitation networks from both fields, and also highest species degree and strength in both pollen networks and visitation networks from field A only, showing oilseed rape was visited by a wide range of pollinators (Table 5.1). As a result, oilseed rape was very dominant in networks from both fields (Figure 5.2). However, other focal species were less generalist in their visitor species; *Fumaria* spp., *Lamium purpureum*, *Ulex europeus* and *Vicia sepium* were predominantly visited by bumblebees, whereas *Ranunculus* spp., *Stellaria holostea* and *Veronica chamedrys* were mainly visited by flies, hoverflies and solitary bees.

Table 5.1. Network parameters for each species, and overall, in both visitation (V) and pollen (P) networks for each site.

Field A	Oilseed rape		*Fumaria* spp.		*Lamium purpureum*		*Stellaria holostea*		*Ulex europeus*	
	V	P	V	P	V	P	V	P	V	P
partner diversity	2	2.2	0.6	0	1.5	1.6	1	1.3	0.4	1.2
no. effective partners	7.4	8.7	1.9	1	4.6	4.9	2.9	3.7	1.5	3.2
species degree	11	19	3	1	8	13	6	8	3	5
strength	6.7	12.7	1.6	0.0004	3.6	1.9	2.2	0.002	0.5	0.05

Field A	V	P
Overall connectance	0.36	0.2
Overall linkage density	3.64	4.7

Field B	Oilseed rape		*Ranunculus repens*		*Stellaria holostea*		*Veronica chamedrys*		*Vicia sepium*	
	V	P	V	P	V	P	V	P	V	P
partner diversity	1.66	1.9	1.45	1.3	1.53	1	1.03	1.2	1.12	0.6
no. effective partners	5.26	6.6	4.27	3.8	4.62	2.8	2.79	3.3	3.08	1.8
species degree	10	16	10	6	6	8	5	9	7	3
strength	4.97	8.8	2.94	1	2.78	1.6	2.25	1.8	5.05	0.8

Field B	V	P
Overall connectance	0.4	0.2
Overall linkage density	2.6	3.6

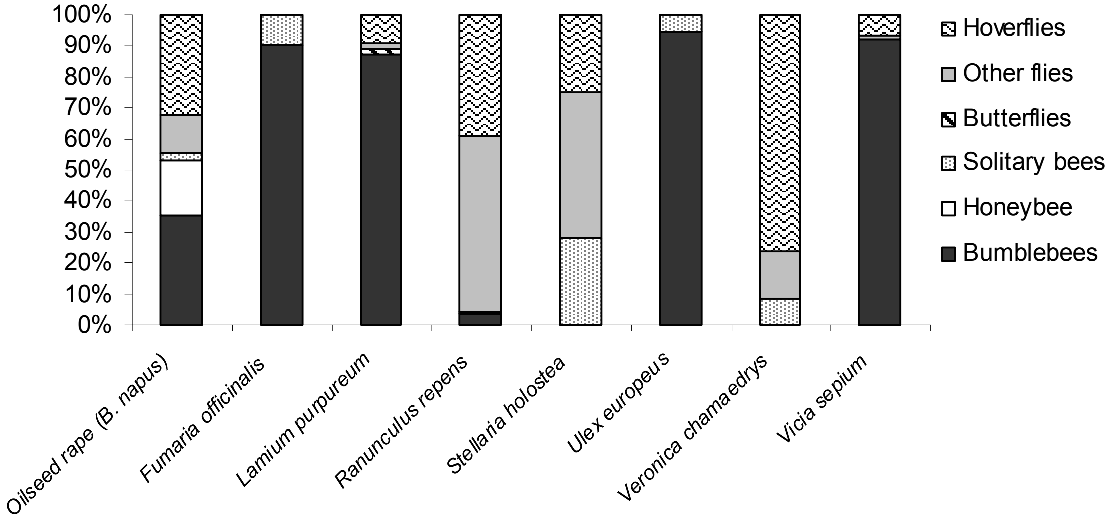


Figure 5.3. The overlap of different pollinator groups between oilseed rape and wild species. The data represented are proportion of visits of each pollinator group to each plant species. Data are from both fields combined.

In addition, there was also variation within pollinator groups in their visitation; the long tongued bumblebees (*B. hortorum* and *B. pascuorum*) mostly visited *V. sepium* flowers, whereas short tongued bees (*B. lapidarius* and *B. sensu stricto*) predominantly visited oilseed rape (Figure 5.4). The hoverfly genus *Eristalis* mainly visited oilseed rape while also visiting *S. holostea,* and *Helophilus* and *Platycheirus* groups visited the crop and a number of other flower types including *R. repens* and *V. chamaedrys* (Figure 5.5).

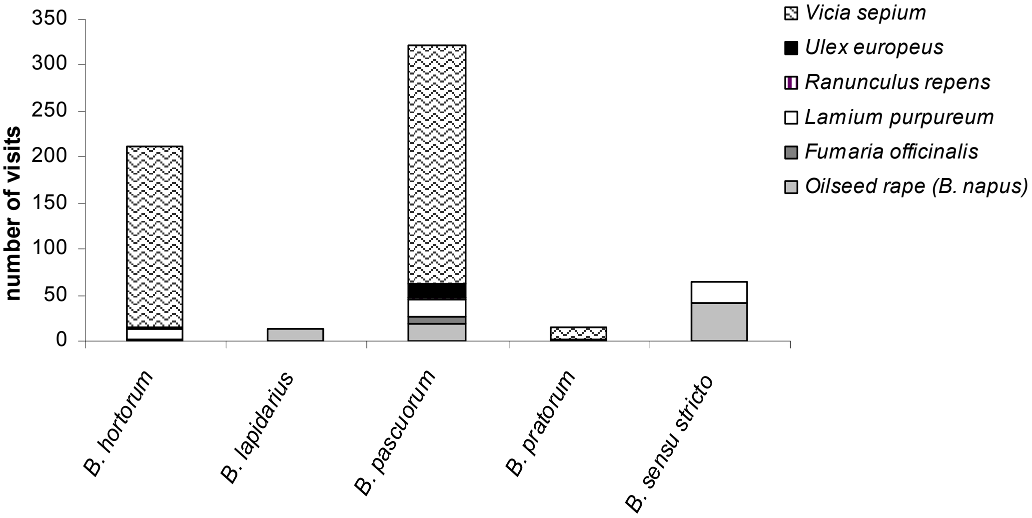


Figure 5.4. The number of visits (to individual floral units) of the different bumblebee species to oilseed rape and wild plant species. Data are totals of all visits observed from both fields combined.

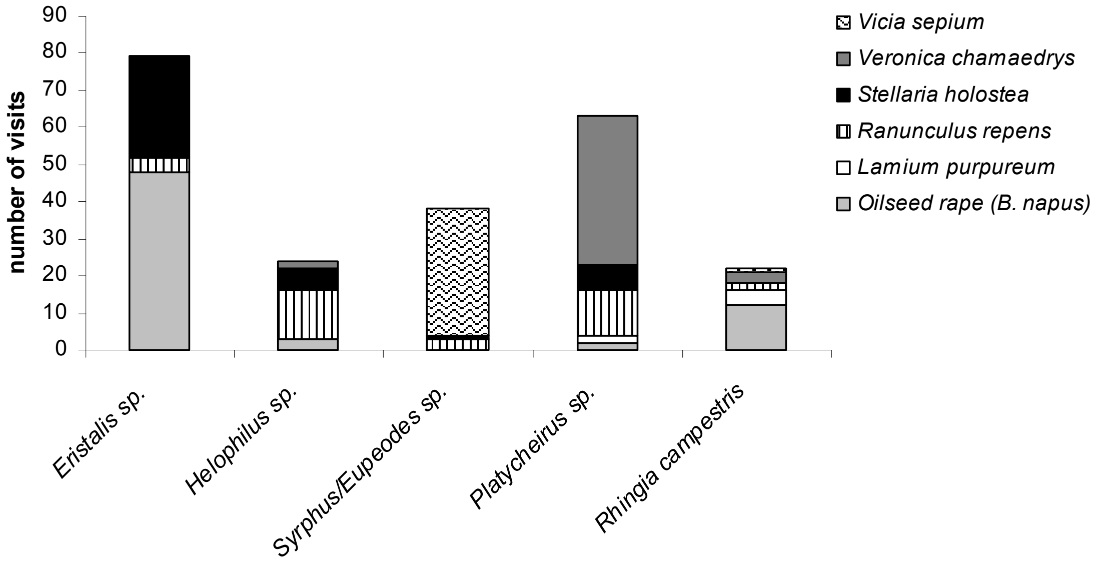


Figure 5.5. The number of visits (to individual floral units) of the different hoverfly groups to oilseed rape and wild plant species. Data are from both fields combined.

The composition of pollen confirmed that oilseed rape was important for defining similarities in pollen loads within all insect taxa, but that wild species were also important. Pollen load composition differed significantly between the different insect groups in both fields (PERMANOVA, field A: Pseudo-F=4.15, p=0.0001, field B; Pseudo-F=4.03, p=0.0001), with all groups differing significantly from each other in field A, and all except the honeybee and solitary bees in field B. Using Simper analyses, in field A oilseed rape pollen contributed to the similarity in pollen loads within each group (64-99%, Table 5.2), and although it was the only species contributing to similarities of hoverflies (97%) and the honeybee (99%), wild species were important in similarities of pollen loads of the other insect groups (Table 5.2). In field B oilseed rape also contributed to the similarity within each group (39-100%, Table 5.2), but again wild species were also important (Table 5.2).

Table 5.2. Total abundance of pollen types found on the bodies of different pollinator taxa, and results of SIMPER analysis, from Field A (top) and Field B (bottom). Figures highlighted in grey represent the species contributing to overall similarities within pollinator taxon. Identification of pollen types were based on the species observed flowering in the area; however, as some pollen grains cannot be identified to species level some types may incorporate closely related species.

pollen types Field A	Bumblebee n=41	Honeybee n=8	Solitary bee n=6	Hoverfly n=27	Other flies n=26
Acer/Prunus sp.	0	0	745	82	0
Aesculus hippocastanum	1364	574	0	0	0
Asteraceae (excl. *Taraxacum*)	0	0	0	101	0
Brassica napus	40703	43456	16119	26201	15669
Capasella bursa-pastoris	0	149	0	2702	0
Cardamine pratensis	100	0	0	0	0
Filipendula ulmaria	6	0	23	49	0
Fumaria spp.	71	0	0	0	0
Geum urbanum	2543	0	0	0	0
Heracleum sphondyllium	34	0	0	50	22
Hippophae rhamnoides	203	0	0	0	0
Hyacinthoides non-scripta	15	0	0	24	0
Ilex aquifolium	172	19	5	9	0
Lamium purpureum	14536	159	555	311	42
Lonicera periclymenum	60	0	0	0	0
Pinus spp.	0	5	0	0	0
Primula vulgaris	18	0	0	0	0
Ranunculus spp.	13	0	0	40	0
Rosaceae	2010	0	2511	151	14
Salix sp.	361	524	7	534	0
Succisa pratensis	40	0	0	0	0
Stellaria holostea	0	0	3782	1334	1752
Taraxacum sp.	202	0	0	23	18
Trifolium repens	20	0	0	0	0
Ulex europeus	168	0	0	71	0
morpho-species 1	22	0	6	0	0
morpho-species 2	0	0	0	7	0
morpho-species 3	0	0	0	5	0
morpho-species 4	0	6	0	0	0
Veronica spp.	6	0	0	0	0
Vicia sepium	67	0	0	0	0
Within group similarity (%)	35.7	75.3	27.82	35.57	29.09

Between group dissimilarity (%)		
	Bumblebee - Fly	76.26
	Bumblebee - Honeybee	63
	Fly - Honeybee	74.91
	Bumblebee - Hoverfly	67.04
	Fly - Hoverfly	70.76
	Honeybee - Hoverfly	65.25
	Bumblebee - Solitary bee	74.42
	Fly - Solitary bee	76.07
	Honeybee- Solitary bee	59.67
	Hoverfly - Solitary bee	74.24

pollen type field B	Bumblebee n=20	Honeybee n=4	Solitary bee n=9	Hoverfly n=50	Other flies n=30
Acer/Prunus sp.	53	0	0	0	0
Asteraceae *(*excl. *Taraxacum)*	14	0	0	0	0
Brassica napus	9776	5162	13198	27876	6956
Brassica sp. 2	0	0	0	74	0
Capasella bursa-pastoris	0	0	0	2205	0
Chenopodium sp.	0	0	0	14	0
Cerastium fontanum	13	0	0	0	0
Digitalis purpurea	133	0	0	87	0
Filipendula ulmaria	0	0	0	123	7
Poaceae	0	0	0	0	43
Heracleum sphondylliym	16	0	14	364	31
Hippophae rhamnoides	0	0	0	9	0
Ilex aquifolium	0	0	0	14	0
Ligustrium vulgare	0	0	0	22	0
Lilium sp.	0	0	61	0	0
Quercus sp.	0	0	0	36	0
Ranuculus repens	1572	0	221	16578	1589
Ranunculus sp. 2	61	0	50	474	135
Rosaceae sp.	568	0	1065	351	0
Salix sp.	0	0	24	507	0
Stellaria holostea	0	0	7746	439	3116
Taraxacum sp.	52	0	224	149	18
Trifolium repens	29	0	0	0	0
Veronica chamaedrys	395	0	153	3215	206
Vicia sepium	8458	0	0	7	0
Within group similarity (%)	42.29	69.27	29,54	24.05	31.8
Between group dissimilarity (%)	Bumblebee - Fly	79.47			
	Bumblebee - Honeybee	63.38			
	Fly - Honeybee	67.44			
	Bumblebee - Solitary bee	77.5			
	Fly - Solitary bee	78.33			
	Honeybee - Solitary bee	63.11			
	Bumblebee - Hoverfly	78.99			
	Fly - Hoverfly	74.32			
	Honeybee - Hoverfly	68.02			
	Solitary bee - Hoverfly	78			

Insect taxa varied in their capabilities to carry and transfer pollen. Bumblebees, hoverflies and solitary bees all carried more types of pollen than flies ($F_{4,216}$ =6.96, $p<0.0001$). All bees carried a higher abundance of pollen grains than hoverflies and flies, with honeybees also carrying more than bumblebees ($F_{4,216}$ =15.6 $p<0.0001$). All bees carried more oilseed rape pollen than flies, but honeybees also carried more than bumblebees or hoverflies ($F_{4,216}$ =18.88, $p<0.0001$). Insects caught foraging on wild species carried more types of pollen than those foraging on oilseed rape ($F_{1,219}$ =66.86, $p=0.009$), but lower abundances of pollen grains ($F_{1,219}$ =29.84, $p<0.0001$).

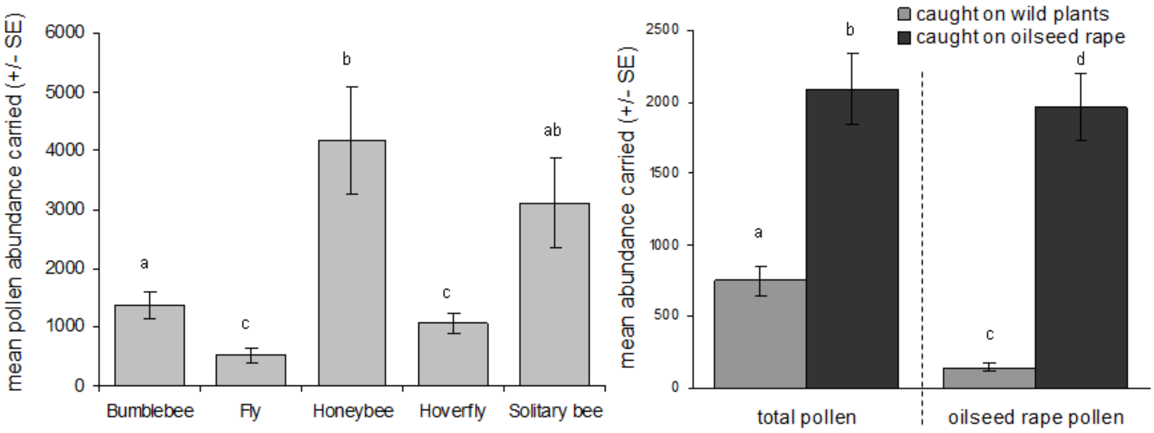


Figure 5.6. The mean abundance of pollen grains carried by the different insect groups (left), and mean abundance of total pollen grains, and oilseed rape pollen grains only, on insects caught on wild species (grey) and those caught on the oilseed rape (black, right). Significant differences ($p<0.05$) are indicated by letters.

5.4.2 Stigma pollen loads

Of the 143 stigmas examined of the seven focal species, nearly all had pollen deposited on them; only 6 had no pollen grains at all. The vast majority of all pollen (97%) was conspecific, with between 1 and 871 grains deposited per stigma. Combining data from both fields, the more closed, complex, zygomorphic flowers *Fumaria* spp., *Lamium purpureum*, *Ulex europeus* and *Vicia sepium* all had very little oilseed rape pollen on their stigmas (0, 0.25, 0.7, 0.06% respectively), whereas the simpler, actinomorphic ones had more (*Stellaria holostea* 7%, *Ranunculus repens* 0.8% and *Veronica chamaedrys* 6.4% oilseed rape pollen). However, amounts of oilseed rape pollen found were overall extremely low (between 1 and 247 grains, 3% in total).

5.5 Discussion

As oilseed rape crops are increasingly planted around Europe to meet bioenergy targets, the provision of pollination services to the crop will be come increasingly important. I found that the majority of insects foraging in the edges of oilseed rape fields do not only visit the crop, but also visit wild plant species growing in the margins and hedgerows around the crop. Although this also has implications for pollination services to these wild species, I found little crop pollen deposited on wild plant stigmas. However, this suggests that to sustain pollination services to mass flowering crops, alternative wild forage in field margins and hedgerows may be important.

Although abundance, diversity and visitation rates of pollinating insects in mass flowering crops has been linked to proximity to semi-natural features (Kremen *et al.* 2002; Garibaldi *et al.* 2011), or habitat connectivity (Steffan-Dewenter 2003), our data show that these trends may be explained by the need for alternative forage resources by insects. All pollinator taxa (except the honeybee) were observed to forage on both the crop and wild plants in the margins and hedgerows, and the majority of individuals also carried pollen from both the crop and wild species. Plant forage resources can vary in terms of their nutritional value (Baker & Baker 1986; Roulston *et al.* 2000). Although experimental studies have shown oilseed rape pollen may be a nutritionally good resource for insects to feed on (e.g. Regali & Rasmont 1995; Cook *et al.* 2003), insects may still need to supplement mass flowering crop monocultures with alternative forage resources from margins and hedgerows. Alternative forage resources may be especially important to sustain pollinator populations after the flowering on the crop; although bumblebees in field margins of mass flowering field bean were more abundant when the crop was in flower, they disappeared after flowering, presumably to exploit alternative resources (Hanley *et al.* 2011). Interestingly, honeybees were observed to exclusively visit mass flowering oilseed rape, and honeybee individuals carried high proportions of oilseed rape pollen. Honeybees are known to be very flower constant (Free 1963), but although honeybees can provide pollination services when wild bees are lacking, services to some crops have been shown to be more stable with visits from other wild pollinator species (Garibaldi *et al.* 2011; Klein *et al.* 2012).

As oilseed rape was visited by the majority of insect taxa and individuals, it was very dominant in both the visitation and pollen networks. Similar patterns have been found for mass flowering invasive species; they are also often highly connected and play central roles in visitation webs (Vila *et al.* 2009). It has been suggested that generalised pollinators can facilitate the integration of alien plants into native plant visitation systems (Lopezaraiza-Mikel *et al.* 2007). Pollinator fauna of agricultural areas is usually depauperate and composed of generalist insect species (e.g. Ekroos *et al.* 2010), and so the potential for mass flowering crops to become integrated into wild visitation systems and share pollinators is high.

However, although they shared pollinators, wild plant species were not as generalised as oilseed rape in the insects they attracted and that picked up pollen. The four species with bilaterally symmetrical flowers with relatively long corolla tubes (*V. sepium*, *U. europeus*, *Fumaria spp.* and *L. purpureum*) were predominantly visited by bumblebees whereas the three more open, shorter-flowered species (*S. holostea*, *R. repens* and *V. chamedrys*) received the majority of visits from hoverflies, other flies and solitary bees. I found more oilseed rape pollen grains deposited on the stigmas of latter. This could be due to similarities in morphology, and therefore pollen placement on pollinators bodies, between oilseed rape and the more open flowers (e.g. Morales & Traveset 2009; Gibson *et al.* 2012), higher susceptibility to pollen deposition by wind which also carries large amounts of oilseed rape pollen on more open flowers (Williams 1984), or the more open flowers may have received less targeted pollen as they are largely pollinated by less specific pollinators (hoverflies and flies). Previous studies on seed set of wild plants beside oilseed rape have investigated long corolla tubed, bee pollinated flowers only (Cussans *et al.* 2010; Diekotter *et al.* 2010; Holzschuh *et al.* 2011); the variable effects seen are therefore unlikely due to crop pollen deposition, and perhaps due to other factors such as visitation rates. Interestingly, other pollen types of non-focal plant species were also abundant on the bodies of insects, including some hedgerow trees. For example, pollen from *Salix* sp., Horsechestnut (*Aesculus hippocastanum*) and Rosaceae sp. (mostly Hawthorn, *Craetagus monogyna*) was also found in abundance. These data may indicate some other useful species for pollinators in farmland in spring, and highlights not only the importance of herbaceous plants for pollinators in farmland (Pywell *et al.* 2005), but also the importance of hedgerow trees.

Insect taxa differ in their efficiency of transferring pollen between flowers partly because they carry different amounts of pollen (Rader *et al.* 2011). In a similar way to previous work (Rader *et al.* 2011), I found bees to carry more pollen than hoverflies or flies, although these other taxa can carry large quantities of oilseed rape pollen (Jauker & Wolters 2008; Chifflet *et al.* 2011). It is commonly known that bumblebees can carry large numbers of pollen types (up to 7; Goulson *et al.* 2002, which is also the maximum found here), and bumblebees have been found to carry more types in suburban areas than in farmland in the UK where alternative forage is available (Goulson *et al.* 2002). Therefore, alternative forage resources may be more important for obligate flower visitors such as the bees, and less so for facultative flower feeders like flies.

Although wild plant species shared pollinators with mass flowering oilseed rape, very little oilseed rape pollen became deposited on wild plant stigmas. Heterospecific pollen deposition has been shown to be common in some systems (Brown & Mitchell 2001), but previous work has also found low quantities of invasive pollen on native plant stigmas (Moragues & Traveset 2005; Bartomeus *et al.* 2008a; Dietzsch *et al.* 2011). Heterospecific pollen may not have become deposited on stigmas for a number of reasons; pollen may be placed in different places on the bodies of pollinators in different species (Muchhala & Potts 2007), or the pollen adhesive capability of hetero and con-specific pollen may differ on the stigmatic surface (Zinkl *et al.* 1999). However, although oilseed rape pollen misplacement on wild plant stigmas may not occur in abundance, there could still be consequences for male fitness of both the oilseed rape and wild species if con-specific loss of pollen occurs in other ways not measured here (Muchhala & Thomson 2012), or if mass flowering oilseed rape alters visitation rates to wild plants (Diekotter *et al.* 2010).

5.5.1 Conclusion

As oilseed rape crops become more frequently planted to satisfy increasing demands for bioenergy, demands for pollination services may increase. I show that pollinators in the edges of oilseed rape crops also visit wild plant species for forage resources during crop flowering in spring. However, very little crop pollen becomes deposited on wild plant stigmas suggesting this is not a mechanism for interruption to pollination services to wild species. Our study suggests that the conservation of field margins and hedgerows in mass flowering crop fields may be beneficial for pollinating insects in provision of alternative forage, and that this is not only important after the flowering of the crop but also during. This could be aided by agri-environmental measures to increase forage availability on farmland (Pywell *et al.* 2005; Carvell *et al.* 2006b) and may help to benefit crop yields and stabilise pollination services to this expanding bioenergy crop in the future. However, oilseed rape is a high input crop in terms of pesticides (DAFF 2004, Appendix 6) which can have negative impacts on bees (Henry *et al.* 2012; Whitehorn *et al.* 2012), and can even be found in the nectar of co-flowering wild species (Krupke *et al.* 2012); therefore, perhaps a more sustainable management of the crop in terms of inputs could also have positive benefits for pollinating insects which widely use resources in the crop and adjacent wild plants.

Chapter 6

Pollinators and pollination of oilseed rape crops (*Brassica napus* L.) in Ireland

To be submitted, with additional caluculations of economic value, as: Stanley, D.A., Gunning, D. & Stout, J.C. Pollinators and pollination of oilseed rape crops (*Brassica napus* L.) in Ireland; an economic incentive for pollinator conservation. *Biology and Environment*

6 Pollinators and pollination of oilseed rape (*Brassica napus* L.) in Ireland

6.1 Abstract

Pollinators are required for the reproduction of the majority of flowering plants worldwide, but also for production of a wide variety of crops. However, although pollinators and the services they provide are in decline and under threat from agricultural intensification, knowledge of the specific pollinators and pollination requirements of certain crops in particular locations is often limited. Oilseed rape is a mass flowering crop that benefits from insect pollination and although its pollination requirements have been studied in other countries, little is known about its pollination in Ireland. We aimed to survey the flower visiting insects found in both winter and spring commercial oilseed rape fields, and investigate the contribution of insect pollination to winter oilseed rape crops. Our data show that oilseed rape in Ireland is visited by a wide variety of insect species including the honeybee, bumblebees, solitary bees and hoverflies. We also found that the crop benefits from insect pollination as more seeds are produced when flowers are visited by insects in both the edges and centres of crop fields, and that exclusion of pollinators resulted in a 28% decrease in the number of seeds produced and a 33% decrease in seed weight per pod. Pollen supplementation did not cause an increase in seed number or weight suggesting that crops currently receive sufficient pollination services, but insect pollination benefits were higher in the edges of fields than the centre. Insect visitors varied in their usefulness as pollinators, with the honeybee and bumblebees (especially *Bombus sensu stricto* and *B. lapidarius*) being the most useful pollinators of winter oilseed rape based on the number of pollen grains they carry, visitation rates per flower and their relative abundance per field (although bumblebees may be more important in spring crops due to a significantly higher abundance). We can suggest the appropriate conservation and management of both honeybees and existing wild pollinators in agricultural areas to ensure continued provision of pollination services to Irish crops.

6.2 Introduction

Pollinating insects are essential for the reproduction of the majority of flowering plants worldwide (Ollerton, Winfree & Tarrant 2011), but they are also required for pollination of a wide variety of world crops (Klein *et al.* 2007). A large portion of the human diet (Klein *et al.* 2007) and essential nutrients (Eilers *et al.* 2011) come from crops pollinated by insects, and there is also increasing dependency on these crops that are increasing in production worldwide (Aizen *et al.* 2008). It has been estimated that the value of pollination in agriculture to the world economy is €153 billion per year (Gallai *et al.* 2009). However, pollinators and the pollination services they provide are under threat from a number of areas, but primarily driven by the intensification of agriculture (Kremen, Williams & Thorp 2002; Klein *et al.* 2007). Declines in many pollinator groups have been recorded (e.g. Biesmeijer *et al.* 2006), and as a result crop pollination may be at risk with economic implications (Gallai *et al.* 2009). The honeybee is often attributed as providing the majority of pollination services, but often pollination services are largely provided by wild taxa (Winfree *et al.* 2008; Rader *et al.* 2009; Breeze *et al.* 2011; Rader *et al.* 2012), and the yield of many crops is increased by higher abundances of wild pollinators (e.g. Kremen, Williams & Thorp 2002; Garibaldi *et al.* 2011). However, surprisingly little is known about the pollinators and pollination services required by some crops (Klein *et al.* 2007).

Oilseed rape (*Brassica napus* L.) is a mass flowering crop commonly planted in Europe, USA, Canada, Brazil, India and China. Initially the crop was used for food oil and animal feed production, but it is now increasing in area planted worldwide due to its use as bioenergy crop; the pure plant oil produced can be used as a liquid biofuel or converted into biodiesel, mainly for use in the transport industry. Traditionally oilseed rape was not a common crop in the Irish landscape, but in recent years it has increased dramatically (e.g. a 99% increase in production between 2010-2011) and now accounts for approximately 4% of arable land area (CSO 2011a).

Oilseed rape is self fertile and is partially wind pollinated (Williams, Martin & White 1986), but as it produces large amounts of nectar and pollen it is visited by a wide range of insects (Bommarco, Marini & Vaissière 2012). Insect pollination has been found to increase seed yield, quality and market value in Sweden (Bommarco, Marini & Vaissière

2012), Canada (Morandin & Winston 2005; Sabbahi, De Oliveira & Marceau 2005), Germany (Jauker & Wolters 2008) and in the UK (Hayter & Cresswell 2006). There are two main forms of oilseed rape; winter oilseed rape is planted in autumn and flowers in early summer while spring oilseed rape is planted in spring and flowers later in the summer, and there are differences in the forms in terms of efficiency of pollination in the UK (Hayter & Cresswell 2006). It has been estimated that the value of pollination to the Irish economy is approximately €53 billion per year (Bullock, Kretsch & Candon 2008), with approximately €1.5 million attributed to oilseed rape alone (Gunning 2010), but this could substantially increase if oilseed rape continues to increase in land area. However, the pollination and pollinators of oilseed rape in Ireland have not been studied previously (but see Gunning 2010), and this information is essential in terms of managing and protecting pollination services for this crop into the future.

The aim of this study was to investigate i) what insects visit oilseed rape crops in Ireland, ii) which are the most useful pollinators, iii) whether the crop benefits from insect pollination and iv) whether the crop is currently limited in production by the amount pollen received. To do this we surveyed pollinators in both winter and spring oilseed rape fields, and in winter oilseed rape only investigated i) differences in pollinator species groups in terms of pollen transport and visitation rates, and ii) measured seed set in response to pollination treatment.

6.3 Methods

6.3.1 Flower visiting insect diversity

Ten winter oilseed rape fields were selected in 2009 and fourteen spring oilseed rape fields in 2010 around south-east Ireland, the region where oilseed rape is predominantly grown (CSO 2011b). All fields were at least 1km apart, and only one field was selected per farm in each year to ensure independence of management. Transects were used to sample pollinator diversity in five of the winter oilseed rape and all fourteen spring oilseed rape fields. Two 100x2m standard belt transects (Pollard & Yates 1993) were walked in centre of each field on one day during peak flowering of the crop, and two transects were also walked in the edge of the winter oilseed rape fields only. Surveys were carried out in warm dry conditions, and all bees (Hymenoptera; Apidae), butterflies (Lepidoptera) and hoverflies (Diptera; Syrphidae) were recorded. Bumblebees and butterflies were identified to species level (except the *Bombus sensu stricto* group as workers cannot be distinguished in the field, Wolf, Rohde & Moritz 2010; Carolan *et al.* 2012), and hoverflies were identified to genus.

Additional pan trap sampling was also carried out in all ten winter oilseed rape fields in 2009, as pan trapping allows the identification of specimens in the laboratory and can be a more useful method for sampling diversity of hoverflies and solitary bees (Westphal 2008). Pan traps were yellow, blue and white coloured UV painted bowls. A set of three bowls (one of each colour) was attached to a stake using a metal clamp and the rim of the bowls adjusted to the height of the oilseed rape flowers. Three stakes were left in the centre of each field, 20m apart, for 48 hours. Specimens were collected and identified in the lab. Type specimens of each species were verified by experts (see acknowledgements) and deposited in Trinity College Dublin.

6.3.2 Visitation rates and pollen transport

Three winter oilseed rape fields were selected in spring 2010; in Shillelagh Co. Wicklow (field 1), Bunclody Co. Wexford (field 2), and Tullow Co. Carlow (field 3). In each field, focal observations were made of insect visitors to the crop to examine visitation rates. Each field was visited seven times during the flowering period of the crop (8th May – 14th June). On each visit, six patches of oilseed rape were observed for 5 minutes each. All flower visitors, the number of flowers visited and the total number of flowers per patch were recorded. This allowed calculation of visitation rates for different species groups, calculated as the proportion of flowers visited per species in a 5 minute period. In fields 2 and 3, insects were also caught for examination of pollen loads. We aimed to catch five individuals of each species group seen visiting oilseed rape. Insects were frozen and returned to the laboratory where each was swabbed with a cube of fuchsin gel which was then melted onto a microscope slide (pollen storage areas on bees were avoided as this pollen is unlikely to be available for pollination). All pollen grains on the slide were identified and counted. Although this did not give a total count of pollen loads of the insects, as all insects were sampled in the same way it gave a relative pollen load.

6.3.3 Seed set

Seed set was examined in four winter oilseed rape fields in 2010; the same three fields used to observe visitation rates and a fourth in Fenagh Co. Carlow (field 4). All fields were of the “Castille” oilseed rape variety, or a mixture of “Castille” and “Excalibur”. Each field was visited in early May and four areas within each were selected, two in the edges of the field along an adjacent hedgerow, and two in the centre of the field (30m from each other and from the field edge). Three pollination treatments were applied to approximately six marked flowers on 4-6 plants per area (mean 85 flowers per treatment per field). Flowers were either 1) left un-manipulated to allow normal pollination, 2) the flower head was bagged using muslin to prevent insect pollinators from accessing the flower (bags were removed as soon as the marked flowers had finished flowering to allow continued growth of the plant) or 3) supplemental pollen from a neighbouring plant was placed on the stigmas of the flowers using a paintbrush to supplement normal pollination. Seed pods were collected 6-8 weeks later when the pods had reached maturity. Pods were dried in the laboratory and the number of seeds, total seed weight per pod and mean seed weight per pod were measured.

6.3.4 Data analysis

Pollinator importance indices were calculated for all insect groups where all data were available:

> Pollinator importance = mean visitation rate per flower in 5 minutes × mean number of oilseed rape pollen grains carried × mean abundance observed visiting oilseed rape per field (using transect data from edge and centre transects combined).

Seed set data were analysed using mixed effects models in the lme package (Pinheiro *et al.* 2012) in R (R Development Core Team 2008). Total seed weight per pod, average seed weight per pod and number of seeds per pod were all tested for differences between treatment (un-manipulated, bagged and supplemental pollination), location within field (edge and centre) and their interaction. To account for the nested design, random terms were specified including field, plot within field, and plant. Models were validated by visual inspection of residuals. If there was a significant treatment effect, Tukey all-pair comparisons were made between treatments using the multcomp package (Hothorn, Bretz & Westfall 2008)

6.4 Results

6.4.1 *Flower visiting insects*

A wide variety of insects were recorded in oilseed rape fields. Bumblebees were the most frequently observed flower visiting insects in both winter and spring oilseed rape; in total five species were recorded with the most abundant being the *Bombus sensu stricto* group, followed by *Bombus lapidarius* (Figure 6.1, Table 6.1). Honeybees were the next most abundant group, and were more dominant in winter oilseed rape than spring. Hoverflies were also commonly recorded, with the genera *Eristalis* and *Helophilus* most abundant (Figure 6.1) Other less commonly observed flower visitors included solitary bees, other hoverfly genera and butterflies.

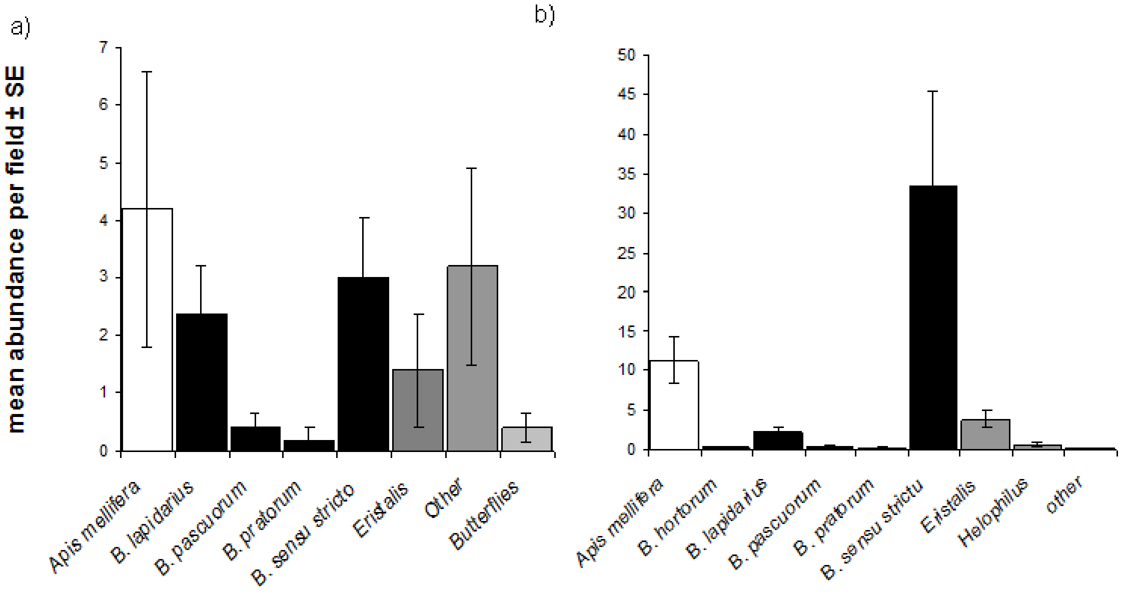


Figure 6.1. The mean (±standard error) abundance of different flower visitor taxa per sampling unit in each field as measured using transects in a) winter oilseed rape (centre transects only) and b) spring oilseed rape. White = honeybee, black = bumblebees and grey = additional taxa. "Other" includes solitary bees and hoverflies not previously mentioned.

Pan traps allowed the recording of additional species in winter oilseed rape. As in the transects, five bumblebee species were recorded, but with *B. pascuorum* recorded instead of *B. hortorum*. Ten species of hoverfly were trapped within the crop, and the most abundant were *Eristalis arbustorum* and *Helophilus pendulus*. Five species of solitary bee were identified, the most abundant were *Halictus rubicundus* followed by various *Andrena* species (Table 6.1).

Table 6.1. Insects identified from the pan traps (and bumblebees from transects) in winter oilseed rape fields, their total abundance, and the number of fields that they were recorded in (out of ten for pan traps and five for transects).

		Total abundance Pan trap (transect)	**No fields present** Pan trap (transect)
Honeybee	*Apis mellifera*	5 (21)	4 (3)
Bumblebees	*Bombus hortorum*	3 (0)	2 (0)
	Bombus lapidarius	5 (13)	4 (4)
	Bombus pascuorum	2 (2)	2 (2)
	Bombus pratorum	11 (1)	3 (1)
	Bombus sensu stricto	14 (15)	7 (4)
Solitary bees	*Andrena angustior*	1	1
	Andrena bicolor	9	2
	Andrena cineraria	1	1
	Andrena fucata	2	2
	Andrena haemorrhoa	2	2
	Andrena scotica	1	1
	Halictus rubicundus	3	1
	Nomada marshamella	1	1
Syrphidae	*Anasimyia lineata*	1	1
	Eristalis abusivus	2	2
	Eristalis arbustorum	55	4
	Eristalis interruptus	4	2
	Eristalis intricarius	7	3
	Eristalis tenax	18	6
	Helophilus hybridus	8	4
	Helophilus pendulus	29	4
	Helophilus trivittatus	1	1
	Platycheirus albimanus	1	1
	Rhingia campestris	5	3
	Syritta pipiens	1	1

6.4.2 *Visitation rates and pollen loads*

Across all three sites the highest visitation rates per flower were by the bumblebees, *B. lapidarius* and the *B. sensu stricto* group, followed by the honeybee. Ninety-eight insects were caught foraging on winter oilseed rape for identification of pollen loads, and 94 carried oilseed rape pollen. On average oilseed rape pollen made up 91% of pollen loads and *Bombus hortorum* carried the most oilseed rape pollen grains followed by *Apis mellifera* and *Andrena* sp.

Pollinator importance indices, based on abundance, visitation rate and pollen load, were highest for *Apis mellifera* (largely due to more pollen grains carried), followed by the bumblebees (Table 6.2). However, *A. mellifera* was not present in all oilseed rape fields surveyed and may be limited to where beekeepers place their hives; therefore in many fields wild bumblebees (in particular *B. sensu stricto* and *B. lapidarius*) would be the most important pollinators.

Table 6.2. Mean (± standard error) values of parameters used to calculated pollinator importance (number of pollen grains carried × visitation rate × relative abundance)

Insect species	**No. pollen grains carried**	**Visitation rate (per flower in 5 min)**	**Relative abundance per field**	**Pollinator importance**
Andrena sp.	4107 ± 1342	0.010 ± 0.003	0.8 ± 0.4	34
Apis mellifera	4350 ± 937	0.03 ± 0.01	5.6 ± 3	730
Bombus lapidarius	1525 ± 684	0.06 ± 0.02	1.6 ± 0.5	150
Bombus pascuorum	1126 ± 241	0.031 ± 0.02	0.4 ± 0.4	14
Bombus sensu stricto	1813 ± 544	0.05 ± 0.01	2.2 ± 0.8	186
Eristalis sp.	2073 ± 444	0.015 ± 0.003	1.4 ± 0.9	43
Helophilus sp.	407	0.013 ± 0.003	0.6 ± 0.6	1
Rhingia campestris	167 ± 57	0.02 ± 0.005	0.6 ± 0.6	2

6.4.3 Seed set

Across all four fields, 1025 seed pods were collected. The number of seeds produced per flower ranged from 0 – 34 seeds per pod, with an average of 12. Flowers that had pollinators excluded (bagged) produced significantly less seeds, and therefore less total seed weight per pod, than those that pollinators could freely visit or those where additional pollen was applied in both the centres and edges of the fields (Figure 6.2, Table 6.3). However, the magnitude of this difference was larger in the edges of the fields than the centres. The mean weight per seed was also higher from flowers that were freely pollinated, and those where supplemental pollen was applied, compared to those with pollinators excluded (bagged), but there was no difference between those flowers in the edge or the centre of the field (Figure 6.3, Table 6.3).

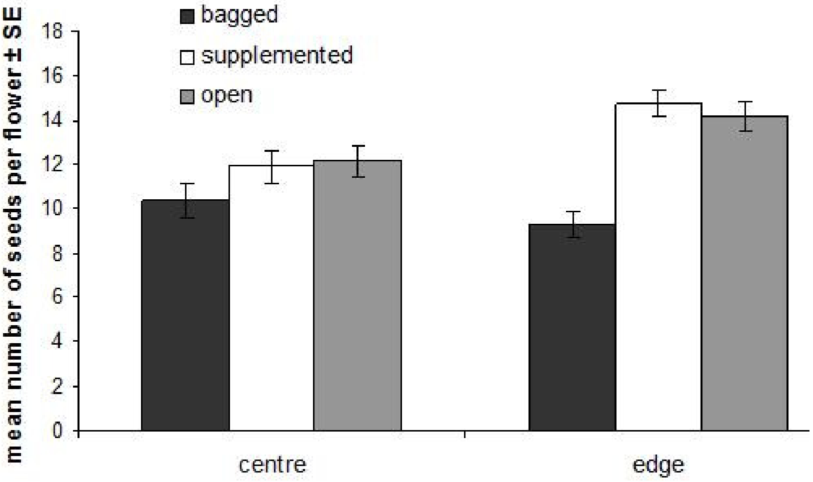


Figure 6.2. Mean number of seeds produced per flower in the three treatments in the centre and edge of the fields, across all four sites. Treatments are as follows: bagged (no pollinators, black), open (normal pollination, grey) and supplemented (extra pollen added to the stigma, white)

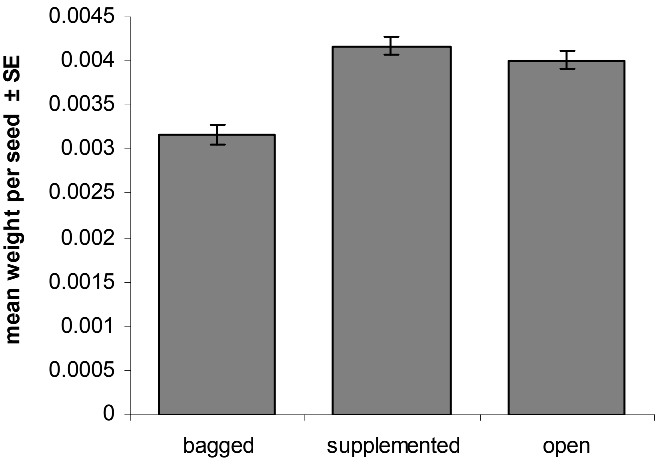


Figure 6.3. Mean weight per seed (± standard error) in the three treatments: bagged (no pollinators), open (normal pollination) and supplemented (extra pollen added to the stigma), across all four sites.

Table 6.3. Final linear mixed effects models describing effects of pollination treatment (un-manipulated, bagged and supplemental pollination) on total seed weight per pod, number of seeds produced per flower, and mean weight per seed.

	df	F	p
Total seed weight per pod			
Treatment	2, 934	12.21	<0.0001
Location in field	1, 9	6.8	0.0284
Treatment * location	2, 934	6.5	0.0017
Number of seeds			
Treatment	2, 934	6.29	0.0019
Location in field	1, 9	8.23	0.0185
Treatment * location	2, 934	4.74	0.0089
Mean weight per seed			
Treatment	2, 936	44.133	<0.0001

6.5 Discussion

Our data show that oilseed rape in Ireland is visited by a wide variety of insect species, and that the crop benefits from insect pollination as more seeds are produced when flowers are visited by insects. More benefits of insect pollination are found in the edges of the fields compared to the centres, which may be due to increased insect numbers in the field edges (e.g. Chapter 2). Insects vary in their usefulness as pollinators, with the honeybee and bumblebees being the most useful pollinators of winter oilseed rape based on the number of pollen grains they carry, visitation rates per flower and their relative abundance per field. Pollen supplementation did not cause an increase in seed number or weight suggesting that crops currently receive sufficient pollination services.

In a similar way to previous studies from other countries (e.g. Morandin & Winston 2005; Bommarco, Marini & Vaissière 2012; Jauker *et al.* 2012) we found a diverse range of insects in oilseed rape fields, with a total of 26 bee and hoverfly species in winter oilseed rape. A diversity of pollinators can improve yield of certain crops (Kremen, Williams & Thorp 2002; Hoehn *et al.* 2008) and can be important for a number of reasons (Klein *et al.* 2008); one pollinator can act as an "insurance policy" for another and so if one declines another may take its place, a higher diversity can result in higher abundance increasing the chances of all flowers being adequately pollinated, or higher diversity of pollinators means that individuals may interact with each other increasing their efficiency (e.g. Greenleaf & Kremen 2006). Therefore supporting a diverse range of pollinators for pollination services is important for maintenance of crop pollination, and wild pollinators are important and efficient pollinators of mass flowering crops (Jauker & Wolters 2008; Rader *et al.* 2009; Jauker *et al.* 2012)

As a partially wind pollinated and self fertile crop, oilseed rape sets some seed without insect visitation. However, we found that flowers where pollinators were excluded produced less seed (and therefore less seed weight per pod and average seed weight) in both the centres and edges of the field than those open to pollination which has also been illustrated in other countries (e.g. Sabbahi, De Oliveira & Marceau 2005; Bommarco, Marini & Vaissière 2012), and which can also lead to differences in oil content, chlorophyll content and market value (Bommarco, Marini & Vaissière 2012). Interestingly, we found no difference in seed set when additional pollen was added to the flowers. This suggests that oilseed rape is not pollen limited in the fields studied in Ireland, and currently receives sufficient pollination services for maximum seed yield from existing wild pollinators.

However, there was a significant interaction between pollination treatment and location within the field (edge and centre). The magnitude of pollination benefit (in terms of number of seeds produced, but not mean weight per seed) was larger at the field edge than in the centres, but the bagged treatment also produced less seed in the edges than the crop centres. Plants at the edges of the field may be less susceptible to wind pollination as they are more sheltered by hedgerows and field boundaries. However, pollinators are more abundant in field edges and this may compensate for reduced wind pollination resulting in comparable overall seed set.

Pollinators can differ in terms of their efficiency of crop pollination (Rader *et al.* 2009; Jauker *et al.* 2012). We find that the honeybee and bumblebees are the most useful pollinators of winter oilseed rape based on the amount of pollen carried, visitation rates and the abundance observed. However, other factors may also influence the importance of pollinator groups such as the stability of pollinator groups over time, the amount of pollen deposited per visit (which may not be linked to the amount of pollen carried), stigmatic contact and pollen export and deposition which can vary among taxa (Thomson & Goodell 2001; Hayter & Cresswell 2006; Rader *et al.* 2012). Also, honeybees may be more sensitive to weather conditions than bumblebees (Willmer, Bataw & Hughes 1994), affecting their importance as pollinators, and wild pollinators can be relevant for stability of crop production even when honeybees are present (Garibaldi *et al.* 2011). Overall abundance of flower visiting insects was higher in spring oilseed rape than in winter oilseed rape which has also been found for social bees in the UK (Hayter & Cresswell 2006); winter oilseed rape is likely to attract bumblebee queens at the beginning of the colony cycle whereas colonies will be established when spring rape begins to flower and so spring crops will be visited by bumblebee workers which will be much more numerous. Although the effect of pollinators on yield of spring oilseed rape was not measured in this study, it is likely that it may be pollinated more efficiently and quickly than winter oilseed rape due to augmented insect numbers; previous work has found flowers of spring oilseed rape to be adequately pollinated after three hours receiving approximately 3 bee visits per hour, whereas winter oilseed rape was only adequately pollinated after five days and only 10% of flowers received a bee visit (Hayter & Cresswell 2006). Therefore it is likely that spring oilseed rape is more efficiently pollinated than winter oilseed rape because of the availability of pollinators, and although honeybees may be important for pollination of winter crops when bumblebees are at the beginning of the colony cycle, bumblebees may be more important for spring crops that flower later on in the season.

Although not the dominant flower visitors found in oilseed rape, hoverflies and solitary bees were also identified. These taxa can also be efficient pollinators of oilseed rape (Jauker & Wolters 2008; Jauker *et al.* 2012a), although they may not be as effective due to lower abundances than the honeybee (Rader *et al.* 2009) or bumblebees. However, if abundances of these taxa were increased in agricultural areas, potentially through agri-environmental schemes (e.g. Haenke *et al.* 2009), they may become more effective pollinators of the crop.

Oilseed rape production in Ireland has increased substantially in recent years and is set to increase further in the future, especially with demands for bioenergy and policy targets (Department of Communications, Marine and Natural Resources, 2007). Crop yields are improved with insect pollination, which is greater in the edges than in the centres of fields, and all the fields studied (which were surrounded by hedgerows) currently appear to receive sufficient pollination services. The majority of insect visitors to the crop are wild pollinators, but the managed honeybee is also a valuable pollinator in fields where it is present. Therefore, to maintain current pollination services, efforts should be made to conserve existing pollinators in Irish farmland and perhaps augment their abundance and diversity to provision for increased pollination service demands in the future.

Chapter 7

General Discussion

From air and soil
from bees and sun,
from others' toil
my bread is won.

And when I bite
the soil, the air,
the bees and light
are still all there.

So I must think
each day afresh
how food and drink
became my flesh.

And then I'll see
the air, the sun,
the earth, the bee
and me, all one.

E. Orteza, (editor)

7 General Discussion

"To manage ecosystem services in a changing world, we need to know how human activities affect the key species or functional groups that provide these services, and the spatial and temporal scales of both disturbance and recovery. How do real-world changes in communities affect the magnitude and stability of ecosystem services?" (Kremen & Ostfeld 2005)

This thesis aimed to improve understanding as to how human mediated changes in agricultural landscapes, specifically the growth of bioenergy crops, can affect pollinators and pollination services, particularly taking into account landscape context. This was achieved through extensive field surveys and observations, landscape mapping, molecular genotyping, experimental manipulations and modelling analyses. In Chapter 2 I initially compared bioenergy crops to the conventional crops they replace at the field scale in terms of their impacts on pollinator abundance, diversity and community composition, as well as on the availability of forage resources and bumblebee nesting sites. Chapter 3 investigated the implications of growing bioenergy crops, and the landscape context they were planted in, on pollination services by examining effects on the structure of plant-pollinator networks. Chapters 4 and 5 focussed on oilseed rape as a mass flowering bioenergy crop, which has particular implications for pollinators and pollination. In Chapter 4 I examined the colony densities and identities of the most abundant visitors to the crop, a cryptic bumblebee complex, and how they were influenced by landscape context. Finally, Chapter 5 studied forage use of pollinators in oilseed rape and associated field margins, and the potential impacts of growing oilseed rape on pollen transfer dynamics and pollination services to wild plant species growing around the crop. In this final Chapter I provide a synthesis of the key results found in the context of previous research, outline methodological considerations and limitations, examine implications for the conservation of pollinators and pollination services and for bioenergy policy and planning, and finally suggest some areas for further research.

7.1 Summary and Synthesis

7.1.1 Insect abundance, diversity and community composition

The growth of crops for bioenergy has the potential to influence biodiversity (Dauber *et al.* 2010). Although the growth of perennial biomass crops has been suggested as potentially beneficial for insects (Dauber *et al.* 2010; Landis & Werling 2010; Werling *et al.* 2011), others have proposed negative impacts (Harrison & Berenbaum in press). In Chapter 2, when comparing the growth of energy crops to the conventional crops they replace at the field scale, I found no declines in pollinator abundance and diversity and increases in some species groups. Bioenergy crops can also have implications for beta diversity by adding more heterogeneity into the landscape (Harrison & Berenbaum in press); in my study both alpha and beta diversity differed between crop types for solitary bees. Although preliminary work has taken place on the *Bombus sensu stricto* group in a variety of habitats in Ireland (Murray *et al.* 2008; Byrne 2011), little is known about the complex in agricultural areas where they are often the most common bumblebee pollinators. I found three of the four species of the complex in mass flowering oilseed rape fields, and different colony densities of each (Chapter 4).

7.1.2 Forage resources

It has been shown that oilseed rape acts as a resource for many insect taxa in other countries (e.g. Cresswell *et al.* 1995; Morandin & Winston 2005; Bommarco *et al.* 2012), and here this is confirmed for Ireland (Chapters 2, 3, 4, 5 and 6). However, I found that it is rare that insects only forage on oilseed rape crops (Chapter 5). Oilseed rape was well connected in terms of pollinators to other plant species in plant-pollinator networks (Chapters 3 & 5), and when the crop stopped flowering, insect abundance and species richness did not change, but insects appeared to switch to other alternative floral resources (both weeds within the crop but primarily species in the field margin and hedgerow, Chapter 3). Even when the crop is in flower, insects appear to supplement their diets with other forage species, as pollen from both oilseed rape and other species was found on the majority of insects foraging on the crop (Chapter 5). Therefore although mass flowering crops can be beneficial for increasing densities of pollinators (Westphal *et al.* 2003; Hanley *et al.* 2011), alternative forage resources are needed during the flowering of the crop, and especially afterwards, to sustain pollinator populations.

Both bioenergy crops oilseed rape and Miscanthus provided more non-crop floral resources than conventional wheat crops, with consistently more floral units in the field margins than centres (Chapter 2). Other bioenergy crops (switchgrass and prairie) in the US can also support more diverse plant communities, which in turn can have positive influences on beneficial insects (Gardiner *et al.* 2010). Previous work has also shown Miscanthus can be rich in weedy vegetation which can be beneficial for many farmland taxa (Semere & Slater 2007a), although flora were also found more abundantly in margins than in field centres. In my work, all floral units were more abundant in field margins than centres, which indicates that the retention of margins and hedgerows during conversion to bioenergy is important for pollinators.

7.1.3 Nesting resources

The availability of nest sites can also be an important determinant of pollinator community structure (Samejima *et al.* 2004; Potts *et al.* 2005). As a perennial crop, it has been suggested that Miscanthus may provide nesting sites for some insects due to increased litter layer and low disturbance (Landis & Werling 2010). However, no differences in abundances or diversity of nest searching bumblebees were found between Miscanthus and other crop types suggesting this is not the case for bumblebees (Chapter 2). Furthermore I confirm that field margins and hedgerows are important nesting sites for bumblebees in agricultural regions (see also Svensson *et al.* 2000; Kells & Goulson 2003), and may be the only sites left in intensive farmland. Solitary bee and wasp species may be more varied in their choice of nest sites (Yeo & Corbet 1983; Cane *et al.* 2007), and significantly more trap nesting solitary bee species were found in Miscanthus than other crop types (Chapter 2). Although this does not directly show a better availability of nest sites for these groups in the crop, it has been suggested that the number of brood cells in trap nests can reflect the number of available nesting sites (Tscharntke *et al.* 1998; Holzschuh *et al.* 2009). Therefore Miscanthus may provide more nest sites for solitary bees and wasps than annual arable crops.

Although oilseed rape provides a mass flowering resource for bumblebees to forage on (Chapters 2, 4 & 5) it does not provide any more nesting resources than other crops (Chapter 2). Chapter 4 shows that *B. terrestris* colony densities are higher per km^2 in mass flowering crops than has been previously shown in conventional agricultural fields in the UK and Germany (Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2005; Kraus *et al.* 2009). Although this may be because this species occurs at a higher density in Ireland, it may also suggest that some bumblebees nest quite far away from oilseed rape fields but will travel longer distances to use the resource. As field margins and hedgerows are the predominant bumblebee nest sites (Chapter 2), these features are required in landscapes near oilseed rape fields to provide nest sites for bumblebee pollinators.

7.1.4 Pollination services

As bioenergy crops can affect the abundance and diversity of pollinating insects (Chapter 2) it is likely that this can have a knock-on impact on the provision of pollination services, both to wild plants but also to bioenergy crops themselves which may require pollination for increased yield (Chapter 6). Plant-pollinator network structure differed between bioenergy crops, with larger differences when arable land was replaced compared to grassland (Chapter 3). Therefore bioenergy production affects species interactions, which potentially has implications for the provision of pollination services.

There are a number of ways in which changes in pollination services may occur; through changes in visitation rates, or through changes in pollen transfer (Morales & Traveset 2008). I found that oilseed rape shares the same species of flower visitor as co-flowering wild species and that the same individuals also visit the crop and wild species (Chapter 5). However, to affect female fitness, oilseed rape pollen would have to become deposited on the stigmas of wild species (Morales & Traveset 2008) but I found very low amounts of oilseed rape pollen were found on native plant stigmas. Therefore although bioenergy production can alter plant-pollinator interactions, deposition of oilseed rape pollen on native plant stigmas is unlikely to affect female fitness.

7.1.5 Landscape scale

As mobile organisms, many pollinating insects experience the landscape at large spatial scales and different resources (e.g. forage, nesting, overwintering) may be separated by space (Steffan-Dewenter *et al.* 2002; Kremen *et al.* 2007). Although previous work has suggested some ecological differences between species of the *B. sensu stricto* cryptic complex (Murray *et al.* 2008; Waters *et al.* 2011b), I found that these cryptic bumblebee species (the main flower visitor group to oilseed rape crops) are affected differently by landscape scale elements including landscape composition and distance to certain features (Chapter 4). As pollinators and insect pollinated plants can be influenced by landscape composition and configuration (e.g. Steffan-Dewenter *et al.* 2002; Power *et al.* 2012), it is likely that plant-pollinator interactions will also be affected at large spatial scales (Burkle & Alarcon 2011), although this has not been investigated until now. Chapter 2 shows that properties of plant-pollinator networks can be affected by crop type at the local scale, but also by the composition of the landscape surrounding the fields. Landscape diversity and hedgerow length seem to be important determinants of network properties.

7.1.6 Pollinators in agricultural systems

Agricultural landscapes have a limited fauna in comparison to other habitat types, and are often dominated by common taxa (Carre *et al.* 2009; Ekroos *et al.* 2010) with higher dispersal capacities and diet breadth (Bommarco *et al.* 2010). As in previous surveys of bumblebees in Irish agriculture (Santorum & Breen 2005; Power & Stout 2011), only a subset of common bumblebee species were found in this study; 11 out of 20 Irish bumblebee species were found in the agricultural sites surveyed, of which eight were least concern (LC) (or data deficient (DD) in the case of the cryptic species) and three of which were near threatened (NT, Fitzpatrick *et al.* 2006). However, none of the vulnerable or endangered species were found (e.g. *B. ruderarius*, *B. sylvarum* or *B. distinguendus*). Out of a potential 82 solitary bee species in Ireland, only 23 were found in study sites for this thesis; 17 of these were least concern (LC), two near threatened (NT) and four vulnerable (VU) but again none of the endangered or critically endangered species were encountered (Fitzpatrick *et al.* 2006). Of the eleven butterfly species recorded of a potential 33, all were of least concern (LC, Regan *et al.* 2010b). Although no threat categories have been assigned to hoverflies, a total of 47 species were recorded for this thesis out of a potential 183 (Speight 2008). Therefore although bioenergy crops can have positive impacts for some pollinator groups in agricultural environments, they are only affecting the common and widespread species and therefore cannot be regarded as a substitution for conservation of semi-natural habitats to preserve the rarer species. Furthermore, replacement of marginal or semi-natural land (which tend to have larger pollinators faunas with more rare species) with bioenergy production could have very different effects.

7.1.7 Climate change

The purpose of growing crops for bioenergy, apart from providing new fuel sources and higher fuel security within countries, is to reduce fossil fuel inputs to mitigate against climate change. My studies show predominantly positive impacts of small scale bioenergy crop growth in existing agricultural areas on pollinator abundance and diversity (Chapters 2 & 4). Climate change could affect pollinators and pollination in a number of ways; changing climate can cause changes range shifts of pollinators and plants (Roy *et al.* 2001; Kuhlmann *et al.* 2012), changes in phenology (O'Neill *et al.* 2012), alterations in plant chemistry (Hoover *et al.* 2012), and many other factors which could all lead to increased extinction with some species being more sensitive than others (Roberts *et al.* 2011). All these climate associated changes could also have knock-on implications for plant-pollinator interactions (Memmott *et al.* 2007; Hoover *et al.* 2012), although climate induced mismatches in some pollinator dependent species may not be occurring (Rafferty & Ives 2011). It appears that the growth of bioenergy crops at a small scale in agricultural regions in this study, as a mitigation against climate change, has the potential to have positive impacts on pollinators and in some cases pollination, while the impacts of the alternative, global warming (although positive in some cases for some species), are quite uncertain and variable. Therefore bioenergy production in its current form could be seen as a preferable alternative for pollinators compared to changes in climate.

7.2 Methodological considerations

For this thesis, a wide variety of methods were employed at various spatial scales to investigate the interactions between pollinators, pollination and bioenergy crops. To evaluate the relevance of the research presented some limitations of methodologies, as well as some particularly useful methods, are highlighted here. This has the potential to aid future work on pollinators and pollination in agro-ecosystems.

The choice of sampling method for pollinating insects is important as different methods can yield contrasting results and be appropriate for different taxa (Westphal 2008; Nielsen *et al.* 2011). Most studies use only one sampling method, as a combination of methods can require more time or different expertise. In Chapter 2 (and Chapter 6) I used a combination of sampling methods to evaluate pollinator abundance and diversity, and some methods were more useful than others for sampling particular pollinator groups. As in previous studies (Westphal 2008; Nielsen *et al.* 2011), pan traps were confirmed as more useful for sampling solitary bees as much higher abundances were recorded compared to transect walks (**Appendix 3**, Plate 11), and trapping allowed identification of specimens to species level in the laboratory. Although some butterflies were caught in pan traps they were not preserved well: therefore transects were more useful for butterflies. Pan traps recorded lower abundances of hoverflies than using transect walks (**Appendix 3**), but as it can be difficult to identify all species in the field, a trapping method may also be preferable for this group. However, more bumblebees were recorded during transect walks than using pan traps (**Appendix 3**) suggesting pan trapping may not be as useful for larger bees, possibly as they can escape from the traps (personal observations). As most bumblebees can be reliably identified on the wing perhaps this non-lethal sampling method may be preferable for future studies.

Plate 11. Pan trap methods: in use in the field, and insect sorting in the laboratory (photos: DS)

Trap nests have been suggested as useful to sample additional species of bees not recorded in other methods (e.g. Westphal 2008; Plate 12). In the regions studied here, the use of trap nests did not increase the overall species richness of bees (Chapter 2) which probably reflects the smaller solitary bee fauna in Ireland than continental Europe; all the cavity nesting bee species recorded in the trap nests (2 species) were recorded using other methods (pan traps). On the other hand, trap nests did allow quantification of solitary wasp diversity which was not recorded elsewhere.

In Chapter 2, all crop types were sampled using pan traps in 50 commercial fields. However, transect sampling was only carried out in 25 fields, which was further reduced to only the arable fields for trap nest sampling (15 fields) and nest searching observations (18 fields). Transects and nest searching observations require large amounts of time spent in the field, and trap nests are labour intensive in terms of assembly and the rearing of individuals from the nests. Ideally, all fields would have been surveyed using all methods, but in reality this was beyond the time and labour capabilities of a single researcher. In a similar way, pollen transfer dynamics between oilseed rape and wild species were only examined in two fields (Chapter 5) and seed set of oilseed rape was only carried out in four winter oilseed rape fields (and not at all in spring fields, Chapter 6) for similar reasons; ideally more replicated fields would have been surveyed for both of these studies to ensure conclusions drawn can be broad and applicable. In addition, as pollinator communities and plant-pollinator networks can vary over time and from year to year (Alarcon *et al.* 2008; Petanidou *et al.* 2008) multi-year sampling is often recommended; although this thesis is comparative and each study provides a within year comparison or snap shot of different effects, data collected over a number of years may have helped to examine inter-annual variability.

Plate 12. Trap nest methods: nest assembly (left), use in the field (centre) and emergence boxes (right) (photos: DS)

In Chapter 4 I estimated the numbers of bumblebee colonies using oilseed rape fields as a forage resource using molecular methods. These methods are a very useful way of studying bumblebee colony densities (Darvill *et al.* 2004; Knight *et al.* 2005; Charman *et al.* 2010), as physically locating bumblebee colonies is notoriously difficult (Osborne *et al.* 2008b; Waters *et al.* 2011a). However, although I based my sample sizes for molecular analyses on those used in previous studies, I identified very few individuals from the same colonies. This indicates that many more colonies were using the mass flowering fields than previous studies of non-mass flowering areas. However, this resulted in large confidence intervals associated with the colony density estimates. Therefore, future work on colony densities of bumblebees in mass flowering habitats should increase the sample sizes of bees to increase the potential of identifying bees from the same colony; this can be done using a non-lethal method to prevent damage to populations (Holehouse *et al.* 2003).

In Chapters 3 and 4 I investigated the role of surrounding landscape composition in patterns seen at the field scale; on plant-pollinator networks and on proportions and colony densities of cryptic bees. For both studies I used a spatial scale appropriate to the organisms studied based on their known foraging ranges. However, different pollinator taxa can respond to landscape context at different spatial scales (Steffan-Dewenter *et al.* 2002), and landscape effects on biodiversity can sometimes be found at very large spatial scales (e.g. up to 5km from sites, Power *et al.* 2012). In comparison to other European countries, agricultural land cover data in Ireland is not freely available to the public or for research use, and available data are limited to the coarse resolution CORINE land cover (EPA 2006) where land parcels have to be larger than 25 hectares to be included. Therefore any landscape data used in this thesis had to be collected and ground-truthed for each landscape individually which can be time consuming and labour intensive. As a result, larger spatial scales were not used. Although the landscape scale results presented in this thesis provide valuable large scale information on the impacts of landscape composition on pollinators, further work on impacts at larger spatial scales (with more easily accessible datasets) could provide extra information on landscape level impacts. In Chapters 3 and 4, I used measures of landscape composition and distances to quantify surrounding landscapes which can be a useful way of elucidating landscape level effects. However, landscape configuration and associated fragmentation of habitats can also be evaluated but have been rarely used in pollinator and pollination studies (Hadley & Betts 2012). These types of measures may also have influences on the biodiversity patterns and processes seen in my studies.

As it is not possible to distinguish between members of the *Bombus sensu stricto* complex in the field, these species were grouped for most studies in this thesis except in Chapter 4 where molecular methods were used to distinguish between them. In fact, these species are grouped for most field studies on bumblebee ecology. However, as these species may have different ecological requirements, they could be affected by bioenergy crops in different ways. Therefore some of the patterns of bumblebee species richness seen in Chapter 2, or network structure in Chapter 3, may be different if these species were treated as individual entities.

This thesis investigated bioenergy production as it currently exists in Ireland. I used the two most commonly grown bioenergy crops as model species, impacts were investigated at the isolated field scale, impacts were assessed when crops replace conventional agricultural land and research was conducted in south-east Ireland where these crops are currently predominantly grown. However, bioenergy crop growth may change from its current model in the future, and different factors and conditions could deserve further study:

- If bioenergy targets are to be met it is likely that bioenergy production will become more widespread and planted at a larger scale. The proportion of the landscape used for bioenergy production and the spatial aggregation of the fields can have different impacts on biodiversity (e.g. Westphal *et al.* 2003; Gevers *et al.* 2011), and the impacts of landscape scale growth of bioenergy crops in Ireland deserves further attention. It is likely that Miscanthus production, to be economically viable as a co-firing material in peat power stations in Ireland, may need to be concentrated and aggregated in certain areas near the stations. This may have different impacts on the magnitude and direction of effects on biodiversity than the occasional replacement of arable or grass fields studied here.
- Bioenergy production may not always replace conventional agricultural land as is currently the case; in certain scenarios it is predicted that semi-natural or marginal land may be used for growing bioenergy crops to reduce direct competition for the production of food (Fargione *et al.* 2008). Agricultural land already often has a sparse and generalist fauna and flora compared to other regions (Carre *et al.* 2009; Ekroos *et al.* 2010), and therefore the growth of new crops in existing agricultural landscapes may not have large impacts on biodiversity. However, replacement of more species rich semi-natural habitats not previously used for intensive agriculture could have conflicting effects on biodiversity and so deserves further attention.
- For this study I provided baseline information on the impacts of growing bioenergy crops on pollinators and pollination using the two most widely grown bioenergy

crops in Ireland as model systems. These crops contrasted in many ways including mass flowering nature, management and longevity, and so represent a wide spectrum of potential crops. Although currently the two most widely grown bioenergy crops in Ireland, other crops such as cereals, hemp, switchgrass or willow are also being grown for bioenergy. These crops may have subtle differences in agronomy to the model crops studied here (for example willow provides nectar and pollen for flower visiting insects, but is also a long lived perennial crop) and therefore could have different impacts on pollinators, pollination and biodiversity which deserve further investigation (e.g. Rowe *et al.* 2011).

- Although work for this thesis was carried out in South-East Ireland which is one of the most intensively farmed areas in the country (CSO 2010), the situation in other countries (or in less intensively farmed parts of Ireland e.g. the west) may be different. Irish agricultural landscapes are traditionally heterogeneous with a large amount of hedgerows, relatively smaller field sizes and relatively large percentages of semi-natural habitat (Chapter 3; Sheridan *et al.* 2011; Sullivan *et al.* 2011) in comparison to other countries (e.g. Westphal *et al.* 2003). Field margins and hedgerows are also relatively species rich; for example, Carvell et al. (2004) found 46 flowering species in naturally regenerated field margins in the UK, while 90 flowering species in field margins and hedgerows have been recorded in this study. Therefore the impacts of bioenergy crops at the field scale may be different in areas farmed in different ways and at different levels of intensity.

7.3 Implications of research for pollinator conservation

As pollinators are important in agricultural regions in terms of the pollination services they provide, it is necessary to sustain pollinator populations in these areas (Chapter 1). There are a number of suggestions for conserving pollinators in agricultural regions as indicated by my research, with further implications for the provision of pollination services both to crops and wild plants.

7.3.1 Field margins

Field margins and associated hedgerows have long been considered important elements for biodiversity in agricultural areas, and are often the only semi-natural habitats left (Marshall & Moonen 2002). High quality hedgerows and field margins can be beneficial for pollinators (Carvell *et al.* 2004; Hannon & Sisk 2009), and more pollinators have been found in margins of fields than in the centres (e.g. Power & Stout 2011). Results from Chapters 2, 3 and 5 all show that field margins and associated hedgerows are important in terms of pollinator abundance and diversity, nesting sites for bumblebees, floral resources and plant-pollinator network structure. Therefore the conservation and maintenance of existing field margins and hedgerows is essential for pollinator conservation in Irish agricultural regions. In addition, augmentation of pollinator abundance and diversity may help to provision for increased pollination requirements in the future. This could be achieved through implementation of agri-environmental schemes which can include measures such as maintenance of existing field margins and hedgerows, increasing size of these features, supplementation of these features with the sowing of additional forage resources for pollinators or reduced agri-chemical inputs to promote diversity (Emerson & Gillmor 1999; Pywell *et al.* 2006).

7.3.2 Landscape scale planning

Bioenergy crops may affect heterogeneity of the landscape with implications for beta-diversity (variation in the species composition of assemblages), and I found heterogeneity of crop types can be important for solitary bees (Chapter 2). In addition, although oilseed rape is visited by many insect groups and colonies of bumblebees (Chapters 2, 3, 4 & 5), the crop does not provide suitable nesting sites and so these resources may be spatially spread across the landscape. Furthermore, both bumblebee proportions and colony densities, and plant-pollinator networks, are affected by surrounding landscape composition (Chapters 3 and 4). In particular, the length of hedgerows and the diversity of habitats appear to be important for network structure, while amount of arable land, forestry and number of soil types can be important predictors of certain bumblebee species (Chapters 3 and 4). Therefore findings from this thesis suggest that the maintenance of landscape and crop type heterogeneity should be considered in agricultural planning, and in the conservation of pollinators and pollination services. Furthermore, when planning agricultural land use or configuration to maximise biodiversity benefits, a landscape scale approach is needed (Gabriel *et al.* 2010).

7.3.3 Pesticide use

Less intensively managed farming systems, especially those with less pesticide use, are generally more beneficial for pollinators and pollination (e.g. Holzschuh *et al.* 2008; Rundlof *et al.* 2010; Power & Stout 2011). Miscanthus is traditionally a low input crop with very little use of agro-chemicals (Lewandowski *et al.* 2000). However, oilseed rape is a high input crop (Stephenson *et al.* 2008, Appendix 6), with insecticides commonly used either as sprays or seed treatments (DAFF 2004). These insecticides (for example imidacloprid) can have negative impacts for bees, even in sub-lethal ways (Arthur *et al.* 2010; Cresswell 2011; Girolami *et al.* 2012; Laycock *et al.* 2012; Stoner & Eitzer 2012; Whitehorn *et al.* 2012). As oilseed rape is visited by a large diversity of pollinators (Chapters 2, 3, 4 & 5) and relies on pollination for increased yields (Chapter 6) the use of pesticides to control crop pests could have knock on implications for both crop productivity and pollinator conservation. Therefore I advocate a more sustainable and limited use of pesticides on this crop, and more research into potential pesticide impacts on different pollinator groups.

7.4 Specific implications for bioenergy policy

The bioenergy sector is currently largely driven by policy. For example, although 6.5% of Irish energy came from renewable sources in 2011 (Dennehy *et al.* 2012), Ireland has an EU target of 16% of energy to come renewable sources by 2020 (Directive 2003/30/EC 2003). Therefore, we can expect further development and expansion of the bioenergy sector in coming years. This thesis presents a number of results that have implications for bioenergy policy and spatial planning, in terms of mitigating impacts on pollinators and pollination.

- Bioenergy crops replacing current agricultural crops (either grassland or arable land) have minimal impacts on pollinators and can have some positive impacts in some cases (Chapter 2); however replacement of semi-natural or marginal habitats may have different effects.

- The former land use replaced by bioenergy crops can have different impacts on pollinators and pollination services (Chapters 2 and 3), as well as other services such as carbon sequestration (Zimmermann *et al.* 2011); larger responses occur when annual arable crops are replaced compared to perennial grassland areas. Therefore, the previous land use replaced by bioenergy should be taken into account for biodiversity and ecosystem service mitigation planning.
- Bioenergy crops at the small scale can improve habitat heterogeneity, which can be beneficial for some pollinator groups (Chapter 2). Large landscape scale growth of these crops may have different effects.
- Oilseed rape benefits from pollination services, and therefore provides an economic incentive for pollinator conservation in farmland (Chapter 6). Efforts to maintain existing pollination services in areas where this crop is grown are essential, especially if production is to increase in the future when pollination services could become more limiting.
- Bioenergy production should not result in field enlargement or destruction of hedgerows and field margins; these areas provide important nesting and forage resources for pollinators (Chapters 2 & 5) that in turn can provide essential pollination services to some bioenergy crops.

7.5 Suggestions for further research

In addition to the research questions proposed at the end of section 6.2 about the impacts of future scenarios of bioenergy production on pollinator and pollination, here I highlight some other potential key areas for future research as suggested by results of this thesis.

In chapter 5 I investigated the potential of oilseed rape to influence pollination services to wild plant species via deposition of crop pollen on stigmas. However, pollination services can also be affected by changes in visitation rates, and differences in visitation and pollen transfer may not translate into changes in seed set (Dietzsch *et al.* 2011). Although this chapter finds little oilseed rape pollen deposited on wild plant stigmas, further work on how mass flowering crops affect visitation rates to native species, and how this affects female fitness in the form of seed set, would further this field (e.g. Cussans *et al.* 2010).

This thesis has shown that oilseed rape provides resources for large numbers and diversity of insects in farmland. Although some studies have carried out nutritional work on the benefit of this resource for some pollinator groups (e.g. Regali & Rasmont 1995; Cook *et al.* 2003; Tasei & Aupinel 2008), further work on the quality of this resource for pollinators would be useful. Pesticides applied to crops can be found in crop nectar (Stoner & Eitzer 2012) and in the nectar of surrounding wild plants (Krupke *et al.* 2012). The effects of some pesticides on bees have been studied (Cresswell 2011; Girolami *et al.* 2012; Henry *et al.* 2012; Laycock *et al.* 2012; Whitehorn *et al.* 2012), but effects on other pollinator taxa are not as clear. Therefore more work on the quality of oilseed rape nectar and pollen as a resource, and the impacts of pesticide application on pollinators and on nectar quality, deserves further investigation before clear recommendations can be made.

In Chapter 6 I investigated the pollinator fauna of both spring and winter oilseed rape crops, but seed set and pollen transfer only in the winter crops. As there are large differences in abundances of insects found between spring and winter oilseed rape, it is likely that there are differences in pollination services between the crop forms in Ireland also, which has been shown in the UK (Hayter & Cresswell 2006). Therefore, future work on the pollination of spring oilseed rape in Ireland would help to determine the benefits of the crop from insect pollinators.

This is the first study to evaluate the proportions of species of the *B. sensu stricto* cryptic complex in agricultural habitats (Chapter 4). Although the idea that the cryptic species are ecologically distinct is gaining evidence (Murray *et al.* 2008; Byrne 2011; Waters *et al.* 2011b), specific forage or nesting preferences of the individual species is not known. Therefore more work on this cryptic complex would be beneficial in the understanding of the distribution and conservation status of these species that provide valuable pollination services in agricultural areas (Chapter 6), and would help in interpreting landscape scale responses observed in this study (Chapter 4).

Although oilseed rape currently appears to receive sufficient pollination services in Ireland, and growth of bioenergy crops seem to have little or even positive effects on pollinator abundance and diversity of the common species, growth of new crops is not likely to significantly augment habitat for pollinators in agricultural areas, or to benefit rarer species. Field margins are particularly important in terms of the provision of both nesting and floral resources and agri-environmental schemes could be implemented to conserve and augment field margins (e.g. Carvell *et al.* 2007), as well as achieve other objectives such as a reduction in pesticide use, which could help to ensure sustained pollination services and to benefit rarer species. The benefits of some of these schemes, such as the planting of pollinator forage mixtures, have been evaluated in some counties (Pywell *et al.* 2006; Carvell *et al.* 2007). The current agri-environmental schemes in operation in Ireland include the Rural Environmental Protection Scheme (REPS; Emerson & Gillmor 1999; this was closed to new applicats in 2009 but existing farms will participate in the scheme until 2014) and the Agri-Environmental Options Scheme (AEOS, which was made available in 2010). At the close of REPS 4, 50% of the utilisable agricultural land in Ireland was being managed under the REPS scheme and in 2011 €257 million was paid to farmers in REPS payments (DAFF 2011). However, there has been no monitoring or critical evaluation as to whether the measures in Irish agri-environmental schemes to date achieve their environmental aims on a large scale (Finn & Huallachain 2012), and evidence suggests that some field margin measures have no biodiversity benefit (Feehan *et al.* 2005). Therefore, further research on how pollinators and the services they provide (especially to bioenergy crops) can be sustained in agricultural areas, by evaluating and implementing different agri-environmental measures, would be beneficial.

Plant-pollinator networks are a useful tool for studying the interactions between plants and their flower visiting insects. However, knowledge about which network properties are beneficial or useful for practical conservation purposes is still under discussion (Tylianakis *et al.* 2010), which makes it difficult to interpret the magnitude and direction of landscape scale effects (Chapter 3). Therefore, further work on what properties of plant-pollinator networks infer robustness, stability or other desirable characteristics could be useful for practical conservation purposes in what, to date, is a largely a theoretical field.

7.6 Concluding remarks

Human modification of the Earth's surface continues to have consequences for biodiversity (Vitousek *et al.* 1997). Although recent interest in halting biodiversity loss is gaining momentum (Ehrlich *et al.* 2012), the rate of biodiversity loss worldwide does not appear to be slowing (Butchart *et al.* 2010). This has many implications for the provision of ecosystem services to humanity (Cardinale *et al.* 2012), and even for human welfare

(Ulrich 1984; Lederbogen *et al.* 2011). Bioenergy production is seen as one alternative to the use of fossil fuels to help to reduce the pressures of climate change on the planet. Although bioenergy production in its current form is unlikely to have the capacity to fulfil global energy requirements, it may be an important contributor in conjunction with other energy sources. What may help to alleviate climate change may, however, have alternative impacts on other factors including biodiversity.

Pollination is an example of a tangible ecosystem service with direct consequences for human food production, as well as indirect consequences through provision of many other ecosystem services. Therefore maintenance of pollinators and pollination services in agricultural areas where pollination services are required is of utmost importance, and provides an economic incentive for biodiversity conservation (Gallai *et al.* 2009). Bioenergy production, as the industry currently stands in Ireland, has little or positive impacts on pollinator abundance and diversity of common taxa and more variable impacts on pollination services, although patterns are influenced by the composition of the surrounding landscape. Furthermore, one of the main bioenergy crops grown in Ireland, oilseed rape, benefits in yield from pollination services. However, future scenarios and predictions for bioenergy production may be different from existing conditions, which has the potential to change the direction and magnitude of effects. Therefore we must endeavour to predict and monitor future impacts and changes in agricultural regions, which may have implications for pollinators and the provision of essential ecosystem services.

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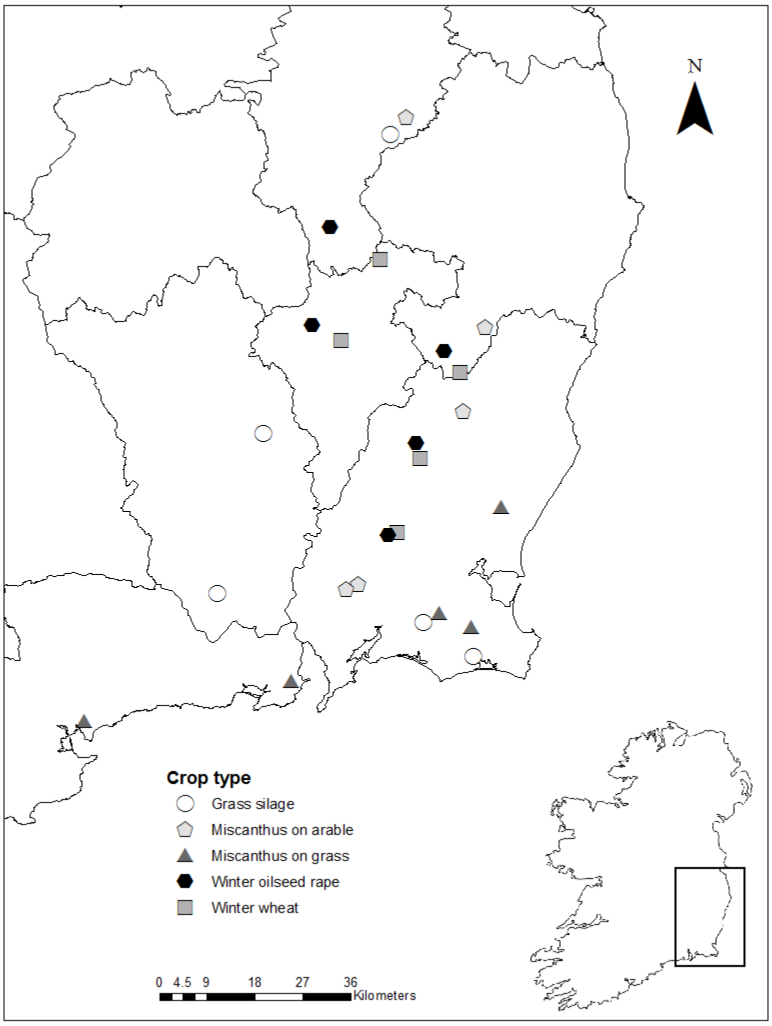
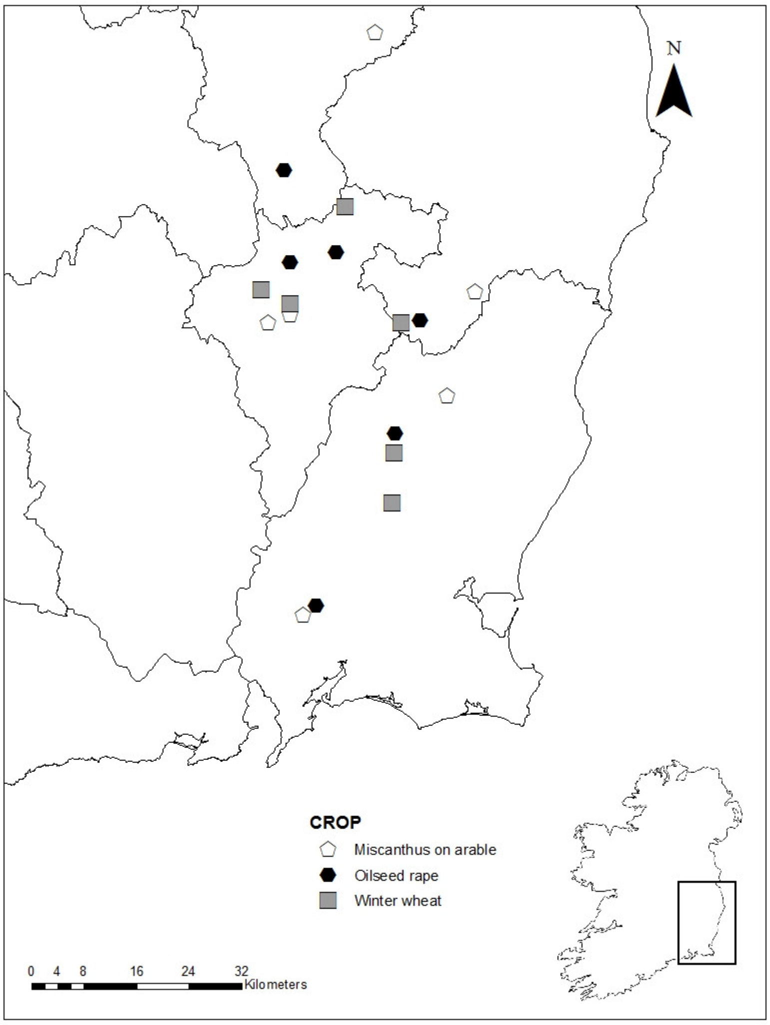
Appendices



Appendix 1. The locations of the subset of 25 sites used for the transect sampling in 2009. The same Miscanthus on arable, oilseed rape and wheat sites were used for trap nest sampling.



Appendix 2. The locations of the 18 sites used for observations of nest searching bumblebees in spring 2010.

Appendix 3. Abundance (A) and species richness (S) of each pollinator group found in each crop type using pan trapping and transect walks. Arable crops: oilseed rape (OS), Miscanthus on arable (MA) and wheat (WW); Grass crops: Miscanthus on grass (MG) and grass silage (GS).

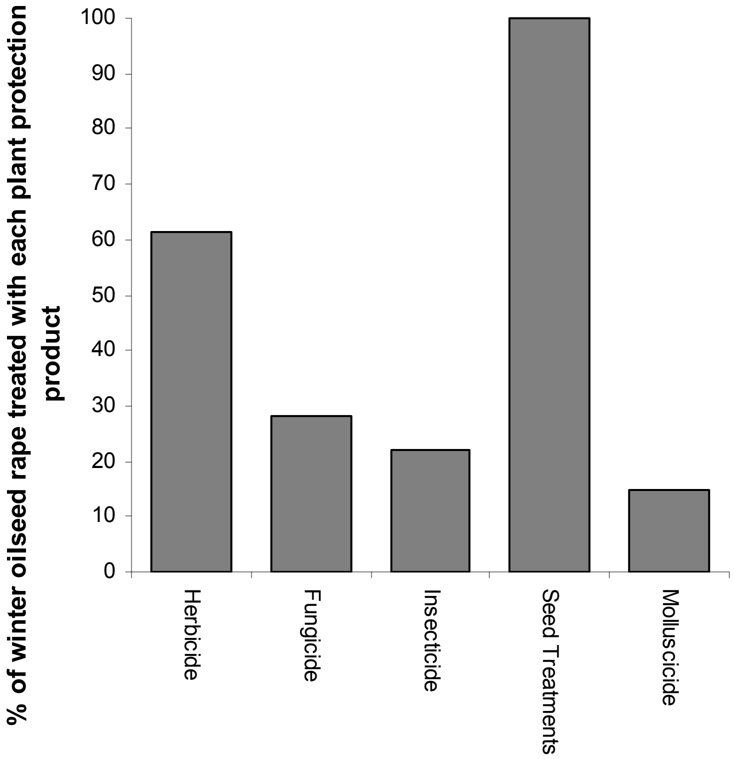
	Pan traps	(x10 fields)	**Transects**	(x5 fields)	**Pan traps**	(x5 fields)
	A	S	A	S	A	S
Bumblebees						
GS	142	6	95	5	100	5
MG	137	5	125	5	74	5
WW	136	7	88	5	78	6
MA	129	6	88	5	59	5
OS	308	8	234	5	228	8
Total	**852**	**8**	**630**	**5**	**539**	**8**
Hoverflies						
GS	211	18	326	------	147	12
MG	332	21	589	------	120	19
WW	315	26	424	------	165	21
MA	177	26	644	------	119	22
OS	484	26	559	------	203	20
Total	**1519**	**43**	**2542**	**------**	**754**	**37**
Solitary Bees						
GS	20	9	3	------	6	6
MG	39	10	6	------	15	6
WW	24	12	10	------	5	4
MA	68	16	5	------	37	12
OS	88	14	18	------	43	11
Total	**239**	**23**	**42**	**------**	**106**	**18**
Butterflies						
GS	------	------	30	5		
MG	------	------	94	9		
WW	------	------	61	8		
MA	------	------	92	9		
OS	------	------	63	8		
Total	**------**	**------**	**340**	**11**		

Appendix 4. Total abundances of the top 27 most abundant hoverfly species collected during pan trap sampling in each of the crop types, and results of SIMPER analysis. Species counts highlighted in grey represent those contributing to overall similarity within crop type. Arable crops: oilseed rape (OS), Miscanthus on arable (MA) and wheat (WW), Grass crops: Miscanthus on grass (MG) and grass silage (GS).

Hoverfly species	**WW**	**MA**	**OS**		**GS**	**MG**
Episyrphus balteatus	22	4	3		4	5
Eristalinus sepulchralis	10		1			
Eristalis abusivus		1	9		6	3
Eristalis arbustorum	30	10	141		9	7
Eristalis horticola			3			
Eristalis interruptus	6	2	10			8
Eristalis intricarius	2	3	12			5
Eristalis pertinax	5	13	1			12
Eristalis tenax	14	4	68		5	10
Eupeodes corollae	14	4	17		2	8
Helophilus hybridus	32	15	32		17	24
Helophilus pendulus	121	96	141		115	224
Helophilus trivittatus	7	1	3		1	
Melanogaster hirtella		1			5	
Melanostoma mellinum	6	2	3		6	3
Myathropa florea	5	1				4
Neoascia podagrica	7	2			7	
Platycheirus albimanus	8	3	3		5	
Platycheirus clypeatus					2	1
Platycheirus granditarsus	3				2	1
Platycheirus manicatus	8		5		2	
Platycheirus nielseni/peltatus	1	1	1			1
Rhingia campestris	5	2	18		21	8
Sericomyia silentis		4	3			1
Syrphus vitripennis	1					3
Volucella bombylans	2	1	2			2
Xylota segnis	1	1	1		1	
within group similarity	39.44	31.17	48.11		31.94	44.74
between group dissimilarity						
	WW-MA	64.26		GS-MG	67.94	
	WW-OS	61.61				
	MA-OS	66.98				

Appendix 5. Total abundances of floral units of the top 32 most abundant flowering plant species collected during pan trap sampling, and results of SIMPER analysis. Species counts highlighted in grey represent those contributing to overall similarity within crop type. Arable crops: oilseed rape (OS), Miscanthus on arable (MA) and wheat (WW), Grass crops: Miscanthus on grass (MG) and grass silage (GS). Plant forms are as follows: a = annual, b = biennial and p = perennial

Plant species	**form**	**WW**	**MA**	**OS**		**GS**	**MG**
Anthriscus sylvestris	p/b	150		575		375	125
Cirsium arvense	p	150	325			175	1250
Cirsium vulgare	b	75	50	275		275	975
Craetagus monogyna	p	525	1225	850		1300	1400
Epilobium angustifolium	p		750				175
Epilobium hirsutum	p		375				350
Epilobium montanum	p		1050	200			1150
Filipendula ulmaria	p	125	475			25	425
Fumaria spp.	a	225		2050			125
Galium aparine	a	1425	275	1600		125	250
Geranium robertianum	a	250	250	125			100
Heracleum sphondyllium	p	625	2350	1100		625	275
Lapsana communis	a	200	50	3300			125
Lathyrus pratensis	p	25	500			150	
Lotus corniculatus	p		1050			350	3050
Myosotis arvensis	a/b	50	50	1300			
Ranunculus repens	p	50	2800	350		2150	3075
Rosa canina	p	200	75	75		150	75
Rubus fruticosus	p	1625	1700	1475		1300	1950
Sambuchus nigra	p	275	850	175		600	350
Senecio vulgaris	a	25	350	500			400
Sonchus asper	a	75	25	1275			350
Stachys palustris	p		25	125			700
Stachys sylvatica	p		425	175		25	925
Stellaria graminea	p	125	150	175		75	125
Trifolium pratense	p		600				75
Trifolium repens	p		125			1450	475
Ulex europeus	p	500	500	650		950	1425
Veronica persica	a			900		50	50
Vicia cracca	p	125	275			125	150
Vicia sepium	p	700		325		525	450
Viola arvensis	a		50	625			
within group similarity (%)		37.69	34.29	39.76		37.34	31.7
between group dissimilarity (%)							
		WW-MA	74.92		GS-MG	70.66	
		WW-OS	68.15				
		MA-OS	76.53				



Appendix 6. The percentage of winter oilseed rape treated with plant protection products (herbicides and pesticides) in 2004.

Data is based in a survey of 10 farms as part of the pesticide usage survey 2004 by the Department of Agriculture (DAFF 2004). Herbicides include diquat (dibromide), glyphosphat and propyzamined, Fungicides include mancozeb, metalaxyl, spiroxamine and tebuconazole, Insecticides include dimethoate and esfenvalerate, seed treatments include beta-cyfluthrin, imidacloprid and thiram, Molluscicides include methiocarb.

Appendix 7. Definitions of network indices, based on Dormann *et al.* (2009)

Qualitative

L	number of realized links in a network
I	number of lower trophic level species (plants)
J	number of higher trophic level species (pollinators)

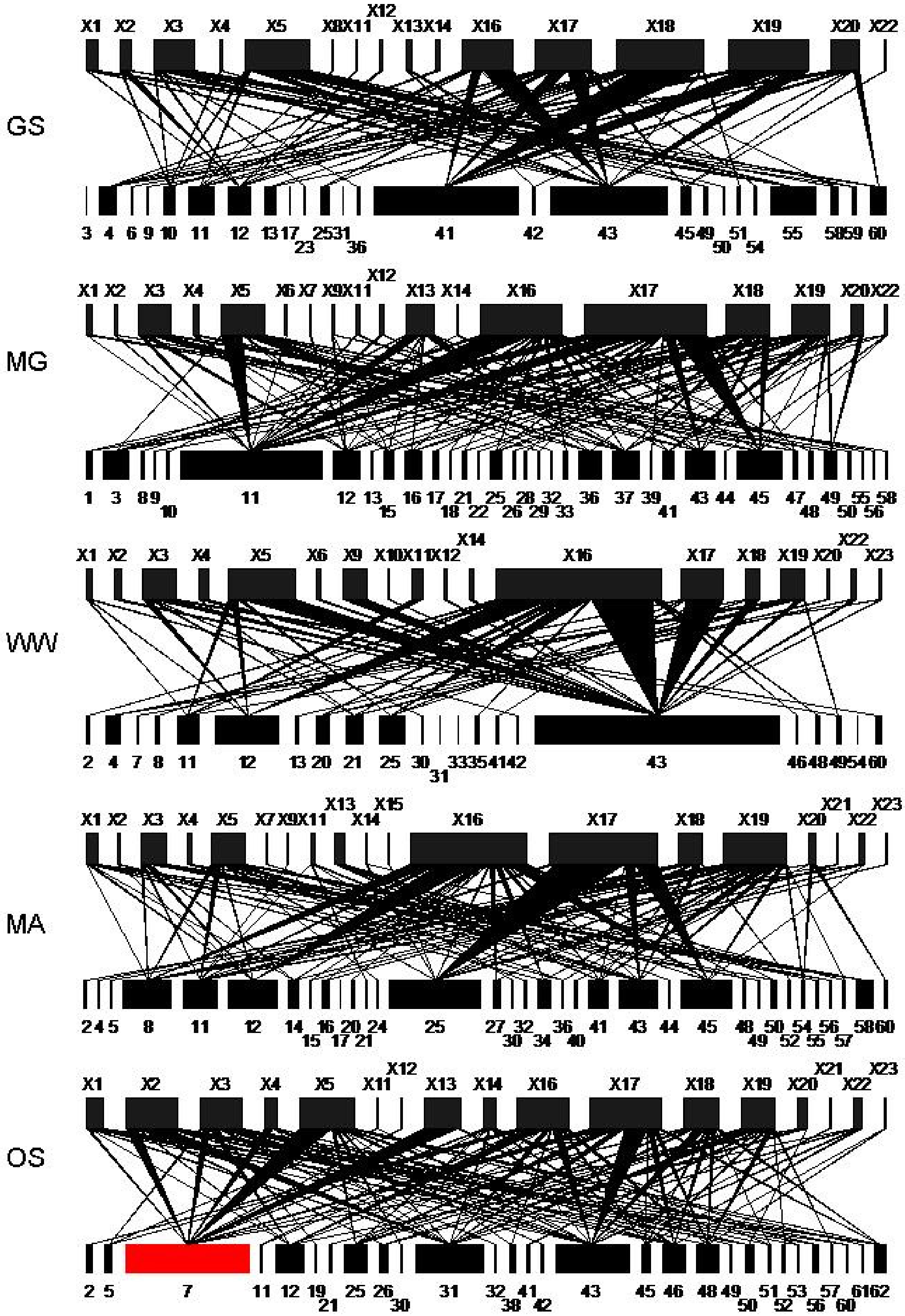
Network size	The number of plant species (I) x number of pollinator species (J) in the network
Connectance	The realised proportion of links in a network: $C = L/(IJ)$
Nestedness	The tendency of specialist species to interact with subsets of more generalist ones (Bascompte *et al.* 2003), or the "departure from systematic arrangement of species by niche width" (Dormann *et al.* 2009). The temperature (0-100) measures distances from perfectly nested (0) to chaos (100).

Quantitative

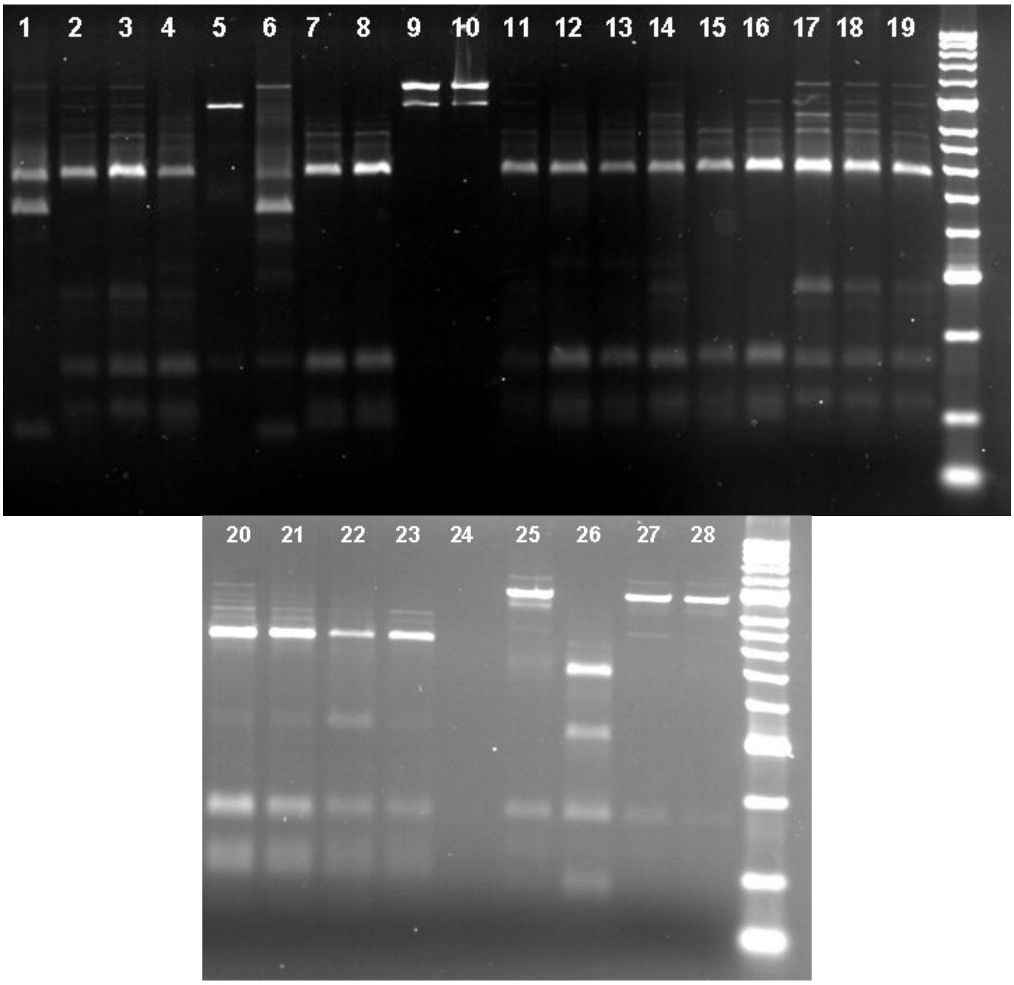
Number of .interactions	The total number of links within a network (L)
Generality	Weighted mean number of plants per pollinator (Bersier, Banasek-Richter & Cattin 2002)
H2	An overall network level measure of specialisation (Bluthgen, Menzel & Bluthgen 2006)
Interaction evenness	Shannons evenness of network interactions
Linkage density	Mean quantitative number of links per species (Tylianakis, Tscharntke & Lewis 2007)
Specialisation asymmetry	Assymetry of specialisation (imbalance) of specialisation in higher vs. lower trophic levels. Positive values indicate a higher specialisation of the higher trophic level (Bluthgen *et al.* 2007).
Vulnerability	Weighed mean number of pollinators per plant (Bersier, Banasek-Richter & Cattin 2002)
Web asymmetry	Balance between numbers of higher and lower trophic species. Positive values indicate more higher trophic level species, negative more lower-trophic level species.

Appendix 8. Species codes for plant-pollinator networks from Chapter 3 and Appendix 9.

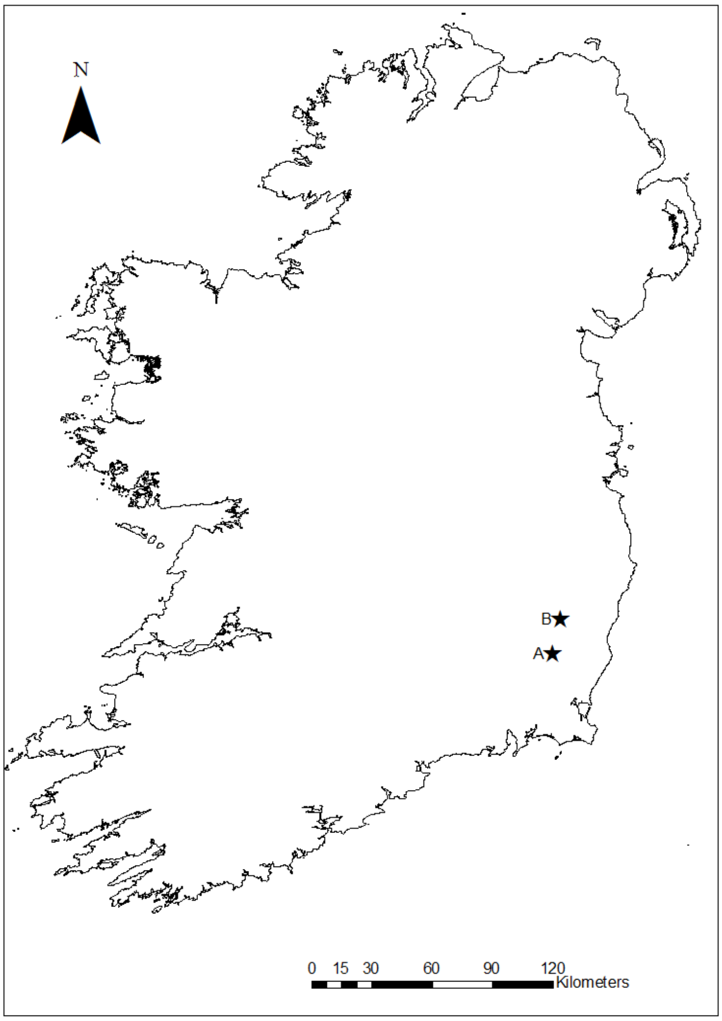
Group	Species	No.	Species	No.	Species (ctd)	No.
Bumblebees	*Bombus hortorum*	X1	*Achillea ptarmica*	1	*Leucanthemum vulgare*	33
Bumblebees	*Bombus lapidarius*	X2	*Aethusa cynapium*	2	Asteraceae	34
Bumblebees	*Bombus pascuorum*	X3	*Angelica sylvestris*	3	*Lonicera periclymenum*	35
Bumblebees	*Bombus pratorum*	X4	*Anthriscus sylvestris*	4	*Lotus corniculatus*	36
Bumblebees	*Bombus sensu stricto*	X5	*Arctium minus*	5	*Lythrum salicaria*	37
Butterflies	*Agalis urticae*	X6	*Bellis perennis*	6	*Papaver rhoeas*	38
Butterflies	*Aphantopus hyperantus*	X7	*Brassica napus*	7	*Persicaria maculosa*	39
Butterflies	*Inachis io*	X8	*Calystegia sepium*	8	*Ranunculus repens*	41
Butterflies	*Maniola jurtina*	X9	*Cardamine pratensis*	9	*Rosa canina*	42
Butterflies	*Parage aegeria*	X10	*Centaurea nigra*	10	*Rubus fruticosus*	43
Butterflies	*Pieris spp.*	X11	*Cirsium arvense*	11	*Sambuchus nigra*	44
Butterflies	*Vanessa carduii*	X12	*Cirsium vulgare*	12	*Senecio jacobaea*	45
Honeybee	*Apis mellifera*	X13	*Conopodium majus*	13	*Sinapsis arvensis*	46
Solitary bee	*Solitary bee spp.*	X14	*Epilobium angustifolium*	14	*Sonchus arvensis*	47
Hoverflies	*Cheilosia spp.*	X15	*Epilobium hirsutum*	15	*Sonchus asper*	48
Hoverflies	*Episyrphus balteatus*	X16	*Epilobium montanum*	16	*Stachys palustris*	49
Hoverflies	*Eristalis spp.*	X17	*Filipendula ulmaria*	17	*Stachys sylvatica*	50
Hoverflies	*Helophilus spp.*	X18	Apiaceae sp.	18	*Stelaria holostea*	51
Hoverflies	*Melanostoma type*	X19	*Teucrium scorodonia*	19	*Stellaria media*	51
Hoverflies	*Rhingia campestris*	X20	*Galium aparine*	20	*Stellaria graminea*	52
Hoverflies	*Sericomyia spp.*	X21	*Gernaium robertianum*	21	*Sysimbrium officinale*	53
Hoverflies	*Syrphus spp.*	X22	*Geum urbanum*	22	*Taraxacum* sp.	54
Hoverflies	*Volucella spp.*	X23	*Glenchoma hederacea*	23	*Trifolium repens*	55
			Poaceae	24	*Ulex europeus*	56
			Heracleum sphondyllium	25	*Veronica* sp.	57
			Hyacinthoides non-scripta	26	*Vicia cracca*	58
			Hypericum perforatum	27	*Vicia sativa*	59
			Hypochaeris radiate	28	*Vicia sepium*	60
			Knautia arvensis	29	*Viola arvensis*	61
			Lamium purpureum	30	*Un-ID 1*	62
			Lapsana communis	31	*Cirsium* sp.	63
			Lathyrus pratensis	32		



Appendix 9. Quantitative plant-pollinator interaction networks from the five crop types: Grass silage (GS), Miscanthus on grass (MG), winter wheat (WW), Miscanthus on arable (MA) and winter oilseed rape (OS). To summarise, networks show data pooled across all five replicate fields for each crop type, although analyses were carried out on a per field basis. Species codes are given in Appendix 9.



Appendix 10. Examples of Restriction Fragment Length Polymorphism (RFLP) fingerprints of cryptic bumblebees in ethidium bromide stained 2% agarose gel with hyperladder IV. *Bombus cryptarum* = lanes 1, 6 & 26. *B. lucorum* = lanes 5, 9, 10, 25, 27 & 28, *B. terrestris* = lanes 2, 3, 4. 7, 8, 11 – 23.



Appendix 11. Locations of the two winter oilseed rape fields used in Chapter 5.

Appendix 12. Nest number estimations for a) *Bombus terrestris*, b) *B. lucorum*, c) *B. cryptarum* and d) *B. lapidarius*. N_{ind} = number of individual worker bees sampled at each site, N_{obs} = number of nests observed, based on sibship reconstruction from Colony. N_{tot} = total number of nests estimated, including unsampled ones, using Two Innate Rate Models (TIRM) or Even Capture Models (ECM) methods in CAPWIRE. ECM methods are equivalent to previously used truncated Poisson methods (Goulson *et al*., 2010). A likelihood ratio test (LRT) was also used to compare between models for each sample, and best model is shown here; this was not used in the main text as it is sensitive to small sample sizes (Miller *et al*., 2005) but is given here for comparison with previous work. N_{sis} = total number of sister pairs found within the sampled individuals using Colony analysis of microsatellite data, and in the oilseed rape field only (OS), adjacent field only (ADJ) and shared between the two (Shared). Values with no upper limit, or where estimates were not possible due to a lack of sister pairs, are marked “n/a”. CAPWIRE models were run in 0.1 increments with capturability ratios of minimum 1, maximum 20; 95% confidence intervals for the estimate on population size based on 1000 bootstrap replicates; a largest population size of 750 for dimensioning; and a likelihood ratio rejection region of 0.2 when conducting likelihood ratio tests.

a) B. terrestris

Site	N_{ind}	N_{sis}	N_{sis} **OS**	N_{sis} **Adj**	N_{sis} **Shared**	N_{obs}	N_{tot} **TIRM (low CI-high CI)**	N_{tot} **ECM (low CI-high CI)**	N_{tot} **LRT (low CI-high CI)**
A	27	7	1	4	2	21	61 (30-119)	49 (30-108)	49 (26-108) ECM
B	73	8	3	0	5	65	332 (182-534)	304 (177-633)	304 (177-633) ECM
D	43	4	4	0	0	39	229 (107-610)	211 (114-437)	211 (114-437) ECM
E	55	15	4	2	9	42	116 (70-172)	95 (63-167)	95 (63-167) ECM
F	63	9	7	1	1	56	329 (164-661)	258 (141-630)	329 (164-661) TIRM
G	17	5	5	0	0	13	37 (15-68)	28 (13-62)	37 (14-92) TIRM
H	32	6	3	4	0	26	81 (42-143)	72 (38-155)	72 (44-155) ECM
K	53	9	5	3	1	44	150 (86-232)	135 (80-258)	135 (80-258) ECM
L	60	10	2	0	8	50	174 (102-297)	157 (97-334)	157 (97-334) ECM
M	47	11	2	7	2	39	152 (80-275)	119 (74-255)	152 (79-244) TIRM
N	21	1	1	0	0	20	213 (58-750)	203 (63-203)	203 (63-203) ECM
R	2	0	0	0	0	2	n/a	n/a	n/a
T	3	0	0	0	0	3	n/a	n/a	n/a
V	35	7	2	3	2	28	81 (44-150)	73 (42-187)	73 (42-137) ECM
Mean							163	142	

b) B. lucorum

Site	**N_{ind}**	**N_{sis}**	**N_{sis} OS**	**N_{sis} Adj**	**N_{sis} Shared**	**N_{obs}**	**N_{tot} TIRM (low CI-high CI)**	**N_{tot} ECM (low CI-high CI)**	**N_{tot} LRT (low CI-high CI)**
A	59	3	0	3	0	56	580 (228-750)	551 (225-551)	551 (225-551) ECM
B	32	1	0	1	0	31	502 (143-750)	485 (155-485)	485 (155-485) ECM
D	18	0	0	0	0	18	n/a	n/a	n/a
E	54	2	0	2	0	52	726 (241-750)	698 (268-698)	698 (268-698) ECM
F	31	6	1	3	2	26	103 (48-237)	83 (41-222)	103 (50-237) TIRM
G	27	0	0	0	0	27	n/a	n/a	n/a
H	44	0	0	0	0	44	n/a	n/a	n/a
K	36	2	0	0	2	34	320 (125-750)	303 (114-618)	303 (114-618) ECM
L	45	5	0	2	3	40	200 (98-502)	183 (95-480)	183 (95-480) ECM
M	62	6	0	5	1	57	428 (197-750)	357 (189-610)	357 (189-610) ECM
N	36	3	3	0	0	33	213 (86-637)	198 (93-618)	198 (78-618) ECM
R	33	2	2	0	0	31	269 (94-750)	253 (94-517)	253 (94-517) ECM
T	79	6	2	2	2	73	522 (267-750)	487 (253-744)	487 (253-744) ECM
V	60	3	0	1	2	57	599 (236-750)	570 (233-570)	570 (233-570) ECM
Mean							406	379	

c) B. cryptarum

Site	**N_{ind}**	**N_{sis}**	**N_{sis} OS**	**N_{sis} Adj**	**N_{sis} Shared**	**N_{obs}**	**N_{tot} TIRM (low CI-high CI)**	**N_{tot} ECM (low CI-high CI)**	**N_{tot} LRT (low CI-high CI)**
A	29	2	4	0	2	22	53 (27-101)	48 (30-126)	48 (30-92) ECM
B	10	1	0	0	1	9	46 (13-750)	42 (12-42)	42 (12-42) ECM
D	0	0	0	0	0	0	0	0	0
E	10	3	0	1	2	8	30 (9-750)	19 (8-42)	30 (8-750) TIRM
F	8	0	0	0	0	8	n/a	n/a	n/a
G	10	0	0	0	0	10	n/a	n/a	n/a
H	5	1	0	0	1	4	10 (4-750)	8 (4-8)	8 (4-8) ECM
K	9	0	0	0	0	9	n/a	n/a	n/a
L	15	0	0	0	0	15	n/a	n/a	n/a
M	5	0	0	0	0	5	n/a	n/a	n/a
N	3	0	0	0	0	3	n/a	n/a	n/a
R	12	0	0	0	0	12	n/a	n/a	n/a
T	38	1	0	1	0	37	710 (203-750)	690 (163-690)	690 (163-690) ECM
V	21	3	0	2	1	18	70 (29-213)	63 (28-203)	63 (28-203) ECM
Mean							153	145	

d) B. lapidarius

Site	**N_{ind}**	**N_{sis}**	**N_{obs}**	**N_{tot} TIRM (low CI-high CI)**	**N_{tot} ECM (low CI-high CI)**	**N_{tot} LRT (low CI-high CI)**
A	0	0	0	0	0	
B	0	0	0	0	0	
D	44	11	33	78 (47-122)	71 (44-120)	71 (48-120) ECM
E	0	0	0	0	0	0
F	51	26	30	48 (32-69)	42 (32-57)	42 (32-57) ECM
G	43	19	26	41 (26-61)	38 (27-49)	38 (27-54) ECM
H	0	0	0	0	0	0
K	52	25	32	58 (36-79)	48 (34-65)	48 (36-65) ECM
L	0	0	0	0	0	0
M	45	13	35	105 (59-164)	84 (50-150)	84 (50-150) ECM
N	44	21	27	48 (30-68)	40 (29-57)	40 (29-57) ECM
R	0	0	0	0	0	0
T	40	20	25	48 (28-69)	38 (26-51)	38 (26-51) ECM
V	0	**0**	0	0	0	0
Mean				61*	52*	

* not including sites where no individuals were found