The Nutritional Ecology of Farmland Bees: a Behavioural & Community Approach

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For the fathers I lost. I miss you both more than I can express. I am the man you made me, and so this is for you.

"*Other kings said I was daft to build a castle on a swamp, but I built it all the same, just to show 'em. It sank into the swamp. So, I built a second one… that sank into the swamp. So, I built a third… that burned down, fell over, and then sank into the swamp. But the fourth one... stayed up*"

Michael Palin as the King of Swamp Castle,

Monty Python and the Holy Grail

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Abstract

Nutritional degradation, attributed to agriculture, is a primary driver of bee declines, yet we know very little about bee larval nutrition. How larvae deal with nutritional variation in their provisions remains relatively unexplored. Additionally, the nutritional requirements of adult and larval bees differ, yet such a distinction is rarely considered when investigating bee-flower communities. Using the Geometric Framework I ask (a) whether solitary bee larvae (*Osmia bicornis*) regulate their nutrient intake, and (b) whether the importance of macronutrients change across development. Second, I investigate (a) how bee and host plants communities change within a flight season on organic and conventional farms, and (b) how bees' foraging decisions shape their interaction networks. Specifically, using DNA metabarcoding of pollen, I separate larval- and adult-focussed foraging interactions. I show that larval bees prioritise carbohydrate over protein, but that the importance of individual macronutrients shifts from protein to carbohydrate throughout development. I also demonstrate that larvae regulate lipid intake, a macronutrient often overlooked in bee nutrition. I show that organic farms support higher abundances, but not higher diversity, of plants and bees, and that nutritional resources vary more with season than farming practice. Lastly, I show that bees forage differentially for their offspring, highlighting the need to consider both adult and larval nutrition when managing landscapes for bees. These findings highlight the importance of a holistic view of bee nutrition. Larval bees are able to regulate their nutritional intake, suggesting a capacity to deal with nutritional variation. However, this ability is limited, with bees perhaps being vulnerable to undetectable changes to their nutritional environment. Nutritional resources also differ phenologically across farming practices, highlighting the need to address key nutritional

gaps for bees. Finally, understanding that bee parental care shapes the way bees interact with their environment is essential to providing quality floral resources that address both adult *and* larval needs.

Contents

1. Introduction

1.1. Bees: a brief background

Bees are a group of Aculeate Hymenopteran insects, a group also including ants and wasps. Widely believed to have originated from wasps (Danforth et al., 2013), bees have switched from a carnivorous lifestyle to one that involves the collection of pollen and nectar from flowers (Falk, 2015). Bees are an often studied group yet, in general, bees are frequently viewed through the lens of common species (Goulson and Nicholls, 2016), such as honeybees and bumblebees, with much of the work studying bee declines being in these groups (Connop et al., 2010; Durant, 2018; Hayes et al., 2008; Kovács-Hostyánszki et al., 2019; Szabo et al., 2012; Williams et al., 2009). However, bees as a group are actually highly varied and speciose (Danforth et al., 2013), with varying morphology, life histories and feeding habits (Falk, 2015; Michener, 2000).

In terms of their lifestyles bees can be highly variable yet can be roughly grouped into two classes, solitary and social (Falk, 2015); although this is a continuum as some bees can be facultatively social (Smith et al., 2018, 2019) i.e. are initially plastic, being able to adopt a social or solitary lifestyle depending on specific conditions, and which once developed is irreversible. The most commonly recognised group of bees, certainly from the perspective of the general public, are the social species, most notably honeybees. Honeybees are termed as highly eusocial (Jones et al., 2018), constructing complex nests (or hives) consisting of sheets of hexagonal wax cells with individual colony members numbering in the tens of thousands (Amdam, 2011). They also have specific castes (queen, worker, drone), as well as division of labour (Amdam et al., 2006), whereby specific individuals have specific roles

within the hive. Although the honeybee is the most commonly thought of eusocial bee, they are not the only group to adopt this highly eusocial behaviour. In the tropics and subtropics stingless bees also share this trait and come in a variety of forms (Baudier et al., 2019; Jailani et al., 2019; Luna-Lucena et al., 2018).

Despite the familiarity we have with the highly social bees, this is actually the least common phenotype, with the majority of species being less social [e.g. bumblebees and sweat bees (Hendriksma et al., 2019; Richards, 2019)] or solitary (Michener, 2000; Richards, 2019).

Solitary bees are, in fact, far more numerous in terms of species, making up the majority of our pollinators (Falk, 2015; Wood et al., 2016). Not only that, they are also more efficient pollinators in many cases (Garibaldi et al., 2013; MacInnis and Forrest, 2019), providing the bulk of our pollination services (Winfree et al., 2008, 2007). Despite this, they are arguably the least understood group, with focus tending to be on the social species (Leach and Drummond, 2018; Wood et al., 2016). A solitary bee is essentially a lone wolf, with each female building her own nest and producing her own offspring with no help from relatives (Bosch, 1994; Field, 2005; Strohm et al., 2002), unlike in the social species. Their nest structures can also vary greatly, with cavity nesters building linear nests of cells in pre-existing cavities such a bore holes or hollow plant stems (Strohm et al., 2002), ground nesters which excavate their own burrows or make use of existing abandoned ones, and even certain *Osmia* species that use empty snail shells (Falk, 2015). A typical solitary life cycle, although this can differ slightly between species, involves the emergence of adults in the spring, mating (after which the males die), then nesting and the provisioning of offspring, which once complete ends the life cycle of the adults. The offspring then develop in their cells, overwinter and then emerge as adults the following

year, starting the cycle again (Bosch, 1994; Radmacher and Strohm, 2011; Strohm et al., 2002).

There are some bees that skip any form of nest building, moving beyond the gathering of nectar and pollen, becoming cleptoparasites (Danforth et al., 2013; Falk, 2015). These 'cuckoo bees' lay their eggs in the nests of other bee species, taking no part in foraging for their offspring (Kilner and Langmore, 2011; Michener, 2000). Cuckoo bumblebees are a particularly interesting group, as not only do they not build their own nests, they hijack the nest of their host, killing the queen and enslaving the workers (Suhonen et al., 2016).

Bees can also vary according to their diet breadth. Although generally speaking bees can forage for nectar across a broad selection of flowers (Falk, 2015) (although they can be morphologically restricted, see tongue length below), in terms of pollen this can vary. Bees tend to range on a spectrum of pollen diet breadth from generalist to specialist i.e. polylectic to monolectic (Haider et al., 2014; Larkin et al., 2008; Ritchie et al., 2016; Vanderplanck et al., 2014; Wood and Roberts, 2017). Polylectic bees, although not indiscriminate, gather pollen from a broad range of flowers (Ritchie et al., 2016) - the most common form of foraging group (Falk, 2015). Oligolectic bees, however, gather pollen from a narrower set of flowers, normally within the same genus or closely related genera (Cerceau et al., 2019; Dötterl et al., 2005). At the most specialised end of the spectrum are the monolectic bees, which gather pollen from only a single species of plant (Cane et al., 1996). This monolecty can also be enforced by the geography. For example, in the UK *Andrena florea* gathers pollen only from White Bryony, yet on the continent there are a broader array of similar plants and therefore *A. florea* is in fact oligolectic in the rest of Europe (Falk, 2015).

The gathering of pollen in bees in primarily for use in feeding their young (Filipiak, 2019), although adults do consume small amounts (Cane et al., 2016). In terms of feeding

their young, bees most commonly mass provision (Richards, 2019) i.e. sealing an egg inside a cell which contains all the required nutrition for the offspring to reach adulthood (Field, 2005). This is the case for almost all solitary bees (Pitts-Singer, 2004; Richards, 2019; Strohm et al., 2002) and even some eusocial species, such as certain stingless bees (Roubik, 2006). However, some bees provision progressively i.e. feed their offspring over the course of their development and often feeding more than one offspring at a time (Field, 2005). This is most notable in social species such as honeybees and bumblebees (den Boer and Duchateau, 2006; Helm et al., 2017), and, in honeybees specifically, specialist nurses feed the larvae until they are ready to pupate (Liu et al., 2019; Lucchetti et al., 2018).

Although bees tend to be more able to utilise a broader set of flowers for nectar than for pollen, a bee's morphology can limit the flowers that they can access for nectar. In particular, the tongue length can restrict which nectaries a bee can access (Falk, 2015). In bumblebees for example, the tongue length dictates the corolla depth of a flower that the bee can utilise. Longer tongued bees are better at harvesting nectar from certain flowers, focusing on inflorescences with deep corollas (Borrell, 2005; Iwasaki et al., 2018), with shorter tongued bees generalising across a wider array of corolla lengths (Miller-Struttmann et al., 2015); although short-tongued species can rob nectar by chewing a hole in the base of an inflorescence with a deep corolla (Heiling et al., 2018; Irwin et al., 2004)

It is clear then that bees are a highly varied group of pollinators and that the differences shown in terms of how they gather food for their young, which flowers they can access, and whether they are social or solitary, could all influence how they interact with their plant mutualists. Being aware of the variation amongst bees, and understanding that such variation will influence how bees interact with the floral resources from which they feed, is important when investigating the nutritional ecology of bees.

1.2. Multiple threats, bees in decline

Bees are amongst our most prolific and important pollinating insects (Wood et al., 2016). They are critical to not only the pollination of our food crops (Klein et al., 2018, 2007; Potts et al., 2016) but also to the survival of the majority of wild flowers as well (Ollerton et al., 2011). However, bees are in decline (Goulson et al., 2015; Goulson and Nicholls, 2016), both in terms of commercial species such as the honey bee (Hayes et al., 2008; Potts et al., 2010b), but also wild bees (Durant and Otto, 2019; Wood et al., 2016) and, as with many wildlife declines, we humans must accept much of the responsibility for their losses (Goulson et al., 2015; Kaluza et al., 2018; Rhodes, 2018). Although the causes of the current declines we see in bees, and insects more generally (Leather, 2018; Sánchez-Bayo and Wyckhuys, 2019), are myriad, almost all of them are caused or exacerbated by human activity (Winfree et al., 2009). The major cause that I focus on in this thesis is the lack of adequate nutrition - arguably the major cause behind bee declines (Roulston and Goodell, 2011; Wood et al., 2016) - and is a topic I will discuss in greater depth throughout this thesis. In brief, it is a scenario primarily brought about through the loss of flower-rich habitats (Goulson et al., 2015; Müller et al., 2006; Wood et al., 2016), which for the most part is attributed to the intensification of agriculture (Nooten and Rehan, 2019; Papanikolaou et al., 2017; Robertson et al., 2013). Such changes have caused the loss of floral resource diversity and abundance through the wide adoption of mass flowering crops (Goulson et al., 2015) and intensive farming practices (Pywell et al., 2005). However, this is by no means the only driver behind bee declines. They also face threats from loss of nesting habitat, disease, pesticide exposure and anthropogenic climate change (Goulson et al., 2015; Goulson and Nicholls, 2016).

Habitat loss not only affects the food supply for bees (Goulson et al., 2015; Wood et al., 2016), but also their nesting resources (Cane, 2001). Human expansion, urbanisation and the conversion of pristine habitat into agricultural land has all contributed to the widespread loss of pollinator-friendly habitat. Almost all bees require the provision of natural materials in their environment in order to create their nests, and this can vary across species (Potts et al., 2005), including hollow plant stems, grassy tussocks, abandoned burrows or even empty snail shells (Falk, 2015; Potts et al., 2005; Svensson et al., 2000). Some bees, such as bumblebee queens, need to also overwinter (Hatfield et al., 2012), and therefore require areas that can provide suitable shelter for them over the winter months. The removal of natural or semi-natural habitats can mean that such sites can be lost, limiting the diversity of bee communities (Potts et al., 2005).

The exposure to pesticides is another anthropogenic threat bees face, and the use of pesticides and its effects on bees has received a great deal of attention (Abraham et al., 2018; Challa et al., 2019; Sponsler et al., 2019). Neonicotinoid insecticides are a prime example of a pesticide that has had demonstrable sub-lethal effects on bees (Ma et al., 2019; Woodcock et al., 2017), affecting their foraging (Henry et al., 2012; Morandin and Winston, 2003), fertility (Fisher and Rangel, 2018; Sgolastra et al., 2018), navigation (Jin et al., 2015), and immunocompetence (Brandt et al., 2016; Grassl et al., 2018). Despite neonicotinoids now being banned (Carreck, 2016; Gross, 2013) other insecticides are still used (Abraham et al., 2018; Raine, 2018), insecticides which we arguable know less about and whose effects may still be unknown. Although insecticides can have direct impacts on bees, herbicides too can cause harm to bees by removing key floral resources, reducing the abundance and diversity of the available food (Roschewitz et al., 2005; Sharma et al., 2018). Chemical fertilisers can have a similar effect, as wildflowers require relatively nutrient poor soils and the artificially enriched soils make it difficult for wildflowers to

grow, with areas often being dominated by very few species (Nowakowski and Pywell, 2016).

Disease has also become a more prevalent problem in recent years and is another driver that has been compounded by human activity (Goulson et al., 2015). The diseases are legion, with deformed wing virus, acute bee paralysis virus and Israeli paralysis virus being just a few examples (Sánchez-Bayo et al., 2016). Parasites are also a significant problem, such as the infamous Varroa destructor (Giuffre et al., 2019; Haber et al., 2019; Tentcheva et al., 2004), and additionally act as vectors for viral pathogens. The threat of disease is particularly prevalent in commercial species (Gisder and Genersch, 2016; Pirk et al., 2016), such as the honeybee (Strobl et al., 2019) and certain bumblebees (Murray et al., 2013; Otterstatter and Thomson, 2008). Diseases and parasites have been spread rapidly through human movement of commercial bees for pollination (Chapman et al., 2017; Goulson et al., 2015; Smith et al., 2013), however, spillover to wild bees is also a concern, as wild bees can pick up parasites from commercial escapees (Colla et al., 2006; Ravoet et al., 2014) by interacting with the same flowers. Moreover, diseases have been becoming more prevalent in recent years (Sánchez-Bayo et al., 2016) and such commercial spillover is thought to be at least partly to blame (Colla et al., 2006; Otterstatter and Thomson, 2008).

Human induced climate change is a threat to all life, affecting biodiversity across the globe (Thomas et al., 2004) and bees are by no means exempt (Le Conte and Navajas, 2008; Lee et al., 2018). Climate change has been linked to shifting phenology in flowers and bee emergence, leading to mismatches in bee emergence and flower availability (Farzan and Yang, 2018; Kőrösi et al., 2018; Miller-Struttmann et al., 2015; Schenk et al., 2018). Bee activity can also be influenced by the weather (Peat and Goulson, 2005; Puškadija et al., 2007; Tuell and Isaacs, 2010), and with the rise in extremes of weather driven by climate change these effects are likely to be exacerbated.

It is clear that bees face a host of threats, but what is becoming clearer and ever more critical is the fact that these threats do not simply act alone, and in fact act in synergy. Specifically, in relation to the other major threat bees face, and the focus of this thesis, poor nutrition can act in concert with other threats to cause greater, compound effects. Poor nutrition can make bees more vulnerable to disease (Foley et al., 2012), being less able to muster an effective immune response. Climate change is also influencing the nutritional quality of pollen resources within flower species (Ziska et al., 2016), possibly degrading nutritional resources in undetectable ways. Conversely, receiving good quality nutrition can reduce bees' sensitivity to pesticides (Schmehl et al., 2014; Tosi et al., 2017) further highlighting the importance of good quality nutrition in bee health. As such, many or all of these threats may interact with the threat of nutritional degradation and therefore studying how bees interact with their nutritional environment and understanding their nutritional needs is crucial to mitigating their continued decline.

1.3. *Osmia bicornis*, the Red Mason bee

Osmia bicornis (=*O.rufa*) is one of the most common cavity nesting bees in the UK, flying from around March to July with wide coverage across southern and central England, growing more sporadic further north (Falk, 2015). The species is univoltine (producing only one brood in a season) and follows a scramble mating system. Females are widely distributed and therefore territorial behaviour by males is not beneficial - males therefore conduct non-competitive searches for females centred around flowering patches (Seidelmann, 1999). Females exhibit choice in males more in terms of vibrational messages

from the males than body size (Conrad et al., 2010) reinforcing the lack of relationship between body size and success in males (Seidelmann, 1999). *O. bicornis* is an excellent model species to use for asking nutritional questions (Nicholls et al., 2017), as it is a well-researched model (Fliszkiewicz et al., 2015; Konrad et al., 2009; Menzel et al., 1988; Ulbrich and Seidelmann, 2001; Wasielewski et al., 2011) and readily nests in artificial nest constructs (Strohm et al., 2002). In addition, they have a growing commercial application with many studies investigating the ability, efficacy and management of *O.bicornis* as a pollinator of an increasing number of commercial crop species (Gruber et al., 2011; Sedivy and Dorn, 2013; Wilkaniec et al., 2004), making them of special significance to agriculture.

The nesting behaviour of *O.bicornis* follows that of a classic cavity-nesting mass-provisioning hymenopteran, with females utilising pre-existing holes and cavities within which to lay a series of individual, linear cells (Bosch, 1994; Strohm et al., 2002). The order of the cells tends to reflect the sex of the offspring, with females provisioned towards the back of the nest - i.e. at the beginning of an adult's laying season - with a transition towards males as the nest progressess (Filipiak, 2019; Strohm et al., 2002). Each cell in a nest is provisioned with a single, independent provision - a 'pollen ball' - upon which is laid a single egg (Strohm et al., 2002). Upon laying, the cell is sealed and the female begins provisioning the next cell, moving from the back of the nest to the front (Nicholls et al., 2017). Upon hatching, larvae consume the pollen provision, spin a cocoon [taking up to a month to reach cocoon completion (Nicholls et al., 2017; Radmacher and Strohm, 2011)] within which they metamorphose into adults by autumn, spending the winter as adults inside their natal nest cells (Radmacher and Strohm, 2011; Strohm et al., 2002). Females will happily use a number of different types of cavity including hollow plant stems (Seidelmann et al., 2015), bamboo internodes (Everaars et al., 2011; Gruber et al., 2011)

and wooden blocks (Dobson et al., 2012), as well as fully artificial structures such as those used by Strohm et al. (2002). In *O.bicornis* specifically, research has been conducted to optimise the use of artificial nest aggregations by identifying the optimal dimensions for artificial nests. Seidelmann et al. (2015) investigated how differing lengths and widths of nesting tubes influenced the nesting behaviour and development of larvae in *O.bicornis* and found that nest cavities of a width between 8-10mm and a minimum length of 150mm to be optimal, producing a balanced sex ratio and largest body sizes. Nesting habitat varies from agricultural areas (Coudrain et al., 2015) to mixed forest margins (Fliszkiewicz et al., 2015).

O.bicornis is polylectic, meaning that it forages on a wide array of flowers from many different families (Dobson et al., 2012). Haider et al. (2014) found pollen from 19 different plant families in the pollen ball of *O.bicornis* with a predominance for *Quercus* and *Ranunculaceae*. However, Coudrain et al. (2015) found that the diet of *O.bicornis* larvae was heavily influenced by both nesting habitat and food availability with parents adjusting their foraging strategy to make up for reductions in preferred host plants, suggesting that despite having preferences the foraging behaviour of *O.bicornis* is flexible. This is further demonstrated by their apparent ubiquity in urban environments, with diets being constructed from private gardens and parks (Everaars et al., 2011). Females use the available floral resources in an area to construct a provision for their young, primarily made up of pollen (~97%) with a relatively low proportion of nectar (Strohm et al., 2002), and it is this that will form the entirety of the larva's nutrition.

1.4. Pollen, bees, and their nutrional interactions

Pollen is essential for the reproduction of all seed plants (Lunau, 2000), its primary function being to deliver male gametes to the female reproductive organs (Konzmann et al., 2019). In many cases, plants must rely on animals to transfer pollen in order to successfully sexually reproduce (Ollerton et al., 2011). This has resulted in coevolution between many flowering plants and their animal couriers, their pollinators (Hu et al., 2008). Bees are a particularly prominent group of pollinators and often show some of the closest evolutionary relationships with their plant hosts (Lunau, 2004; Ramírez et al., 2011; Shimizu et al., 2014). A particular trait developed by plants to encourage these pollinator partnerships is the provision of a floral reward, most commonly in the form of nectar (Fowler et al., 2016; Wright and Schiestl, 2009) and/or pollen (Russell et al., 2017). This mutualistic relationship between bees and plants can often give the romanticised impression that bees and flowers are just helping each other out. In truth, the relationship is not quite so altruistic, especially in terms of pollen, as bees and plants have conflicting interests and therefore the relationship should be viewed more in terms of mutual balanced exploitation (Praz et al., 2008; Westerkamp, 1996). Bees do not gather pollen from plants in order to pollinate them but in order to consume it, mainly through delivery to their offspring (Filipiak, 2019). Pollination, however, is highly inefficient, with typically <1% of pollen removed from flowers reaching conspecific stigmas (Harder and Johnson, 2008; Hargreaves et al., 2009). Therefore, pollen consumption can be a considerable cost to plants, both metabolically through its production (Wäckers et al., 2007), but also reproductively by the removal of pollen resources from pollination (Harder and Routley, 2006). Of the two major floral rewards, the consumption of pollen is likely to be more costly to plants than nectar consumption, as it has a more direct reproductive impact

(Hargreaves et al., 2009). Thus, bees and plants have conflicting interests (Konzmann et al., 2019; Westerkamp, 1996), and plants carry adaptations to limit overexploitation of their pollen resources (Konzmann et al., 2019; Lunau et al., 2015; Praz et al., 2008), as they benefit from maximising pollen transfer to their conspecifics, i.e. pollination, whereas bees benefit from gathering the most nutrition with the least effort, i.e. foraging. Within foraging there is a further split; that between adult foraging and foraging for larval provisions, which likely results in bees making different foraging decisions based on whom they are foraging for (Filipiak, 2019). Recognising and teasing apart the different nutritional interactions from those of pollination is important if we are to provide a true representation of bee-flower relationships.

Understanding nutritional relationships is of particular importance ecologically as nutrition shapes the way in which animals interact with their environment (Simpson and Raubenheimer, 2012a). In bees, nutrition has been shown to influence behaviour (Walton et al., 2018), growth and development (Fischman et al., 2017; Lawson et al., 2017), caste determination (Luna-Lucena et al., 2018), immunocompetence (Spivak et al., 2019) and even the mitigation of harmful toxins such as pesticides (Schmehl et al., 2014). Despite this, we still know relatively little about the nutritional requirements of most bees (Leach and Drummond, 2018), with research traditionally restricted to a small set of focal species (Corby-Harris et al., 2019; Démares et al., 2016; Li et al., 2019; Ruedenauer et al., 2018; Tanaka et al., 2018; Vaudo et al., 2018; Walton et al., 2018); although work in other groups in increasing (Filipiak, 2019; Fischman et al., 2017; Müller et al., 2006). On top of this lack of a broad species representation, we also know comparatively little about the larval nutrition of bees, with work tending to focus on adults (Altaye et al., 2010; Archer et al., 2014a; Pirk et al., 2010; Vaudo et al., 2015). However, the nutrition received at the larval stage is critical in insects, as it is where all growth occurs (Nijhout et al., 2014), and in

bees, the larval diet is markedly different to that of adults (Filipiak, 2019). Although studies are beginning to highlight these differences (Filipiak, 2019; Helm et al., 2017), our understanding of larval nutrition in bees still lags far behind that of adults.

The larval provisioning relationship in bees is particularly interesting; social species progressively provision, having regular contact with their offspring, yet mass-provisioning species, which form the majority of bees, have the challenge of gathering food for their offspring without ever coming in contact with them (Field, 2005). Moreover, pollen is highly variable in its nutrient content (Roulston and Cane, 2000a) and is a particularly difficult resource to assess nutritionally (Nicholls and Hempel de Ibarra, 2016), making the question of how bees deal with this critically important in terms of bee health, both from an adult and a larval perspective. Evidence for whether adult bees are able to assess the nutrient content of pollen and adapt their foraging accordingly is mixed (Corby-Harris et al., 2018; Muth et al., 2016; Nicholls and Hempel de Ibarra, 2016; Roulston and Cane, 2002; Ruedenauer et al., 2018, 2016), yet many larval insects also have the ability to assess and regulate their nutrient intake (Lee et al., 2012, 2004b; Wilson et al., 2019) and therefore perhaps bee larvae have a similar ability. However, experimental tests of such an ability have yet to be completed in a manner that allows for the accurate tracking of nutrient intake in bee larvae (Helm et al., 2017). The variety in nutritional composition of pollen, and the difficulty that bees face in assessing this variation, means that the nutritional landscape within which parents forage is not only highly variable but also likely extremely challenging to navigate. How this is achieved in adults is still being explored, but how this may also influence larval behaviour has gone almost unconsidered.

Bees are in decline globally (Bartomeus et al., 2013; Cameron et al., 2011; Potts et al., 2010b; Sirohi et al., 2015), which is of great concern as they are crucial to ecosystem health (Goulson and Nicholls, 2016), as well as our future food security (Potts et al., 2016).

Considering that one of the major causes behind these declines is thought to be the degradation of their nutritional landscapes (Goulson et al., 2015; Roulston and Goodell, 2011), understanding bees' nutritional relationships has never been more important. This change in bees' nutritional landscapes is primarily driven by human-induced landscape change, which is affecting the quantity, quality, and diversity of bees' nutritional resources (Donkersley et al., 2014; Goulson and Nicholls, 2016; Hass et al., 2018; Rhodes, 2018; Ziska et al., 2016). Of the myriad forms of human-induced landscape change, the intensification of agriculture is the major change affecting bees (Fuller et al., 2005; Goulson and Nicholls, 2016), and its growth and expansion means farmland is an increasingly major habitat for bees (Falk, 2015). The acknowledgement of this negative impact of agriculture has led to more ecologically-friendly methods of farming, such as organic farming, receiving much attention (Kovács-Hostyánszki et al., 2017; Meena et al., 2017; Pywell et al., 2015) as a way to mitigate the damage done by intensive practices. Organic farms have been shown to support different, and often more abundant, plant communities than similarly located conventional farms (Gabriel et al., 2006; Hawes et al., 2010; Rundlöf et al., 2009), with often similar benefits shown on bee communities (Kennedy et al., 2013; Le Féon et al., 2010; Rundlöf et al., 2008). Farming practice may play a significant role in the structure of bees' local nutritional landscape, possibly providing different quantities and qualities of pollen nutrition. The foraging decisions bees make may therefore differ depending on farming practice.

Nutrition also plays an important part in shaping biological communities as a whole (Simpson and Raubenheimer, 2012a), with only a limited suite of nutritional resources available at any given time. On farmland in particular, floral composition can shift across a bee's flight season (Nowakowski and Pywell, 2016), yet often, investigations into bee communities on farmland neglect such fine-scale phenology (Holzschuh et al., 2008;

Kehinde et al., 2017; Power et al., 2012). Phenology is a critical component influencing community architecture (Encinas-Viso et al., 2012; Morente-López et al., 2018) and, in bee-flower relationships in particular, their phenology is mutually dependent (CaraDonna et al., 2018; Lawson et al., 2018; Morente-López et al., 2018). A change in the flowers available through a season will also mean a change in the nutritional resources available to bees. The landscape dictates what resources are available, but the nutritional decisions bees make influence how they interact with those available resources. These interactions can be incorporated into "ecological" or "interaction" networks and used to investigate the structure of communities (Tylianakis et al., 2010), and how they may react to losses of particular species (Banza et al., 2015). Such networks are perhaps most commonly investigated for bees in terms of pollination (Ballantyne et al., 2017; Bendel et al., 2019; Power and Stout, 2011), however, these studies exclude pollen destined for larval nutrition (Alarcón, 2010; Forup and Memmott, 2005; Popic et al., 2013) and so do not give a full account of how bees interact with their floral hosts. Although a number of studies have investigated pollen preferences in bees (Danner et al., 2017; Eltz et al., 2001; Ison et al., 2018; Sinu and Bronstein, 2018), with some indirectly indicating differential larval and adult foraging (Haider et al., 2014; Kraemer et al., 2014; Saunders, 2018), little concern has been given to the separation of these two foraging decisions from a network perspective. As adult and larval diets differ (Carr et al., 2015; Filipiak, 2018; Filipiak et al., 2017), it seems plausible that these differing requirements would be reflected in the foraging decisions made by the adults, and therefore influence network structure.

The acquisition of nutrients is a key component of ecological interactions, at both the individual and community level, and yet ecology and nutrition are often explored in isolation from each other (Simpson et al., 2010). This research aims to contribute towards the unification of these areas by adopting two complementary approaches to the question of

how bees get adequate nutrition from modern farmland, investigating (a) the parental provisioning behaviour of bees, specifically how offspring cope with varying levels of parentally-provided nutrition, and (b) how changing farmland resources affect the structure of nutritional interactions for the bee communities that depend on them. Community ecology can be valuably viewed from a nutritional perspective, as nutrition fuels many of the interactions between organisms (Simpson and Raubenheimer, 2012a) and provisioning behaviour and community ecology are intimately linked when viewed from the perspective of nutrition. Parents forage on behalf of offspring within the community; the foraging behaviour of the parent will determine the trophic interactions within that community, especially where the parental diet is markedly different from that of the offspring, as in bees (Roulston and Cane, 2002). Equally, the structure of a community will affect the quality of the nutrition available to parents for offspring provisioning; an effect that is even more pronounced in systems where the food sources vary widely in nutritional quality, as in pollen (Roulston et al., 2000). Given that degradation of the nutritional environment is thought to be a primary driver of global bee declines (Goulson et al., 2015; Roulston and Goodell, 2011), understanding how bees cope with nutritional changes, and how their nutritional decisions shape the way they interact with their hosts, is important if we are to understand, and hopefully reverse, such declines.

1.5. Chapter of this thesis

I am interested in how nutrition influences the development and drives the behaviour of bees. Specifically, I am interested in how bee larvae cope with varying levels of nutrition and how this may influence their subsequent fitness, along with how the nutritional decisions made by adult bees, based on whether they are foraging for themselves or for their young, influence how they interact with their nutritional environment on farmland.

In the first half of this thesis I take a behavioural approach, focussing on how bee larvae deal with differing levels of nutrients in their diet, in particular, whether larval bees are able to regulate their nutrient intake and what nutritional decisions they make when optimal nutrition is not available. A powerful technique exists with which to investigate nutritional decisions in animals: The Geometric Framework for Nutrition (Simpson and Raubenheimer, 2012b, 1993). The Geometric Framework (GFN) can be used to determine an organism's intake target [the optimal amount and balance of macronutrients that an animal should aim to consume in order to achieve maximal fitness (Simpson and Raubenheimer, 1993)], as well as whether it forages selectively in order to achieve this target when offered imbalanced foods (Fig. 1), and, what decision it makes when it cannot achieve that target with the nutritional resources it has available (Fig. 2) i.e. its rule of compromise (Simpson and Raubenheimer, 1995). In addition, by raising organisms on fixed diets it is possible to compare their performance using predetermined fitness proxies. Such proxies can be visualised, in relation to nutrient intake, as a response surface (Fig. 3) and used to determine whether such proxies are constrained by certain nutrients in the diet.

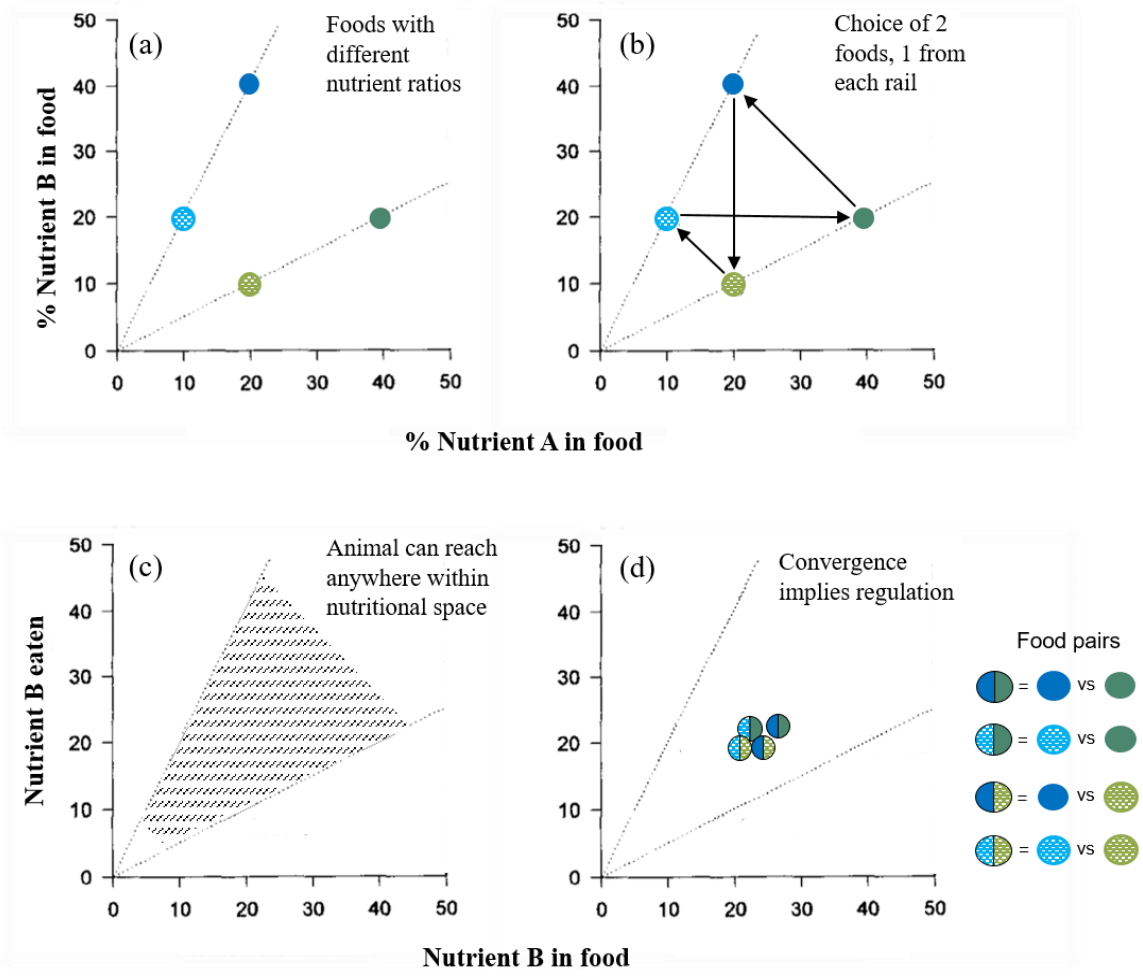


Fig. 1. Determining an animal's intake target, modified from (Simpson and Raubenheimer, 1995). (a) each coloured point represents a different food, blue and green points indicate different A:B macronutrient ratios between which the intake target is predicted to be, solid colours indicate higher concentration and hashed colours indicate lower concentration e.g. dashed blue and solid blue points have the same macronutrient ratios but at different overall concentrations. (b) animal is offered a choice between two foods, each with a different macronutrient ratio, which when eaten in specific amounts would allow for the animal to reach the predicted intake target. (c) animal can reach anywhere in nutrient space (dashed fill) i.e. anywhere within the range encompassed by the macronutrient ratios of the food. (d) experimental groups can be offered, in this case, the four possible combinations of choices, convergence on an area implies the animals are differentially consuming the foods in order to defend an intake target.

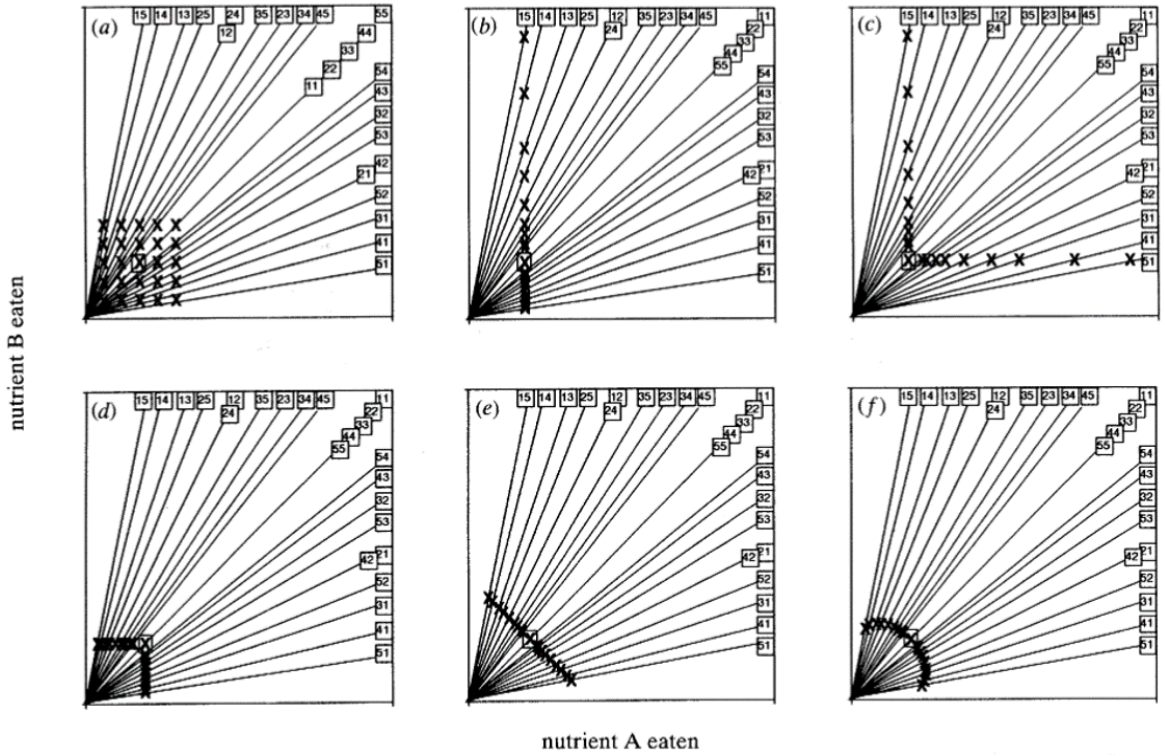


Fig. 2. Consumption of nutrient B plotted against nutrient A on fixed diets with examples of decision rules, taken from Simpson and Raubenheimer (1993). Differing arrays indicate different decision rules i.e. the animal's 'rule of compromise'. Lines, or 'rails', indicate specific nutrient ratios. Boxed numbers indicate specific diet treatments, first digit (1-5) gives level of nutrient A, second number gives levels of nutrient B (1-5). (a) animal eats the same amount of food, irrespective of nutrient content. (b) animal eats to a target for A, irrespective of under- or overeating B. (c) animal eats until at least the target of A and B is met, irrespective of overeating the more abundant nutrient. (d) animal eats until the target for A or B is met, irrespective of undereating the less abundant nutrient. (e) animal consumes food until the amount of A and B eaten equals the sum of the intake target. (f) 'closest distance optimisation', whereby the animal eats to the point that is geometrically closest to the intake target.

The GFN has been used in multiple insect groups previously to investigate nutritional intake and decisions (Lee et al., 2008; Noreika et al., 2016; Simpson et al., 2004), as well as in adult bees (Paoli et al., 2014a; Vaudo et al., 2016), but has yet to be fully applied for the purposes of larval bee nutrition (Helm et al., 2017). A likely reason for this is that larvae of the traditional social focal species, such as honeybees and bumblebees, feed their larvae progressively and/or modify the nutrition they gather from the environment significantly prior to provisioning, making the accurate tracking of nutritional intake by larvae difficult. To circumvent this issue I use the solitary, mass-provisioning model bee species *Osmia bicornis* L. (described above), a polylectic cavity-nesting species (Fig. 4, Dobson et al., 2012; Falk, 2015) which is relatively well researched for a solitary bee (Fliszkiewicz et al., 2015; Konrad et al., 2009; Ulbrich and Seidelmann, 2001) and has growing commercial significance (Gruber et al., 2011; Sedivy and Dorn, 2013; Wilkaniec et al., 2004), making them particularly relevant to agriculture. Their willingness to readily nest in artificial constructs (Strohm et al., 2002) combined with their mass-provisioning method of providing larval nutrition, makes them an excellent study system for asking nutritional questions, as it is possible to swap out the nutrition provided by the mother.

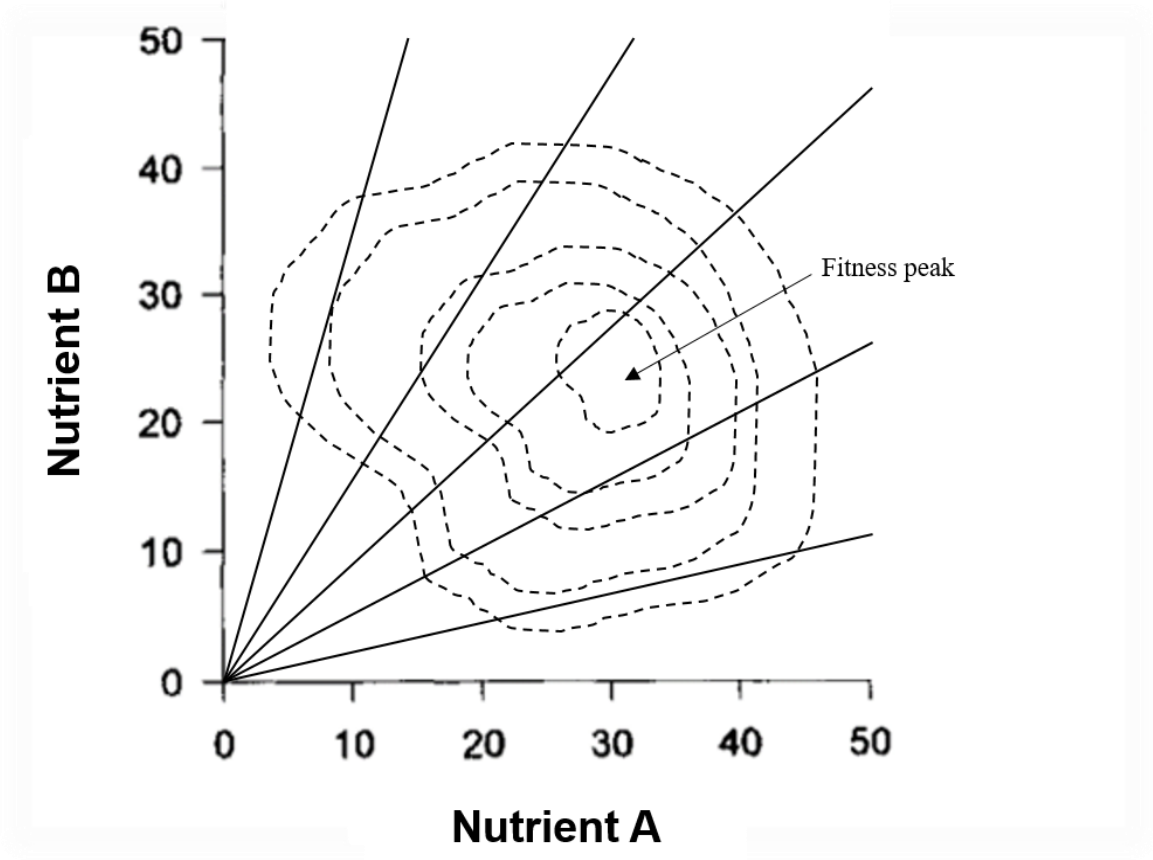


Fig. 3. A hypothetical example of the variation of a predetermined fitness proxy (e.g. body weight), with respect to nutrient intake, visualised as a response surface. Solid lines (rails) represent the fixed diets, differing in the ratio of nutrient A to nutrient B, given to experimental organisms. Organisms on each diet can only move along the diet rail from 0. Arrow indicates the 'fitness peak', where the proxy in question is maximised. Dashed lines represent changes in the value of the fitness proxy in respect to nutrient intake. In the case of body weight for example, values increase inwards towards the 'fitness peak'.

Fig. 4. *Osmia bicornis* (Red Mason Bee) female. Photo by S. Rae (https://www.flickr.com/people/35142635@N05), shared via Creative Commons Attribution 2.0 Generic license: https://creativecommons.org/licenses/by/2.0/deed.en

In Chapter 2, I ask whether *O. bicornis* larvae are able to regulate their intake of protein and carbohydrate, two major macronutrients considered critical to insect development (Behmer, 2009; Clissold and Simpson, 2015; Huang, 2012; Scriber and Slansky, 1981), in order to achieve an intake target. In addition, I investigate what rules of compromise larvae employ if their desired intake target cannot be reached. In chapter 3, I extend this by asking whether *O. bicornis* larvae also regulate their lipid intake. Recent findings in bumblebees demonstrate that adult bees seem to regulate their lipid intake (Vaudo et al., 2016), however, no study as yet has demonstrated this in bee larvae. Secondly, as intake targets can change through time (Simpson and Raubenheimer, 2012b), I

investigate whether the relative importance of each macronutrient changes as *O. bicornis* larvae develop.

The second half of the thesis focuses on bee-plant communities and interactions. I investigate floral communities across different farming practices, and how the way in which bees interact with such communities differs, depending on whether they forage for themselves or for their young. In Chapter 4, I investigate whether bee communities, and the nutritional resources upon which they depend, change across farming practices, asking whether organic farms support different, more diverse and more abundant plant resources than their conventional counterparts. I ask whether such patterns change over time, with the aim of identifying key areas for focused pollinator support interventions. Bees must cope with gluts and droughts in floral resource (Goulson and Nicholls, 2016; Nowakowski and Pywell, 2016); highlighting when such droughts occur across farming systems will provide valuable practice-specific management information for farmers. In chapter 5, I delve deeper into the bee and flower communities on farmland. I ask whether bees interact with different floral resources when foraging for themselves compared to foraging for their offspring, and whether these interactions differ across farming practices. To achieve this, I employ the powerful molecular technique of DNA metabarcoding to create interaction networks. DNA metabarcoding is a fast, high-throughput method of DNA-based identification allowing for the discrimination of Molecular Operational Taxonomic Units (mOTUs) from a collection of multiple specimens (Cristescu, 2014), and is increasingly being used to construct interaction networks (Bell et al., 2017; Pornon et al., 2017, 2016). Though DNA metabarcoding has previously been used to investigate how bees interact with their floral hosts (Danner et al., 2017; de Vere et al., 2017), it has yet to be used to investigate how such networks change depending on whether bees forage for themselves or for their

offspring - a distinction that could have a profound impact on the way we view bee-plant interactions in the future.

2. The geometry of dependence: bee larvae prioritise carbohydrate over protein in parentally provided pollen

2.1. Abstract

Bees, important pollinators, have declined significantly in recent decades, and human-induced changes to nutritional landscapes are partly responsible. Changes to nutritional quality rather than quantity are relatively overlooked as a threat to bee health. Yet knowledge of bee nutrition is currently largely restricted to adults of social species. Larval stages, where most growth occurs, are relatively understudied - perhaps because most social bees provision progressively and collectively, making nutrition difficult to trace. In mass-provisioning solitary bees (*Osmia bicornis* L.), we can manipulate and follow larval nutrition, and thereby determine effects of changes in diet quality. Under the Geometric Framework for Nutrition, we restricted larvae to 6 diets: 3 protein:carbohydrate ratios and 2 nutrient concentrations. We asked: (a) which combinations of nutrients maximise body size and survival, (b) what rule of compromise do larvae follow when nutrients are imbalanced? Finally, we gave larvae a choice of complementary diets, asking (c) are larvae able to reach their intake target? Larvae raised on fixed diets pupated after consuming a fixed carbohydrate amount, but tolerated a wide range of protein. Contrary to similar findings for adult bees, in our study, larvae that consumed the most carbohydrate survived best and grew most. Although larvae did not all converge on an overall amount of each nutrient (i.e. an intake target), when eating freely from complementary diets, larvae ate a common P:C *ratio* of about 1:1.8. However, like the larvae given fixed diets, these

larvae maintained approximately stable carbohydrate intake, while protein intake varied. Our results suggest that carbohydrate is the more important macronutrient for growth and survival of solitary bee larvae, and accordingly that carbohydrate is regulated most closely. Carbohydrate may also be important for overwinter survival, and/or may be more limiting than protein. Tolerance of variable protein, despite its importance to development, suggests bee larvae may be reliant on parents to regulate protein - and are therefore vulnerable to landscape changes. Our results highlight bees' potential vulnerability to a "nutritional trap", i.e. where rapid changes in their nutritional environment outstrip their evolved capacity to detect those changes, impairing their fitness.

Keywords: pollination, foraging ecology, agriculture, nutritional geometry, limiting nutrient, diapause, ecological trap, environmental change, bee health

2.2. Introduction

Bees are critical not only to global ecological stability but also to humans' food security, as major pollinators for 90% of the world's food crops (Klein et al., 2018, 2007; Potts et al., 2016) and many wildflowers (Ollerton et al., 2011). Many wild and domesticated bees have seen marked declines in recent decades, with both significant range contractions and extinctions (Ollerton et al., 2014). Honeybees have suffered huge losses across Europe and North America (vanEngelsdorp et al., 2008; Potts et al., 2010b) and wild bees have similarly struggled (Wood et al., 2016). These declines have been driven by a suite of reasons including, amongst others, nutritional stress (Goulson et al., 2015; Roulston and Goodell, 2011). Nutritional stress suffered by bees is mainly driven by human induced changes (Robertson et al., 2013), such as habitat fragmentation (Söderman et al., 2018), causing rapid changes to floral diversity, quantity and quality (Goulson et al., 2015; Robinson and Sutherland, 2002; Ziska et al., 2016). In particular, agriculture, and the spread of intensive practices specifically, has severely altered the floral landscape, with farmland often supporting lower floral diversity (Letourneau et al., 2011; Poggio et al., 2013). Agriculture also affects temporal availability, with mass-flowering crops creating brief, monotonous gluts of food followed by periods of resource scarcity (Goulson and Nicholls, 2016). This change in the quantity of nutrition is a common attribute of human-altered systems; however, a largely under-recognised risk for global ecosystems is the wholesale change in *quality* of nutrition, rather than just quantity (Ziska et al., 2016). A change in nutritional quality could be of particular concern for bee larvae, as their diet, pollen, varies widely in nutrient content (Roulston and Cane, 2000a), with recent studies also showing changes in pollen nutrient content within plant species due to environmental change (Ziska et al., 2016). Wholesale changes to the nutritional quality of landscapes are

of critical concern, as nutrition mediates animals' ability to grow, reproduce, and maintain themselves (Simpson and Raubenheimer, 2012a). Understanding how animals cope in a changing nutritional environment requires us to understand not only how animals gather the correct balance of nutrients they need, but also how they adjust their foraging when resources are imbalanced (Simpson and Raubenheimer, 2012b).

In bees, the larval stage is where almost all growth occurs (Nijhout et al., 2014) as well as resource accumulation for diapause (Giejdasz and Wasielewski, 2017) - the way larval bees behave in the face of variable nutrition may be critical for bee health generally. Thus, bee nutrition research should focus on larvae at least as much as on adults. Unfortunately, we know relatively little about the nutritional ecology of most bee species, whether as larvae or adults (Roulston and Cane, 2002; Vanderplanck et al., 2014), with findings generally restricted to the latter (Altaye et al., 2010; Archer et al., 2014a; Pirk et al., 2010; Vaudo et al., 2015). Findings in adult bees cannot necessarily be applied to their larvae; larval bees have a distinctly different diet to adults, adults primarily feeding on nectar (although see Cane, 2016), and larvae feeding almost solely on pollen (Filipiak, 2019; Muth et al., 2016).

The Geometric Framework for Nutrition (GFN) allows us to investigate foraging decisions made by animals in multi-dimensional "nutrient space" (Simpson and Raubenheimer, 2012b, 1993). The GFN can be used to determine an organism's intake target [the optimal amount and balance of macronutrients that an animal should aim to consume in order to achieve maximal fitness (Simpson and Raubenheimer, 1993)] as well as how that target is achieved. Additionally, we can use the GFN to investigate the *rule of compromise* - that is, the rules governing consumption that an animal uses when it is unable to reach its intake target with the nutritional options available (e.g. Lee et al., 2004b; Simpson and Raubenheimer, 2001).

The GFN has provided profound insights into broad topics from ant agriculture (Shik et al., 2016) to human obesity (Simpson and Raubenheimer, 2005). The GFN has been used to investigate the nutrition of some highly social hymenopterans such as ants (Arganda et al., 2014; Cook et al., 2010; Dussutour and Simpson, 2012, 2009), bees (Altaye et al., 2010; Archer et al., 2014a; Paoli et al., 2014a; Stabler et al., 2015; Vaudo et al., 2016) and more recently termites (Poissonnier et al., 2018). However, those studies have focused almost invariably on adults rather than larvae (although see Helm et al., 2017). It is extremely difficult to investigate larval nutrition in social species, principally because larvae in many social systems are difficult to feed directly, and it is rarely possible to accurately track nutrition within a colony - food brought in by workers is often shared and/or modified within the nest, and is then continually fed to the larvae (Field, 2005). This means that nutritional insights from GFN studies into the parent-offspring relationship are currently limited.

In solitary bees, by contrast, typically each reproductive female provisions each of her offspring individually with a single, independent "pollen ball" before sealing the cell and leaving. This pollen ball contains all the resources that the larva will need to grow to adulthood. This behaviour makes solitary bees a better model for larval nutritional studies, and studies of parental provisioning, than social species - once the female has left, both larva and pollen ball can be manipulated, and larval development monitored.

In this study, we used a commercially important solitary bee species, *Osmia bicornis*, to investigate how larval bees cope with varying nutrition: different diets, and different diet choices. Bee larvae are typically entirely sedentary and parents supply all their nutritional demands (Field, 2005). Yet we have little knowledge about whether parent bees consistently provide offspring with a ready-balanced diet, whether pollen ball composition varies passively with the flowers available to foraging adults in the landscape,

or somewhere in between. Adults forage in a heterogeneous nutritional environment, but we know that, despite this, honeybee foragers are nevertheless able to collect food that balances out deficiencies in colony nutrients (Hendriksma and Shafir, 2016). In general, though, evidence is scarce and mixed on whether adult bees can directly detect pollen quality at the flower (Nicholls and Hempel de Ibarra, 2016).

Given that parents may bring pollen of variable quality, the question of whether offspring are able to regulate their own nutrition to compensate for deficiencies in their provisions is a fundamental, but overlooked, component of bee nutrition. Even if parents cannot provide consistently balanced nutrition, larvae may still be able to eat selectively in order to achieve a nutritional target. Such regulation has been demonstrated in other insect larvae that develop independently of parents (Lee et al., 2002; Merkx-Jacques et al., 2008) but is unstudied in bees.

We used a classic GFN design (Jensen et al., 2012) with two experimental phases: in the first "no-choice" phase we raised *O. bicornis* larvae on fixed diets of differing protein to carbohydrate (P:C) ratios (two macronutrients regarded as critical to non-carnivorous insects; Behmer, 2009; Clissold and Simpson, 2015; Huang, 2012; Scriber and Slansky, 1981) in order to determine their rules of compromise and the diet composition that maximised fitness. In a second "choice" phase, we then provided larvae with targeted choices between sets of two imbalanced diets that differed in their P:C ratios to determine whether larvae consistently aim for an intake target. Sterile adult workers of some social bee species have been shown to have carbohydrate-biased intake targets (Paoli et al., 2014a; Stabler et al., 2015); however, we focus here on the growing larvae of *O. bicornis*, whose adults are all reproductive. Given the traditionally assumed importance of protein for growth and reproduction in insects and animals generally (Chapman and Chapman, 1998; Simpson and Raubenheimer, 2012a), we predicted (1) that protein would be a key driver of

fitness in larval *O. bicornis*, (2) that larvae would accordingly aim for a relatively protein-biased intake target, and (3) that larvae would prioritise protein intake over carbohydrate in their rule of compromise.

2.3. Methods

Study organism

Osmia bicornis is a common, univoltine, cavity-nesting solitary bee native to Europe (Falk, 2015), and a commercially important pollinator of multiple crops (Jauker et al., 2012; Schulze et al., 2012). It is polylectic, feeding from a wide variety of flowers, and flies from March to July with males emerging a few weeks prior to females (Falk, 2015). Females nest in a variety of pre-existing cavities but can also be encouraged to nest in artificial constructs (Strohm et al., 2002).

All brood care in bees is performed by the female (Field, 2005) and *O. bicornis* larvae are entirely dependent on the food supply provided by their mother (Seidelmann et al., 2010) who builds a ball of pollen upon which she lays an egg. These provisions are stored in linear mud-lined cells each containing a single larva with each larva receiving a pollen provision directly from the mother.

Study population

O. bicornis larvae were obtained as diapausing adults in cocoons (Mauerbienen®, Germany), and released at the nesting site at the University of Hull in April 2017. Nesting material consisted of Styrofoam blocks (Styrodur 3035 CS), with a 9x9mm furrow and polycarbonate lid, housed within a wooden frame (modified from Strohm et al., 2002; Fig. S1a,b). Completed nests, signified by a mud plug at the entrance, were then brought into the laboratory. Early trials revealed that fresh eggs and newly emerged larvae were too fragile for manipulation. Therefore, newly emerged larvae were left alone for two days before we transferred them to a single-occupancy nest and assigned each to an experimental treatment.

The majority of nests were completed and sealed by the adult before the larvae hatched. When this was not the case, and some eggs hatched whilst the adult was still provisioning other cells in the nest, larvae reaching the two-day-post-hatching stage were removed at the nest site, taken to the lab and placed into a single-occupancy nest.

Diet Formulation & Treatments

We used the Geometric Framework of Nutrition (Simpson and Raubenheimer, 2012a), as described above, to investigate the intake target and rule of compromise employed by *O. bicornis* larvae. In the no-choice treatment, larvae were restricted to one of six diets, consisting of three different protein:carbohydrate (P:C) ratios (Diet A = 1:1.2, Diet B = 1:2.3 & Diet C = 1:3.4) and two total macronutrient concentrations (concentration

1 = 90% nutrients, 10% diluent, or concentration 2 = 70% nutrients, 30% diluent; see table S1 for amounts of macronutrients). To our knowledge, there is no precedent for composing artificial pollen diets for larval solitary bees, so these diet ratios were chosen based on a combination of the nutrient ratios in honeybee-collected pollen loads and published data for protein in *O. bicornis* pollen balls (Budde and Lunau, 2007). All diets contained an equal amount of honeybee-collected pollen and honey to which was added specific amounts of protein (micellar casein) and carbohydrate (trehalose), creating differing P:C ratios. The two diet concentrations were achieved by adding sporopollenin (see S1 for protocol), a major component of the outer wall of pollen considered largely indigestible by bees (Nepi et al., 2005; Roulston and Cane, 2000a; Suárez-Cervera et al., 1994). Sporopollenin was chosen rather than the more commonly used α-cellulose (Lee et al., 2004a; Muth et al., 2016; Pernal and Currie, 2002) as (1) initial trials showed high larval mortality when fed α-cellulose, and (2) sporopollenin more closely resembled the natural fibre found in larval bees' diet, and is indigestible (Roulston and Cane, 2000a).

In the choice treatment, larvae were provided with two alternating diets (A [1:1.2] and C [1:3.4]) each of which was at one of two possible concentrations (90% or 70%; see Fig. 1), together forming 4 separate treatment groups. Because *O. bicornis* larvae are sedentary, it is biologically inappropriate to allow access to both diets simultaneously (Chambers et al., 1995; Shik et al., 2014; VanOverbeke et al., 2017). Therefore, choice was offered temporally by swapping the provision every other day, allowing the larvae to differentially feed over the course of the experiment. All larvae were kept on the same treatment from two days post-hatching up to pupation, whereupon diet replenishment ceased and cocoons were weighed. For larvae that died before pupation, date of death was recorded.

Experiment 1: No-choice phase

Two day old larvae, randomised according to parentage, were allocated to one of 6 treatments, corresponding to our 6 artificial diets (n = 20/treatment). The larva was removed from its natal nest onto a scoop, within a single-occupancy nest, containing one of the diets. The scoop was used to facilitate removal of food material and prevent the food from soiling the nest block. The single-occupancy nest, scoop and provision were weighed prior to use. The nest was then weighed (OHAUS Pioneer, PA-213) when containing the scoop, with the scoop and the provision, and then finally with the larva added to the provision. This ensured that the weights of all individual components could be separated, allowing for the monitoring of provision consumption. Initial provision weight was not tightly controlled as the diets were provided in excess (i.e. regularly replenished), but were made to resemble the size of natural provisions (mean initial artificial provision weight = 0.323g +/- 0.034g).

Once provisioned, larvae were placed in an incubation chamber (Gallenkamp, IH-270) at 23°C and 80% RH. Provisions were replaced weekly, to avoid desiccation and mould formation, or when fully consumed by larvae. Weight of provision consumed was recorded upon provision replacement. To confirm that our method did not adversely affect survival, we included a control group of larvae which underwent the same manipulation but were supplied with natural provisions, i.e. a bee-collected pollen ball. Should they finish this provision, it was replenished with a fresh pollen ball, making the simplifying assumption that all parentally provided provisions were of equal composition. A “water control” group, containing artificial diets but no larvae, was used to track water loss from the diets, going through the same weighing regime as above with weight loss recorded at each swap.

Nests were checked daily to ensure the health of the larvae. Final provision consumption was calculated once larvae had pupated by summing consumption across diet changes. Protein and carbohydrate consumed by each larva across the course of the experiment was then back-calculated from the final provision consumption. Cocoon weight was also recorded at the completion of pupation.

Experiment 2: Choice phase

Larvae received two diets, presented one at a time, in alternating order. 32 two-day-old larvae of mixed parentage were randomly divided among four complementary diet pairings (see Table 1) consisting of the 1:1.2 and 1:3.4 P:C diets at the 70% and 90% concentration (Table 2). The diet that the larvae would be fed first was randomly assigned prior to the experiment. The paired diets were designed so that larvae would need to differentially feed from each in order to converge on an intake target. A control group underwent the same manipulation protocol but were fed natural pollen balls. Performance criteria were recorded as in Experiment 1. Total provision consumption per larva was calculated by summing the consumption of each of the paired diets across the duration of the experiment, and macronutrient consumption was back-calculated as for Experiment 1.

Table 1. Sample sizes for each diet combination used for choice phase (allocated by random coin toss). "Order" refers to diet order - e.g. for A1C1, Order 1 would receive A1 first whereas Order 2 would receive C1 first. Surviving larvae are in parentheses.

	Order 1	Order 2
A1C1	1 (1)	6 (5)
A1C2	5 (5)	4 (3)
A2C1	3 (2)	5 (5)
A2C2	5 (2)	3 (1)

Table 2. Diet combinations used for choice phase.

	Concentration 1 (90%)	Concentration 2 (70%)
P:C Ratio:		
A (1P:1.2C)	A1	A2
C (1P:3.3C)	C1	C2

Statistical Analysis

All analyses were conducted in R version 3.4.2 (R Core Team, 2017). For the no-choice experiment, raw diet consumption data were first adjusted for water loss and dilution, and then total nutrient content (P and C) calculated from adjusted figures based on the known nutrient percentages in the dry diets. Values were then summed for each larva and plotted onto nutritional space. Response surfaces were calculated for cocoon weight and visualised using non-parametric thin-plate splines. Larvae that died pre-pupation were not used in the calculation of the mean P and C consumption for diets in either experiment, but were used in analyses involving survival.

In the choice experiment, mean final consumption of each nutrient was investigated using linear models with diet combination, dilution and their interaction as predictors. The minimal model was determined using reverse stepwise model selection and pairwise differences among groups were examined using Tukey's Post Hoc tests. We additionally tested whether larvae were exercising a choice at all, i.e. whether they were consuming the available diets non-randomly. We calculated the expected protein and carbohydrate that would be consumed under random consumption of each diet by assigning exactly half the total amount of food consumed by each larva to each of the two diet choices offered to that larva. We then re-ran our models, using "random vs. observed consumption" as a predictor variable.

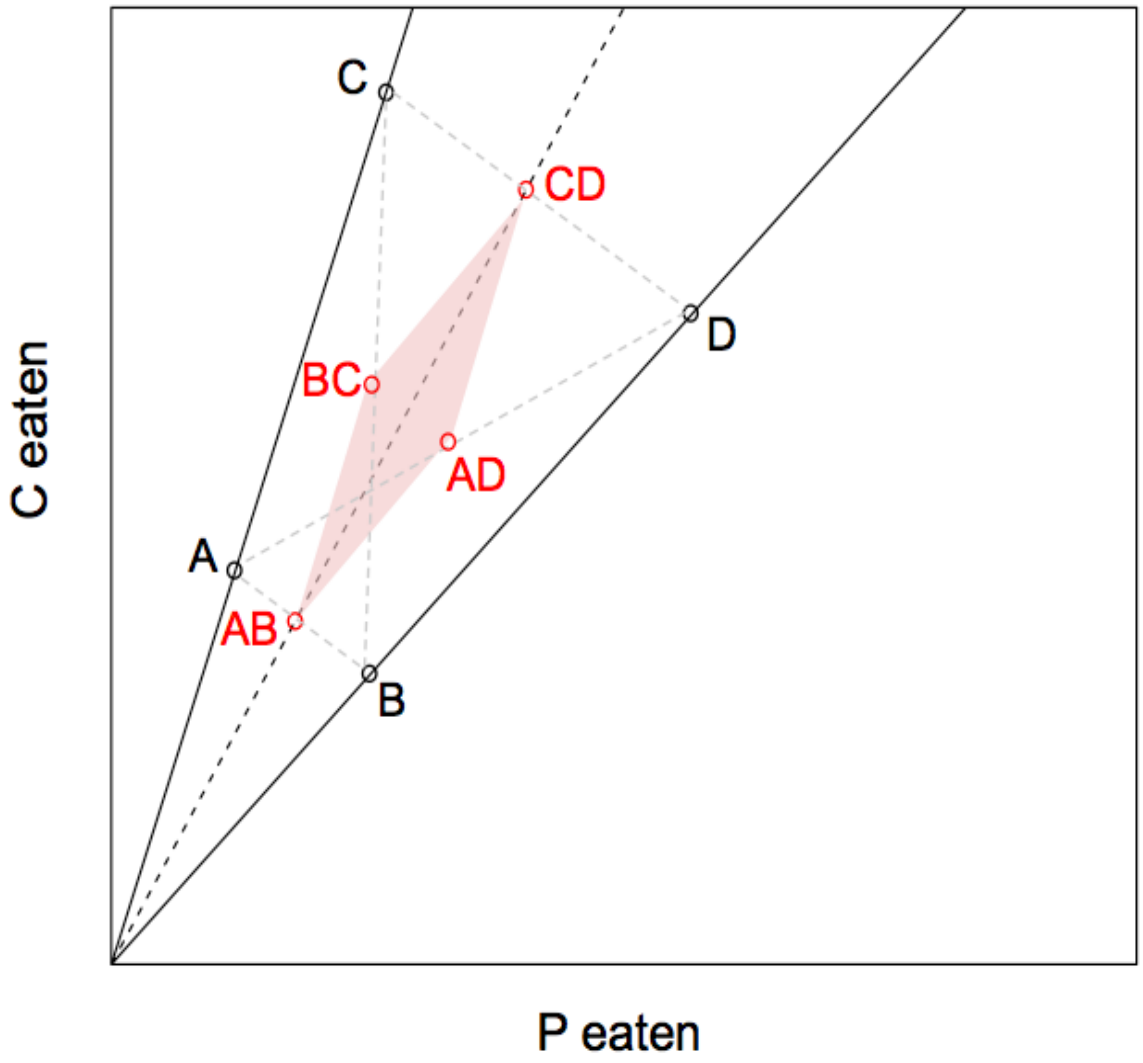


Fig. 1. The expected amounts of protein and carbohydrate consumed if larvae hypothetically eat indiscriminately between two diets. Diet choices are pairwise combinations of diets A, B, C and D, which each contain protein and carbohydrate at different ratios and concentrations. Solid lines represent the P:C ratios of the individual diets; black points represent actual nutrient content of each diet, which depends upon dilution as well as P:C ratio. Red points represent the expected nutrient consumption if larvae eat randomly (i.e. equally) from each of a choice of two diets (choices denoted by the red point labels). Note that random consumption patterns resemble a diamond shape surrounding the line that bisects the two rails.

2.4. Results

No-choice phase

Dietary P:C ratio had a significant effect on the overall amount of provision consumed (linear model with diet ratio and diet concentration as predictors, dropping main effect of diet ratio, $F_{1,79} = 21.46$, $p<0.0001$), with larvae consuming more provision on high P:C ratio diets (Fig. 2a). The concentration of the diet also influenced the total amount of provision consumed by larvae (linear model with diet ratio and diet concentration as predictors, dropping main effect of concentration, $F_{1,79} = 14.52$, $p = 0.0003$), with those larvae on less concentrated diets consuming more provision (Fig. 2a).

Dietary P:C ratio also had a strong effect on the total amount of P eaten (linear model using P:C ratio and diet concentration as predictors, dropping the main effect of ratio, $F_{2, 81}=74.16$, $p<0.0001$). More protein was eaten by those larvae that were raised on the higher P:C diets (Fig. 3). Larvae seem to compensate for dilution with diet concentration having no effect on the amount of P consumed (linear model, dropping main effect of concentration, $F_{1,79}=1.85$, $p=0.18$) and neither was there a ratio × concentration interaction ($F_{2,78}=<0.01$, $p=0.99$). In contrast, larvae consumed similar amounts of carbohydrate across all diets with neither concentration nor dietary P:C ratio having an influence on the amount of C consumed (linear model dropping interactions and main effects of P:C ratio and concentration, all NS; minimal model contained no terms). C was consumed to similar levels across all diets (Fig. 3). All ANOVA models showed a good fit.

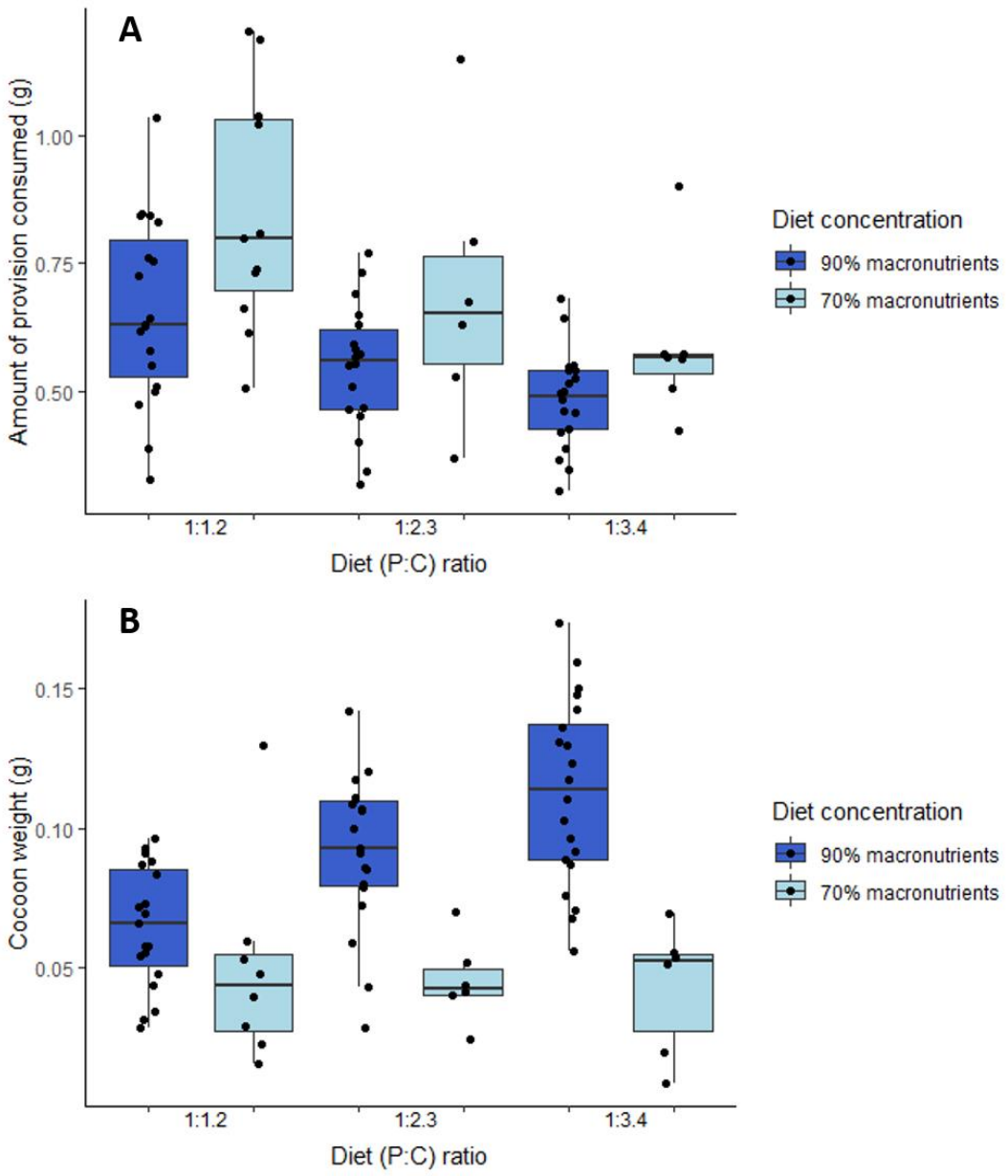


Fig. 2. (A) Amount of provision in grams consumed by larvae raised on the 3 different P:C ratio artificial diets at the 2 different macronutrient concentrations (90% and 70% macronutrient content). (B) The weight in grams of *Osmia bicornis* cocoons after being raised on artificial diets differing in P:C ratio (A = 1:1.2, B = 1:2.3, C = 1:3.4) and macronutrient concentration (90% or 70% macronutrient concentration).

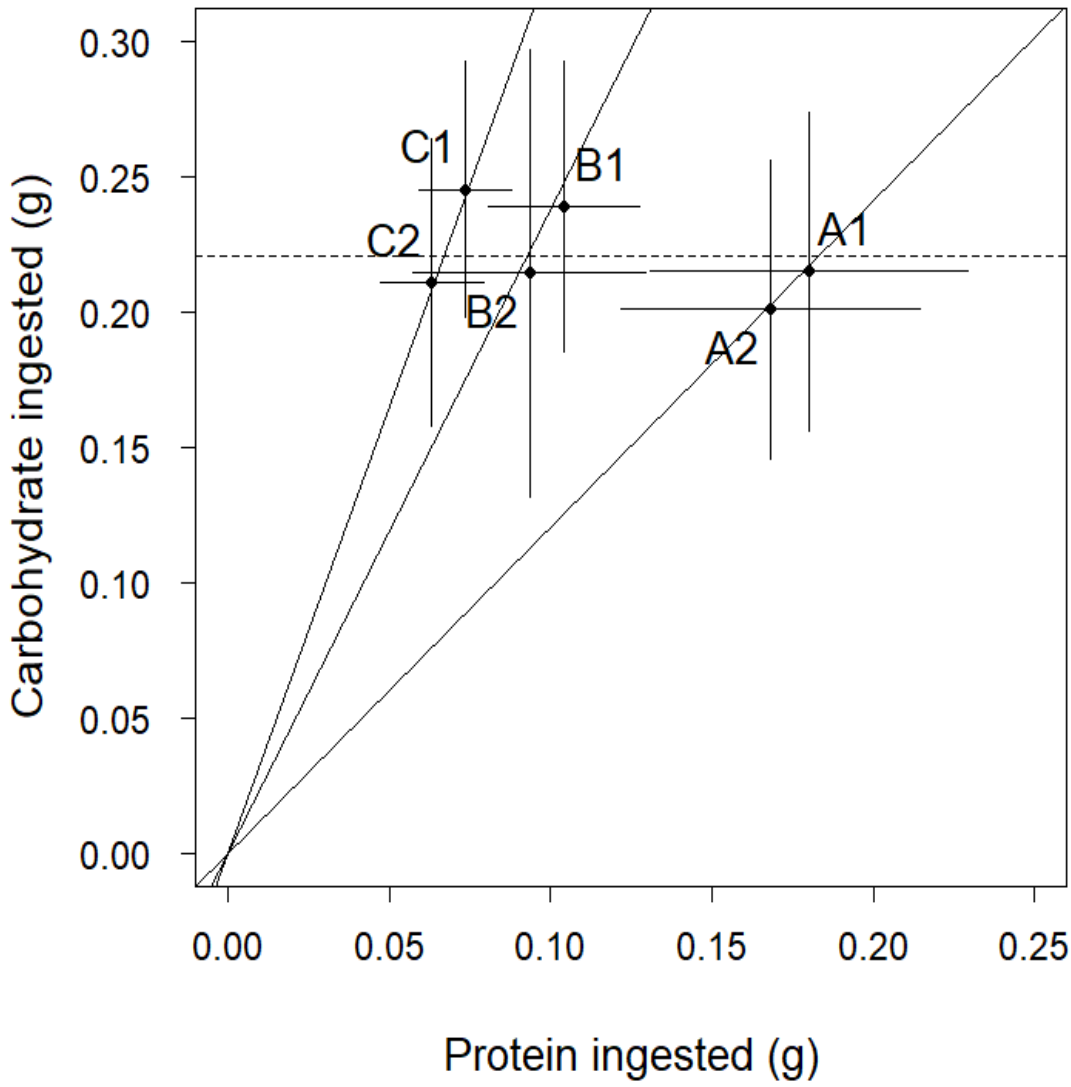


Fig. 3. Mean total (+/- 1 SD) amount of P and C consumed in grams by larvae on each diet across the duration of development. Solid lines and letters represent three P:C ratios (A = 1:1.2, B = 1:2.3, C = 1:3.4). Numbers following letters denote diet concentration (1 = 90%, 2 = 70%).

Both the amount of protein and the amount of carbohydrate consumed were correlated with cocoon weight (linear model using protein and carbohydrate as predictors ($R^2 = 0.28$) dropping the main effect of protein, $F_{1,76}= 12.44$, $p<0.001$, and the main effect of carbohydrate, $F_{1,76} = 29.28$, $p<0.001$). Higher amounts of protein resulted in lower cocoon weights whereas higher amounts of carbohydrate resulted in higher cocoon weights (Fig. 4). The linear model showed a good fit. For our range of diets, the greatest weights were obtained by larvae that ate above approximately 0.3g C and below 0.15g P (Fig. 4). However, note that no non-linear effects were observed for the range of diets used - that is, we did not identify an optimal amount of protein or carbohydrate that maximised cocoon weight. Additionally, although cocoon weight increased with the carbohydrate content of the artificial diet, those larvae raised on the control treatment achieved the highest cocoon weights overall (Fig. 2b).

Diet also influenced the survival of larvae, with those raised on the more dilute diets (A2, B2, C2) suffering greater mortality (parametric survival regression, $\chi^2_1=53.2$, $p<0.0001$, Fig. 5). When analysed according to amounts of carbohydrate and protein actually consumed, survival depended upon carbohydrate consumption ($\chi^2_1=19.00$, $p<0.0001$). Those larvae that consumed high amounts of carbohydrate saw the lowest mortality irrespective of how much protein was consumed. Interestingly, however, at lower levels of carbohydrate, mortality showed a marginal increase with decreasing amounts of protein (dropping the interaction of protein × carbohydrate, $\chi^2_1=3.74$; $p=0.053$ns, Fig. 6). As all linear models showed a good fit, we did not look for non-linear patterns in larval survival in relation to protein and carbohydrate consumption.

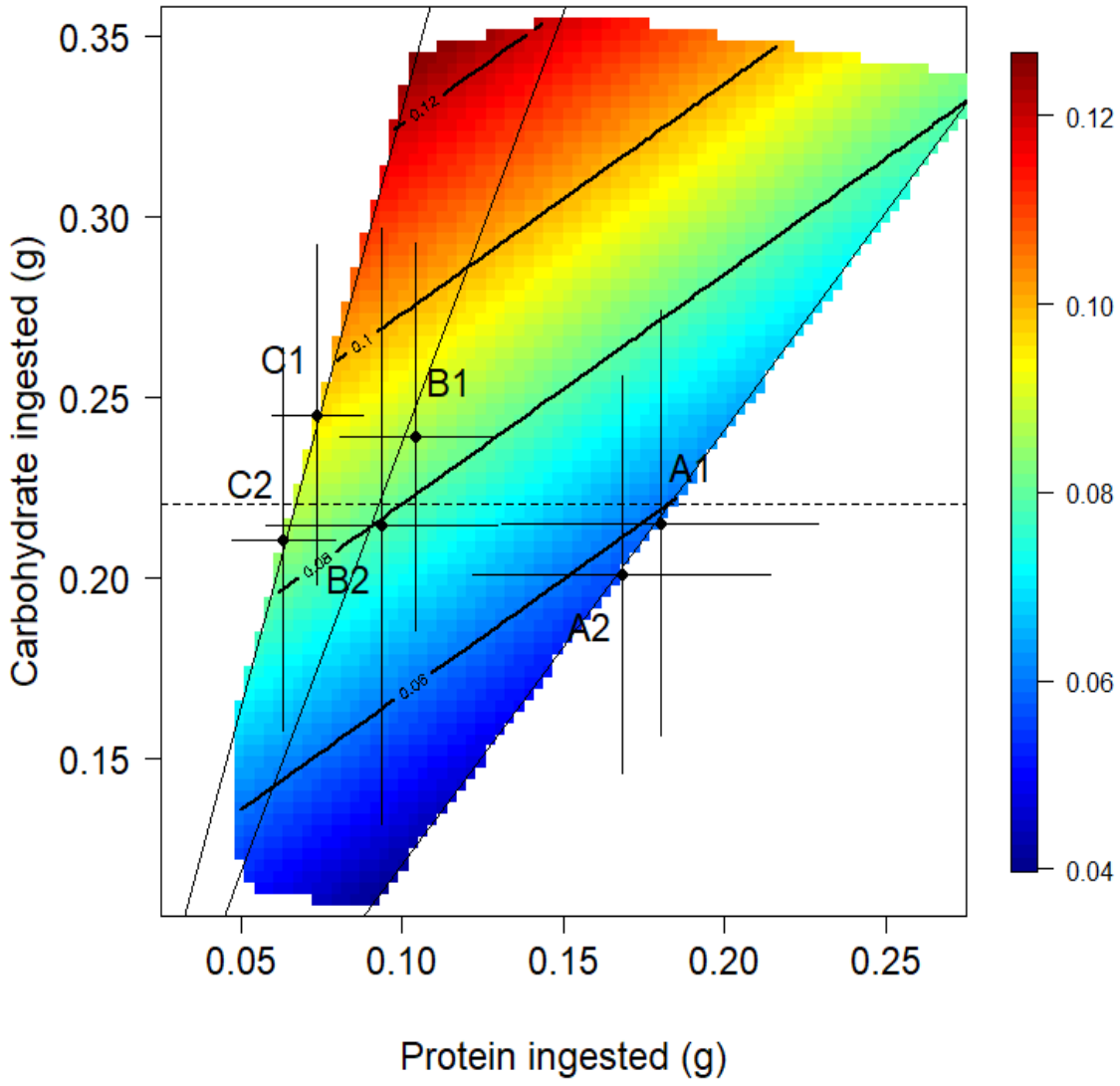


Fig. 4. Response surface showing the effects of the amount of P and C consumed on Cocoon weight (g). Transition from blue to red indicates heavier cocoons. For context, mean total consumption of P and C for each diet is plotted (data as in Fig. 2). Solid lines and letters represent three P:C ratios (A = 1:1.2, B = 1:2.3, C = 1:3.4). Numbers following letters denote diet concentration (1 = 90%, 2 = 70%).

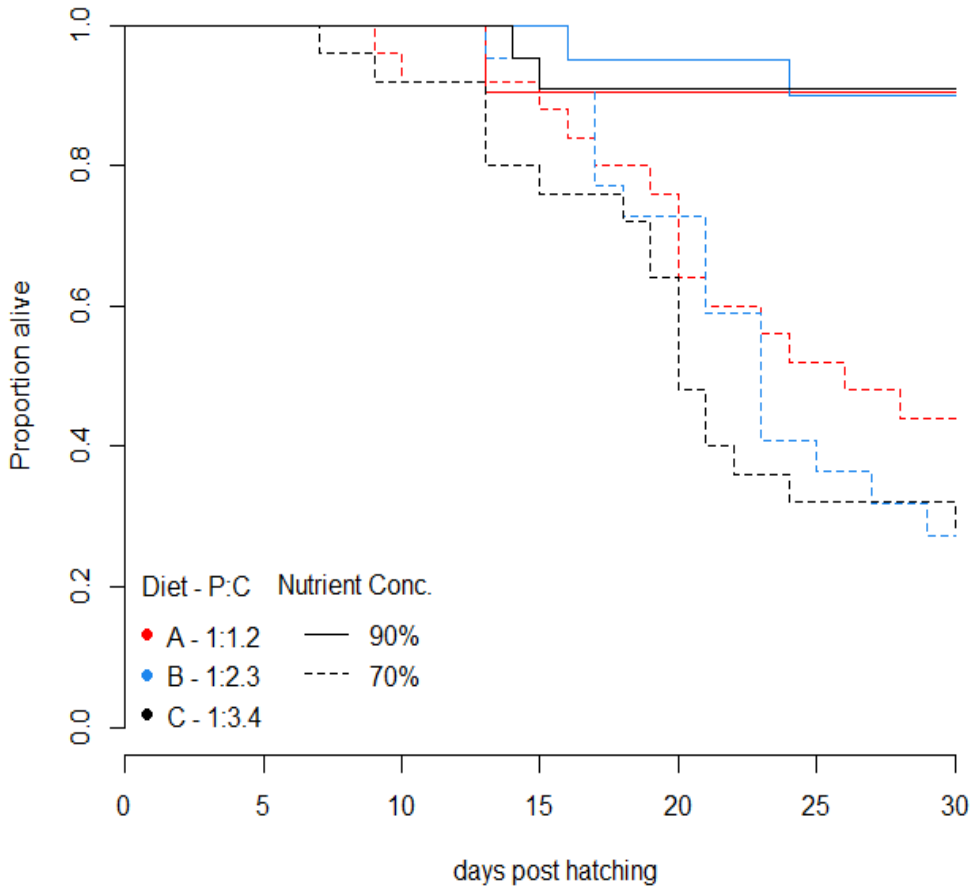


Fig. 5. Survival of larvae on fixed diet treatments according to the diet they were fed. Letters represent three P:C ratios (A = 1:1.2, B = 1:2.3, C = 1:3.4). Lines represent the proportion of larvae still alive at a given time point. Solid lines indicate diets at 90% macronutrient concentration, dashed lines indicate diets at 70% macronutrient concentration.

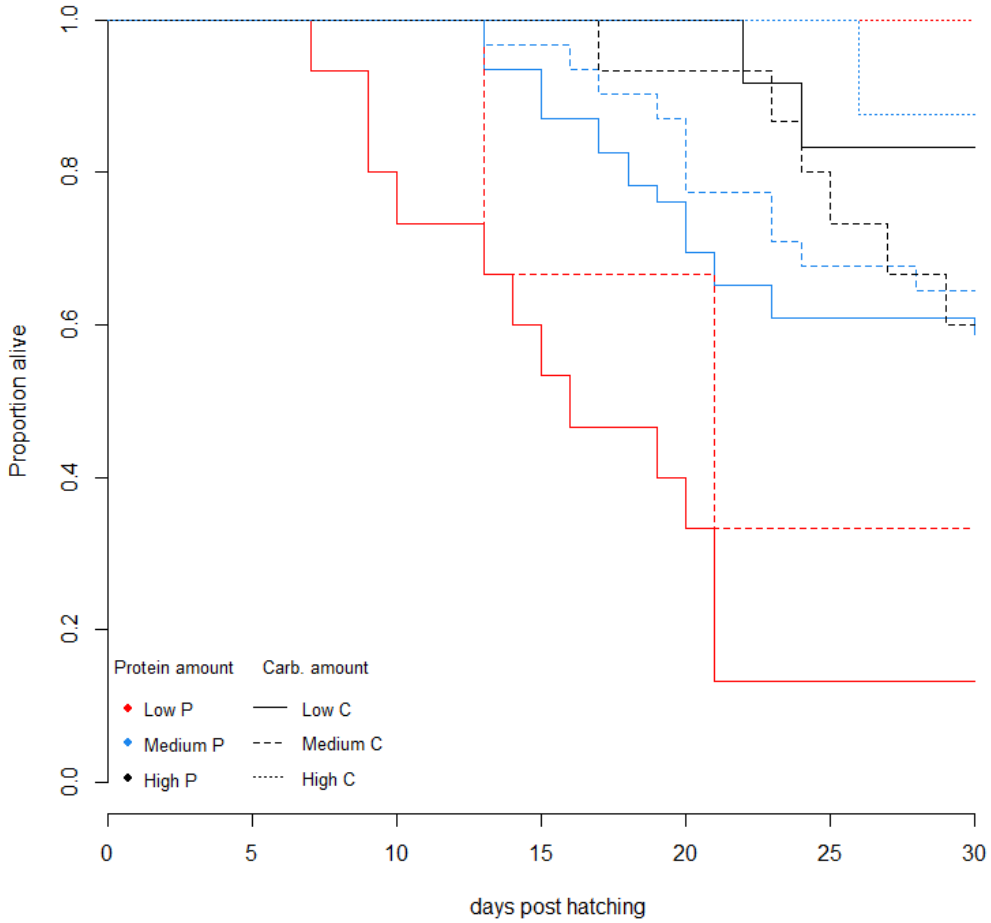


Fig. 6. Survival of fixed-diet groups broken down by amounts of macronutrients actually consumed. Lines represent the proportion of larvae alive at a given time point. Key: P = protein, C = carbohydrate; colour denotes protein amount, line type denotes carbohydrate amount; High = top 33% of consumption, Medium = middle 33% of consumption, Low = bottom 33% of consumption

Choice phase

In the previous experiment (the no-choice phase), our prediction was that we would see total P and C eaten reflect a nutritional intake target that optimised fitness, with no difference among diet treatments. However, over the range of diets we used, we did not detect a peak in fitness and therefore there was no expected intake target. Correspondingly, we found no evidence of larvae defending such an intake target at the choice phase (Fig. 7).

The amount of protein consumed by larvae during the choice experiment was significantly affected by diet combination (linear model using diet combination as a predictor, dropping the main effect of diet combination, $F_{3,23}$= 7.43, p=0.0016) with more protein consumed in both diet combinations that contained the more concentrated diets (Tukey's Post Hoc tests; A2C2-A1C2, p=0.015; A1C1-A2C1, p=0.019; A2C2-A1C1, p=0.0031). Similar results were seen for carbohydrate, with consumption being significantly affected by diet combination ($F_{3,23}$= 4.58, p=0.013). However, unlike with protein, this appeared to be driven solely by the diets at the extreme, with significantly more carbohydrate being eaten only by those in the most concentrated diet pair compared to the least concentrated pair (Tukey's Post Hoc test; C2A2-C1A1, p=0.016). Other pairwise comparisons of diet treatments were not significant.

Despite not converging upon an intake target in nutritional space, larvae were nevertheless not consuming the diets at random, instead seeming to align on a P:C ratio of about 1:1.8 (Fig. 7). For both carbohydrate and protein we saw differences in consumption from what would have been expected for each larva based on random consumption, and this effect was dependent on the specific set of diet choices (carbohydrate: linear mixed models with ID as a random effect and "diet combination" and "random or observed" as

predictor variables; dropping the interaction, χ^2_3=42.76, p<0.0001; protein: dropping the interaction, χ^2_3=16.91, p<0.001, Fig. 8).

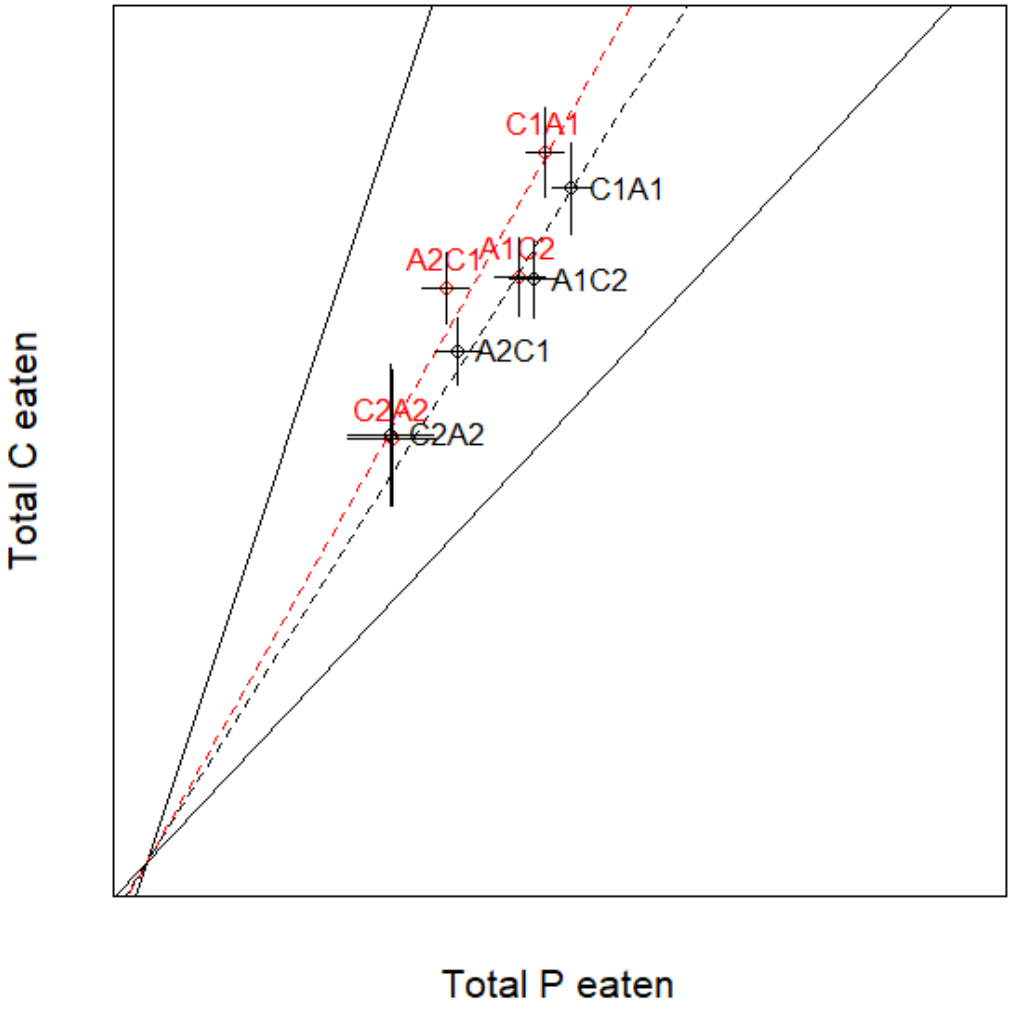


Fig. 7. The mean total (+/- 1 SE) amount of protein (P) and carbohydrate (C) eaten by larvae in the choice experiment. Each point label denotes a choice of two diets, one A and one C; black labels show observed intake, red labels show expected intake under random (i.e. equal) consumption. Letters in diet names represent two P:C ratios (A = 1:1.2, C = 1:3.4). Numbers in diet names (e.g. A1, A2) represent the concentration of each individual diet within a pairing (1 = 90%, 2 = 70%). Solid lines represent the P:C ratio of the individual diets within the pairings (Top line = Diet A, Bottom line = Diet C). Dashed red line shows the expected average

P:C ratio for all larvae, if the larvae eat randomly between the diets within their pairing (random). Dashed black line shows the P:C ratio to which the larvae conformed based on their observed intake.

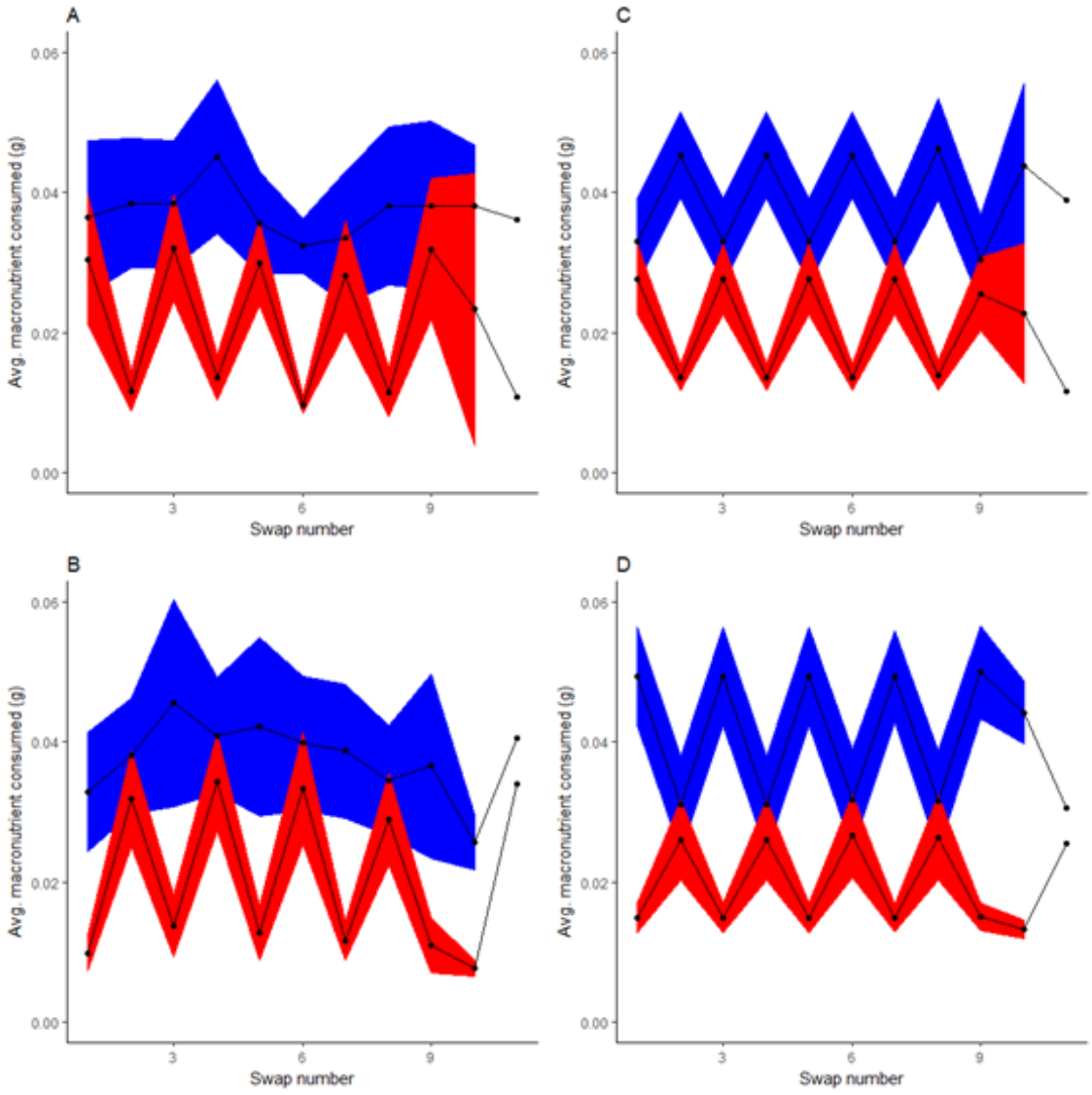


Fig. 8. Mean (+/- 1SD) intake of protein (red) and carbohydrate (blue) over successive diet swaps for observed larval consumption (A & B), versus the expected nutrient intake under random consumption of diets (C & D), irrespective of the concentrations of the diet choices

(for calculations of expected consumption, see text). A & C show larvae starting on diet A, B & D show larvae starting on diet C.

Moreover, when visualised as the amounts of P and C consumed during each 48h treatment period (Fig. 8a, b), it is clear that larvae were achieving a degree of homeostasis in C consumption compared to what would be expected under random consumption of each diet choice (Fig. 8c, Fig. 8d). In contrast, their consumption of P aligned very closely with what would be expected under random consumption (Fig. 8).

2.5. Discussion

When fed a diet with a fixed protein:carbohydrate ratio, larval *Osmia bicornis* pupated after eating a particular amount of carbohydrate, irrespective of whether they over- or under-ate protein, i.e. when eating diets of a sub-optimal P:C ratio, they exhibited a no-interaction rule of compromise (de Carvalho and Mirth, 2017), prioritising C over P intake. Although we did not identify an optimal (fitness-maximising) intake amount for either nutrient, carbohydrate was positively associated with both cocoon weight and survival. Conversely, at low carbohydrate levels, survival instead became dependent on protein (i.e. survival increased with increasing P:C ratio when absolute C levels were low), a pattern more or less opposite to those observed in adult insects (Lee, 2015) and honeybee larvae (Helm et al., 2017). When larvae were given a choice of complementary foods, they partially adjusted their intake of each food to compensate for the variation in nutrient content. In this choice phase, although consumption of both protein and carbohydrate

differed significantly from expected based on random consumption of each diet choice, larvae did not converge on an intake target - perhaps unsurprisingly, as no intake target had previously been identified over the same range of dietary ratios during the no-choice phase. Nevertheless, larvae converged on a common P:C ratio of approx. 1:1.8, and showed tighter control over carbohydrate consumption than over protein consumption, adding weight to the findings from the no-choice phase that suggest that *O. bicornis* larvae prioritise carbohydrate over protein consumption. Here we argue that overwintering and the regulation of the typically limiting nutrient may explain these findings.

Within the range of diets studied, cocoon weight was positively related to carbohydrate consumed, with little influence of protein (Fig. 3). Increased body size is related to the size of nutrient stores in *Osmia* (Bosch et al., 2010) and other insects (Briegel, 1990; Hahn, 2005; Strohm, 2000) - and may reflect the size of the fat body, where carbohydrate-derived fat is stored in insects (Arrese and Soulages, 2010). The fat body is critically important to species such as *O. bicornis* that undergo diapause - not only during diapause (Giejdasz and Wasielewski, 2017; Wasielewski et al., 2013) but also afterwards (Hahn and Denlinger, 2007). It would now be interesting to determine the specific relationships between larval nutrition and fat body size, overwintering success and subsequent fitness in *O. bicornis*. It is also important to note that increased body size may have several other benefits, e.g. larger females may be more robust to changeable weather conditions (Bosch, 2008; Bosch and Vicens, 2005). Whilst carbohydrate-biased (and protein-poor) intake targets have traditionally been seen as detrimental to female fitness (Lee et al., 2008), lipid is a key component in insect oocytes, comprising 30-40% of the dry weight (Kawooya and Law, 1988; Ziegler and Van Antwerpen, 2006) of which the vast majority comes from the fat body reserves of the female (Arrese and Soulages, 2010). Female *O. bicornis* may therefore prioritise carbohydrate intake in order to provide

adequate lipid stores to meet energy demands of their developing eggs (Beenakkers et al., 1985). Nevertheless, (Bosch and Vicens, 2005) found little correlation between body size in *Osmia cornuta* and fecundity, which was instead more related to longevity and provisioning rate. It is also worth noting that, although we found that high carbohydrate increased cocoon weight, we did not identify a fitness peak at which cocoon weight was maximised. Further work could employ a wider range of diet ratios in order to locate such a peak.

Dietary macronutrients also had noticeable effects on survival to pupation within our range of diets (Figs. 5, 6): the larvae consuming most carbohydrate had the greatest survival rates. Among *adult* insects, high carbohydrate (or more specifically, low P:C ratio) has often been associated with increased lifespan (Fanson et al., 2009; Le Couteur et al., 2016; Lee et al., 2008), including in both honeybee and bumblebee adults (Paoli et al., 2014a; Pirk et al., 2010; Stabler et al., 2015). Conversely, high P:C ratios have been linked to reduced lifespan in the adults of many insects (Dussutour and Simpson, 2012; Lee, 2015; Lee et al., 2008). Focussing on larvae, we saw somewhat different survival patterns with respect to protein consumption. Although high-carbohydrate consumption increased survival to pupation, among those larvae that ended up eating low quantities of carbohydrate, *protein* consumption mediated survival, with those on higher protein diets (higher P:C) experiencing greater survival (Fig. 6). This pattern may be driven by alternative mechanisms for surviving diapause. Well-fed larvae that have eaten sufficient carbohydrate may survive the winter via lipogenesis of carbohydrate-derived fat body reserves (Arrese and Soulages, 2010). However, if carbohydrates are limited, larvae may instead utilise dietary protein in order to provide lipid stores via deamination and gluconeogenesis of amino acids, as in some caterpillars (Lee et al., 2003, 2002; Merkx-Jacques et al., 2008). Interestingly, in the only other study to have used the GFN to

investigate nutrition in larval bees, in this case honeybees (Helm et al., 2017), precisely the opposite effect was found. *In vitro* feeding revealed that, at high carbohydrate, larval survival was dependent on the amount of dietary protein, but at low carbohydrate survival was independent of protein. Larvae showed significantly reduced survival on high carbohydrate, low protein diets and the highest growth rates on diets that contained a medium level of protein but low carbohydrate - suggesting that worker recruitment could suffer in situations where protein is limited (Helm et al., 2017). The reasons behind this stark difference are unclear but it is worth noting that honeybee workers have a different role as adults from individuals of *O. bicornis*; all female *O. bicornis* are destined to be reproductives, unlike the honeybee larvae in Helm et al's study. Additionally, the honeybee larvae used in Helm et al's study were 'summer bees' (Steinmann et al., 2015) and therefore would not need to accrue nutrient reserves in order to enter diapause over the winter. If nothing else, these contrasting findings highlight not only the importance of understanding larval *as well as* adult nutrition for our general understanding of bee health, but also that knowledge of the nutritional ecology of more commonly studied social species cannot necessarily be applied to the more numerous, mass-provisioning solitary bees that collectively provide the bulk of our pollination services (Winfree et al., 2007).

Interestingly, when allowed to self-select their diets, *O. bicornis* larvae did not cluster in nutritional space but were instead spread out along a P:C ratio of 1:1.8 (Fig. 6a), approximately according to overall diet concentration. Notwithstanding the variable amounts actually eaten, assuming at least that this 1:1.8 *ratio* reflects the ratio of the larvae's true intake target, this would differ markedly from that reported for adult honeybees (1:12; Altaye et al., 2010), and would lie closer to that of ants foraging for offspring (1:1.5; Dussutour and Simpson, 2009). Unlike sterile adult honeybees, all *O. bicornis* larvae are destined to be reproductive, and do not require fuel for immediate flight,

so might be expected to require more protein. Unlike larval ants, however, *O. bicornis* larvae need to compile enough stores to survive winter diapause (Fliszkiewicz et al., 2012) so may require higher amounts of carbohydrate. The fact that we did not observe the expected clustering in nutrient space could potentially be explained by the fact that the more dilute diets contained more indigestible pollen husks (Roulston and Cane, 2000a), increasing the proportion of indigestible material passing through the gut. Some insects may be volumetrically limited when consuming dilute foods (Lee et al., 2008), so the additional fibrous material may have effectively limited the ability of bee larvae to regulate their diet by compensatory feeding. This may also explain why, although on average larvae ate more of the dilute diets on no-choice treatments (Fig. 2b), they were clearly unable to compensate for the reduced nutrient concentration, consuming less of each macronutrient than larvae fed more concentrated diets (Fig. 2a). Despite the fact that total food consumption was similar across treatments in the choice phase (Fig. 6b), larvae on less concentrated diets may have been unable to reach the same point in nutritional space due to consumption rate limitations.

The apparent lack of protein regulation shown by *O. bicornis* larvae is perhaps surprising given that, (1) the opposite is seen in larvae of other insects e.g. *Drosophila* flies (de Carvalho and Mirth, 2017) and *Heliothis virescens* caterpillars (Telang et al., 2001), (2) protein is important for somatic growth and survival (Lee, 2007; Povey et al., 2009; Roulston and Cane, 2002; Tasei and Aupinel, 2008), (3) bees primarily receive their protein (and lipid) requirements from pollen (Vaudo et al., 2016), and (4) larval bees feed primarily on pollen (Muth et al., 2016). Although adult workers of social bees have also been shown to prioritise carbohydrate over protein, their need for protein is relatively low (Paoli et al., 2014b; Stabler et al., 2015), requiring large amounts of carbohydrate to fuel flight (Darveau et al., 2014) and their high metabolism (Harrison and Roberts, 2000). Considering that

growth is concentrated in the larval stage, it would seem reasonable to expect that protein acquisition would drive nutritional decisions in larval bees, even if not adults.

Tolerance of wide variation in dietary protein, such as we saw in *O. bicornis* larvae, is typically seen in predators - both vertebrates, e.g. cats (Hewson-Hughes et al., 2011) and mink (Mayntz et al., 2009), and invertebrates (Kohl et al., 2015; D. Raubenheimer et al., 2007) - i.e. organisms with an excess of protein in their normal diets. However, bees are considered herbivorous (Larkin et al., 2008); herbivores tend to have protein-based decision rules, including pollen-foraging adult bumblebees (Vaudo et al., 2016) and many other herbivores [e.g. caterpillars (Lee et al., 2002; VanOverbeke et al., 2017)] and also omnivores [e.g. humans (Simpson and Raubenheimer, 2005)]. While clearly not predators, bees do share a common ancestor with predatory social wasps and ants (Johnson et al., 2013; Peters et al., 2011), and ant workers have been shown to tolerate varying protein levels in favour of a carbohydrate target (Dussutour and Simpson, 2009). Furthermore, "nutrient-generalist" species (i.e. those that tolerate wide ranges of dietary compositions) tend to be more able to tolerate swings in the particular nutrient which is least limiting (Raubenheimer and Simpson, 1999; Simpson and Raubenheimer, 2012b). Larval bees feed on pollen, which is amongst the most protein-rich of plant tissues (Mattson, 1980), and, as such, carbohydrate may be the more limiting nutrient for larvae. It is likely that starch, the standard form of digestible carbohydrate storage in pollen (Pacini, 1996), rarely exceeds the protein content of pollen; Roulston and Buchmann (2000) found that average starch content of pollen ranged from 0-22%, considerably less than the range for protein (2-60%; Roulston and Cane, 2000a). Thus, the relative abundance of protein in pollen may help to explain why *O. bicornis* larvae appeared to eat to a carbohydrate target whilst tolerating varying levels of protein.

Furthermore, the fact that larvae consumed very different amounts of protein, despite its importance to larval insects, suggests that bee larvae may be vulnerable to environmental variations in the protein:carbohydrate ratio of pollen - in a similar but opposite manner to humans, who will consume excesses of carbohydrate in order to eat enough protein, rendering them vulnerable to variation in protein:carbohydrate ratio of food (Simpson and Raubenheimer, 2005). Since bee larvae appear to be able to regulate carbohydrate, they may therefore rely on parents to regulate protein on their behalf. Given the natural variation in pollen protein content in the environment, bee parents may be able to sense the macronutrient composition of pollen directly at the flower and thus actively regulate the composition of pollen provisions they provide to offspring. Some studies support this idea (Muth et al., 2016; Ruedenauer et al., 2016, 2015), yet many studies have found otherwise (Konzmann and Lunau, 2014; Roulston and Cane, 2002, 2000b; for review, see Nicholls and Hempel de Ibarra, 2016). Conceivably, though, adults may instead simply collect pollen for young indiscriminately, relying on (1) larval tolerance of varying protein (this study), which may carry costs unmeasured here, and (2) variation in pollen quality balancing out owing to the historically rich diversity of the floral environment (Bukovinszky et al., 2017). In modern agricultural landscapes, where floral diversity is reduced in favour of brief gluts of monotonous crops, this strategy may no longer be effective and may indeed be deleterious. Under this scenario, the ways larvae deal with excesses and deficiencies of protein would be of paramount importance for bee health. With few exceptions (see Helm et al., 2017), we know very little about larval nutrition in bees, as most studies focus on adults, whether foraging for nectar (Altaye et al., 2010; Kriesell et al., 2017; Paoli et al., 2014a; Ruedenauer et al., 2015; Vaudo et al., 2016) or pollen (Altaye et al., 2010; Kriesell et al., 2017; Paoli et al., 2014a; Ruedenauer et al., 2015; Vaudo et al., 2016). Considering that (1) all the nutrients required to reach adulthood are

accrued during the larval stage, and (2) larvae and adults have different nutritional requirements (Cridge et al., 2017), the way larval bees deal with macronutrient imbalances is a considerable knowledge gap. In particular, further studies that assess whether *O. bicornis* larvae employ post-ingestive processing to regulate protein intake, as shown in some other insects (Lee et al., 2004a; Raubenheimer and Simpson, 2003; Rho and Lee, 2017; Telang et al., 2001), may help to explain the large variation of protein tolerated by larvae.

That (1) larvae seem not to regulate protein, (2) parents are likely unable to assess protein content for their young, and (3) human activity, particularly intensive agriculture, is reducing floral diversity, potentially make for a toxic combination for bees. Changes to the composition of available nutrition, driven by loss of floral diversity (Goulson et al., 2015), or potentially through changes in the nutritional composition of pollen *within* a plant species in response to human activity (Ziska et al., 2016), may cause mother bees to unwittingly feed their offspring nutrient-deficient diets. This would mean that, despite otherwise favourable environments, *O. bicornis* and possibly bees more generally, would become caught in a "nutritional trap", gathering food that no longer provides offspring with appropriate nutrition. More research is needed into the nutrition of larval bees, especially solitary species where knowledge is sparse, in order to inform conservation management and stewardship schemes. Further studies should focus on whether larval bees have physiological adaptations to overcome nutrient imbalances, e.g. post-ingestive processing, and whether mother bees are able to adjust the provisions they provide their offspring in order to counter changing nutritional composition of pollen available in the environment.

2.6. Supplementary Material

S1. Pollen husk (sporopollenin) preparation

To produce the exines 500g of raw sunflower pollen (Henan Shengchoa Apiculture Co., China) was suspended in 9M aqueous HCL solution (2250ml) and heated at 94-97°C for one hour. The solution was then neutralised with a 50% KOH solution, the resulting fluid filtered out using a Buchner funnel under vacuum, and the filtrate discarded. The residue was then washed with water, methanol (100%), and acetone (100%) sequentially under vacuum to remove any remaining solution. Each wash was repeated until the effluent ran clear before moving to the next washing stage i.e. filtrate was first washed with water until effluent ran clear, then methanol etc. Once the final wash was completed, filtrate was allowed to dry overnight. These ‘husks’ were then subjected to SEM imaging to ensure that only the outer pollen shell, the husk, remained.

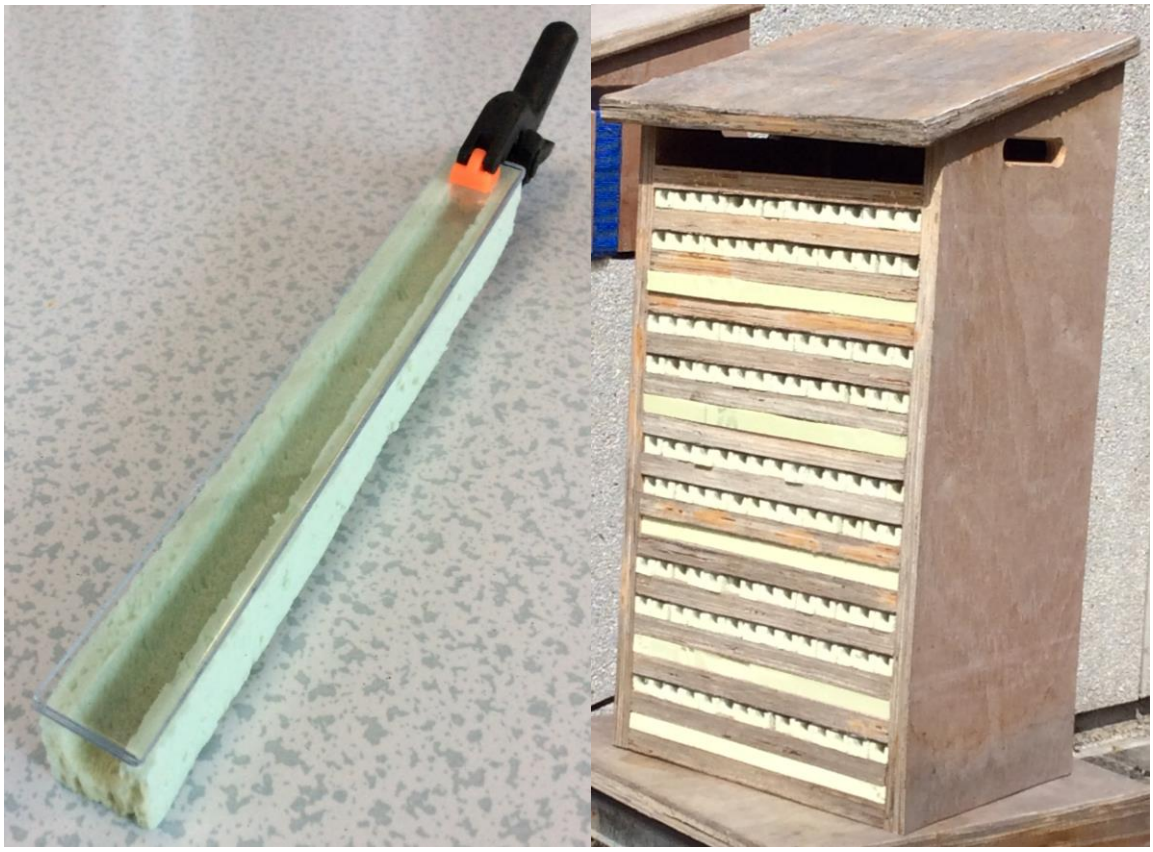


Fig. S1. Nesting material provided to *O. bicornis* adults during 2017 season. A) Styrofoam (Styrodur, 3035CS) nest block with polycarbonate lid and clamp. B) Wooden housing containing nesting material.

Table S1. The amount (g) of each macronutrient, protein or carbohydrate, in each diet treatment per 1 gram of dry diet (inclusive of diluent for all but control).

Diet	Protein (g)	Carbohydrate (g)
A1	0.322	0.392
A2	0.250	0.305
B1	0.220	0.505
B2	0.171	0.392
C1	0.166	0.563
C2	0.129	0.438

Table S2. Average amounts of protein and carbohydrate in grams eaten by larvae raised on fixed diet treatments

	Protein consumed (g)	Carbohydrate consumed (g)
Diet		
A1	0.180	0.215
A2	0.168	0.201
B1	0.104	0.239
B2	0.094	0.214
C1	0.074	0.245
C2	0.063	0.211

3. Nutritional needs and preferences of solitary bee larvae shift during development

3.1. Abstract

Bees are key to ecosystem health and food security, yet have declined globally, driven partly by human-induced landscape change. We have limited knowledge of how nutritional quality, not simply quantity, influences bee health, with most studies focussed on adults. The few studies of larval nutrition focus on a snapshot that may miss important developmental changes. In mass-provisioning solitary bees (*Osmia bicornis* L.) we can manipulate and track nutrition accurately. Using the Geometric Framework we conducted two experiments investigating, respectively, effects of manipulating dietary protein:carbohydrate (P:C) or protein:lipid (P:L) ratio. In each experiment, larvae were restricted to one of 10 diets, at 5 different nutrient ratios and 2 concentrations. We investigated: (a) which rules of compromise larvae employ on varying P:C and P:L diets, (b) at what macronutrient combinations is weight gain maximised, and (c) how macronutrient ratio influences survival. Larvae appeared to consume nutrients to a certain level for each macronutrient, irrespective of overeating one or the other; this was true for both P:C and P:L treatments - to our knowledge the first demonstration of lipid regulation in larval bees. Weight gain in the P:L treatments was driven by neither P nor L alone, but seemed limited by the total amount of both nutrients consumed, despite larvae on low P:L diets achieving the highest maximum weight. In P:C treatments, weight gain during early larval stages was driven by protein consumption, but this shifted to carbohydrate in the

latter stages. Similar to findings in other insects, survival increased with increasing carbohydrate in P:C treatments, and with increasing lipid in P:L treatments. Our results highlight the complexity and time-dependence of nutritional requirements in bee larvae, and suggest that lipid content of pollen is an important but overlooked variable in the floral landscape. As larval bees are entirely reliant on parental provisions, such complexity demonstrates the risks to larval nutrition in increasingly fragmented habitats.

Keywords: pollination, foraging ecology, habitat fragmentation, nutritional geometry, lipid, environmental change, bee health, macronutrient, development, nutritional quality

3.2. Introduction

Bees are important pollinators (Klein et al., 2018), especially in temperate regions (Hanley et al., 2015; Klein et al., 2007). They boost productivity of crops (Nicholson and Ricketts, 2019; Ollerton et al., 2016) and wildflowers (Ollerton et al., 2011), and are essential to ecosystem health (Goulson and Nicholls, 2016), and food security (Potts et al., 2016). Both wild (Bartomeus et al., 2013; Cameron et al., 2011; Goulson et al., 2015; Sirohi et al., 2015) and domestic bees (vanEngelsdorp et al., 2008; Potts et al., 2010b) however, are in global decline. These declines are widely viewed as human-induced (Goulson et al., 2015), with a major contributor being the intensification of agriculture (Fuller et al., 2005; Goulson and Nicholls, 2016), leading to habitat fragmentation (Nagamitsu et al., 2017; Söderman et al., 2018), the rise of monoculture (Kovács-Hostyánszki et al., 2017; Letourneau et al., 2011; Nicholls and Altieri, 2013), and loss of wildflower resources (Goulson et al., 2015). Such alterations of the floral landscape have led to nutritional stress; a major cause of bee declines (Filipiak et al., 2017; Goulson et al., 2015; Woodard and Jha, 2017; Wood et al., 2016). As appropriate nutrition is key to animals' growth, reproduction, and survival (Simpson and Raubenheimer, 2012a), we need to understand how such changes to bees' nutritional landscape affects their fitness.

Pollen is arguably the most important nutritional resource for bees, providing the majority of bees' protein and lipid intake (Vaudo et al., 2016), and is of particular importance to larvae, whose diets consist almost solely of parentally-gathered pollen (Filipiak, 2019; Muth et al., 2016). The larval stage is where all growth (Nijhout et al., 2014) and nutrient storage (Giejdasz and Wasielewski, 2017) occurs in bees, yet most nutritional studies focus on the adults of social species (Altaye et al., 2010; Hendriksma and Shafir, 2016; Pirk et al., 2010; Ruedenauer et al., 2018; Tanaka et al., 2018; Vaudo et al.,

2018), with the larvae receiving comparatively less attention (Fischman et al., 2017; Helm et al., 2017). Larval *solitary* bees in particular are poorly studied in terms of their nutrition (Filipiak, 2019; Roulston and Cane, 2002; Sedivy et al., 2011; Vanderplanck et al., 2014), which is important in the light of recent studies that suggest the nutritional knowledge gained in social species cannot necessarily be applied to solitary species (Chapter 2; Helm et al., 2017). Thus, it is important to study the nutrition of bees not only at different life stages but also across different ecologies, especially considering the importance of solitary bees to pollination (Winfree et al., 2008, 2007).

The Geometric Framework (GFN) has been used to determine how animals, including bees (Altaye et al., 2010; Archer et al., 2014a; Paoli et al., 2014a; Stabler et al., 2015; Vaudo et al., 2016) navigate their nutritional environment (Simpson and Raubenheimer, 2012a, 1993). The GFN can be used to determine an organism's "intake target"; the optimal amount, and specific ratio, of nutrients an organism should consume to maximise fitness (Simpson and Raubenheimer, 1995, 1993). Additionally, the GFN can help determine the rules animals use to govern their nutritional intake when the desired target cannot be reached, i.e. their *rule of compromise* (Lee et al., 2004b; Simpson and Raubenheimer, 1993). Yet, the GFN has seen only limited use in bees at the *larval* stage, with only two studies using it to investigate larval nutrition (Chapter 2; Helm et al., 2017).

Currently, studies in larval bees have focussed on a single point in time, often looking at overall consumption once subjects reach a certain point (Chapter 2; Helm et al., 2017). For example, in a previous study, we demonstrated that larvae of the solitary bee *Osmia bicornis* prioritised carbohydrate intake over that of protein (Chapter 2). Mass-provisioned bee larvae receive a fixed pollen provision upon which they must develop, and we have shown previously that carbohydrate appears to be the most important macronutrient overall. However, nutritional needs, as well as the relative importance of

individual nutrients, can change during development (Al Shareefi and Cotter, 2018; Cohen et al., 1987; Simpson et al., 2002; Simpson and Raubenheimer, 1993) and therefore an organism's intake target may shift to reflect this (Simpson and Raubenheimer, 2012b). Additionally, any changes in nutritional optima during development would have to be balanced by the parent when making nutritional choices for their offspring.

Lipids and their derivatives have been shown to be important for bees (Vaudo et al., 2016; Zarchin et al., 2017), yet, this has not been investigated in bee larvae. Given that (1) all dietary lipids for bees come from pollen (Vaudo et al., 2016), (2) bee larvae feed primarily on pollen (Muth et al., 2016), (3) it is at the larval stage where most fat stores, as well as other nutrients, are acquired (Bosch et al., 2010; Dmochowska et al., 2013), and (4) the building of fat stores is essential for surviving through diapause in insects (Raubenheimer et al., 2007), including solitary bees (Giejdasz and Wasielewski, 2017; Wasielewski et al., 2013), it seems plausible that bee larvae may show some sort of regulatory capacity when it comes to lipid intake.

In this study we use *O. bicornis*, a commercially important pollinator (Fliszkiewicz et al., 2011; Gruber et al., 2011; Jauker et al., 2012; Wilkaniec and Radajewska, 1996), to investigate how larval bees deal with both differing protein:carbohydrate (P:C) and protein:lipid (P:L) ratio diets during development, and how the regulation of nutrients changes across the larval period. Using a well-established GFN design (Lee et al., 2002; VanOverbeke et al., 2017), we raised *O. bicornis* larvae on two distinct diet treatments: one group raised on fixed diets differing in P:C ratio, and another on fixed diets differing in P:L ratio. We assessed rules of compromise in relation to both nutrient pairs; and, in both cases, rather than simply looking at total nutrient consumption, we tracked how consumption changed across the developmental period. Additionally, we also mapped nutrient consumption against weight gain across larval development. Since all growth occurs at the

larval stage (Nijhout et al., 2014), achieving appropriate stores is crucial for overwinter survival in *O. bicornis* (Giejdasz and Wasielewski, 2017), and larvae pupating into small adults have reduced survival (Radmacher and Strohm, 2010). Greater weight gains can therefore be attributed to greater fitness. We predicted that, (1) as previously shown for total consumption (Chapter 2), larvae would regulate C over P on P:C diets, (2) as both carbohydrate and lipid can be used to create fat stores (Al Shareefi and Cotter, 2018; Arrese and Soulages, 2010), larvae would regulate lipid over protein, and (3) that those larvae on the lowest P:C and P:L diets would gain the most weight over development.

3.3. Methods

Study Organism and Population

We used the solitary bee *Osmia bicornis*, a cavity-nesting solitary bee, for our model. *O. bicornis* is univoltine, and is common across northern Europe and the UK (Falk, 2015). Overwintering adults hatch out in the spring (March/April), and fly until early July (Falk, 2015). *O. bicornis* are mass-provisioners, and utilise pre-existing cavities for their nests, which they divide into mud cells, each provisioned individually with a single pollen ball onto which a single egg is laid (Giejdasz et al., 2016). Their reproductive biology coupled with their willingness to nest in artificial constructs (Strohm et al., 2002) makes *O. bicornis* an excellent model for manipulative experimentation, being successfully used to investigate larval bee nutrition previously (Chapter 2; Filipiak, 2019).

A previously established population of *O. bicornis* at the University of Hull was used to provide larvae for the experiments and was supplemented with additional diapausing adults in cocoons (Mauerbienen®, Germany). Styrofoam blocks (Styrodur 3035CS) housed within a wooden frame were provided as nesting material as described in Chapter 2. Nests were checked daily once females began laying and were brought into the laboratory once sealed by the female or when, under favourable weather conditions, no more progress had been made on a nest during a week. Any eggs that hatched prior to nest completion were individually collected along with their pollen provision. Due to initial high larval mortality (attributed to premature manipulation) over the first few (<3) days post hatching larvae were allowed to feed for 4 days on their natural provision before being allocated to treatment groups, and were then transferred to single-occupancy nests (Fig. S1) on one of ten diet treatments. Larvae were kept in an environmental chamber (Sanyo MLR-351H; 20°C, 70% RH) in complete darkness for the duration of larval development.

Treatments & Diet Formulation

We use the Geometric Framework (GFN) to investigate how larval *O. bicornis* manage their intake of macronutrients: lipid and protein, or carbohydrate and protein, specifically their rules of compromise. We then mapped macronutrient consumption against established fitness proxies to indicate a possible intake target.

Larvae were assigned to either the protein:lipid (P:L) or protein:carbohydrate (P:C) group (see Table 1). Each group was fed one of 10 diets at 5 different macronutrient ratios (see Table S1) at one of two different macronutrient concentrations (95 and 80%). All diets

consisted of a fixed base of honeybee-collected pollen and honey with a fixed amount of added protein (micellar casein, Sigma-Aldrich). Differing amounts of lipid (soy lecithin, Agros Organics) were added to create differing P:L ratios and differing amounts of carbohydrate (trehalose, trehalose.co.uk) were added to create different P:C ratios. To standardise the ingredient list for each diet, fixed amounts of carbohydrate and lipid were added to diets where that component was not being altered e.g. fixed carbohydrate amount added to all P:L diets. Differing concentrations were achieved by diluting the diets, by weight, with sporopollenin (as in Chapter 2), the major indigestible component of the outer wall of pollen (Roulston and Cane, 2000a). All dry components of the diets were premixed and then batches of each diet were set in equal volumes of 3% agar solution and kept frozen until required. 10% methyl paraben solution was added to the agar solution, at 1% of total agar solution volume, to discourage mould growth. This differed slightly from our previous study (Chapter 2), as due to the increased number of larvae used here, food needed to be pre-prepared in batches, as opposed to made fresh, in order to allow for all necessary diet swaps within a day. Provisions were replaced at regular intervals to ensure a constant supply of food and to avoid desiccation or degradation of the provisions.

Table 1. Number of larvae assigned to each diet treatment

	Sample size		Sample size
Diet: PC		**Diet: PL**	
A1	16	F1	14
A2	18	F2	19
B1	14	G1	14
B2	19	G2	16
C1	16	H1	13
C2	19	H2	19
D1	14	I1	13
D2	17	I2	15
E1	15	J1	15
E2	18	J2	16

Experimentation

Larvae of mixed parentage were randomly allocated to each diet treatment and placed into single occupancy nests as in Chapter 2. All nest components were weighed prior to use. The larva was then added back to the new provision and the entire construct

weighed again to ascertain any change in larval mass. Both larva and remaining provision (minus frass) were weighed to 0.1 mg at each swap (AND® BM-252 balance).

Once provisioned, larvae were placed into the environmental chamber and allowed to develop. Nests were checked on a daily basis. Once larvae began spinning cocoons, diet swapping was ceased, and upon completion, the cocoon was removed and weighed.

A second treatment, to which no larvae were assigned, was set up to monitor moisture loss of all experimental diet provisions over the course of 4 days (the maximum time between diet swaps in experimental groups). These were weighed daily to record by-day moisture loss.

Statistical Analysis

All analyses were conducted in R version 3.5.0 (R Core Team, 2018) and all nutrient consumption data were analysed "by swap", i.e. for each period between successive provision replacement, building on the results of Chapter 2 by looking at how intake changes throughout the duration of development. Prior to analysis, all consumption data was adjusted for water loss and dilution. Larvae that died before the initial diet swap were excluded from subsequent analysis.

Nutrient consumption was analysed via Linear Mixed effects Models (LMMs) with swap number, nutrient ratio (P:C or P:L), and concentration as predictors, with larval ID as a random factor. Minimal models were determined via reverse stepwise comparison.

To maximise the ability of the data to detect broad patterns in weight gain, we first used a process of reverse stepwise merging of adjacent swaps to establish major

breakpoints in the relationship between weight gain and nutritional intake. To do this we used Generalised Additive Mixed effects Models (GAMMs), under the mgcv package in R (Wood, 2011), with weight change per swap as the response variable and swap number as a predictor, with a bivariate smoother fitted to protein and lipid (or carbohydrate) consumption for each level of swap number. A GAMM was initially fitted with all swap numbers left separate. Swap numbers were then merged, starting with the final swaps, and compared. Model selection was based on AIC because competing models were non-nested. Once the best pattern of breakpoints among merged swaps was established, multiple separate response surfaces were calculated for larval weight change based on these breakpoints, and each was visualised using non-parametric thin-plate splines.

3.4. Results

Protein:Carbohydrate

Rule of compromise

Larvae on less concentrated diets consumed less protein overall than those on concentrated diets irrespective of the diet P:C ratio (Linear Mixed effects Model with P:C ratio, swap number and diet concentration as predictors, dropping ratio × concentration interaction, with larva as a random effect, $\chi^2_{9,13} = 37.87$, $p<0.001$; Table 2), however, protein consumption was more negatively affected by concentration on high P:C diets (see Table S2 for minimal model estimates). No other interactions showed significant effects

(swap number × diet ratio × concentration, $\chi^2_{18,22} = 2.1$, $p = 0.72$; swap number × concentration, $\chi^2_{17,18} = 0.14$, $p = 0.71$; swap number × diet ratio, $\chi^2_{13,17} = 2.17$, $p = 0.71$).

As with protein, larvae raised on less concentrated diets consumed less carbohydrate than those on concentrated diets (LMM with P:C ratio, swap number and diet concentration as predictors, dropping concentration, $\chi^2_{8,9} = 69.73$, $p<0.001$; Table 2). Larvae also consumed less carbohydrate as development progressed (LMM with P:C ratio, swap number and diet concentration as predictors, dropping swap number, $\chi^2_{8,9} = 4.86$, $p = 0.028$) and larvae broadly consumed more carbohydrate on lower P:C diets (LMM with P:C ratio, swap number and diet concentration as predictors, dropping ratio, $\chi^2_{5,9} = 57.95$, $p<0.001$). No interaction term showed a significant effect (swap number × diet ratio × concentration, $\chi^2_{18,22} = 4.88$, $p = 0.3$; swap number × concentration, $\chi^2_{17,18} = 0.5$, $p = 0.48$; swap number × diet ratio, $\chi^2_{13,17} = 4.49$, $p = 0.34$; diet ratio × concentration, $\chi^2_{9,13} = 8.9$, $p = 0.064$). Broadly speaking, consumption of P and C appeared to follow an "equal distance" rule of compromise (Simpson and Raubenheimer, 1999) evidenced by an approximately negative linear relationship among related points in Fig. 1a-b. However, it could also be argued that points in Fig. 1 follow more of an L shape, which would indicate that there is a minimum level for *both* protein and carbohydrate, whereby larvae will overeat *whichever* macronutrient is in excess in order to achieve the target for the other (Simpson and Raubenheimer, 1993).

Table 2. Average amounts of total protein and carbohydrate in grams eaten by larvae raised on fixed diet treatments. SD indicates standard deviation. N represents the number of larvae used.

	Protein consumed (g)	SD	Carbohydrate consumed (g)	SD	N
Diet					
A1	0.213	0.0020	0.423	0.0040	(16)
A2	0.167	0.0017	0.331	0.0034	(18)
B1	0.146	0.0009	0.438	0.0027	(14)
B2	0.072	0.0010	0.215	0.0029	(19)
C1	0.098	0.0011	0.391	0.0044	(15)
C2	0.056	0.0012	0.222	0.0049	(19)
D1	0.123	0.0009	0.612	0.0047	(14)
D2	0.078	0.0005	0.388	0.0025	(17)
E1	0.092	0.0008	0.552	0.0048	(15)
E2	0.064	0.0008	0.384	0.0046	(18)

Weight gain during development

The effect of consumption of carbohydrate and protein upon larval weight gain was similar from swaps 4 to 11, so we merged them for analysis, resulting in 4 distinct periods: swaps 1, 2, 3, and 4-11. A single surface was fitted for each of these combined stages. It is worth noting that we did not find any non-linear patterns i.e. we did not identify an optimal amount of carbohydrate and protein that maximised weight gain at any given time during larval development.

The effects of protein and carbohydrate on larval weight changed throughout larval development. Up to the first three days (i.e. swap 1), weight gain was driven by protein consumption (Fig. 1c). However over the course of development this shifted towards total energy intake (Fig. 1d,e), finally shifting to weight gain being driven by carbohydrate acquisition in the later stages (Fig. 1f).

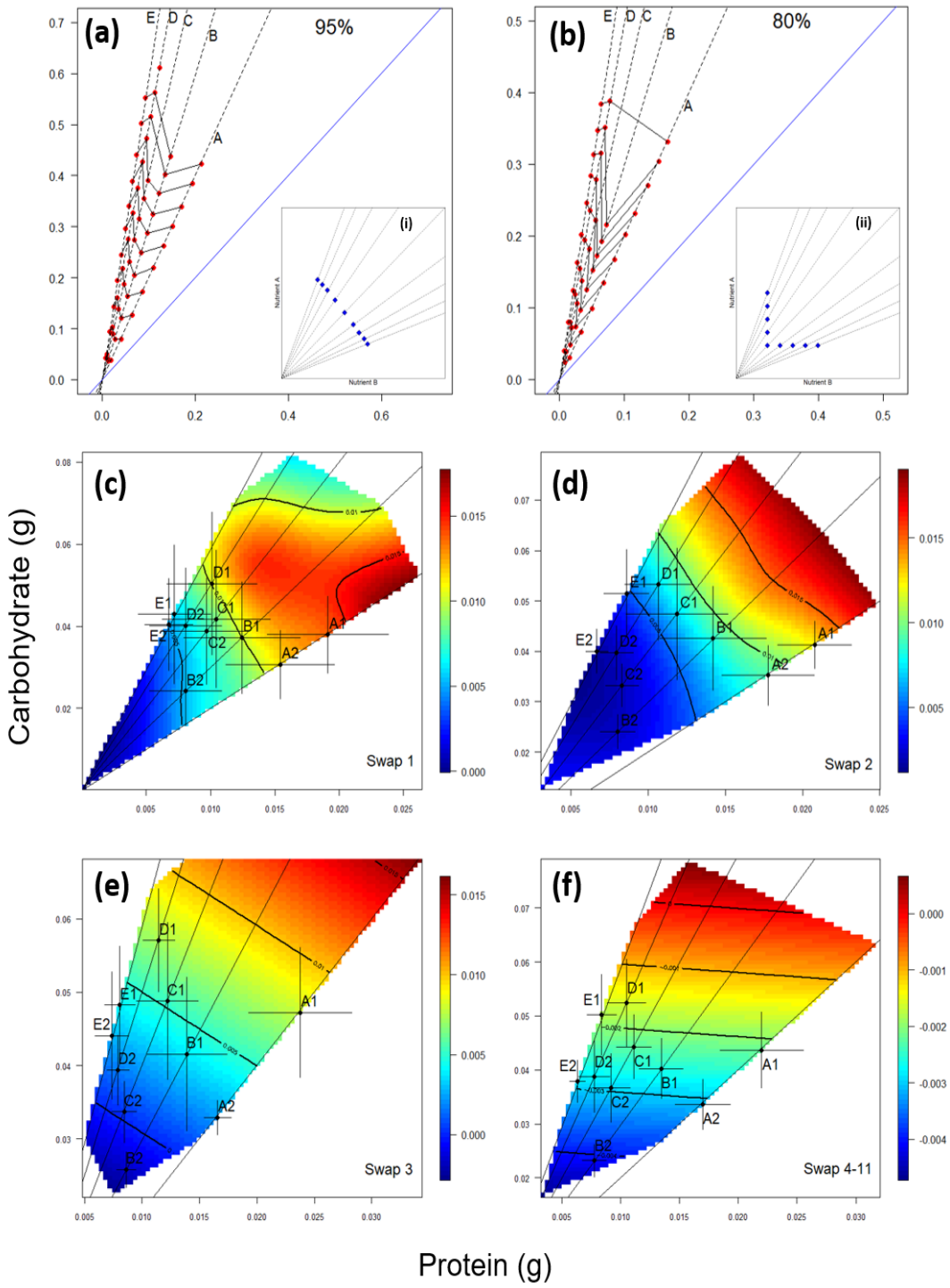


Fig. 1. (a-b) - Mean total (+/- 1 SD) amount of P and C consumed in grams by larvae on each diet at each swap across the duration of development. Insert (i) illustrates the 'perfect' example of the equal distance decision rule, insert (ii) shows a decision rule where larvae aim for a target for both nutrients, irrespective of overeating either. Note that either rule may apply to either (a) or (b). (c-f) - Response surfaces showing the effects of the mean amount of

P and C consumed (+/- 1 SD) on larval weight gain (g) across diet swaps. Transition from blue to red indicates greater weight gain. Solid lines and letters represent the five P:C ratios (A = 1:2, B = 1:3, C = 1:4, D = 1:5, E = 1:6). Numbers following letters denote concentration (1 = 90%, 2 = 80%).

Survival

Viewed in terms of diet composition, survival of larvae was influenced by diet concentration, but not diet ratio, with those on more concentrated diets living longer than those on the least concentrated diets (parametric survival regression, $\chi^2_1 = 18.56$, $p<0.001$, Fig. 2d-f). When analysed in terms of actual amounts of protein and carbohydrate eaten, survival was driven by the interaction between carbohydrate and protein ($\chi^2_1 = 33.31$, $p<0.001$), whereby those larvae that consumed greater amounts of carbohydrates lived longer (Fig. 2a), and this remained true when split into the two differing diet concentrations (Fig. 2b, c). However, at medium and low levels of carbohydrate those larvae on medium levels of protein achieved the greatest survival.

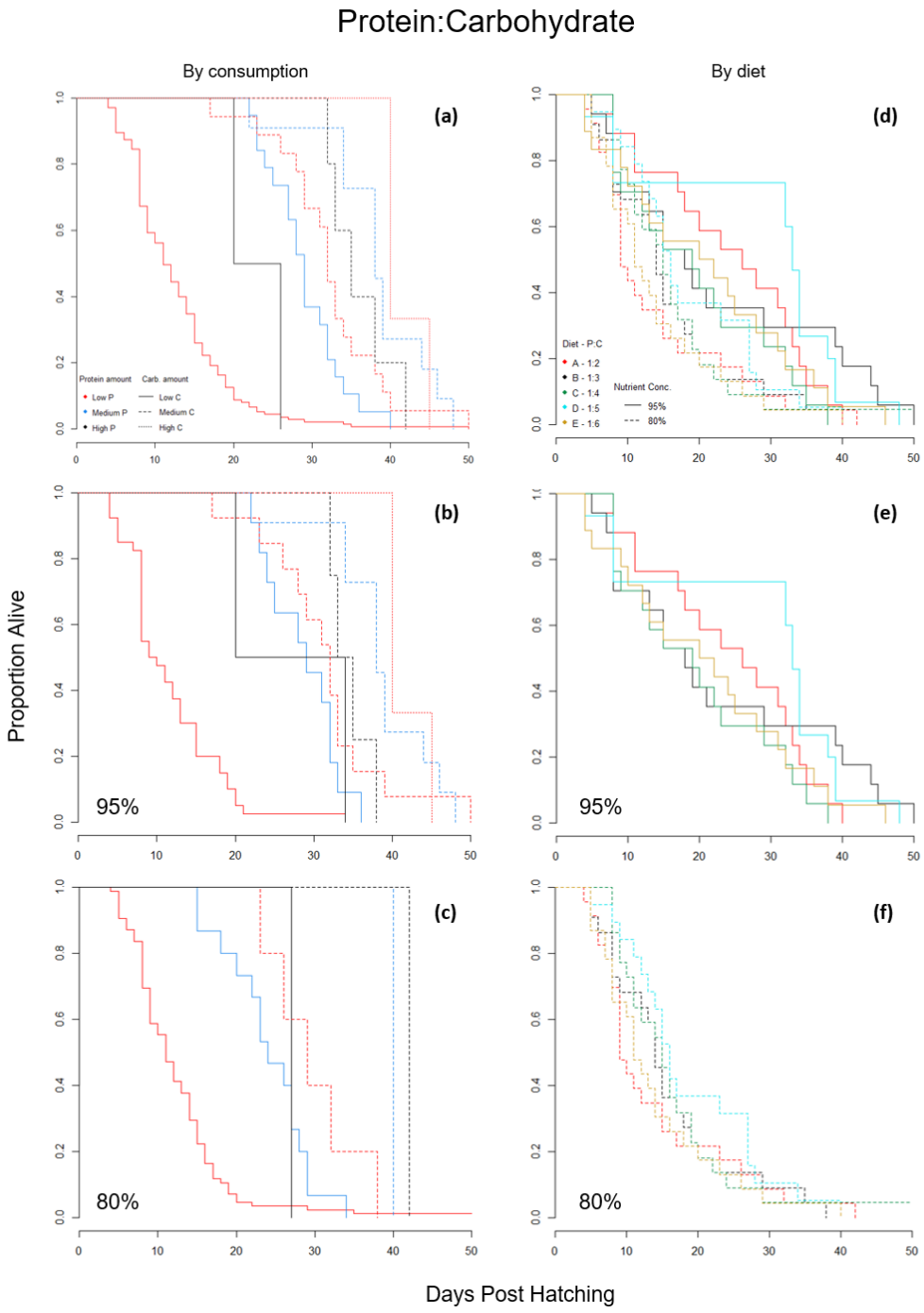


Fig. 2. a-c - Survival of P:C diet treatments broken down by amounts of macronutrients actually consumed. Lines represent the proportion of larvae alive at a given time point. Key: P = protein, C = carbohydrate; H = high, M = medium, L = low consumption (High = top 33% of consumption, Medium = middle 33% of consumption, Low = bottom 33% of consumption) . d-f - Survival of larvae on P:C diet treatments according to the diet they were fed. Letters

represent 5 P:C ratios (A = 1:2, B = 1:3, C = 1:4, D = 1:5, E = 1:6). Lines represent the proportion of larvae still alive at a given time point. Solid lines indicate diets at 95% macronutrient concentration, dashed lines indicate diets at 80% macronutrient concentration.

Protein:Lipid

Rules of compromise

Dietary P:L ratio had a significant effect on the amount of protein consumed by larvae at each swap, with more protein consumed on the higher P:L diets (Linear Mixed effects Model of protein consumption with P:L ratio, swap number and diet concentration as predictors, with larva as a random effect, dropping effect of P:L ratio, $\chi^2_{5,9} = 36.3$, $p<0.001$). Swap number also had a significant effect on the amount of protein consumed per swap ($\chi^2_{8,9} = 18.84$, $p<0.001$), with larvae broadly consuming less protein towards the end of development. Protein consumption was also affected by the concentration of the diet ($\chi^2_{8,9} = 150.01$, $p<0.001$), with larvae on less concentrated diets consuming less protein (Table 3). Note that no interactions had significant effects when dropped from the full models in any case (swap number × diet ratio × concentration, $\chi^2_{18,22} = 0.62$, $p = 0.96$; swap number × diet ratio, $\chi^2_{13,17} = 1.39$, $p = 0.84$; swap number × concentration, $\chi^2_{17,18} = 0.27$, $p = 0.61$; diet ratio × concentration, $\chi^2_{9,13} = 9.13$, $p = 0.06$).

For lipid consumption in the P:L treatments, as with protein consumption in the P:C treatments, models revealed an interaction between diet ratio and diet concentration (Linear Mixed effects Model of lipid consumption with P:L ratio, swap number and diet

concentration as predictors, with larva as a random effect, dropping the P:L ratio × diet concentration interaction, $\chi^2_{9,13} = 49.64$, $p<0.001$), with lipid consumption more negatively affected by concentration on comparatively low P:L diets (see Table S3 for minimal model estimates). No other interaction terms showed significant effects (swap number × diet ratio × concentration, $\chi^2_{18,22} = 0.8$, $p = 0.94$; swap number × concentration, $\chi^2_{17,18} = 0.53$, $p = 0.47$; swap number × diet ratio, $\chi^2_{13,17} = 2,04$, $p = 0.73$). Overall, larvae on less concentrated diets consumed less lipid than those on more concentrated diets (Table 3). Relative consumption of protein and lipid by larvae changed little over the developmental period at either concentration, resulting in similarly shaped rules of compromise over time (Fig. 3a-b). Broadly, consumption followed what we term an "equal leverage rule?" rule whereby the target for each nutrient would be reached, irrespective of over consumption of either (Simpson and Raubenheimer, 1993). Protein consumption was similar across diets at low P:L ratios, however, at higher P:L ratios, lipid consumption became more constant and protein consumption varied.

Table 3. Average amounts of total protein and lipid in grams eaten by larvae raised on fixed diet treatments. SD indicates standard deviation. N represents the number of larvae used.

	Protein consumed (g)	SD	Lipid consumed (g)	SD	N
Diet					
F1	0.257	0.0021	0.102	0.0008	(13)
F2	0.176	0.0008	0.070	0.0003	(14)
G1	0.303	0.0024	0.060	0.0005	(13)
G2	0.156	0.0020	0.031	0.0004	(15)
H1	0.257	0.0019	0.034	0.0003	(12)
H2	0.140	0.0013	0.019	0.0002	(17)
I1	0.252	0.0022	0.025	0.0002	(13)
I2	0.181	0.0019	0.018	0.0002	(16)
J1	0.245	0.0018	0.020	0.0001	(11)
J2	0.182	0.0015	0.015	0.0001	(15)

Weight gain during development

Stepwise amalgamation of swaps using GAMMs showed that protein:lipid consumption was similar at swaps 4 & 5, swap 6 & 7, and swap 8 to 13. Therefore, a single surface was modelled for each of these groupings. The dietary P:L ratio actually consumed had little effect overall on larval weight gain with weight gain being primarily dictated by energy acquisition (i.e. the total amount of P+L consumed; Fig. 3c-h). Interestingly however, larvae maintained on the lowest P:L diets did achieve higher maximum weights (Fig. 4). As with P:C we did not identify an optimal amount of protein and lipids that maximised weight gain.

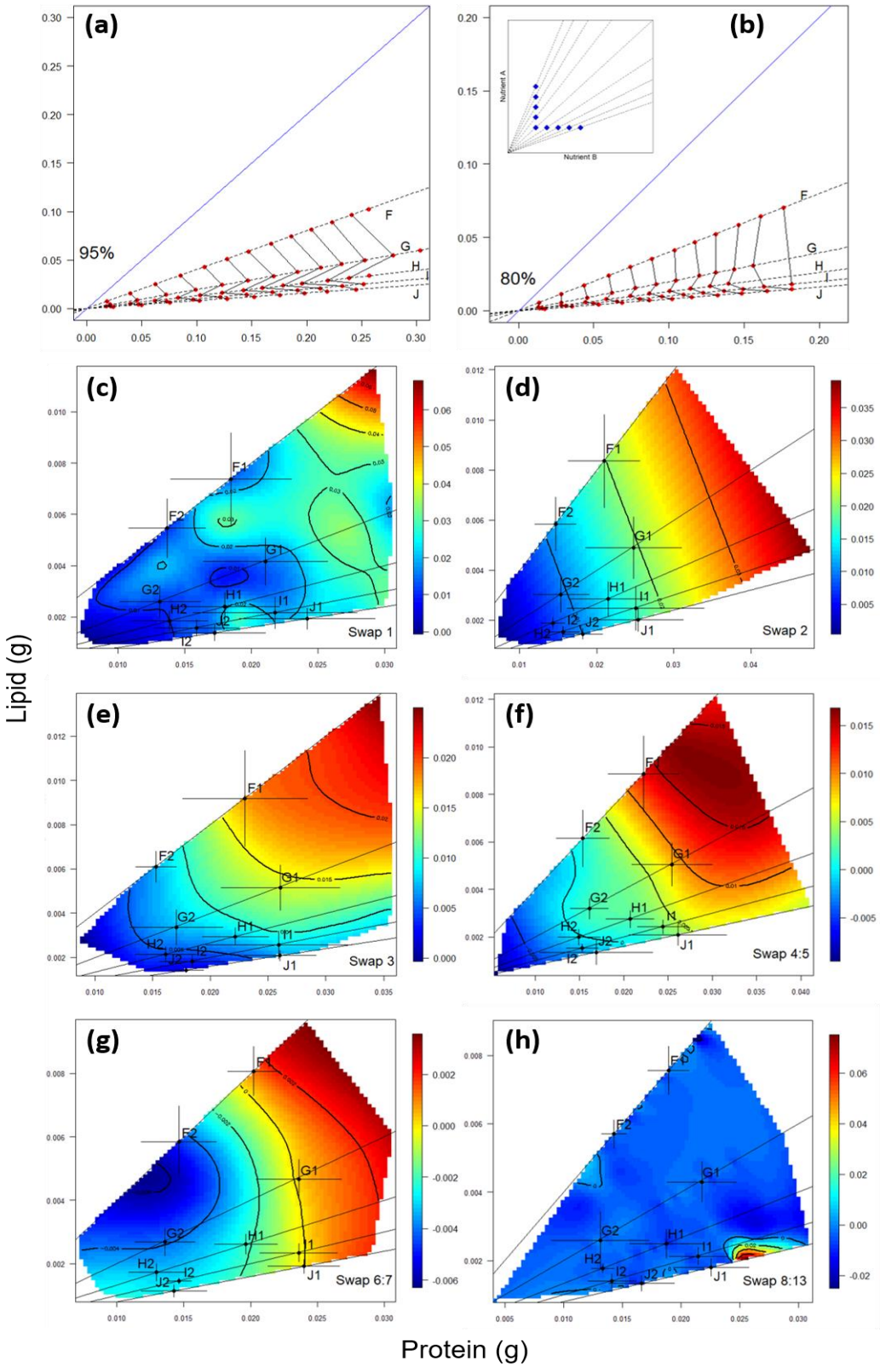


Fig. 3. (a-b) - Mean total (+/- 1 SD) amount of P and L consumed in grams by larvae on each diet at each swap across the duration of development. Insert illustrates 'perfect' example of

the decision rule employed. Note: the decision rule may apply to either (a) or (b). (c-h) - Response surfaces showing the effects of the mean amount of P and L consumed (+/- 1 SD) on larval weight gain (g) across diet swaps. Transition from blue to red indicates greater weight gain. Solid lines and letter represent the five P:L ratios (F = 2.5:1, G = 5:1, H = 7.5:1, I = 10:1, J = 12.5:1). Numbers following letters denote concentration (1 = 90%, 2 = 80%).

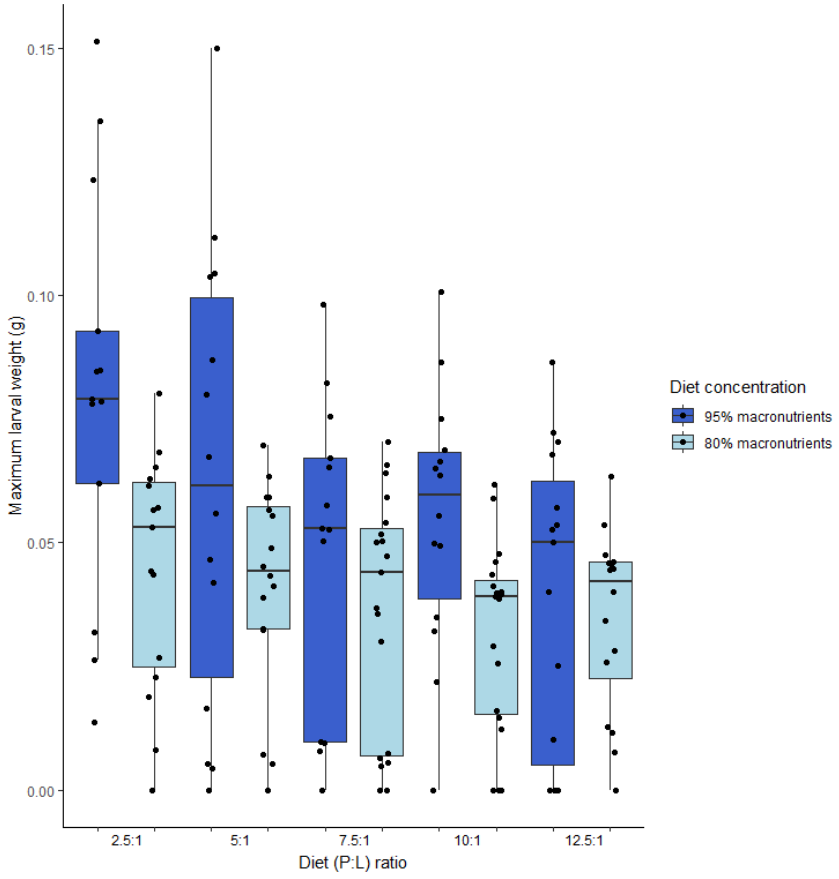


Fig. 4. Maximum larval weight (g) achieved on each P:L diet irrespective of larval age. Colour denotes macronutrient concentration (dark blue = 95%, pale blue = 80%).

Survival

Survival of larvae was not affected by P:L ratio but was influenced by concentration, with those larvae on the more concentrated diets achieving better survival rates (parametric survival regression, $\chi^2_1 = 16.28$, $p<0.001$, Fig. 5d-f). When split into the actual amounts of protein and lipid consumed, survival depended on the interaction between protein and lipid ($\chi^2_1 = 22.55$, $p<0.001$) with those consuming the most lipid living the longest no matter their protein consumption (Fig. 5a), a relationship that persisted despite diet concentration (Fig. 5b-c). However, at low lipid levels survival seemed to be mediated by protein consumption, with those consuming the most protein surviving longer (Fig. 5b-c).

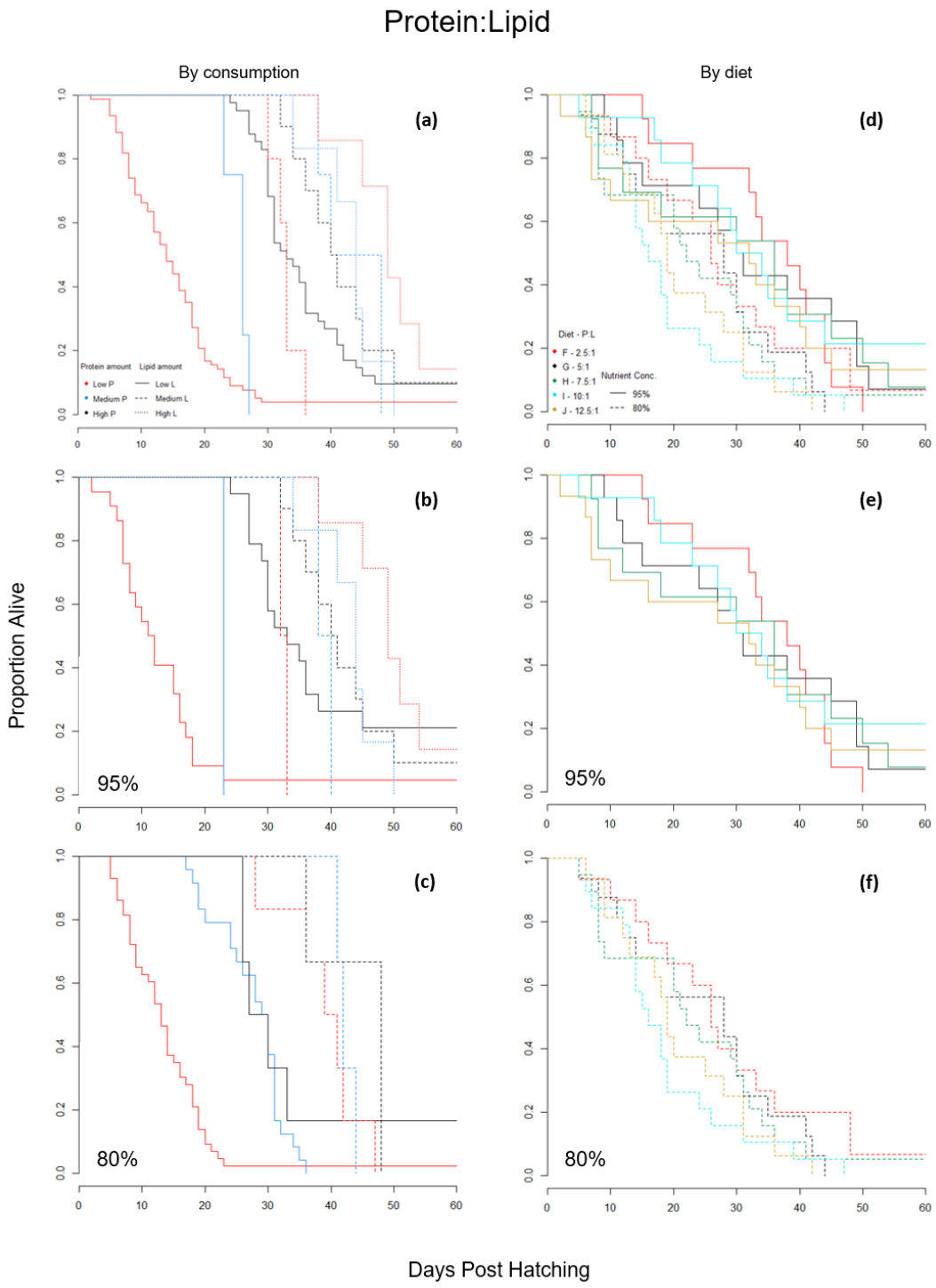


Fig. 5. a-c - Survival of P:L diet treatments broken down by amounts of macronutrients actually consumed. Lines represent the proportion of larvae alive at a given time point. Key: First letter; P = protein, L = Lipid. Second letter; H = high, M = medium, L = low consumption (High = top 33% of consumption, Medium = middle 33% of consumption, Low = bottom 33% of consumption) . d-f - Survival of larvae on P:L diet treatments according to the

diet they were fed. Letters represent 5 P:L ratios (F = 2.5:1, G = 5:1, H = 7.5:1, I = 10:1, J = 12.5:1). Lines represent the proportion of larvae still alive at a given time point. Solid lines indicate diets at 95% macronutrient concentration, dashed lines indicate diets at 80% macronutrient concentration.

3.5. Discussion

It is important when discussing these results to clarify that although initial larval mortality was low, the majority of larvae did not reach the pupal stage. Although this is not an uncommon occurrence with the *in vitro* rearing of bee larvae (Helm et al., 2017), it is worth keeping this in mind when interpreting our findings. This mortality likely came about due to a change in the formulation of diets compared to chapter 2. Unlike in chapter 2, diets in this chapter were set into agar as the previous method, though relatively successful, was highly labour intensive and so limited the number of larvae that could realistically be raised by a single experimenter. The switch to setting the diets in agar allowed for the mass formulation of diets prior to the provisioning of the offspring, markedly reducing the time taken to process larvae, meaning more larvae (and more diet rails) could be included in the experiment. This is a method that has been used successfully with larval insects previously, namely caterpillars (Lee et al., 2004a; Povey et al., 2009). However, setting the diets in agar also further reduces the nutrient density per gram. Caterpillars are used to dealing with low nutrient density food as their food source, leaves, contains little nutrients. Bee larvae however, have arguably evolved to consume far higher nutrient density foodstuffs, as their main food source, pollen, can contain up to 60% protein and 20% carbohydrate (Roulston

and Buchmann, 2000; Roulston and Cane, 2000a). As such, diluting the diets by setting them in agar may have deceased the nutrient density to such a degree that larvae were unable to make up for such dilutions with increased feeding rate, causing a nutrient deficit, resulting in larvae failing to reach pupation.

When dietary P:C ratio was manipulated, protein dictated larval weight gain in the first few days but then shifted to carbohydrate in the latter stages. Interestingly, larvae arguably demonstrated a rule of compromise whereby they over-consumed the non-limiting nutrient, irrespective of whether that was protein or carbohydrate. Although no fitness peaks were identified across larval development, carbohydrate was positively related to larval survival and to weight gain later in development, mirroring the findings in Chapter 2. Similar patterns were seen when varying the dietary P:L ratio, with larvae on high lipid diets surviving longer, and larvae once again appearing to overeat whichever nutrient was in excess. Patterns of weight gain differed from those seen when varying the dietary P:C ratio, showing a surface more closely resembling that of an energy-constrained system (Cotter et al., 2011), however, those larvae on the highest fat diets did achieve the highest maximal body weight (Fig. 7). Here, we argue that such patterns may be explained by bee larvae differentially utilising carbohydrate and lipids dependent on their levels in the diet. However, our results also reveal a complex interplay between the three macronutrients (protein, carbohydrate and lipid) that requires further investigation.

Larvae on the P:C treatments that consumed greater amounts of carbohydrate lived longer. The link between high carbohydrate (and low protein) and increased lifespan is well documented in adult insects (Archer et al., 2017; Dussutour and Simpson, 2012; Le Couteur et al., 2016; Lee et al., 2008), including bees, where adult bumblebees (Stabler et al., 2015) and both adult and larval honeybees (Helm et al., 2017; Paoli et al., 2014a) show

higher survival on high carbohydrate diets. This has previously shown to be broadly true for larval *O. bicornis* reaching the pupation stage (Chapter 2), whereby larvae focus on carbohydrate acquisition - which may be to ensure adequate fat body stores for diapause. Here however, few larvae reached this stage [a common issue with *in vitro* rearing of bee larvae (Helm et al., 2017)] and yet carbohydrate still dictated survival, suggesting that it also plays a crucial role across larval development. Whilst protein is seen as important for somatic growth and reproduction (Lee, 2007; Lee et al., 2008; Povey et al., 2009) carbohydrate is considered more important to somatic maintenance (Fanson et al., 2009; Fanson and Taylor, 2012; Le Couteur et al., 2016). Further studies could focus on whether the higher survivorship on high carbohydrate diets is negatively correlated with ovarian development in surviving adults. Similarly, larvae in the P:L treatments also saw increased survival when consuming high amounts of lipid. High lipid stores have been linked with longer lifespan (Hansen et al., 2013; Judd et al., 2011; Lee and Jang, 2014). This is contrary to patterns seen in adult bumblebees, however, where adults consuming high amounts of lipid died sooner (Vaudo et al., 2016). Adult bumblebee workers likely do not require high levels of lipid stores as they do not diapause or reproduce, both of which require the utilisation of lipids (Giejdasz and Wasielewski, 2017; Hansen et al., 2013), and therefore high levels of lipid may lead to obesity (Warbrick-Smith et al., 2006) or possible excretory-related costs (Lee et al., 2002). In diapausing insects, like *O. bicornis*, lipid is the primary source of stored energy (Raubenheimer et al., 2007), is primarily gained through pollen consumption (Cane et al., 2016), and is an essential component of vitellogenin, the central storage lipoprotein in bees (Wegener et al., 2018). Although often linked to ovary maturation (Amdam et al., 2003), vitellogenin also occurs in larvae, and across sexes, suggesting additional roles (Guidugli et al., 2005). What is more, vitellogenin is also important in adult *Osmia* (Cane, 2016; Dmochowska et al., 2013) and is linked to increased

lifespan in honeybees (Amdam, 2011). Therefore, it seems possible that higher fat allows for greater longevity is *O. bicornis* larvae through adequate production of vitellogenin. These results highlight the critical importance of species ecology - for example in this case the occurrence of diapause - in both interpreting and predicting nutritional requirements.

Interestingly, both high carbohydrate and high lipid resulted in increased lifespan. It is possible that carbohydrate and lipid are to some extent interchangeable, as carbohydrate can be converted into fat stores in insects (Arrese and Soulages, 2010; Warbrick-Smith et al., 2006), and lipid storage has been shown to increase with increased carbohydrate intake (Lee et al., 2002). However, directly ingested lipids can also be stored (Raubenheimer et al., 2007), therefore under high lipid conditions more carbohydrate may be available for somatic maintenance. Perhaps *O. bicornis* larvae have some degree of plasticity when it comes to the assimilation of carbohydrates and lipids, whereby each can be used for somatic maintenance. For example, it has been shown that *Drosophila* flies will utilise carbohydrate initially for energy demand and then switch to lipids when these are depleted (Lee and Jang, 2014). This may help to explain why larvae lived longer on either high lipid or high carbohydrate levels, both of which have been associated with longer life spans in insects previously (Judd et al., 2011; Lee et al., 2008; Stabler et al., 2015).

Larvae in the P:C treatment seemed to demonstrate a rule of compromise whereby they consumed food until the intake target for both protein and carbohydrate were met, irrespective of overeating either nutrient (Raubenheimer and Simpson, 1993; Simpson et al., 2004). This pattern suggests that larvae regulate both their level of protein and their level of carbohydrate over their development, possibly dependent on which is limiting at a given time. This is similar to that seen in adult honeybees where bees consistently overate essential amino acids in order to achieve their target for carbohydrate and vice versa (Paoli et al., 2014a). Unlike honeybees, *O. bicornis* larvae are mass provisioned, yet temporal

changes in nutrient limitation may still occur through changes in *demand* for each nutrient, even if the supply is a fixed pollen mass. Previously, in Chapter 2, little evidence for protein regulation in *O. bicornis* larvae was found, with larvae tolerating varying amounts of protein whilst maintaining a steady level of carbohydrate intake. However, this previous study only looked at the larvae once they had pupated; not over the entire period of development, and as (1) unlike in Chapter 2, most larvae did not reach pupation, and (2) nutritional targets shift through time i.e. are specific to certain life history stages (Simpson and Raubenheimer, 1993), it is possible that the relative importance of each nutrient shifts through time, with carbohydrate only becoming dominant at the pupation stage. Protein is essential for somatic growth (Lee, 2007; Povey et al., 2009; Roulston and Cane, 2002; Tasei and Aupinel, 2008) and may be key to building up essential tissues during the early stages. As development continues however, carbohydrate demand may increase for overwintering purposes (Giejdasz and Wasielewski, 2017); therefore carbohydrate becomes the priority (Fig 2). This shift towards carbohydrate may explain the apparent lack of protein regulation seen when assessed at the pupation stage in Chapter 2, and highlights the importance of investigating the nutritional requirements of bees not just at the two major stages, larval and adult, but also at finer scales within these two broad categories.

As with P:C, those larvae raised on the P:L treatments overate the more limiting nutrient in order to regulate the nutrient that was less limiting. Similar findings were seen in adult bumblebees, where adults overate lipid on high fat diets to achieve a target for protein and vice versa, even to the point of toxicity (Vaudo et al., 2016). These findings, along with our own, are more similar to predaceous arthropods than to herbivorous ones (Vaudo et al., 2016), bees typically being considered herbivores (Larkin et al., 2008). Herbivores and omnivores tend to prioritise protein acquisition (Simpson and Raubenheimer, 2005; VanOverbeke et al., 2017). However, as bee larvae feed primarily on pollen, which for

plant tissue is relatively proteinaceous [as much as 60% (Roulston and Cane, 2000a)], it has been suggested that a larval bee's diet composition may be more similar to that of a predatory insect (Chapter 2). Predatory insects also tend to prioritise lipid intake (Al Shareefi and Cotter, 2018; Jensen et al., 2012, 2011; Raubenheimer et al., 2007) and although lipid was not necessarily prioritised on all diets, on higher P:L ratio diets larvae did appear to overeat protein, suggesting tighter regulation of lipid than protein on these diets. Bee larvae are not predatory, but as pollen is protein rich (Roulston and Cane, 2000a) and as bees get all their lipids from pollen (Vaudo et al., 2016), lipids may be the more limiting nutrient in many pollen types (Roulston and Cane, 2000a).

However, larvae also seemed to overeat lipid on low P:L diets. Insects have been shown to be at risk of obesity on low P:L diets when overeating lipid to reach a protein target (Al Shareefi and Cotter, 2018). Indeed, larvae raised on low P:L diets in this study achieved the highest maximal weight during development, possibly suggesting obesity in bee larvae fed high lipid diets (Fig. 7). Obesity can lead to deleterious effects in larval insects (Raubenheimer and Lee, 2005; Raubenheimer and Simpson, 1999) and, as the effects of larval diet are often only realised at the adult stage (Runagall-McNaull et al., 2015), further studies could assess the effects of high lipid larval diets on the lifespan of the subsequent adults. Broadly speaking, weight gain on P:L diets seemed to be driven by overall energy acquisition, showing a surface comparable to that of energy-constraint (Cotter et al., 2011). Both protein and lipids are important for development (Feingold and Grunfeld, 2018; Guidugli et al., 2005; Lee, 2007; Povey et al., 2009) and both lipids and protein can be used to create fat body stores, directly from dietary lipids or indirectly from dietary protein via deamination and gluconeogenesis of amino acids (Lee et al., 2003, 2002; Merkx-Jacques et al., 2008), which could explain the relationship with weight gain. Nevertheless, larvae on the P:L treatments did show a broadly similar rule of compromise

to those larvae raised on differing P:C diets, perhaps unsurprisingly given the partial overlap in carbohydrate and lipid utility (Arrese and Soulages, 2010; Lee et al., 2002; Raubenheimer et al., 2007; Warbrick-Smith et al., 2006).

Why, then, do the P:L treatments not show the same temporal shift in the predominant nutrient driving weight gain that we saw in the P:C treatments? Both carbohydrate and lipid tend to be the primary macronutrients for laying down fat in insects (Al Shareefi and Cotter, 2018; Arrese and Soulages, 2010) and so could be expected to have a similar influence on weight gain. Levels of fat stored in insects tend to remain relatively stable, whereas carbohydrates can fluctuate across development (Arrese and Soulages, 2010). Alternatively, perhaps bee larvae assimilate, or prioritise the use of, carbohydrate differently at different developmental stages; adult *O. bicornis* do utilise macronutrients differently throughout diapause for example (Wasielewski et al., 2013). Perhaps in the case of larvae, carbohydrate is used for maintenance initially with a switch to additional fat storage in the latter stages. This may help to explain the lack of change seen in their rule of compromise despite the shift in weight gain for those larvae on P:C treatments.

Despite the shift in the nutrient driving weight gain, we nevertheless saw little change in macronutrient consumption over time. It is possible that excess nutrients are dealt with post-ingestively (Rho and Lee, 2017; Simpson and Raubenheimer, 2012b; Telang et al., 2001; Warbrick-Smith et al., 2006), whereby excess carbohydrates are excreted early on, when not required, and then retained during the latter stages for pre-winter storage. It is also worth noting that carbohydrate was added to all P:L diets at a fixed P:C ratio (1:2) and at a level deemed to be non-limiting, which may mask some effects of low lipid as carbohydrate may have been utilised under low fat scenarios with excess fat on low P:L diets regulated post-ingestively. Further work could focus on assessing the ability of bee

larvae to post-ingestively process excess macronutrients as has been shown for multiple nutrients in other larval insects (Lee et al., 2004a; Telang et al., 2001; Warbrick-Smith et al., 2006). Additionally, investigating all dietary macronutrients simultaneously, for example, via the right-angle mixture triangle (Judd et al., 2017; Raubenheimer, 2011) may allow for the intricate relationship between these three macronutrients to be unraveled in bee larvae.

Lipid has received limited attention in relation to bee nutrition, and our knowledge is restricted to adult studies (Vaudo et al., 2016). Typically, protein is seen as an indicator of pollen quality (Beekman et al., 2015; Carr et al., 2015; Leonhardt and Blüthgen, 2011; Ruedenauer et al., 2015). Our study suggests that lipid likely also plays a key role in bee development. Pollen varies greatly in nutrient content and ratios across species (Roulston and Cane, 2000a) and landscapes (Donkersley et al., 2014). Thus, when designing floral improvement schemes for farmland, such as seed mixes and wildflower strips, consideration must be given to quality of the resources provided in terms of *all* the nutrients constituents, not simply protein content, and to the fact that bees' nutritional requirements can change over developmental time.

Our study not only stresses (1) the need to study the nutrition of larval bees across development in order to understand their changing needs, and (2) the necessity for more research into the interplay between major macronutrients in bee nutrition, but also (3) the need to understand whether adult bees are able to navigate these fragmented nutritional environments in order to ensure that their larvae receive the quality of pollen they require for their complex nutritional needs.

3.6. Supplementary Materials

Fig. S1. A single occupancy nest containing the scoop, cotton wool plug and polycarbonate lid

Table S1. Macronutrient ratios for P:C and P:L diets

P:C Ratio		P:L Ratio	
Diet: PC		**Diet: PL**	
A	1:2	F	2.5:1
B	1:3	G	5:1
C	1:4	H	7.5:1
D	1:5	I	10:1
E	1:6	J	12.5:1

Table S2. Table of coefficients covering the outputs from the minimal model for protein consumption in the P:C treatments. Non-significant model predictors are not shown.

Term		estimate	se
Fixed effects			
Intercept (Diet Ratio A (1:2), Concentration 95%)		$2.208x10^{-02}$	$4.463x10^{-04}$
Swap Number		$-1.086x10^{-04}$	$4.125x10^{-05}$
Diet ratio (P:C)			
	B (1:3)	$-8.49x10^{-03}$	$6.196x10^{-04}$
	C (1:4)	$-1.096x10^{-02}$	$6.203x10^{-04}$
	D (1:5)	$-1.126x10^{-02}$	$6.104x10^{-04}$
	E (1:6)	$-1.38x10^{-02}$	$6.1x10^{-04}$
Concentration (%)			
	80%	$-5.973x10^{-04}$	$6.376x10^{-04}$
Diet ratio × concentration			
	B:80%	$5.667x10^{-04}$	$9.064x10^{-04}$
	C:80%	$3.983x10^{-03}$	$9.031x10^{-04}$
	D:80%	$3.322x10^{-03}$	$8.854x10^{-04}$
	E:80%	$4.579x10^{-03}$	$9.031x10^{-04}$
Random effects		**Variance**	**sd**
Sample ID		$1.682e^{-06}$	$1.297e^{-03}$
Residual		$4.879e^{-06}$	$2.209e^{-03}$

Table S3. Table of coefficients covering the outputs from the minimal model for lipid consumption in the P:L treatments. Non-significant model predictors are not shown.

Term		estimate	se
Fixed effects			
Intercept (Diet Ratio F (2.5:1), Concentration 95%)		$8.323x10^{-03}$	$1.397x10^{-04}$
Swap Number		$-3.644x10^{-05}$	$9.12x10^{-06}$
Diet ratio (P:L)			
	G (5:1)	$-3.579x10^{-03}$	$1.906x10^{-04}$
	H (7.5:1)	$-5.564x10^{-03}$	$1.981x10^{-04}$
	I (10:1)	$-5.791x10^{-03}$	$1.914x10^{-04}$
	J (12.5:1)	$-6.217x10^{-03}$	$1.993x10^{-04}$
Concentration (%)			
	80%	$-2.392x10^{-03}$	$1.894x10^{-04}$
Diet ratio × concentration			
	G:80%	$6.82x10^{-04}$	$2.688x10^{-04}$
	H:80%	$1.634x10^{-03}$	$2.731x10^{-04}$
	I:80%	$1.535x10^{-03}$	$2.732x10^{-04}$
	J:80%	$1.765x10^{-03}$	$2.767x10^{-04}$
Random effects		**Variance**	**sd**
Sample ID		$1.63x10^{-07}$	$4.037x10^{-04}$
Residual		$4.476x10^{-07}$	$6.690x10^{-04}$

4. Plugging the hunger gap: Organic farming supports more abundant nutritional resources for bees at critical periods

4.1. Abstract

Understanding the decline in bee populations and their plant mutualists is of paramount concern for ecosystem health, as well as our future food security. Intensive farming practices are one of the major drivers behind such declines. Organic farming is one of the principal alternatives to conventional practices yet the evidence for its effects are mixed, with some studies showing limited benefits. We conducted bee and floral surveys on 10 paired organic and conventional farms across Yorkshire, UK, to investigate how farming practice influenced the abundance, richness and community composition of bees and plants. Firstly, we found that species richness for plants and bees was similar across organic and conventional farms. Plant species composition differed between organic and conventional farms, but these differences were mainly driven by the months of May and June, whereas both farm practices supported similar bee communities. Secondly, both bee and plant abundance were higher in organic farms. Spikes in plant abundance, and corresponding bee abundance, again showed a strong temporal effect, most notably in July, with abundance during the rest of the season being similar across both farming practices. Our results suggest that higher floral availability on organic farms corresponds with increased bee abundance. Of particular importance was the higher floral abundance during spring, in the pollinator '*hungry gap*', where floral resources are traditionally scarce. However, conventional farms performed comparably across the rest of the season, as well

as showing similar levels of species richness, diversity and species composition for both plants and bees. We suggest that targeted management on conventional farms, aimed at boosting floral abundance in the spring, when plant abundance is low, could allow conventional farms to make up the shortfall. Additionally, focusing on increasing the diversity of plants, in terms of both phenology and nutritional quality, for both adult bees and their larvae, could improve bee community diversity across both farming systems.

Keywords: Conventional farming, wildlife-friendly farming, pollinators, wildflowers, bee conservation, community composition, phenology, nutritional quality, wild bees, targeted management

4.2. Introduction

Insects are in the midst of a global crisis, with unprecedented reductions being seen across insect taxa (Sánchez-Bayo and Wyckhuys, 2019). Some of the strongest evidence for declines occurs within pollinating insect guilds (Potts et al., 2010a; Rhodes, 2018), and more specifically, from bees, a key pollinator group (Durant, 2018; Goulson et al., 2015, 2008; Goulson and Nicholls, 2016; Klein et al., 2017; Kosior et al., 2007; Papanikolaou et al., 2017). Similar patterns are also seen in plants (Niedrist et al., 2008), with losses in agricultural habitat particularly prominent (Benton et al., 2003; Sotherton and Self, 2000). In bees, as with plants, there are multiple drivers involved in their declines (Goulson et al., 2015); however, intensive agriculture is widely regarded as one of the largest contributors (Fuller et al., 2005; Goulson and Nicholls, 2016). This loss of bee species, along with other pollinators, is of particular concern as they are essential for the pollination of not only many wild plants (Ollerton et al., 2011) but also the majority of commercially important crop species (Aizen et al., 2009; Goulson and Nicholls, 2016; Klein et al., 2003).

Ecologically-friendly farming methods have the potential to mitigate the effects of intensive agriculture upon pollinator declines and, as such, have received much attention (Kovács-Hostyánszki et al., 2017; Meena et al., 2017; Pywell et al., 2015). Organic farms tend to (1) use little to no insecticide (Gabriel et al., 2013; Rigby and Cáceres, 2001), which have well documented negative effects on bees (Cham et al., 2018; Fisher and Rangel, 2018; Raimets et al., 2017; Raine, 2018), (2) have reduced fertiliser inputs (Pimentel et al., 2005), which is important for many wildflower species which rely on relatively nutrient-poor soils (Nowakowski and Pywell, 2016), and have (3) increased habitat heterogeneity (Fuller et al., 2005; Krebs et al., 1999) which has been shown to support greater levels of bee and plant diversity (Basu et al., 2016; Rader et al., 2014). This less intensive practice

means that organic farms should have a positive effect on plant and bee communities and, indeed, many studies have demonstrated benefits (Bengtsson et al., 2005; Hole et al., 2005; Tuck et al., 2014), with evidence for higher species richness and abundance on organic farms. For example, Gabriel and Tscharntke (2007) showed that arable weed richness was consistently higher in organic fields compared to conventional fields, and Kennedy et al. (2013) found that bee richness was higher on organic farms amidst high-quality landscapes. Rundlöf et al. (2008) showed that bumblebees were more abundant on organic farms when compared to conventional farms, and in a meta-analysis of the effects of farming practice on biodiversity Bengtsson et al. (2005) found a positive relationship between organic farming and plant abundance. Yet these studies do not show how these patterns shift across a season, and as we know that both plant and bee phenology are mutually dependent (CaraDonna et al., 2018; Lawson et al., 2018; Morente-López et al., 2018) it is important to address this gap in knowledge in order to gain a holistic view of plant and bee communities on farmland. For example, spring is often referred to as the pollinator *'hungry gap'* on farmland (Nowakowski and Pywell, 2016), where floral resources can be scarce at a critical time for emerging bees. Yet, any differences between these early months and later summer months may be lost when analysed together.

However, positive effects of organic farming are not universal, with results often mixed (Brittain et al., 2010; Holzschuh et al., 2008; Rundlöf et al., 2009; Weibull et al., 2003). These conflicting findings suggest that other factors may confound effects of farming practice (Roschewitz et al., 2005). Differences can be seen at the farm scale (Weibull et al., 2003), demonstrating the importance of individual farmers in any effects seen within their particular farming system. Farms can also vary greatly within their designation in terms of management practices, intensity, and adoption of stewardship schemes, which themselves can have levels of intensity (Wood et al., 2016). These

discrepancies between studies highlight the need for further research into the area and to conduct studies across a broad array of farming landscapes.

In this study, we investigated how organic and conventional farming practices influenced the plant and bee communities in the East Riding of Yorkshire, UK, across the season from spring through to late summer. By conducting floral surveys and capturing bee specimens across the farms we aimed to investigate whether the abundances, richness and community composition of bees and plants differed based upon farming practice. Few studies have specifically looked at how the benefits (or lack thereof) of different farming practices change within the bee flight season (Holzschuh et al., 2008; Kehinde et al., 2017; Power et al., 2012), despite the importance of phenology in structuring pollinator communities (Encinas-Viso et al., 2012; Morente-López et al., 2018). This temporal aspect is of particular importance for bees, as it is known that farmland floral resources often suffer during particular months of the year (Nowakowski and Pywell, 2016). Therefore, investigating the effects of farming practice with a phenological mindset is important for understanding effects of farming at finer temporal scales, and for allowing targeted management interventions that maximise plant-pollinator community benefit. As the effects of organic farming can also be influenced by other factors such the size of the farm (Fuller et al., 2005) the surrounding habitat (Roschewitz et al., 2005), and crop type (Tuck et al., 2014) we paired conventional and organic farms together to reduce confounding effects as far as possible.

Given that in many cases species richness does not differ between farm types (Power and Stout, 2011) and that organic farming in isolation i.e. when organic farms are situated in an otherwise intensively farmed landscape, has been shown to have little benefit for bee and plant communities (Brittain et al., 2010), we first predicted that richness and community composition would not differ between farming types. Second, as organic farms

use more of the crop area for additional floral resources due to restricted herbicide and fertiliser use (Pimentel et al., 2005), and weed species are more common (Gabriel and Tscharntke, 2007; Romero et al., 2008), we predicted that abundances of plants and consequently bees would be higher on organic sites when compared to conventional sites. Third, we predicted that organic farms would show higher floral abundance peaks (Morente-López et al., 2018), and correspondingly higher peaks in bee abundances, across months, specifically in spring, an often challenging time for bees in terms of nutrient availability. This study will provide valuable information to both farmer and local policy makers as to the current status on plant and bee communities on differing farm types and give insights into where improvements can be made to bolster or improve such communities where necessary.

4.3. Methods

Study Sites

Ten matched pairs of conventional and organic farms were recruited in the East Riding of Yorkshire and North Yorkshire, UK. Initial contact was made with farmers via letter, email or telephone and, upon response, face-to-face meetings were arranged to allow both site visits and the exchange of information. Organic farming is relatively uncommon in the East Riding of Yorkshire in comparison to other parts of the UK therefore organic farms were recruited first and then conventional farms local to the organic farms were contacted afterwards. Farms were paired together based on soil type and local

environmental conditions (see Fig. S1 and Table S1 for farm pair locations and details) and were paired as closely as possible, in terms of distance. All survey locations within a pair were at least 4 km apart, minimising the chance of any bees caught being able to have foraged at both locations (Knight et al., 2005; Zurbuchen et al., 2010). Eight of the ten farm pairs were arable or arable/pastoral mixed farms and two pairs were dairy/beef farms. Sample sites on arable/pastoral mixed farms were all located in the arable region of the farm.

Organism Surveys & Sample Identification

A single field was chosen at each farm within a pair to be the sampling site. Where possible, fields within a pair were matched by crop or, when this was not possible, by crop type i.e. winter wheat with winter wheat or cereal with cereal. Cereals were the primary crop at all farm sites (barring dairy/beef). Each field within a pair was sampled five times from May to September 2016, between 09:00 and 16:00 on dry, sunny days with only moderate wind speeds (Forup and Memmott, 2005). The order in which each farm within a pair was visited was alternated every sampling session to ensure that, a) farms within pairs were sampled at the same time of day, and b) each farm was sampled during both the morning and afternoon (Power and Stout, 2011). An 80 x 1 m transect was walked at a steady pace during each sampling session at each site. The transect consisted of crop, margin and hedgerow habitat types and was ‘E’ shaped with the spine running along the hedgerow/margin and the arms stretching out into the crop. Both spine and arms were 20 x 1 m each. This shape was chosen to reflect the relative land-use of the farm as margins and

hedgerows make up comparatively less of the area of a farm than does the crop (Evans et al., 2013). By adopting this shape and walking the transect at a steady pace, more time was spent sampling within the crop compared to the hedgerows and margins. The initial location of the transect was chosen using a random number generator where the northern edge of the field was assigned 1, the eastern 2 etc. Subsequent visits then followed a clockwise fashion, to ensure that each start point was random whilst also ensuring each margin was sampled at least once.

Flowering plant surveys were conducted at the beginning of each site visit. Any flowering plant species (excluding grasses) along the transect were identified (using Rose and O'Reilly, 2006) and the number of floral units taken to provide a measure of abundance. A single floral unit was defined as any inflorescence or group of inflorescences that could be navigated without a medium sized bee needing to fly between them (Dicks et al., 2002; Power and Stout, 2011). Flowering plant species that were not in flower at the time of sampling were not recorded during that visit.

To sample the bee community, the transect was walked for an hour (total sampling effort) using a stopwatch, catching all bees possible in a sweep net. Upon capture, the stopwatch was paused for sample processing to ensure the full hour was spent walking the transect. All captured bees were stored singly in 50 ml falcon tubes, the time of capture recorded (along with any plant they were interacting with at capture), and then placed in a cool bag containing ice packs. Once the transect was complete, all captured samples were placed into a freezer (-20°C) on-site to euthanise and store them. Thawed bees were identified to species level using Falk (2015), and then sexed, under a light microscope.

Statistical Analysis

All analyses were performed with R statistical software v3.5 (R Core Team, 2018) and bee and plant data were analysed in the same way. Community indices (species richness, Simpson's index, and Shannon's index) were calculated from abundance data per site per sampling visit using the package iNEXT (Hsieh et al. 2019). The extrapolated values for all indices were used as species accumulation curves fell short of an asymptote.

Generalised Linear Mixed effect Models (GLMMs) were used to investigate whether farm type influenced bee and plant abundance as well as community indices. Farming type and visit month, along with their interaction, were used as predictors, and farm pair as a random factor in the maximal model. Minimal models were determined by reverse stepwise model selection. GLMMs were also used to investigate whether bee abundance was influenced by the plant abundance on a farm, with bee abundance as the response variable and plant abundance, farm type and visit month, as well as all interactions, as predictor variables.

Community structure was analysed within the package vegan (Oksanen et al. 2018) in R. Community similarity of both bees and plants was investigated using PERMANOVA (ADONIS) of relative species abundances with farm type and visit month, plus their interaction, as predictors. Permutations were restricted to within-farm-pair comparisons only. Nonmetric multidimensional scaling (NMDS) ordination on Bray-Curtis distance matrices was used to visualise differences.

4.4. Results

A total of 1332 bees (780 from organic and 552 from conventional) from 39 species were caught across all farm sites (Table 1), with 4 species unique to conventional sites and 6 to organic sites (Table S2). 67,401 floral units were recorded from 105 flowering plant taxa across farm types (Table 2), with 31 taxa unique to conventional sites and 21 unique to organic sites (Table S3).

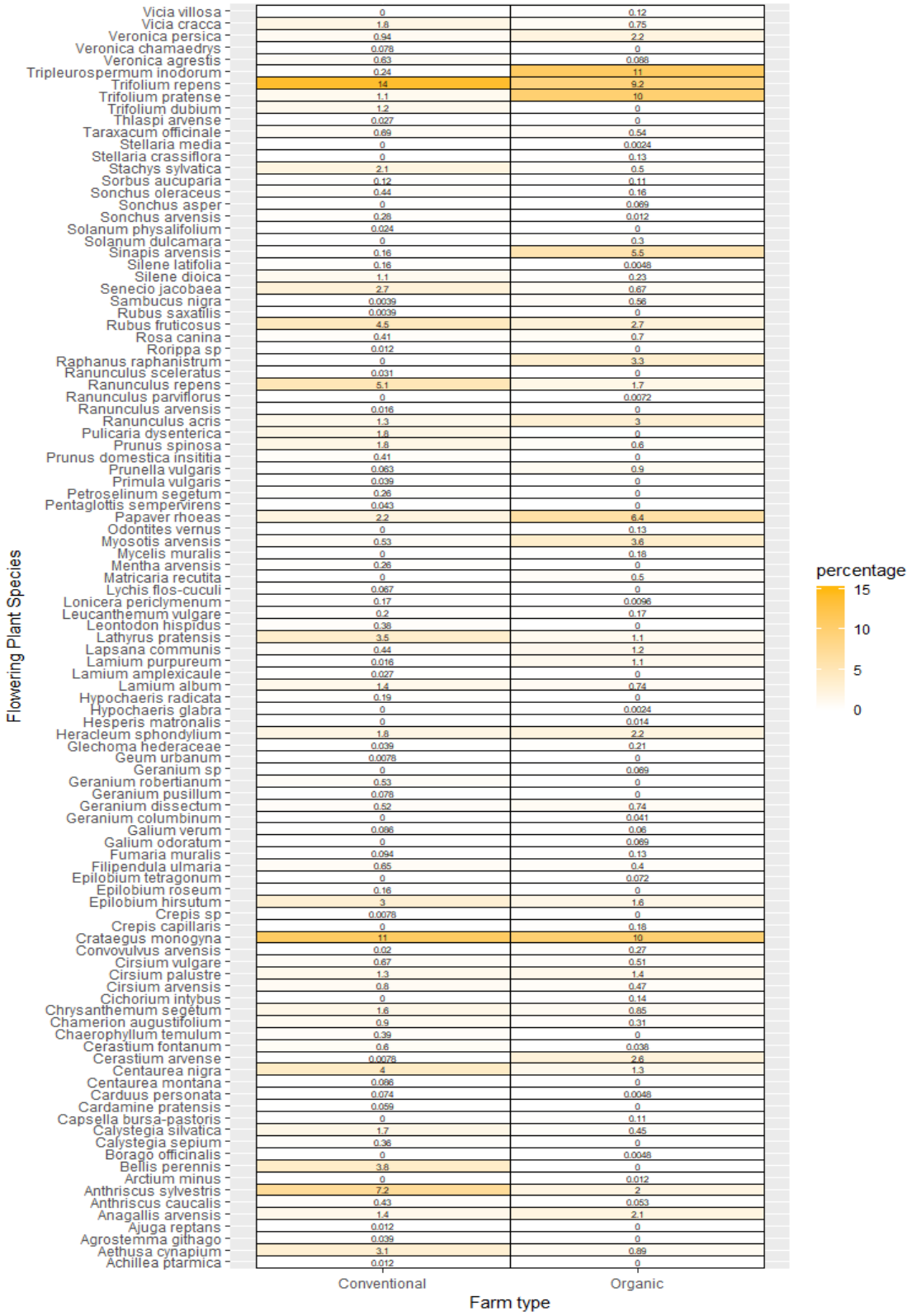
Table 1. The proportion of each bee species caught on organic and conventional farms as a percentage of the total number of bees caught per farming type.

Bee Species	Conventional	Organic
Sphecodes gibbus	0.18	0
Nomada ruficornis	0.18	0
Nomada panzeri	0.18	0.26
Nomada leucophthalma	0	0.38
Nomada flavoguttata	0.36	0.13
Nomada flava	0.72	0.26
Melecta albifrons	0	0.13
Megachile willughbiella	0	0.13
Lasioglossum villosullum	0.18	0.13
Lasioglossum sp.	0.18	0
Lasioglossum morio	0.36	0
Lasioglossum calceatum	2.2	2.3
Lasioglossum albipes	1.6	1.5
Hylaeus communis	0	0.13
Halictus rubicundus	0.36	0.13
Coelioxys rufescens	0	0.13
Bombus vestalis	2.4	2.6
Bombus terrestris	13	9.9
Bombus rupestris	1.4	2.3
Bombus pratorum	1.6	1.8
Bombus pascuorum	23	26
Bombus lucorum	1.1	0.51
Bombus lapidarius	28	30
Bombus jonellus	0.18	0.13
Bombus hypnorum	1.3	0.64
Bombus hortorum	5.6	6.8
Bombus campestris	1.3	0.51
Bombus barbutellus	2.4	0.77
Apis mellifera	3.4	1.9
Andrena subopaca	1.4	1
Andrena scotica	0.54	1.3
Andrena nigroaenea	0.36	0.13
Andrena minutula	0	0.13
Andrena helvola	0.9	0.38
Andrena haemorrhoa	3.6	6.3
Andrena fulva	0.18	0.26
Andrena fucata	0.18	0.64
Andrena chrysosceles	1.3	0.26
Andrena barbilabris	0.18	0

Farm type

percentage: 30, 20, 10, 0

Table 2. The proportion of each plant taxa recorded on organic and conventional farms as a percentage of the total number of plants recorded per farming type.



Community diversity indices

The number of bee species caught overall did not differ between farm types (GLMM, dropping effect of farm type. $\chi^2_{1,7} = 0.85$, $p = 0.36$). Shifts were seen, however, between months (GLMM , $\chi^2_{4,6} = 12.24$, $p = 0.016$), with notable peaks in richness being seen in the months of July and August (Fig. 1a). The same trends were seen in the number of plant species, with farm type having no effect on plant species richness (GLMM, $\chi^2_{1,7} = 2.01$, $p = 0.16$) and only the visit month showing a significant effect ($\chi^2_{4,6} = 24.50$, $p<0.001$), with higher richness being seen in the summer months (Fig. 1b).

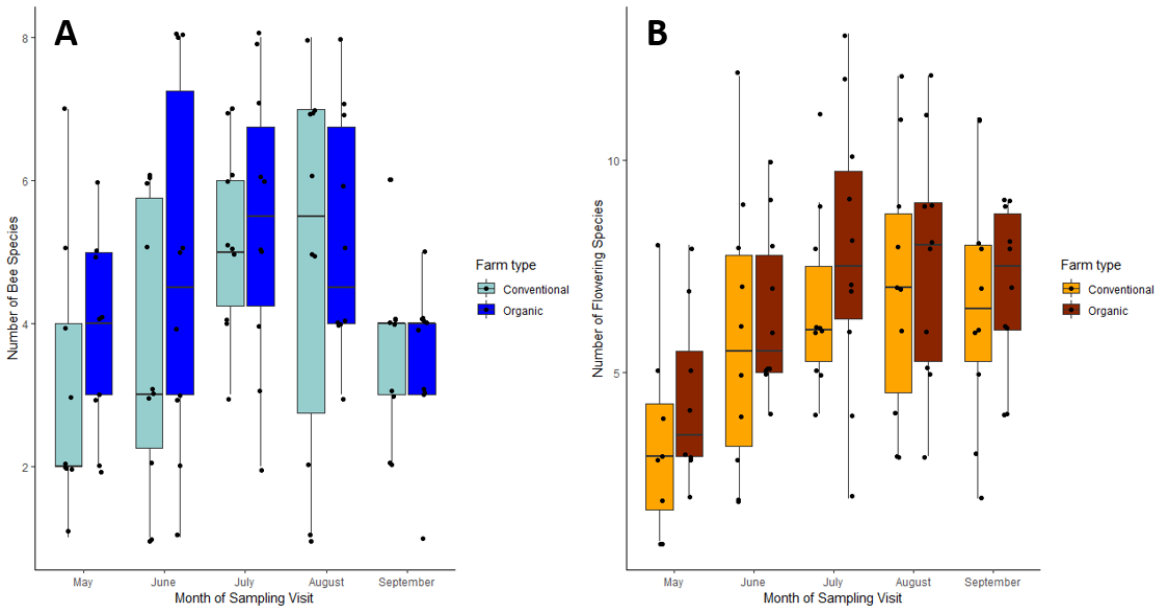


Fig. 1. Species richness of bees (A) and plants (B) recorded on organic and conventional farms plotted by sampling month

As the same patterns were seen for both bees and plants for Shannon's Index and Simpson's Index, the results for Shannon's Index only are visualised here. In accordance with the results for richness, Shannon's Index was not influenced by farm type for either bees (GLMM, $\chi^2_{1,8} = 0.88$, $p = 0.35$) or plants ($\chi^2_{1,8} = 0.075$, $p = 0.78$), but was significantly influenced by visit month (Bees, $\chi^2_{1,8} = 17.55$, $p = 0.0015$, Fig. 2a; Plants, $\chi^2_{4,7} = 17.36$, $p = 0.002$, Fig. 2b). For bees, lower index values were seen in September ($t_{88} = 3.94$, $p<0.001$) but with plants, May was the lowest diversity month ($t_{89} = 9.87$, $p<0.001$), with higher values seen across all other months.

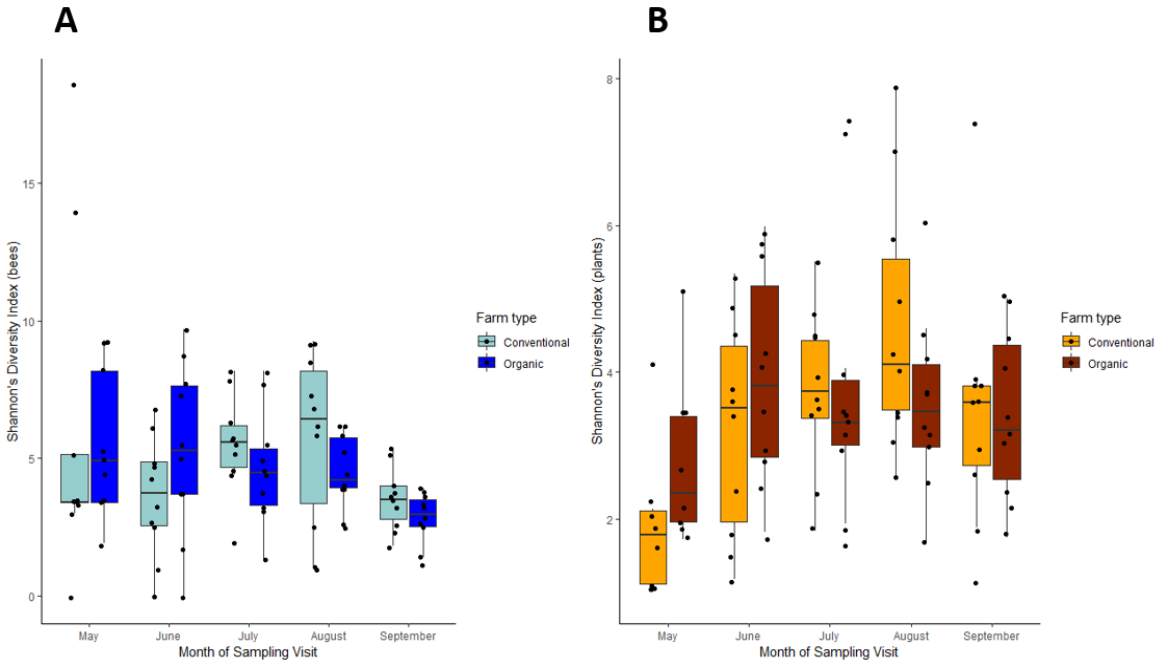


Fig. 2. Shannon's Diversity Index for bees (A) and plants (B) on organic and conventional farms plotted by sample month

The same results were seen for Simpson's Index for both bees and plants, whereby farm type had no effect (Bees, $\chi^2_{4,8} = 2.68$, $p = 0.1$; Plants, $\chi^2_{1,8} = 0.06$, $p = 0.81$), with all variation being driven the by visit month (Bees, $\chi^2_{4,8} = 14.21$, $p = 0.0067$; Plants, $\chi^2_{4,8} = 12.77$, $p = 0.012$). Again, as with Shannon's Index, Simpson's Index was significantly lower in September for bees ($t_{91} = 2.86$, $p = 0.0042$) and significantly lower for plants in May ($t_{89} = 9.87$, $p<0.001$).

Community composition

The bee community composition at farm sites was not influenced by farm type (ADONIS: $R^2 = 0.002$, $p = 0.986$) but was significantly affected by visit month (ADONIS: $R^2 = 0.176$, $p<0.001$), clustering together by the visit month when visualised with NMDS ordination (Fig. 3a,b).

The plant community, however, was influenced by both farming type (ADONIS: $R^2 = 0.018$, $p = 0.0042$) and visit month (ADONIS: $R^2 = 0.13$, $p<0.001$). This influence of farming type remained even when one outlying conventional community sample in May was removed (ADONIS: $R^2 = 0.019$, $p = 0.0044$, Fig. S2a). Under NMDS, plant community samples clustered together by visit month, as with the bee communities (Fig. 3c,d). Note that one visit to a conventional site, was removed from ordination analysis (due to only a single specimen being recorded for both bee and plants) along with its organic partner. NMDS visualisation with this point included can be seen in Fig. S2. The same conventional site was removed from both bee and plant data and the removal did not influence the outcome of ADONIS analyses.

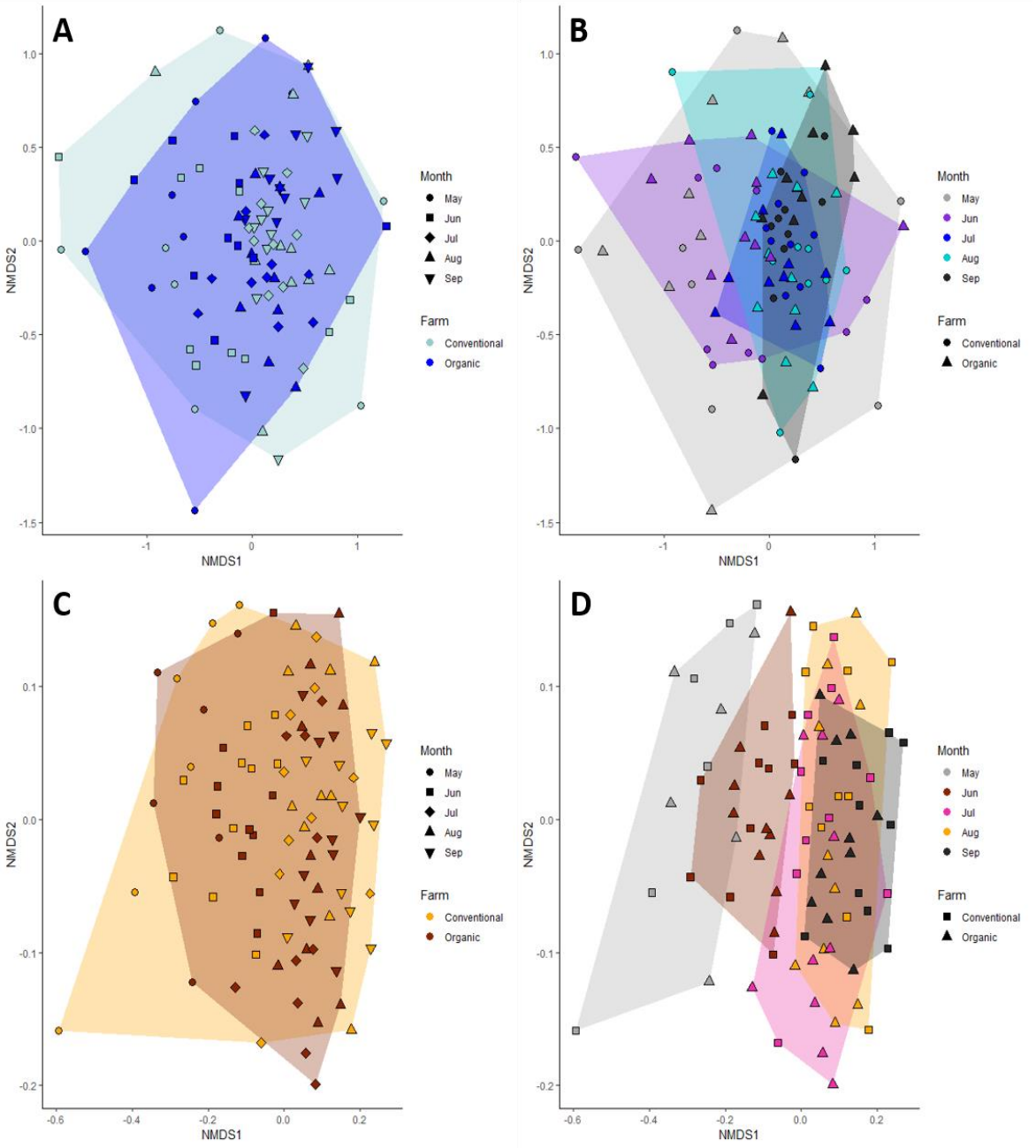


Fig. 3. NMDS plot for counts of bee species at each site during each sampling month (raw data converted to percentages), shaded by farm type (A) and sampling month (B). Counts of plant species at each site during each sampling month shaded by farm type (C) and sampling month (D). Note: visit one from one conventional farm was removed as an outlier, its paired organic site was also removed.

Bee & floral abundance

The abundance of bees was influenced by both farming type and visit month with a significant interaction between the two predictors (Generalised Linear Mixed effects Model - GLMM - containing farm type and visit month as predictors with farm pair as a random factor, dropping interaction. $\chi^2_{4,11} = 87.727$, $p<0.001$). Overall, more bees were caught on organic farms than on conventional farms (Fig. 4a) with this difference primarily being driven by the months of July and August (Fig. 4b).

There was a significant interaction between farm type and visit month when analysing the floral abundance (GLMM, dropping the interaction. $\chi^2_{7,11} = 2312.8$, $p<0.001$). Overall, there was a higher number of floral units (greater floral abundance) recorded on organic farms (Fig. 4c) and, as with bee abundance, floral abundance was higher on organic farms in the summer months of July and August, as well as in May (Fig. 4d).

There was a significant 3-way interaction (all predictors) when modelling bee abundance against plant abundance (GLMM, $\chi^2_{4,21} = 10.71$, $p = 0.03$), with bee abundance being positively associated with floral abundance to different degrees based on month and farm type (Fig. 5a-e,f). However, it seems clear that the month of May is driving this interaction (Fig. 5a), with bee abundance being negatively associated with floral abundance on organic sites but not on conventional sites.

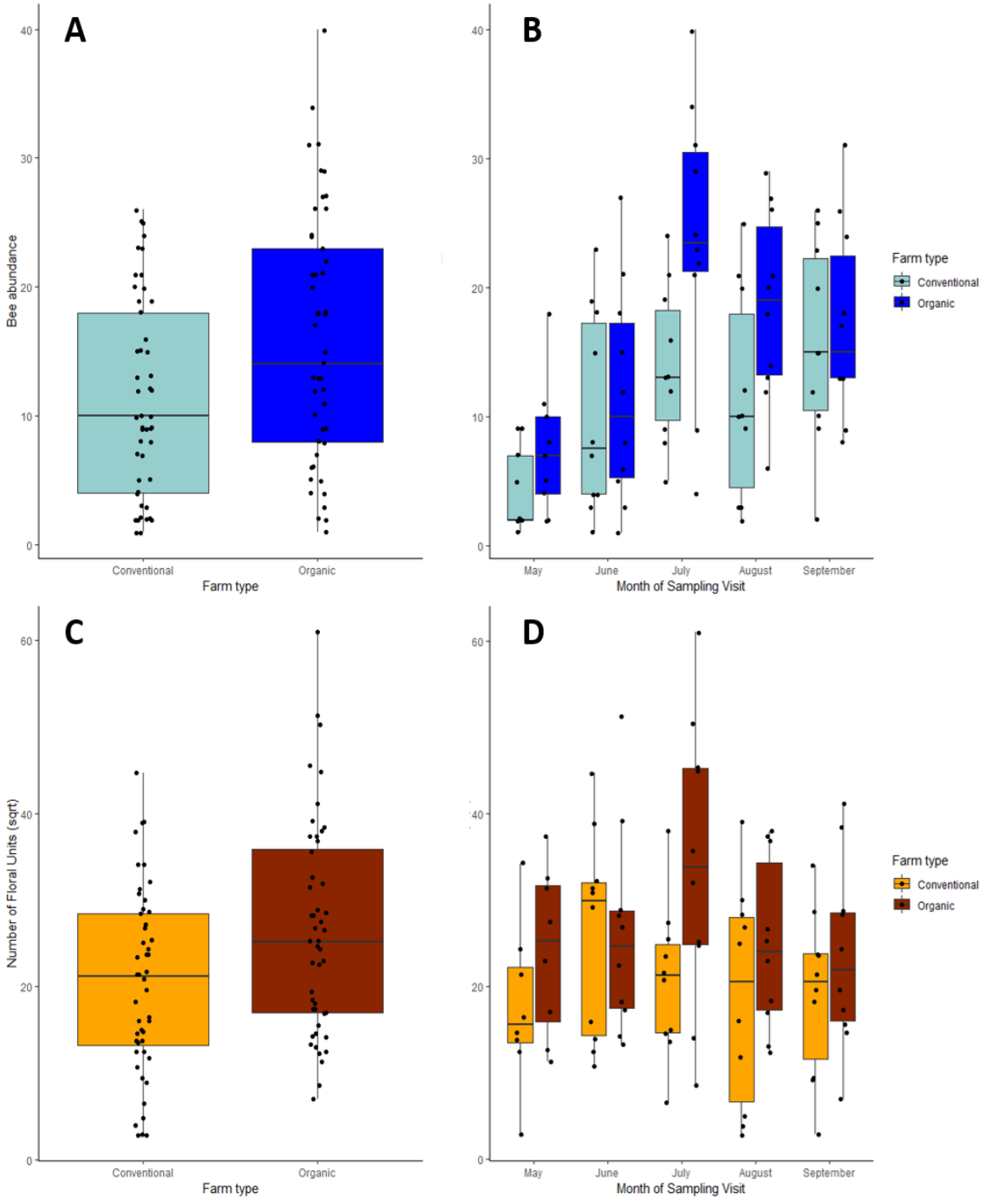


Fig. 4. Abundance of bees and plants recorded on farm sites. Number of bees recorded on organic and conventional farms in totality (A), and within each visit month separately (B). Number of floral units (square root transformed) recorded on organic and conventional farms in totality (C), and within each visit month separately (D).

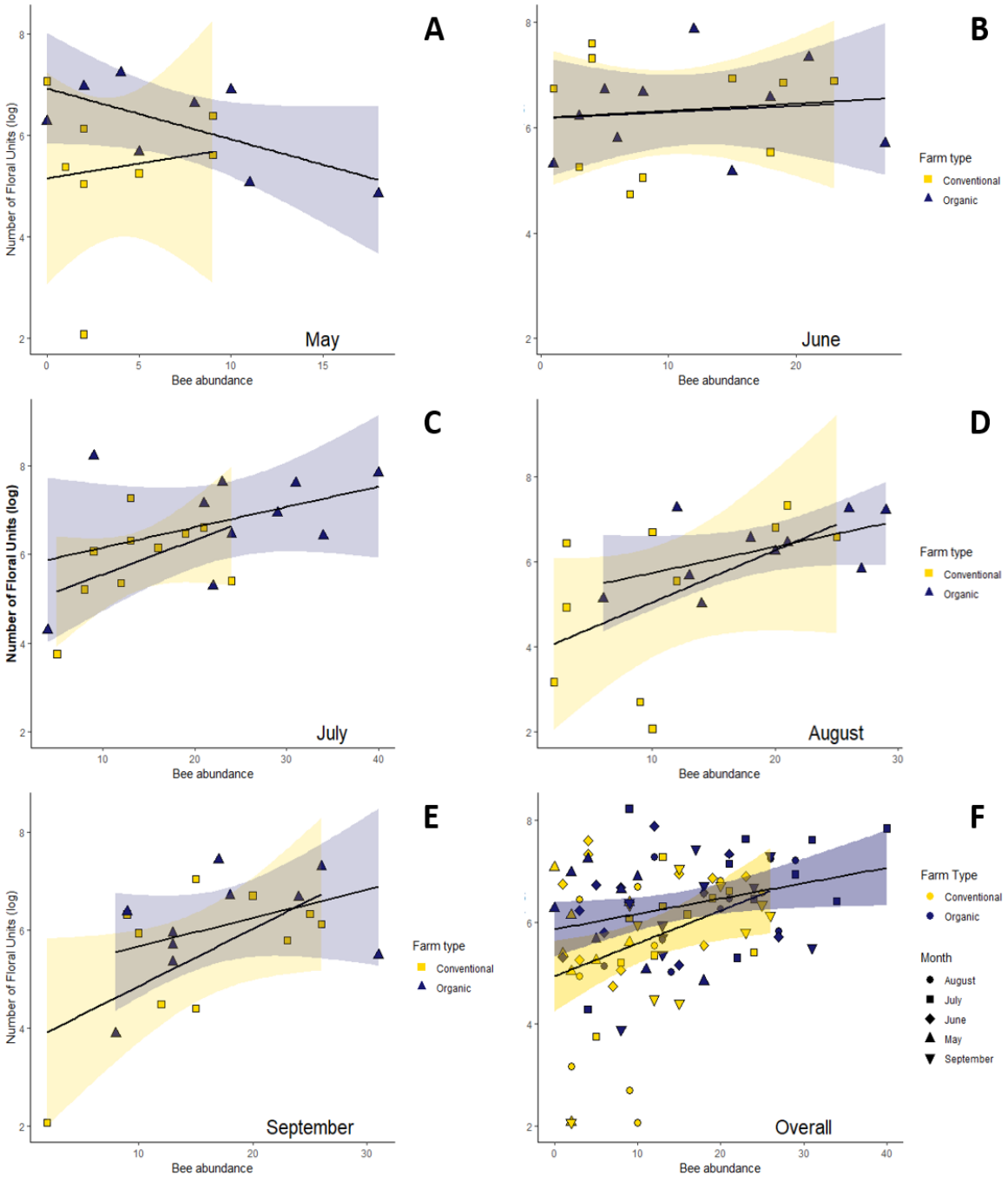


Fig. 5. (A-E) the number of bees and the corresponding number of floral units recorded on conventional and organic farms plotted by sampling month. Each point represents a single farm. (F) The total number of bees and corresponding floral units recorded on organic and conventional sites pooled across sampling months. Each point represents a single month at a single farm.

4.5. Discussion

Primarily, farming practice influenced abundance of both bees and flowers, with organic farms supporting higher numbers than conventional farms, corroborating previous studies (Hole et al., 2005; Kremen et al., 2002). However, there was a strong temporal component, with increases being driven only by particular months (Fig. 4) and the number of floral units being positively related to the number of bees caught in both farming systems across the majority of the season (Fig. 5). Despite the higher abundance on organic farms for both bees and flowering plants, species richness and diversity in both groups was unaffected by farming practice, which is counter to many studies (Gabriel and Tscharntke, 2007; Kennedy et al., 2013). Moreover, bee community structure did not differ between organic and conventional farms, despite seeing differences in plant community structure. Although plant communities differed in structure between farm practices, with organic farms clustering more closely than conventional farms (Fig. 3c), these differences, again, were mainly driven by the sampling month. Whilst the benefits of organic farming have received much attention (Hole et al., 2005; Power et al., 2016; Rigby and Cáceres, 2001) the relative similarities found here between organic and conventional sites suggests that with focussed management interventions the differences in abundance between farm types could be reduced. We suggest that with targeted management in key months and improved support to farmers, bee and plant abundances can be boosted in conventional systems.

Farming practice had a significant effect on the abundance of both plants (floral units) and bees, with organic farms supporting greater abundances as predicted (Fig. 2). Organic farms have previously been shown to support higher floral abundance (Bengtsson et al., 2005; Fuller et al., 2005; Hole et al., 2005; Rundlöf et al., 2009, 2008). As organic farming practices inherently use less intensive management practices, i.e. absence or

significant reduction in herbicide use (Pimentel et al., 2005; Rundlöf et al., 2009; Tuck et al., 2014), and as a result also utilise the crop area for supporting additional non-crop flowers (Power et al., 2012; Power and Stout, 2011), they are able to support greater floral abundances. Accordingly, organic farming is associated with an increase in bee abundance (Gabriel et al., 2013; Kremen et al., 2002; Power and Stout, 2011). These less intensive practices, e.g. reduced pesticide use (Rigby and Cáceres, 2001), as with plants, likely allow organic farms to support greater bee abundances. Indeed, bee numbers are strongly linked to resource availability, be that nesting habitat or food resources (Goulson et al., 2015; Goulson and Nicholls, 2016; Meyer et al., 2017; Papanikolaou et al., 2017; Rhodes, 2018). Interestingly, the higher abundances in organic farms only occurred in certain months. Organic farms showed the highest abundance relative to conventional farms in July, in both bees and plants, with modest increases in May and August; however, during June and September conventional farms were comparable. May is of particular interest, as spring is often a pollinator '*hungry gap*' on farmland (Nowakowski and Pywell, 2016). This period is critically important for bees but is traditionally linked with poor floral availability on farmland, suggesting that organic sites may support bees better during this crucial time. As a general pattern, an increase in floral abundance is related to increases in bee numbers, save for May on organic farms.

Increases in bee numbers were related to higher floral unit abundance overall (Fig. 3). As organic farms tend to support the highest floral abundances (Batáry et al., 2013; Hawes et al., 2010; Rundlöf et al., 2009) it could be expected that they also support the highest numbers of bees (Gabriel et al., 2013; Kennedy et al., 2013; Le Féon et al., 2010) and indeed our results support this. So, do more flowers equal more bees? Broadly speaking this tends to be the case (Biesmeijer et al., 2006; Morandin and Winston, 2005; Potts et al., 2010a; Power and Stout, 2011; Rundlöf et al., 2008). This positive association

does not simply apply to organic farms, however; the same is true for conventional sites [Fig. 3; Power and Stout (2011)], suggesting conventional farms will also be able to support higher number of bees, if floral availability is improved. Moreover, the increase in bee abundance on organic farms could also be driven by additional factors such as more nesting habitat or reduced exposure to harmful pesticides (Goulson et al., 2015; Potts et al., 2010a). It is worth noting here that the positive association between bee and plant abundance was true for all months except May, when organic farms showed a negative relationship (Fig. 3a). This is perhaps surprising given the previously mentioned overall trends of organic farms supporting more flowers and bees (Gabriel et al., 2013; Kennedy et al., 2013). Floral resources are not the only resource necessary for supporting bees however; without appropriate nesting sites or overwintering sites bee populations can also struggle (Kells and Goulson, 2003; Sardiñas et al., 2016). Alternatively, resources on farmland tend to be scarcer in May (Nowakowski and Pywell, 2016) so perhaps in situations, where floral availability is low, bees need more time to forage and are therefore more likely to be encountered when sampling. In summer months, when availability is high, more bees can be supported and therefore more bees are caught during sampling. However, the true reason behind this result, and whether it is an anomaly or not, requires further investigation.

The bee and plant communities found on organic and conventional farms were similar, running counter to the results found in some studies (Holzschuh et al., 2010, 2007; Kremen et al., 2002) but agreeing with others (Brittain et al., 2010; Kehinde and Samways, 2012). Specifically, Brittain et al. (2010) showed that isolated organic farming within an intensively farmed mosaic landscape had no influence on the bee community i.e. the positive benefits of organic farming may only be apparent when such land makes up a threshold proportion of the agricultural landscape. As organic farms in Yorkshire are also

isolated, and far fewer in number than in other parts of the UK, this could explain the uniformity in bee community structure in this case.

In contrast, the major difference in bee and plant communities was seen between the different sampling months (Fig. 1). This seasonal difference is not surprising given that flower phenology differs markedly between species (Morente-López et al., 2018) and that bee emergence also varies (CaraDonna et al., 2018; Lawson et al., 2018) and is often timed to exploit particular floral resources (Falk, 2015). In mutualistic communities in particular, phenology dictates species interactions (Morente-López et al., 2018) and is a crucial driver of community structure and diversity (Encinas-Viso et al., 2012). Our results support this, with community composition being driven by the sampling month more so than other factors.

Unlike with bees, we found that farming practice did influence plant communities, whereby (a) a significant difference in the plant community was seen between organic and conventional sites, and (b) organic sites seemed to be more similar to each other than their conventional counterparts (Figs. 3, S2). This suggests that conventional and organic farms differ in their plant communities, but that conventional farms also show greater among-farm variance, and that these patterns are not necessarily reflected in the bee community. Similar patterns have been shown previously, where conventional field centres were both more varied and different in plant community structure compared to organic equivalents (Power et al., 2012), yet the same bee species were found across farming practices (Power and Stout, 2011). Although they do share plant species, organic and conventional farms have been shown to support different plant taxa also (Gabriel et al., 2006; Hawes et al., 2010; Rundlöf et al., 2009). For example, Power et al. (2012) found 20 plant species unique to organic sites and 7 unique to conventional sites. Organic farms support more weed species (Gabriel and Tscharntke, 2007; Romero et al., 2008), due to their less intensive pest

and weed control practices (Tuck et al., 2014), which may go some way to explaining the community differences. The bias towards unique plants on organic farms was not found in this study however, with conventional farms supporting 31 unique species and organic farms only 21. Despite this, that different farming practices supported differing plant species yet similar bee species is interesting. The vast majority of the bees caught in this study were polylectic, i.e. plant generalists (Falk, 2015); such generalist bee species have been shown to dominate agricultural landscapes (Ahrenfeldt et al., 2019; Amy et al., 2018; Hall et al., 2019). This generality may help to explain the similarity in bee communities found here despite floral differences, as generalist bees would be able to use a wider selection of flowers that encompasses those found in both conventional and organic farming systems. Despite the difference seen in the plant communities between farm types it is important to note that the differences seen between months were far greater, suggesting that differences in farmland plant communities during the bee season are affected more by phenological drivers than by farming practice *per se*, as stated above. Therefore, understanding the effects of phenology should not be overlooked in farmland bee-plant communities as it likely has a stronger influence on the health of such systems than the farming practice itself.

Despite the differences seen in plant community structure across sampling months, there was no difference in overall species richness or diversity between farming practices. Previous studies show mixed results for both plants and bees, with some showing higher levels of richness and diversity on organic farms (Batáry et al., 2013; Belfrage et al., 2005; Bengtsson et al., 2005; Boutin et al., 2008; Gabriel and Tscharntke, 2007; Kennedy et al., 2013) but others not (Brittain et al., 2010; Gibson et al., 2007; Kehinde and Samways, 2012; Power and Stout, 2011; Weibull et al., 2003; Winfree et al., 2008). As with community structure, this may explain the similarity between plant and bee species richness

found here. Although statistically there was no difference between farming practices, it is worth noting that plant richness and diversity do seem to fluctuate across months, with May in particular showing higher diversity and species richness - coinciding with that of bees (Fig. 4, 5) - and that such early floral resources are important for supporting emerging bees (Lye et al., 2009; Moquet et al., 2015) and initial colony growth (Williams et al., 2012).

We found little difference in the richness and diversity of plants, or bees, between farming practices (Fig. 4b, 5b) and although higher floral abundance may mean more bees it does not necessarily mean more species of bee, as bee diversity is often linked to greater plant *diversity rather than abundance* (Scheper et al., 2015). Indeed, studies have found that declines in bee community diversity coincide with a reduction in plant diversity (Biesmeijer et al., 2006) and vice versa (Nicholls and Altieri, 2013). What is clear from this study however is that spikes in floral abundance correspond with spikes in bee abundance (Fig. 4b, d) and although these gluts in floral abundance are useful sources of nutrition, bee communities require floral constancy across the season in order to support them throughout their life cycles (Goulson and Nicholls, 2016). This is something that both farming systems could improve. Additionally, an important and often overlooked factor is the *quality* of available floral resources. There is increasing support for the importance of quality as well as quantity of food resources for bees (Fowler et al., 2016; Kaluza et al., 2018; Nicholls and Hempel de Ibarra, 2016), in particular that of the pollen (Donkersley et al., 2017), which primarily supports the larvae (Filipiak, 2019; Nicholls and Hempel de Ibarra, 2016) and can vary greatly in its nutrient content (Roulston and Buchmann, 2000; Roulston and Cane, 2000a). Therefore, more flowers equals more bees could be seen as a rather simplistic view of a complex issue.

Organic farming appears to support greater abundances of floral resources and bee pollinators in Yorkshire, yet, in line with some previous studies, does not seem to support

higher levels of diversity in either group (Brittain et al., 2010; Gibson et al., 2007; Power and Stout, 2011; Weibull et al., 2003). The higher floral abundances coincide with higher abundance in bees, and these levels are substantially higher during the months of May and July on organic farms. However, plant and bee communities on both farm types are similar and conventional sites may be able to plug the gaps by creating flower-rich habitats (Carvell et al., 2007). Additionally, as organic farms only showed increases in floral resource during specific months, targeted management towards these periods of scarcity (Nowakowski and Pywell, 2016) on conventional farms may allow then to catch up. However, as we have seen from our study, along with others, increased floral and bee abundance does not correspond to an increase in diversity (Power and Stout, 2011). Therefore ensuring that management practices for both farming methods take into account diversity and quality (in terms of nutrition) of floral resources will likely support a greater number and diversity of not only plants (Gibson et al., 2007) but bees also (Pywell et al., 2012). Additionally, individual farmers can make a difference, as many of these effects can be seen at the farm scale (Weibull et al., 2003), especially for solitary bee species, which respond to relatively small management improvements at smaller scales (Steffan-Dewenter et al., 2002). Our findings, along with previous studies that show mixed results for organic and conventional farm comparisons (Brittain et al., 2010; Kennedy et al., 2013), highlight the fact that biodiversity support in agriculture is more complex than simply organic or conventional. Other parameters, such as farm size and surrounding landscape complexity can influence the effects seen from different farming practices (Belfrage et al., 2005; Nicholls and Altieri, 2013; Williams and Kremen, 2007). Perhaps then, such pigeon-holing may be of little benefit, and encouraging farmers to adopt wildlife-friendly farming practices (Pywell et al., 2015) irrespective of their designation should be the focus, as well as communicating the benefits of such methods directly to the end user i.e. the farmers

themselves. For example, wildlife-friendly farming methods do not have to coincide with decreased yield (Gabriel et al., 2013) and have in some cases been shown to improve crop yields (Pywell et al., 2015). Additionally, maintaining wild pollinators can provide the full pollination service required on farmland with no need for managed species (Carvell et al., 2007; Kremen et al., 2004). Both making farmers aware of such benefits, as well as continuing to research new ways to improve management schemes, should be a priority. For example, pollinator seed mixes have been shown to benefit certain species (Blackmore and Goulson, 2014), yet provide little support during certain periods (Pywell et al., 2011) or for some groups, such as solitary bees (Wood et al., 2016). Additionally, an increased focus on the nutritional quality of such resources should be used to inform the inclusion of particular flowers into such seed mixes. Improvements to these schemes, coupled with targeted management, could not only allow for conventional farms to plug the gap in plant and bee abundances seen in comparison to organic farms, but also lead to improvements in the diversity of bee (and wider pollinator) communities across all farm practices.

4.6. Supplementary Materials

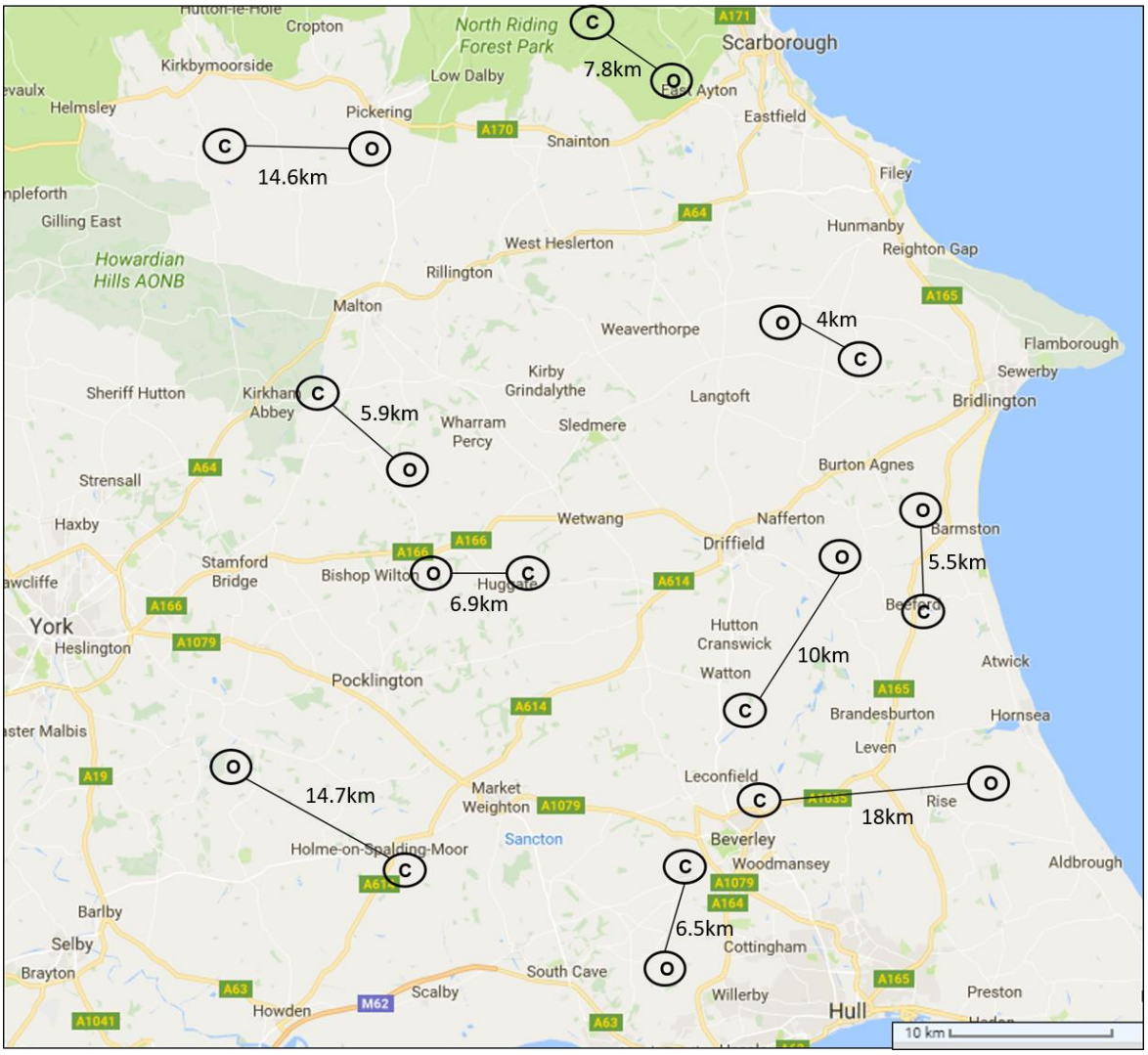


Fig. S1. Map of farm sites sampled during the study. Letters represent farm type (c = conventional, o = organic) and lines represent site pairings, with approximate distances between farms.

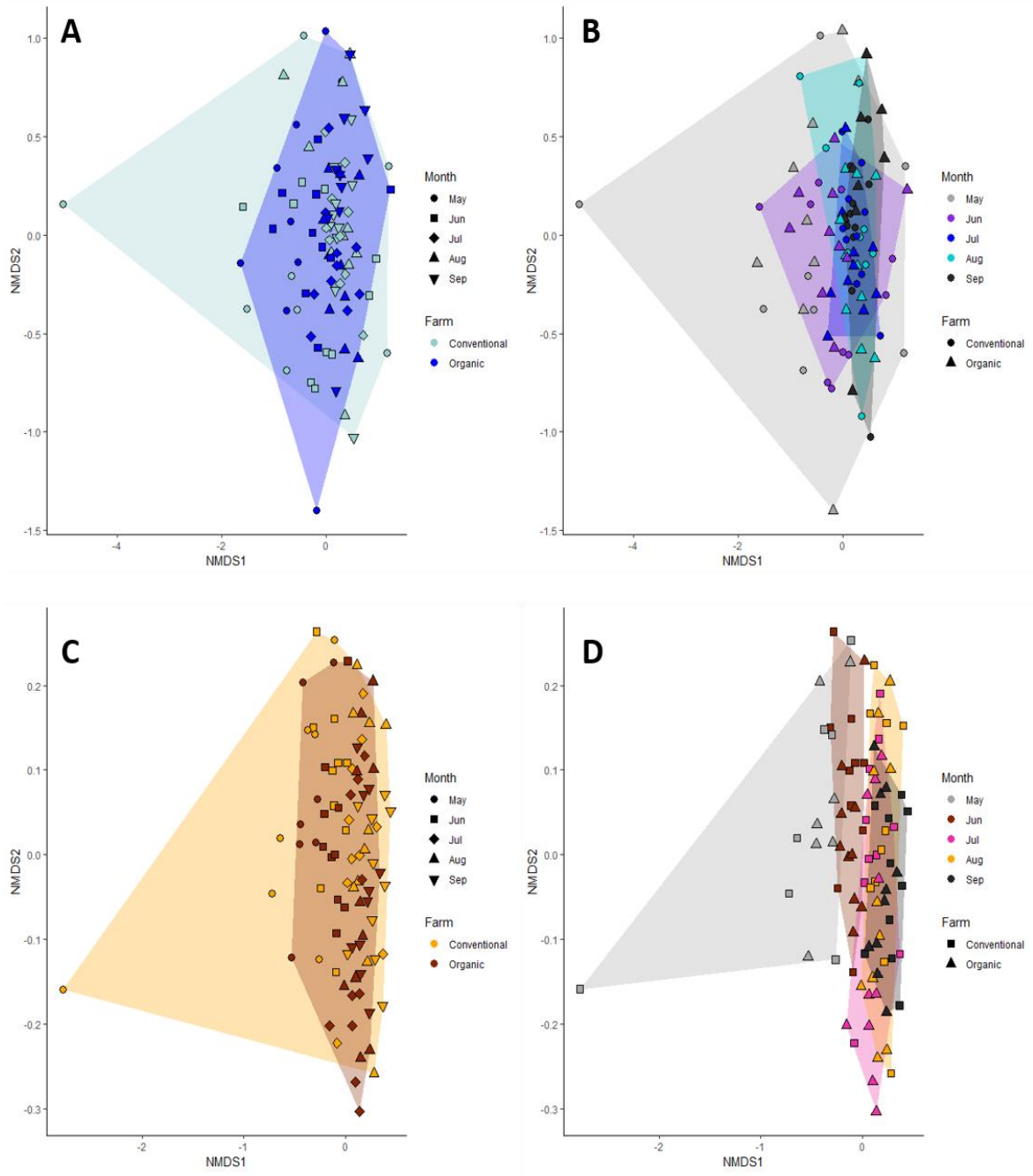


Fig. S2. NMDS plot for counts of bee (A & B) and plant (C & D) species at each site during each sampling month (converted to percentages), shaded by farm type (A & C) and sampling month (B & D) with the outlying point from the single conventional farm in May included.

Table S1. List of individual farm sites with corresponding details of practice, soil types and crops farmed

Farm Code	Farm Pair	Farming Practice	Farm Type	Soil Type	Crop
DEN	P1	Organic	Mixed	clay - slightly acid	Winter Wheat
MLS	P1	Conventional	Arable	clay - slightly acid	Winter Wheat
BES	P2	Conventional	Arable	clay - slightly acid	Winter Wheat
CAR	P2	Organic	Arable	clay - slightly acid	Spelt Wheat
BAR	P3	Organic	Mixed	clay - slightly acid	Winter Wheat
BEE	P3	Conventional	Arable	clay - slightly acid	Winter Wheat
COL	P4	Organic	Mixed	shallow - chalk/limestone	Winter Barley
LIT	P4	Conventional	Mixed	shallow - chalk/limestone	Winter Barley
FRE	P5	Conventional	Arable	Free-draining loamy	Winter Barley
STR	P5	Organic	Arable	Free-draining loamy	Winter Oats
BEC	P6	Conventional	Arable	Free-draining loamy	Spring Barley
BRO	P6	Organic	Mixed	Free-draining loamy	Spring Oats
WAL	P7	Conventional	Arable	Free-draining loamy	Winter Wheat
YOR	P7	Organic	Arable	Free-draining loamy	Winter Wheat
HAS	P8	Conventional	Arable	Wet sandy loam - acid	Winter Wheat
STO	P8	Organic	Arable	Wet sandy loam - acid	Winter Wheat
BXA	P9	Conventional	Pastoral	Free-draining acid loam	grassland - cattle

THO	P9	Organic	Pastoral	Free-draining acid loam	grassland - cattle
LEA	P10	Organic	Pastoral	clay - slightly acid	grassland - cattle
THR	P10	Conventional	Pastoral	clay - slightly acid	grassland - cattle

Table S2. Names of unique bee taxa according to farming method. Numbers in brackets represent incidences. Note: all these species are considered generalist in their flower usage

Conventional only	Organic only
Andrena barbilabris (1)	*Andrena minutula* (1)
Lassioglossum morio (2)	*Coelioxys rufescens* (1)
Nomada ruficornis (1)	*Hylaeus communis* (1)
Sphecodes gibbus (1)	*Megachile willughbiella* (1)
	Melecta albifrons (1)
	Nomada leucophthalma (3)

Table S3. Names of unique plant taxa for each farming method. Numbers represent number of floral units

Conventional only	Organic only
Ajuga reptans (3)	*Solanum dulcamara* (126)
Prunus domestica (105)	*Borago officinalis* (2)
Hypochaeris radicata (49)	*Arctium minus* (5)
Ranunculus sceleratus (8)	*Hypochaeris glabra* (1)
Pulicaria dysenterica (458)	*Cichorium intybus* (60)
Ranunculus arvensis (4)	*Stellaria media* (1)
Centaurea montana (22)	*Geranium sp.*(29)
Mentha arvensis (66)	*Hesperis matronalis* (6)
Petroselinum segetum (67)	*Vicia villosa* (52)
Agrostemma githago (10)	*Geranium columbinum* (17)
Rorippa sp. (3)	*Sonchus asper* (29)
Cardamine pratensis (15)	*Odontites vernus* (56)
Bellis perennis (963)	*Matricaria recutita* (208)
Thlaspi arvense (7)	*Capsella bursa-pastoris* (44)
Veronica chamaedrys (20)	*Ranunculus parviflorus* (3)
Pentaglottis sempervirens (11)	*Crepis capillaris* (77)
Crepis sp.(2)	*Epilobium tetragonum* (30)

Calystegia sepium (91)

Lamium amplexicaule (7)

Geranium robertianum (135)

Trifolium dubium (315)

Solanum physalifolium (6)

Epilobium roseum (41)

Primula vulgaris (10)

Lychis flos-cuculi (17)

Chaerophyllum temulum (100)

Leontodon hispidus (98)

Geranium pusillum (20)

Achillea ptarmica (3)

Rubus saxatilis (1)

Geum urbanum (2)

Stellaria crassiflora (55)

Mycelis muralis (75)

Raphanus raphanistrum (1383)

Galium odoratum (29)

5. As mother says not as mother does: bees forage differentially for themselves and for their offspring

5.1. Abstract

Nutritional resource degradation is linked to bee declines. Measures such as organic farming seek to mitigate this degradation. Yet we know little about the needs of most bee species. For example, bees' nutritional needs differ between adults and larvae, suggesting that parents need to make different nutritional decisions when foraging for themselves compared to their young; a distinction rarely considered when investigating bee foraging ecology on farmland, hampering mitigation. We used DNA metabarcoding of pollen samples from the pollen storage areas of bees ('baskets' - destined for larvae) and from areas more likely indicative of adult foraging ('swabs' - face and upper thorax) to investigate whether host plant communities, and bee-flower interactions, differed between adult- and larval-focussed foraging on organic and conventional farmland. Host plant community composition differed primarily between sample types (swabs vs. baskets), but also between farming types. Swabs contained higher pollen diversity than basket samples on conventional farms, with little difference seen between sample types on organic sites. Networks showed that bees used flowers differentially between sample types, with plant genera from Asteraceae being prominent in swab samples and genera from Fabaceae and Apiaceae more so in baskets; this persisted across farming practice. Additionally, networks showed that bees forage differently for their young on organic and conventional farms, but that such differences were not reflected in adult-focussed swab samples, which showed uniformity across farming practice. These findings suggest bees make different nutritional

decisions when foraging for themselves or for their young. This may reflect the differing nutritional needs of adults and larvae, suggesting that bees require a diverse yet different set of floral resources for larval nutrition. Our results highlight needs for a deeper understanding of floral resource use by bees, specifically for their offspring, as well as the need to mitigate nutritional deficits by providing resources that satisfy both adult and larval requirements.

Keywords: agriculture, pollen, bee nutrition, bee larvae, pollen transport networks, foraging ecology, pollination, agri-environment schemes, wild bees, pollen metabarcoding

5.2. Introduction

Bees are amongst the most important pollinators (Praz et al., 2008), pollinating the majority of our crops and wildflowers (Klein et al., 2018, 2007; Ollerton et al., 2011; Potts et al., 2016), an ecosystem service critical to humanity's food security (Archer et al., 2014b; Woodcock et al., 2014). This makes bees not only highly valuable ecologically (Bartomeus et al., 2013; Burkle et al., 2013), but also economically (Carreck and Williams, 1998; Hanley et al., 2015; Losey and Vaughan, 2006) . However, bees are in global decline (Goulson et al., 2008; Jacobson et al., 2018; Potts et al., 2010a, 2010b; Rhodes, 2018), with many seeing significant range retractions or even extinction (Ollerton et al., 2014).

Nutritional deficit is thought to be one of the major drivers of bee declines (Goulson et al., 2015; Roulston and Goodell, 2011). Nutrition is crucial in every aspect of bee biology, affecting behaviour (Walton et al., 2018), growth and development (Fischman et al., 2017), caste (Luna-Lucena et al., 2018), and bees' ability to fight disease (Spivak et al., 2019) and mitigate toxins such as pesticides (Schmehl et al., 2014). Despite these findings however, we know comparatively little about the foraging decisions of the majority of bees (Roulston and Cane, 2002; Vanderplanck et al., 2014), and what we do know is often limited to a handful of focal species (Leach and Drummond, 2018).

Farmland makes up a considerable proportion of many bees' habitat, so this environment in particular is of critical importance when considering bee conservation. Human-induced landscape change, particularly the intensification of agriculture, is largely responsible for the reduction in bees' nutritional resources (Robertson et al., 2013), primarily via ecological disruption (e.g. habitat fragmentation) and the subsequent shifts in floral resource quantity, quality and diversity (Goulson et al., 2015; Robinson and Sutherland, 2002; Ziska et al., 2016). The adoption of organic farming practices has been

one of the major suggestions for mitigating the negative effects of intensive agriculture (Crowder and Reganold, 2015). As organic farming is thought to be more ecologically beneficial (Hole et al., 2005; Tuck et al., 2014), using reduced amounts of agrochemicals (Gabriel et al., 2013; Pimentel et al., 2005) and possessing greater levels of habitat heterogeneity (Fuller et al., 2005; Krebs et al., 1999), such practices are thought be beneficial for pollinators such as bees (Holzschuh et al., 2008). In addition, other environmental management schemes have also received attention, such as the sowing of pollinator-focussed seed mixes, and the establishment of wildflower strips (Crowder and Reganold, 2015; Nowakowski and Pywell, 2016). However, many of these schemes, as well as organic farming practices, often have limited effectiveness for certain bee groups (Balzan et al., 2016; Brittain et al., 2010; Feltham et al., 2015; Wood et al., 2016) and rarely consider the nutritional quality of the resources they provide (Filipiak, 2018). Thus, despite current interventions, bees face a possible nutritional crisis on farmland, where they may struggle to fulfil their nutritional needs.

Understanding how bee communities navigate and utilise nutritional resources on farmland is of great importance. Bee-flower interaction networks have been studied previously, often within the context of pollination (Ballantyne et al., 2017; Bendel et al., 2019; Power and Stout, 2011). Traditionally, this is often done via observations of visitations to flowers as a proxy for an interaction (Theodorou et al., 2016; Traveset et al., 2017), but flower visitation does not necessarily imply that pollen is transferred (Pornon et al., 2016). Identifying pollen found on the body of bees is a more direct method for detecting foraging interactions, as each pollinator carries its visitation history with it (Bosch et al., 2009). Pollen metabarcoding has been successfully used to investigate bee foraging ecology (Danner et al., 2017; de Vere et al., 2017; Gresty et al., 2018; Hawkins et al., 2015)

and is increasingly being used to construct plant-pollinator interaction networks (Bell et al., 2017; Galliot et al., 2017; Macgregor et al., 2018; Pornon et al., 2017).

A number of studies have investigated pollen preferences in bees (Haider et al. 2014; Ison et al. 2018; Eltz et al. 2001; Sinu and Bronstein 2018), and demonstrated, for example, strong seasonal variation in pollen diversity (Danner et al. 2017; de Vere et al. 2017). Bees have been observed to forage from different plants for pollen and nectar (Raw 1974; Cripps and Rust 1989; Kraemer et al. 2014) and even from wind-pollinated plants (which do not contain nectar) (Saunders 2018; Haider et al. 2014). This suggests that bees may be making crucial foraging choices based on whether they are feeding their larvae or themselves (Filipiak 2018; Filipiak 2019) as pollen is the main food source for larvae (Filipiak et al. 2017; Larkin et al. 2008; Carr et al. 2015), while adults primarily feed on nectar (Filipiak 2018; although see Cane 2016). In addition to this, pollen is highly variable in nutrient content (Roulston and Cane 2000) and is likely far more difficult for bees to nutritionally assess than is nectar (Nicholls and Hempel de Ibarra 2016). Pollen harvesting is also more costly than nectar harvesting to the host, so plants have numerous ways to protect against overconsumption of pollen (Lunau et al. 2015; Praz et al. 2008). Combined, these are likely to have a considerable effect on how bees gather pollen for their young. To our knowledge, no study has yet directly investigated the rules bees use when foraging selectively for themselves or their offspring.

Here we use DNA metabarcoding to investigate differences in bee-flower interaction networks between adult- and larval-focussed foraging. Organic farming often supports differing and more abundant plant communities (Bengtsson et al., 2005; Gabriel et al., 2006; Gabriel and Tscharntke, 2007; Power et al., 2012; Rundlöf et al., 2009), so we investigated whether foraging decisions made by bees differed between farming practices as well as between adult- and larval-focussed nutrition. Most bees store pollen gathered for

their young in specialised areas of their bodies, usually on the legs or sometimes underneath their abdomen (Falk, 2015) (although some also carry pollen in their crop (Wilson et al., 2010)). By extracting DNA from pollen destined for larvae (henceforth referred to as 'baskets') versus the pollen on the rest of the body, which is more likely to indicate adult nectar foraging and pollination ('swabs'), we investigated whether host plant composition and diversity differed between adult- and larval-focussed foraging. We then constructed and analysed interaction networks from the different sample types, and across farming practices, in order to investigate differences in resource use (Gresty et al., 2018; Tylianakis et al., 2010).

We predicted that (1) the host plant communities as well as the network interactions would differ between adult- and larval-focussed foraging (i.e. between swabs and baskets) due to the differing nutritional needs of adults and larvae (Filipiak, 2018), (2) a greater diversity of flowers would be utilised on organic farms, reflecting the increased resource abundance on organic farms (Batáry et al., 2013; Rundlöf et al., 2009), and (3) network structure and diversity would differ based on sample type, with bees utilising a greater number of floral resources when foraging for their young, in order to overcome the variable nutritional content (Roulston and Cane, 2000a) and defenses of pollen (Konzmann et al., 2019; Praz et al., 2008). Alternatively, bees may instead avoid certain pollens due to such defences, which may lead them to actually use a narrower set of plants when foraging for offspring.

5.3. Methods

Study Sites & Experimental Design

Ten pairs of organic and conventional farms were used as study sites across East and North Yorkshire, UK. Farms were either arable or arable mixed (n=16), or dairy/beef (n=4). Organic farms were recruited first, due to the limiting number of organic farms, with local conventional farms recruited afterwards. Farms were paired based on soil type and environmental conditions as well as distance. Farms within a pair were a minimum of 4 km apart (max distance of 15km) so as to limit the chance of individual bees being able to forage at both farms (Greenleaf et al., 2007). Single fields per farm were sampled, and pairs matched to crop or crop type i.e. barley with barley or cereal with cereal.

Bees were collected from each site 5 times between May and September 2016. An 80 x 1m E-shaped transect was walked for 1 hour (total sampling effort) during each sampling session and bees captured in a sweep net. The spine of the transect (20 x 1m) ran along the field margin, with the arms (20 x 1m each) running into the crop, resulting in greater time spent sampling in the crop area, compared to the margins, and reflecting the land-use of the farm. Captured bees were placed singly into empty 50 ml falcon tubes and kept in a cool bag then placed into a freezer (-20°C) on-site. Bees were transferred to a laboratory freezer (-80°C) at the end of the sampling day.

Data on floral species richness and abundance was gathered along each transect prior to bee collection (for data, see chapter 3). Each flowering plant species (excluding grasses) that was in flower at the time of collection was recorded and included in the subsequent reference database.

Bee Identification & Pollen Sampling

1332 bees were available as possible samples, 780 from organic farms and 552 from conventional farms. Of bees that possessed filled pollen baskets, 253 were organic and 133 were conventional. Bees were allowed to thaw in a fridge then placed under a light microscope to be sexed and identified to species level using (Falk, 2015). Pollen from corbiculae/scopae (hereafter referred to as pollen baskets) were removed with a sterile needle and placed into a sterile 0.2 ml PCR tube, which was then stored at -80°C until DNA extraction. In cases where pollen loads, or the bee itself, were particularly small, the leg was removed entirely. A subset (n=300) of these harvested baskets were taken for DNA extraction. The subset was chosen proportionally, reflecting the differing numbers of bees caught between organic and conventional farms and across sample visits per site e.g. as more bees containing pollen baskets were caught in total on organic farms they made up a greater proportion of those samples used for DNA analysis (equating to approximately 65% of the 300 basket subset coming from organic farms; see Table S1-2, 4 for details). Prior to species identification, an additional 300 bees were swabbed for pollen on their bodies, specifically the head and upper thorax, which were then used for DNA extraction. As with baskets, the subset was chosen proportionally (equating to approximately 58% of the 300 swab subset coming from organic farms; see Table S1, 3-4 for details), however bees with filled pollen baskets (or bees that had no pollen on their bodies at all) were not swabbed. Such specimens were replaced randomly with another bee caught on the same site as well as during the same sampling visit where possible. A cube of jelly (traditionally used for swabbing pollen for slide mounting (see (Alarcón, 2010), lacking stain, was used to remove

pollen, avoiding the pollen basket area and focusing on the head, mouthparts and upper thorax. Swabs were then placed into sterile 0.2 ml PCR tubes and frozen at -80°C prior to DNA extraction. For full details of all pollen sampling, see Supplementary Materials.

DNA metabarcoding

Detailed descriptions of all DNA extraction, amplification and sequencing methods are given in Supplementary Materials. Briefly, DNA was extracted from all samples following the 'Mu-DNA - Tissue lysis' protocol followed by an inhibitor removal step as outlined in Sellers et al. (2018). DNA was then captured and purified using a Solid Phase Reversible Immobilisation (SPRI) method adapted from (Rohland and Reich, 2012).

A 506 bp fragment of the rbcL gene region of chloroplast DNA was amplified using a two-step PCR nested tagging protocol (Kitson et al., 2018) and previously published primers: rbcL-Z1aF (Hofreiter et al., 2000) and rbcL-3CR (Macgregor et al., 2018). Amplified DNA was then sequenced on an Illumina MiSeq (Illumina, Inc., San Diego, CA, USA) using V3 chemistry. Final sample size was n=300 baskets, n=300 swabs, with n=50 negative controls (including extraction blanks) and n=22 positive controls.

Bioinformatics

A custom database of reference sequences was created from a base list of plausible flora, as in Macgregor et al. (2018), which was previously recorded in the vice-county of South-East Yorkshire. Raw sequencing data were processed and taxonomic assignment was performed using the metaBEAT pipeline (version 0.97.10; https://github.com/HullUni-bioinformatics/metaBEAT), based on a BLAST and Last Common Ancestor approach (see Supplementary Materials for full details on bioinformatic data processing); as has previously been used to successfully identify plants using the rbcL locus (Bell et al., 2017; Koyama et al., 2018). Prior to taxonomic assignment, OTUs were end-trimmed based on read quality, PCR primers clipped, and only reads greater than 50 bp were retained for further analysis (see Supplementary Materials for details).

Statistical Analysis

We analysed the sequence read data at 3 taxonomic levels: family, genus, and species, with all other reads assigned at higher taxonomic levels removed from further analysis. Analyses at the genus level included those reads assigned to species level rolled back into their respective genera. The same was true for family level analyses, with all genus and species reads rolled back into their respective families. Presence or absence of DNA was used as opposed to read count as read count does not necessarily reflect abundance (Pornon et al., 2016; Yu et al., 2012). All data analyses were performed using the statistical software R 3.5.0 (R Core Team, 2018). Community indices (Simpson's index,

Shannon's index, and richness) were calculated for each taxonomic level using the R package iNEXT (Hsieh et al. 2019). The estimated value for each index, calculated within iNEXT, was used based upon the extrapolated accumulation curve asymptote.

Generalised Linear Mixed effects Models (GLMM) were used to investigate factors affecting host plant community indices (Simpson's index, Shannon's index, and richness) with farm type, sample type, visit month and all their interactions as predictors plus farm pair and bee species as random factors. Models were fitted with a Gamma family and an inverse link function. Minimal models were determined via reverse stepwise comparisons.

Host plant community similarity was assessed using PERMANOVA (ADONIS) of relative plant abundance in the package vegan (Oksanen et al. 2018). Two separate PERMANOVA were performed differing in restricted permutation structures, each with two levels. Firstly, both permutation structures contained farm pair as their upper level. Permutations were then restricted within this level to only within bee species or within visit month respectively. Restricted permutations were used so as to only permute within and not across matched farm pairs, and similarly so with either visit month or bee species, as plant communities change phenologically and different bee species use different plant hosts. Differences in host plant community structure were visualised using nonmetric multidimensional scaling (NMDS) ordination on Bray-Curtis distance matrices, grouping data by farm type and by sample type.

Interaction network metrics were calculated using the bipartite package (Dormann et al., 2009) and networks were visualised using Food Web Designer v.3.0 (Sint and Traugott, 2016). All networks were created using the interaction frequency data between bee species and plant species i.e. presence-absence of plant DNA, as interaction frequency has been shown to positively relate to interaction strength (Vázquez et al., 2005). The following quantitative network metrics were created for all 20 farms and both sample types

separately to investigate the stability and structure of pollinator networks (Gómez et al., 2007; Gresty et al., 2018; Valdovinos et al., 2016): connectance; nestedness; generality (of bee species); and linkage density. Connectance is the number of links available in a network that are actually realised. Nestedness is the degree to which the plant interactions of specialist pollinators exist wholly within a subset of the interactions of the generalist pollinators. Generality is the number of interactions with differing plant species an individual bee species has. Linkage density is the mean number of links per species within a given network.

Linear Mixed effects Models (LMMs) were used to investigate how sample type affected network metrics. Due to issues with model complexity, we did not explicitly compare farm types in these models. Instead, metrics from both farming types were first modelled together with the predictor variables: sample type, plant richness, and bee species richness, as well as every two-way interaction containing sample type, with farm pair included as a random factor. Metrics from each farm type were then analysed separately, using the same predictor variables, in order to investigate factors affecting the metrics within each farming practice. Models in both cases were simplified using reverse stepwise comparisons. A gaussian error structure was specified for all models and model assumptions were met in all cases.

5.4. Results

Summary

In total, 8,957,540 reads were assigned (96% of total reads), of which 6,981,750 were retained after quality control. Only reads assigned to family level and lower were retained for further analysis, resulting in 5,864,871 final reads. Of these reads, 26.5% were from conventional swabs, 16% from conventional baskets, 22.6% from organic swabs, and 34.9% from organic baskets. Average read depth per sample, excluding negatives, was 14,572 prior to trimming. In total, we sampled 30 different bee species across 8 genera, upon which we identified the pollen of 99 plant species, 82 plant genera, and 37 plant families (see Supplementary Materials for list of bee species and unique plant species by farm and by sample type - Table S5,6). Of the 30 bees species sampled, 20 contributed basket samples. For consistency, and as patterns were similar across taxonomic groups, results are reported at the genus level. However, results from other taxonomic levels can be seen in Supplementary Materials.

Host plant community dissimilarity: communities differ primarily by sample type

Firstly, we observed 4 clusters with NMDS, which correspond to farm and sample type (Fig. 1a). Broadly, for both permutation structures (by bee and by visit respectively), host plant communities differed more between the sample types (basket vs. swab) than they did by farming practice (Sample type; PERMANOVA by bee, $R^2 = 0.03$, $p<0.001$; by visit,

R^2 = 0.03, p<0.001: Farm type; by bee, R^2 = 0.015, p<0.001; by visit, R^2 = 0.015, p<0.001). Specifically, when restricted to permutations within bee species, basket samples differed more between farming practice than did swab samples (PERMANOVA, sample type × farm type interaction, R^2 = 0.013, p<0.001, Fig. 1a). Additionally, dissimilarity also changed differentially between farm types across the sampling months (PERMANOVA, R^2 = 0.034, p<0.001) as may be expected from the different communities seen on organic and conventional farms (Chapter 3). The effect of sample type continued when restricting permutations to only those within each visit month (farm type × sample type, R^2 = 0.015, p<0.001; Fig. 1a) Additionally, effects of farm and sample type both differed across bee species (farm type × bee species, R^2 = 0.055, p<0.001; sample type × bee species, R^2 = 0.025, p = 0.004), possibly reflecting different bee species' foraging ecologies (a topic discussed in more detail in Chapter 3).

Host plant community indices: swabs show higher diversity on conventional farms

Swabs were more diverse than baskets collected from bees on conventional farms, whereas on organic farms swabs and baskets showed little difference in diversity. As similar patterns were seen across indices, we report only Shannon's Index here (see Supplementary Materials for other indices; Fig. S1, S2). Overall, Shannon's index was higher for conventional swabs compared to conventional baskets, and for conventional compared to organic farm types. By contrast, Shannon's index was similar for organic swabs and baskets (Fig. 1b). Shannon's Index was influenced by the three-way interaction between farming type, sample type and visit month; while conventional swabs were

consistently more diverse than conventional baskets across the season, organic baskets were more diverse than organic swabs at the beginning and end of the season (May and September; GLMM, $\chi^2_{4,23} = 11.47$, p = 0.022; Fig 1c).

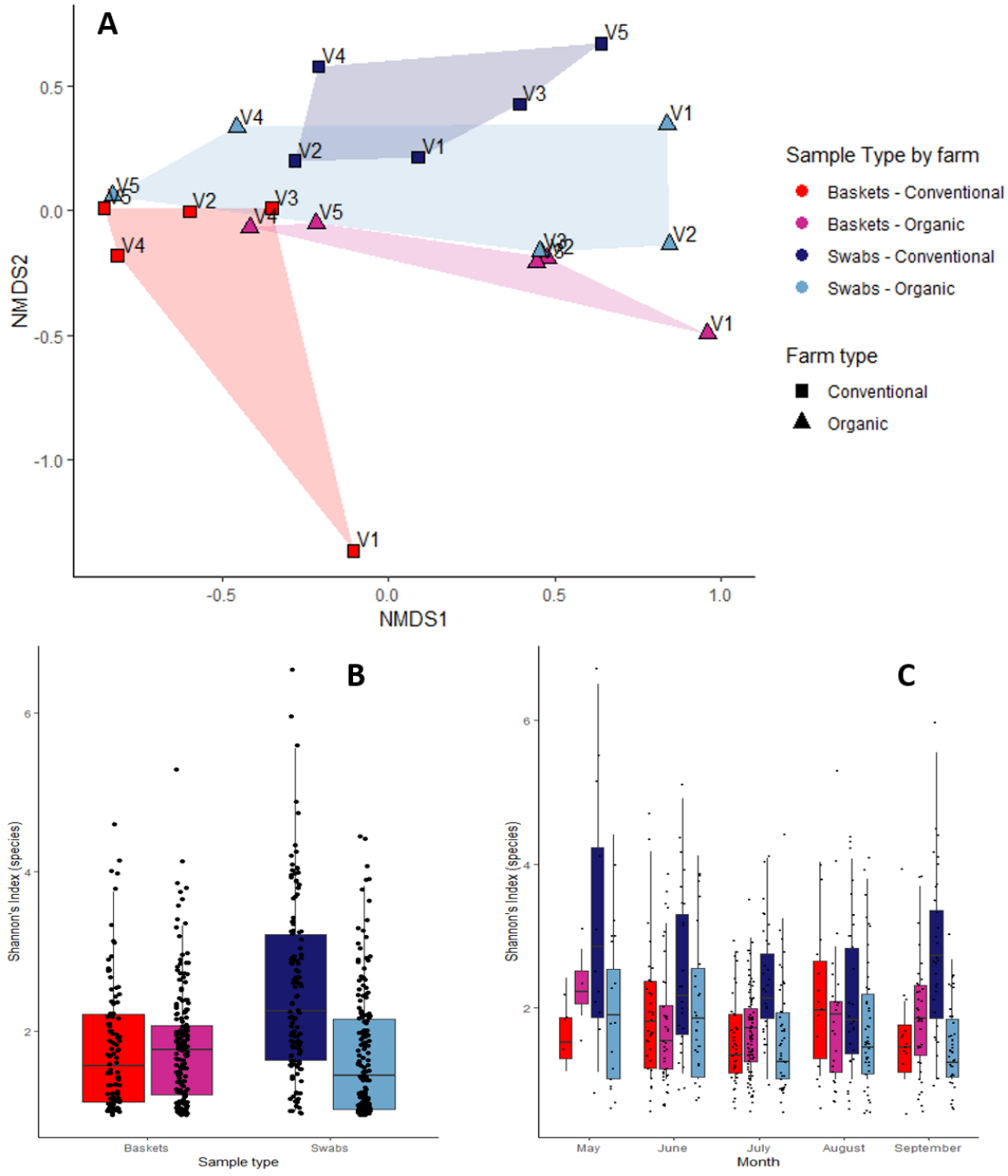


Fig. 1. Host plant community composition at the genus level. Colour shades represent sample type; swabs (Blue) & baskets (Red). (A) NMDS plot for plant genera found across farming

practices for both swab and baskets sample. (B) Boxplot of Shannon's Index by farm type & sample type pooled across the sampling season. (C) Boxplot of Shannon's Index by farm type and sample type split by sampling month. Points represent plant DNA from pollen taken from an individual bee sample.

Bee-flower networks: bees forage differentially for their offspring

Clear differences could be seen between networks constructed using swab and basket samples. For example, a higher proportion of Fabaceae was observed in basket than swab samples on both organic (Fig. 2) and conventional (Fig. 3) farms. Conversely, a higher proportion of Asteraceae was observed in swabs than in baskets in both farming types (Fig 2, 3). Furthermore, those interaction networks that were constructed specifically from baskets on organic farms differed from baskets on conventional farms (Fig. 5). For example, the Papaveraceae and Solanaceae were major contributors to baskets on organic farms, yet were less so on conventional farms, where Apiaceae, Lamiaceae and Rosaceae played a larger role. In contrast, differences between farming types were less pronounced in the swab samples (Fig. 4).

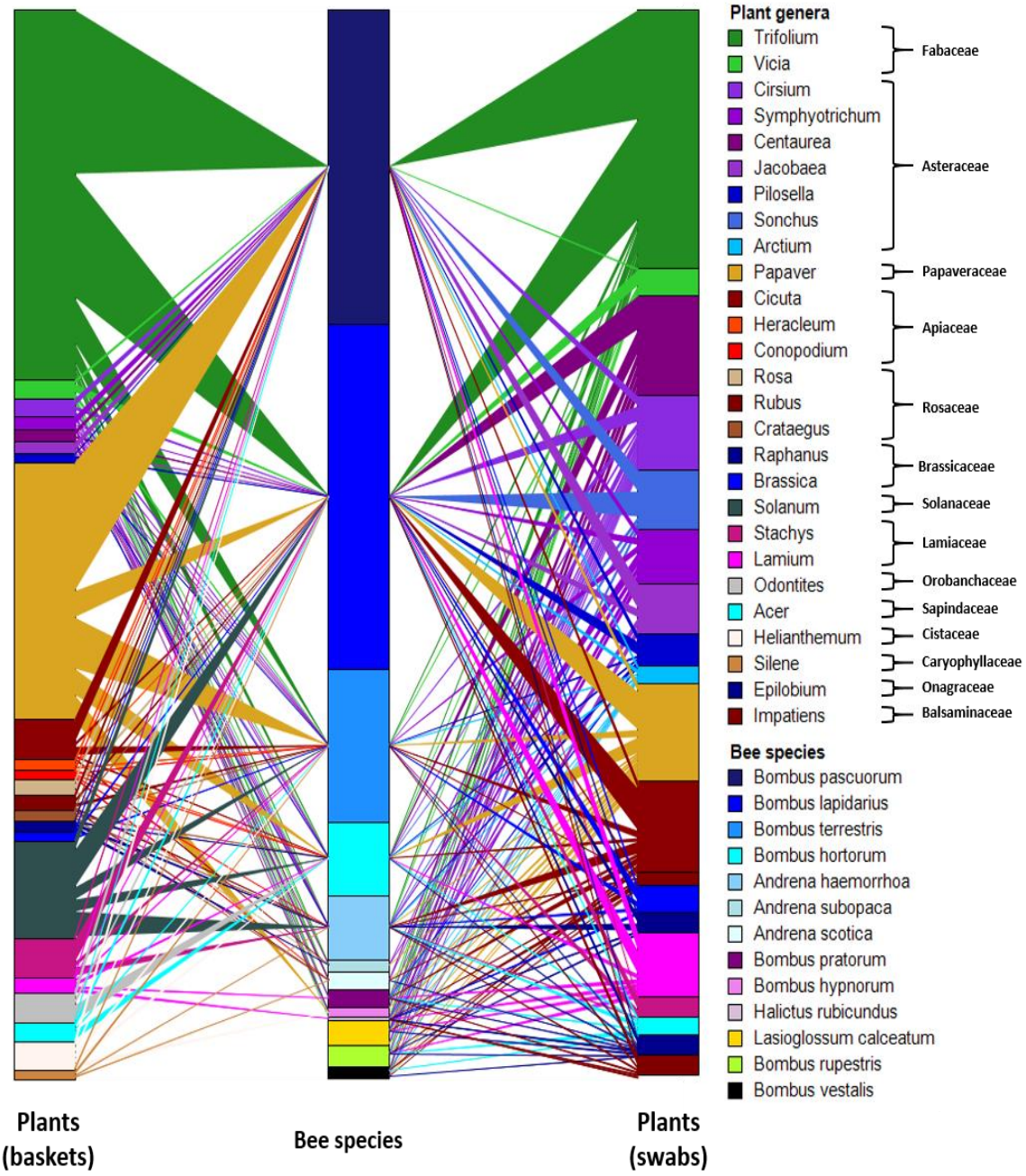


Fig. 2. Network constructed from pollen taken from bees on organic farms, split between swab and basket sample. Plant genera are colour shaded by family and bees are coloured by species (see key). Plant genera occur top to bottom as shown in the key. For bees bar depth denotes the species abundance. Bar depth for plants denotes the incidence of DNA occurrence on bees. Links represent the proportion of each plant genera found on each bee species.

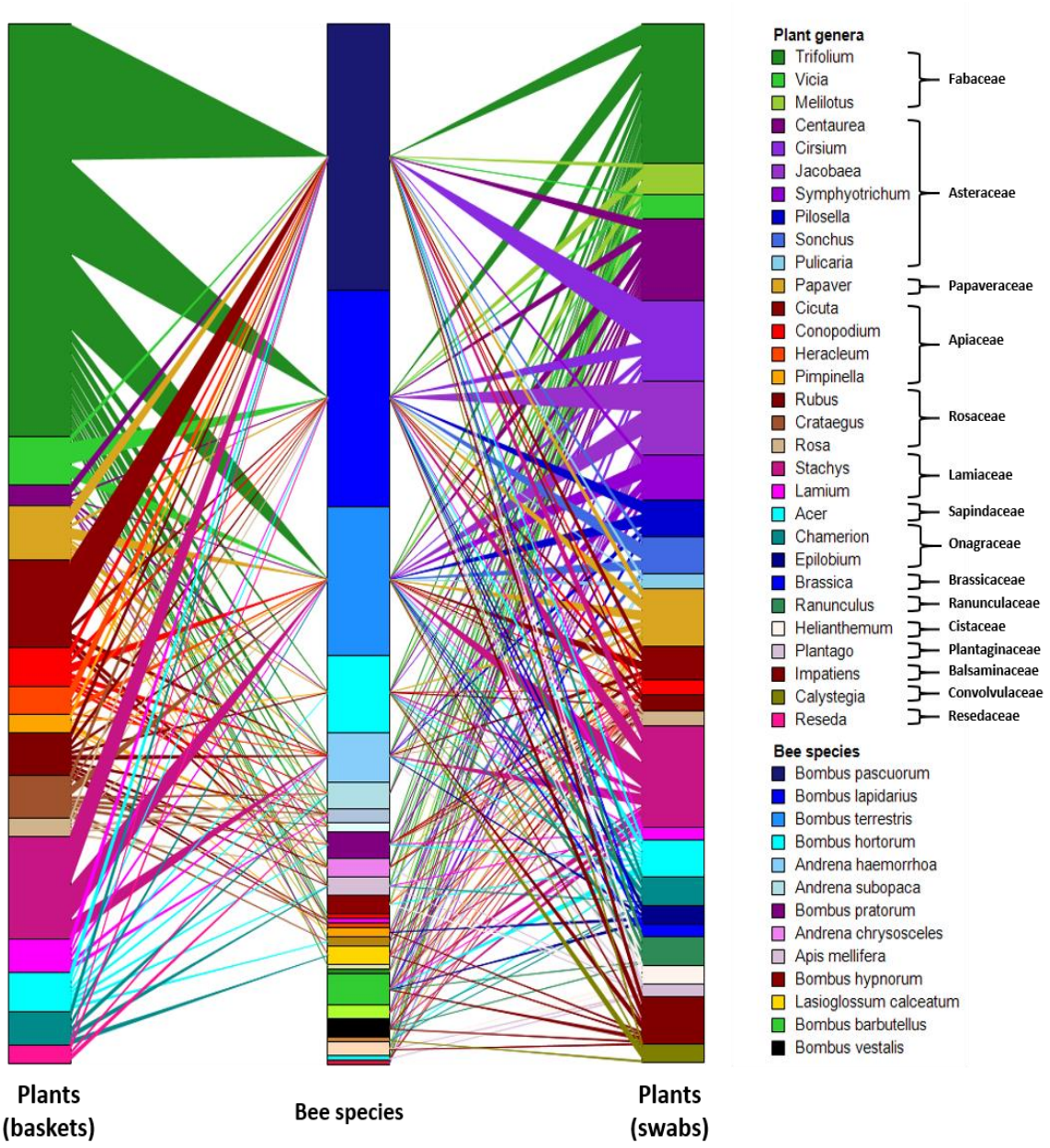


Fig 3. Network constructed from pollen taken from bees on conventional farms, split between swab and basket sample. Plant genera are colour shaded by family and bees are coloured by species (see key). Plant genera occur top to bottom as shown in the key. For bees bar depth denotes the species abundance. Bar depth for plants denotes the incidence of DNA occurrence on bees. Links represent the proportion of each plant genera found on each bee species.

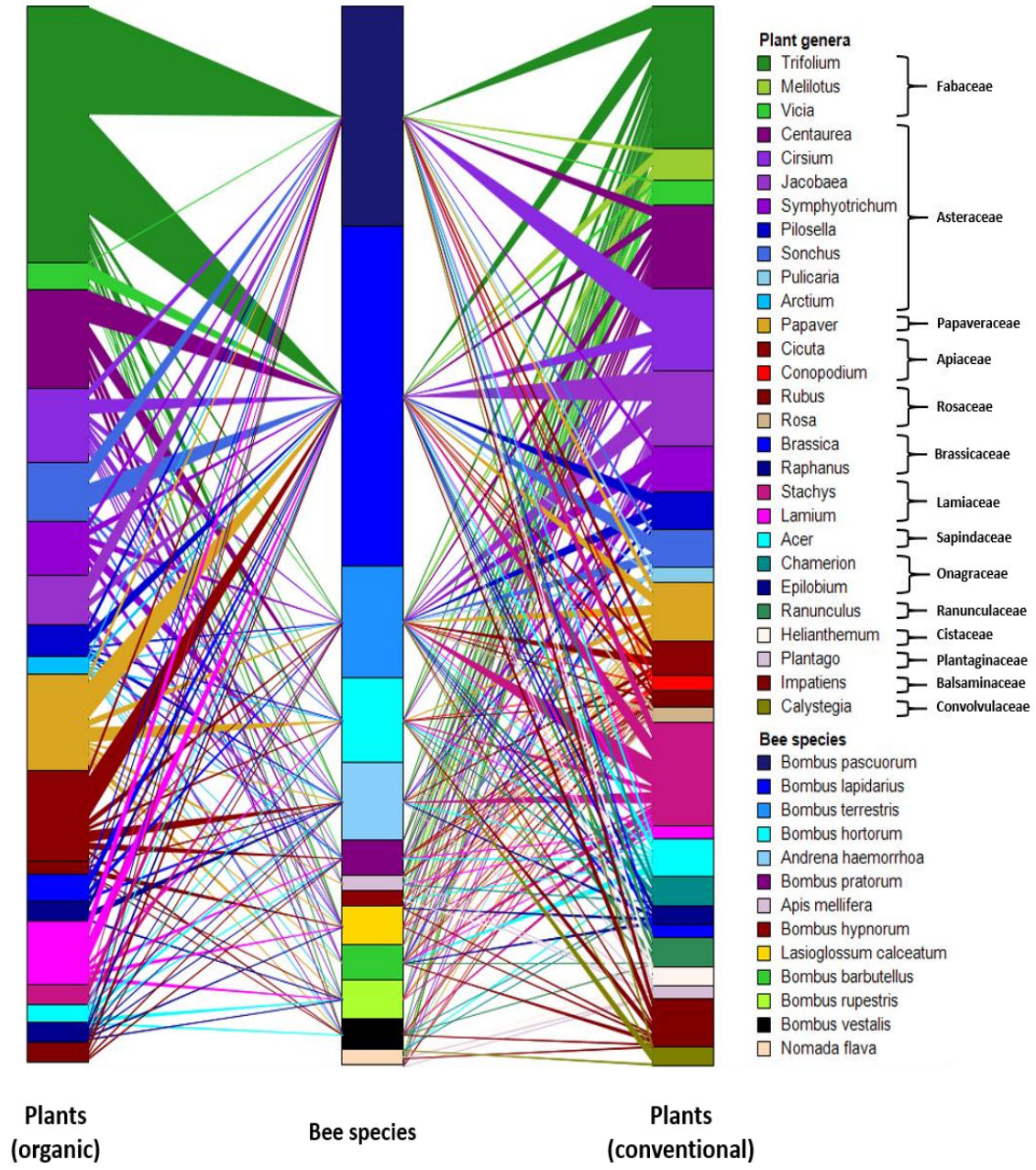


Fig. 4. Network constructed from pollen analysed via swabs from bees, split by farming practice. Plant genera are colour shaded by family and bees are coloured by species (see key). Plant genera occur top to bottom as shown in the key. For bees bar depth denotes the species abundance. Bar depth for plants denotes the incidence of DNA occurrence on bees. Links represent the proportion of each plant genera found on each bee species.

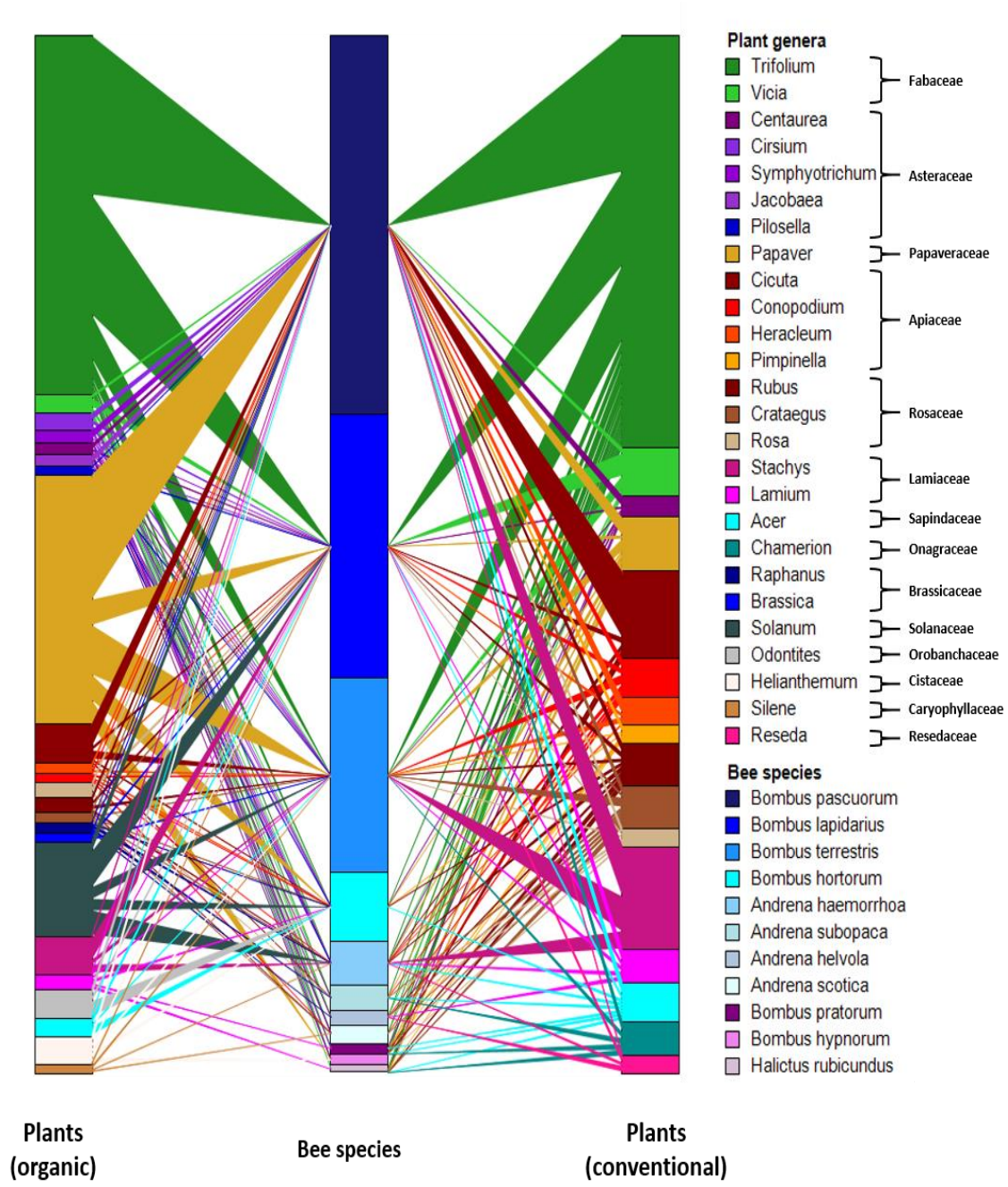


Fig. 5. Network constructed from pollen analysed from pollen baskets of bees, split by farming practice. Plant genera are colour shaded by family and bees are coloured by species (see key). Plant genera occur top to bottom as shown in the key. For bees bar depth denotes the species abundance. Bar depth for plants denotes the incidence of DNA occurrence on bees. Links represent the proportion of each plant genera found on each bee species.

Bee-flower network metrics - by sample type

In brief, across our 3 analyses (first irrespective of farm type, then for each farm type separately), metrics differed broadly between sample types when comparing raw values. Connectance showed similar values across sample types, with baskets showing marginally higher values (Fig. 6a). Both linkage density and generality showed higher values for swabs than baskets (Fig. 6b,c), although this was marginal for generality. Sample type (along with all other predictor variables) had no effect on network nestedness at any taxonomic level and so is not shown here.

When analysed irrespective of farming type connectance and generality were broadly explained by bee richness, and linkage density by plant richness. However, sample type did influence generality at the genus level (LMM, χ^2=10.02, p = 0.0015). Generality increased with the more plant genera and bee species in the network (LMM: Plant, χ^2=4.04, p = 0.044; Bee, χ^2=33.83, p<0.001) with higher values for basket samples than for swab samples when taking account of plant and bee richness. See Table S7-9 for all metric values. A full description of all metrics and model outputs, across all taxonomic levels, can be seen in the Supplementary Materials.

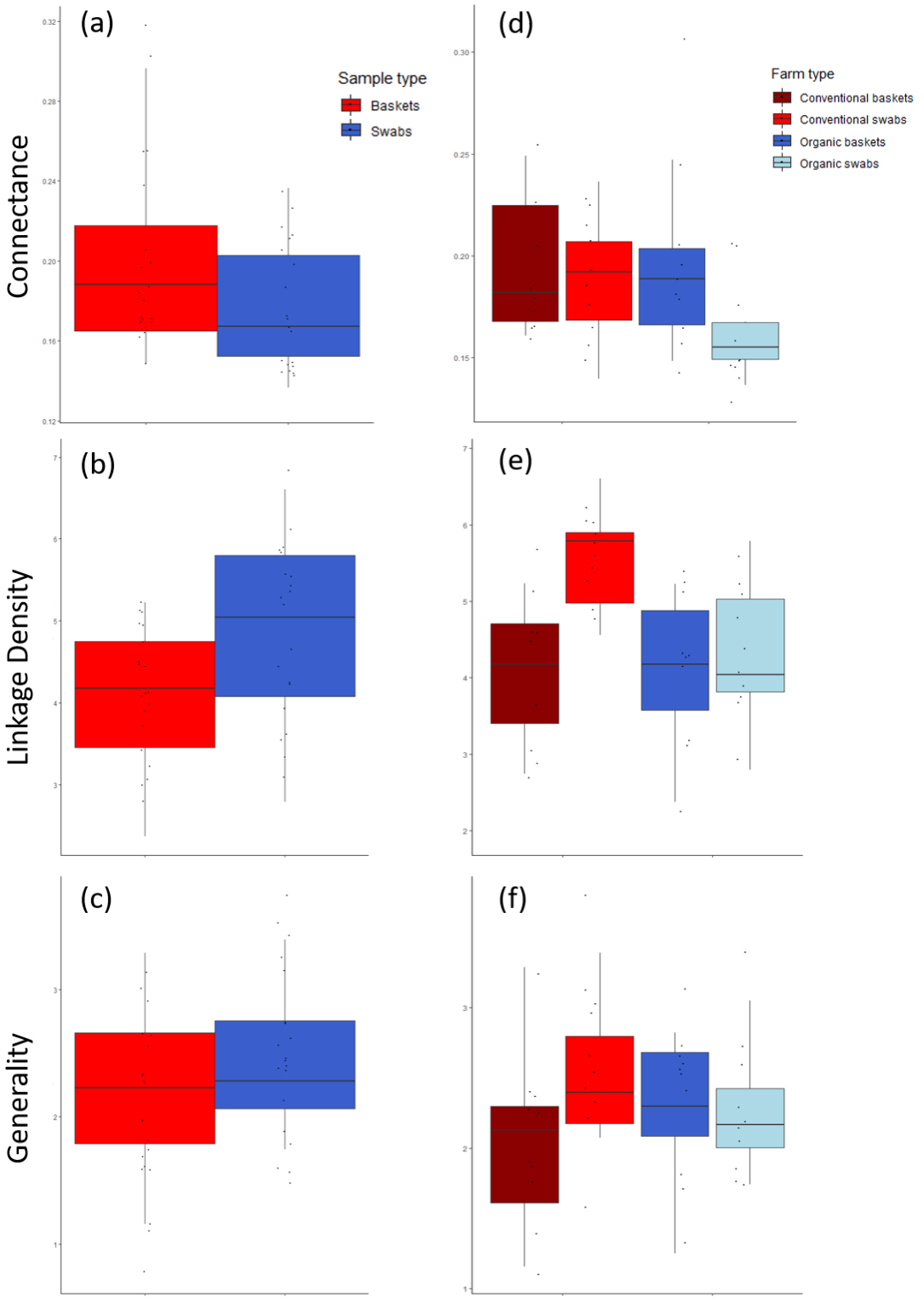


Fig. 6. Metric values, based on plant genera, by sample type only (left) and by farm type and sample type (right). (a) and (d) network connectance, (b) and (e) network linkage density, (c) and (f) network generality

Bee-flower network metrics - split by farming method

When comparing raw values, metrics differed between farming practice and between sample type. Connectance showed similar levels across sample type on conventional farms, with baskets seeming slightly higher than swabs, whereas on organic farms this difference was more pronounced (Fig. 6d). Linkage density tended to be higher for swabs than baskets, a pattern more pronounced on conventional farms (Fig. 6e). Generality also varied most on conventional sites, with swabs seeing higher levels than baskets, whereas on organic farms values were more comparable with baskets being slightly higher than swabs (Fig. 6f).

Model outputs for the metric analysis split by farming method can be seen in Table 1. In summary, linkage density was broadly explained by plant richness, with linkage density increasing with increasing plant richness. At the genus level on conventional farms however, linkage density was also influenced by sample type. Swabs showed higher linkage density than baskets at the plant genus level, however, swabs increased less sharply with increasing bee richness than did baskets.

Connectance, however, was broadly explained by bee richness, with connectance decreasing with increasing bee richness. Interestingly, effects of sample type were only seen alongside effects of plant richness, which was associated negatively with connectance. Taking plant richness into account, swabs showed lower connectance on organic farms compared to baskets yet the reverse was true on conventional sites, with baskets being less connected.

Generality, like connectance, was primarily driven by bee richness, with generality increasing with increasing bee richness. However, at the plant genus level on organic farms,

alongside the effect of bee richness, generality was also influenced by sample type, with swabs showing lower generality than baskets.

Nestedness, unlike the other metrics, showed no effect of any of the predictors across all taxonomic levels and both farm types.

Table 1. Significant effects found on network metrics across taxonomic levels. All models were Linear Mixed effects Models (LMM) with bee richness, plant richness and sample type as predictors. All two-way interaction with sample type were also included in the models, along with farm pair as a random factor. Metrics affected by sample type are highlighted.

Network Metric	**Organic farms**	**Conventional farms**
Plant Species Level		
Linkage Density	Plant Richness (χ^2=13.66, p<0.001)	**Sample Type** × Plant Richness (χ^2=3.94, p=0.047)
Connectance	Bee Richness (χ^2=11.07, p<0.001)	Bee Richness (χ^2=16.48, p<0.001)
Generality	Bee Richness (χ^2=7.63, p=0.006)	Bee Richness (χ^2=19.09, p<0.001)
Nestedness	No effect of predictors	No effect of predictors
Plant Genus Level		
Linkage Density	Plant Richness (χ^2=16.77, p<0.001)	**Sample Type** × Bee Richness (χ^2=8.71, p=0.0032)
Connectance	**Sample Type** × Plant Richness (χ^2=4.8, p=0.028)	Bee Richness (χ^2=16.97, p<0.001)
Generality	**Sample type** (χ^2=7.45, p=0.0063)	Bee Richness (χ^2=36.66, p<0.001)

	Bee Richness (χ^2=16.69, p<0.001)	
Nestedness	No effect of predictors	No effect of predictors
Plant Family Level		
Linkage Density	**Sample Type** (χ^2=4.69, p=0.03) Bee Richness (χ^2=15.42, p=0.02) Plant Richness (χ^2=4.55, p=0.033)	Plant Richness (χ^2=8.04, p=0.005)
Connectance	**Sample Type** (χ^2=12.81, p<0.001) Plant Richness (χ^2=11.11, p<0.001)	**Sample Type** (χ^2=5.43, p=0.02) Bee Richness (χ^2=11.32, p<0.001) Plant Richness (χ^2=8.02, p=0.005)
Generality	Bee Richness (χ^2=22.23, p<0.001)	Bee Richness (χ^2=38.58, p<0.001) Plant Richness (χ^2=5.06, p=0.024)
Nestedness	No effect of predictors	No effect of predictors

5.5. Discussion

Here we demonstrate that bees forage differentially for pollen and nectar. Host plant communities, represented by their pollen, clustered together primarily by sample type, highlighting the distinct host plant communities found within swab and basket samples. Second, we show that host plant communities differed by farming practice, suggesting that organic and conventional farms may support different nectar *and* different pollen resources. Although diversity of host plant communities differed between swabs and baskets, these differences were not consistent across farming practices. Bees showed evidence of having visited different plant communities in pollen baskets as opposed to pollen swabs irrespective of farming type, strongly suggesting that bees make different decisions based on whether they forage for themselves or for their young. Sample type broadly influenced network metrics, with linkage density being higher for swabs on conventional farms only, generality being lower for baskets than swabs but only on organic farms, and swabs showing lower connectance than baskets on conventional farms. We believe these findings have profound implications for the conservation management of bees, highlighting the need to accommodate nutritional thinking from both the larval and adult perspective in future schemes.

Bees forage differentially between plant hosts for their offspring and themselves

Host plant communities associated with swab and basket samples showed clear dissimilarity, suggesting that the floral community structure differs depending on adult- or

larval-focussed foraging behaviour. The construction of interaction networks supported this difference in floral community, demonstrating that bees use flower groups differentially depending on whether they are foraging for themselves or for their offspring. For example, Asteraceae were used heavily from an adult perspective but were present in comparatively tiny amounts in the pollen baskets destined for larvae.

These findings suggest that bees may be making different decisions when it comes to larval nutrition; differences that may reflect the differing needs of adults (Darveau et al., 2014; Harrison and Roberts, 2000) and larvae (Giejdasz and Wasielewski, 2017; Nijhout et al., 2014). Although adults do eat some pollen (Cane, 2016; Cane et al., 2016) they primarily feed on nectar (Filipiak, 2018), with the larvae being the principal pollen consumer (Carr et al., 2015; Filipiak et al., 2017; Larkin et al., 2008; Muth et al., 2016). As adults and larvae have different nutritional needs (Filipiak, 2018) this may explain why the swab and basket samples consist of different plant communities.

Adults tend to have diets predominantly driven by carbohydrates (Altaye et al., 2010; Paoli et al., 2014a) in order to fuel flight and a high metabolism (Darveau et al., 2014; Harrison and Roberts, 2000). Larvae, however, require nutrients for growth, development and often diapause (Giejdasz and Wasielewski, 2017; Helm et al., 2017; Roulston and Cane, 2002; Tasei and Aupinel, 2008). Plants themselves also differ in both the type (Bergström et al., 1995; Hicks et al., 2016; Russell et al., 2017, 2016) and quality (Fowler et al., 2016; Hanley et al., 2008; Roulston and Cane, 2000a; Schaeffer et al., 2016; Somme et al., 2014) of the rewards they offer. Therefore, bees may need to forage differentially when visiting flowers for their offspring versus for themselves. This complexity would also likely be compounded by differences between bee species in their nutritional needs and preferences (Haider et al., 2014; Sedivy et al., 2011; Woodcock et al., 2013; Wood et al., 2018), as well as differences between social and solitary systems, for

example, the creation of honey (de Vere et al., 2017) and nutritional manipulation of larval food in honeybees (Corby-Harris et al., 2018; Lucchetti et al., 2018).

While not itself a nutritional interaction *per se*, pollination is also likely to be profoundly influenced by these different decisions. Pollen gathered in baskets is destined for the bees' offspring (Thorp, 2000) and as such, it is likely to have a very limited role in pollination (Konzmann et al., 2019; Michener, 2000; Popic et al., 2013). The swab samples however are more likely to indicate pollination interactions (Alarcón, 2010; Forup and Memmott, 2005; Popic et al., 2013) and can be seen as more indicative of adult foraging. The mutualistic relationship between bees and flowers is perhaps better viewed as mutual exploitation (Praz et al., 2008; Westerkamp, 1996). Basket samples could be viewed as a more antagonistic relationship, as bees take pollen with very little of it ending up in pollination (Konzmann et al., 2019; Schlindwein et al., 2005), incurring a cost to the plant (Hargreaves et al., 2009). Understanding how bees interact differentially when visiting different plants could have important implications for our understanding of the effectiveness of pollination between plant groups, especially considering that animal pollination is intrinsically inefficient (Hargreaves et al., 2009).

Metric differences were also seen between sample types. When considering plant genera, swabs showed higher linkage density but only on conventional farms, perhaps suggesting that there is greater support for consumer needs at such sites (Blüthgen and Klein, 2011), at least in terms of nectar provisions. Basket samples had higher connectance than swab samples, suggesting a greater resilience to species loss (Gresty et al., 2018; Popic et al., 2013; Thébault and Fontaine, 2010), and that bees may be more able to effectively switch between pollen hosts via rewiring (CaraDonna et al., 2017; Gresty et al., 2018). However, it could be that such resilience is illusory, as due to the intrinsic nutritional variation found in pollen resources (e.g. nutrients, secondary compounds; Filipiak et al.,

2017; Praz et al., 2008; Roulston and Cane, 2000a) perhaps bees must forage from a broad but similar set of flowers in order to compensate for plant species loss when foraging for larvae, meaning that individual floral resources are not necessarily interchangeable. Further work could investigate the structure of these networks in greater depth and scale in order to see whether the drivers suggested here are reflected across farmland bee communities in general.

Bees forage differently on organic and conventional farmland

Within sample type the floral community also showed clear dissimilarity between farming practices, suggesting that not only do organic and conventional farms support different floral communities, as shown in previous studies (Gabriel et al., 2006; Gabriel and Tscharntke, 2007; Hawes et al., 2010; Power et al., 2012; Rundlöf et al., 2009), but that they also support different adult- and larval-focussed host plant communities. However, network construction revealed far more variation in larval-focussed (baskets samples) foraging interactions between farm types as opposed to adult-focussed (swab samples) interactions. For example, on organic farms Papaveraceae contributed considerably more to basket samples than on conventional farms, whereas Apiaceae and Rosaceae were more important on conventional farms. Interestingly, the host plant diversity differed between swab and basket samples but only on conventional farms, suggesting the adults use a more diverse set of floral hosts for themselves but not their larvae on conventional farms.

Our findings suggest that there are differences not just between farming practices but differences *within* these communities, between plants targeted specifically for larval nutrition and those used more from an adult perspective. Although little difference was seen between swab samples between farming types, basket samples did differ. Pollen is a highly

variable resource, differing widely in macro- (Roulston and Cane, 2000a) and micronutrient (Filipiak and Weiner, 2017) content, which may make it a more challenging resource to harvest than nectar (Nicholls and Hempel de Ibarra, 2016). Bees may or may not be able to assess the quality of pollen (Nicholls and Hempel de Ibarra, 2016; Roulston and Cane, 2002; Ruedenauer et al., 2016, 2015), therefore it is plausible that bees have limited abilities to assess nutritional content of pollen during foraging. Bee larvae, however, require a precise balance of nutrients in order to develop, the vast majority of which comes from pollen sources (Filipiak et al., 2017; Larkin et al., 2008). As such, generalist bees may actively gather pollen from a wide array of plants in order to hedge their bets (Bukovinszky et al., 2017; Kaluza et al., 2017), i.e. have the greatest chance of achieving nutritional balance, whilst also avoiding any negative effects of secondary pollen compounds (Eckhardt et al., 2014). What we do not know, however, is whether the nutritional quality of nectar and pollen available on these two farm type communities differ, and whether these differences are more or less impactful for larval or adult foraging. Further work could investigate this by building nutritional profiles of farmland flora (Pamminger et al., 2019) and comparing this against the differences in host plant communities between farms to ascertain the nutritional impact on bees.

We also found that the diversity of plants found differed between farming practices, but only for swab samples, with the flowers used on organic farms different to those used on conventional farms. This is interesting as there is often little difference in overall diversity between farming types using traditional vegetation surveys (Gibson et al., 2007; Weibull et al., 2003). There is however often a difference in abundance, with conventional farms usually being less abundant than organic farms (Bengtsson et al., 2005; Fuller et al., 2005; Hole et al., 2005; Rundlöf et al., 2009, 2008). Perhaps then, resources are more limited, i.e. each plant species is less abundant, on conventional farms, a scenario often

associated with intensive farming (Roulston and Cane, 2000a; Sedivy et al., 2011), and therefore the increase in diversity of swab samples on conventional vs. organic farms is a result of bees needing to use more varied, but individually more limited resources. Basket samples however are not more diverse between farms. Pollen not only differs in nutritional content (Filipiak and Weiner, 2017; Roulston and Cane, 2000a) but also in morphology (Konzmann et al., 2019; Lunau et al., 2015) and secondary protective compounds (Praz et al., 2008), and even generalist bees can struggle to gather certain pollens (Lunau et al., 2015) or even fail to develop on different pollen types altogether (Sedivy et al., 2011). In light of this, perhaps bees cannot be as flexible when gathering pollen for their offspring as with nectar for themselves, and can therefore not necessarily make up for a reduction in abundance by increasing the diversity of flowers from which they forage for pollen. It would be interesting to see if the differences seen here relate to changes in bee reproductive output and sex ratio, as we may expect that under limiting conditions bees may produce less young (Kim and Thorp, 2001; Peterson and Roitberg, 2006) or skew the sex ratio towards the least expensive sex (Bosch, 2008; Kim, 1999).

The patterns in diversity described above persisted across sampling months. Flower phenology drives structure in plant pollinator communities (Encinas-Viso et al., 2012; Morente-López et al., 2018) and it is therefore important to view the nutrition available to bees in the same manner. Farmland conservation interventions for bees, and all pollinators, need to focus on providing resources for a wide variety of bees across the entire flight season (Nowakowski and Pywell, 2016; Wood et al., 2016), as key periods can often be comparatively barren for many pollinators. Indeed, we found that baskets from organic farms showed higher diversity than their conventional counterparts in May. Spring is often referred to as the '*hungry gap*' on farmland (Nowakowski and Pywell, 2016), where floral resources are in particularly short supply. Seeing as May also showed low abundance of

flowers for conventional farms (see previous chapter) it seems possible that organic farms may provide better pollen resources during the spring months, which is an essential time for newly emerged queens and spring solitary bees (Falk, 2015).

Bees appear to forage differentially for pollen versus nectar. Adult-focussed foraging (swabs) appeared more uniform across farms in their floral utilisation than larval-focussed foraging (baskets). This suggests that pollen diets for larvae may be highly varied dependent on the landscape within which they were provisioned, possibly due to high variations in pollen nutritional quality (Roulston and Buchmann, 2000; Roulston and Cane, 2000a) and the differing plant communities between organic and conventional farms (Gabriel et al., 2006; Gabriel and Tscharntke, 2007; Hawes et al., 2010; Power et al., 2012; Rundlöf et al., 2009). This variation highlights the specific need for measures to enhance diversity of pollen as well as nectar resources. Future conservation practices need to reflect these differences within bee-flower interactions in order to optimally support bees. Currently, such schemes on farmland have limited effectiveness (Wood et al., 2016) with little consideration of nutrition, particularly that of the larvae (Filipiak, 2018). A greater focus on providing floral resources that reflect the diverse pollen requirements of bees is therefore of vital importance. Additionally, our networks highlight the differences in the nutritional interactions with plants for adult- and larval-focussed foraging. However, we do not yet know how variation in the specific nutrition of the pollens influences the network structure. Although networks have been investigated with foraging in mind previously (Bendel et al., 2019; Pasquaretta et al., 2017; Valdovinos et al., 2016), the relative importance of these interactions may be shaped by the quality of nutrition available from different flowers. As such, future research could concentrate on combining nutritional

assessment of different pollens within a network with the interactions themselves, to shed light on the importance of links in terms of nutritional contributions.

Bee-plant interactions on farmland have received much attention (Power and Stout, 2011), however, rarely are they viewed specifically through the lens of larval nutrition, nor to such a depth as we present here. Moreover, bees' nutritional needs are complex, knowledge of which is lacking for most species (Leach and Drummond, 2018), and although interaction networks often incorporate varied bee pollinators (Ballantyne et al., 2017; Carman and Jenkins, 2016; Pauw et al., 2017) nutritional studies have historically been less inclusive, often focusing on social groups (Leach and Drummond, 2018; Li et al., 2019; Ruedenauer et al., 2018). However, solitary bees make up the bulk of the species (Falk, 2015) and as such investigating such data as we present here in respect to particular bee groups is an important next step (Filipiak, 2019). Bees currently face a number of risks, with lack of nutrition being one of the most critical (Goulson et al., 2015; Roulston and Goodell, 2011). Further study into the nutritional relationships between bees and their host plants is vital if we are to understand their complex nutritional needs, and so ensure their continued contribution to global pollination.

5.6. Supplementary Materials

5.6.1. Pollen DNA metabarcoding workflow

Pollen sample preparation

Baskets:

1. Line a petri dish with greaseproof paper
2. Remove specimen from the freezer and place in the petri dish; sex and identify using microscopy
3. Dip a manipulating needle and forceps into 10% bleach solution, dry, rinse, and then dry again
4. With the needle, scrape the pollen basket from the bee's leg/pollen brush into a sterile 0.2ml PCR tube
5. Label and freeze the tube for later DNA extraction
6. Return specimen to the freezer
7. Discard greaseproof paper
8. Re-sterilise needle and petri dish with 10% bleach solution

Swabs:

1. Create jelly (100ml):
    a. Dissolve 15.4g of gelatin in 54ml of double distilled (dd) H_2O in a 250ml glass beaker using a microwave at a low energy setting
    b. Add 46ml of glycerol and stir
    c. Decant into petri dishes to approximately 3mm in depth

d. Seal the dish and refrigerate to set

2. Cut jelly into cubes within the petri dish using a sterile scalpel
3. Replace lid and sterilise under UV light for 1 hour
4. Line a petri dish with greaseproof paper
5. Remove specimen from the freezer and place in the petri dish; sex and identify using microscopy
6. Dip a large pinning needle and forceps into 10% bleach solution, dry, rinse, and then dry again
7. Using the needle, skewer a cube of jelly and swab the mouth parts, head and upper thorax of the specimen, taking special care to avoid any pollen carrying areas
8. Place the jelly cube into a sterile 0.2ml PCR tube
9. Steps 5 through 8 of Baskets protocol

SPRI based pollen DNA extraction

Stock solutions:

***0.5M EDTA (pH 8)*:** Add 18.6g of EDTA to 75ml of double distilled (dd) H_2O. Adjust to pH 8 with 5M NaOH until dissolved. Bring to 100ml final volume with ddH_20.

***1M Tris HCl (pH 8)*:** Dissolve 15.7g of Tris HCl in 75ml ddH_2O. Adjust to pH 8 with 5M NaOH. Bring to 100ml final volume with ddH_20.

***20% SDS*:** Dissolve 20g of sodium dodecyl sulphate in 75ml of ddH_2O, bring to 100ml with ddH_2O.

***50% PEG 8000*:** To 50g of polyethylene glycol 8000 add ddH_20 to a final volume of 100ml. Repeatedly invert at room temperature until dissolved.

***5M NaCl*:** Dissolve 29.2g of sodium chloride in 75ml of ddH_2O. Bring to 100ml final volume with ddH_20.

5M Ammonium acetate: Dissolve 38.6g of ammonium acetate in 75ml of ddH_20, bring to 100ml with ddH_20.

180 mM Aluminium ammonium sulphate dodecahydrate: Dissolve 8.2g of aluminium ammonium sulphate dodecahydrate in 75ml of ddH_20, bring to 100 mL with ddH_20.

3% Calcium chloride: Dissolve 3g of calcium chloride dihydrate in 75ml of ddH_20, bring to 100ml with ddH_20.

Working solutions:

Lysis Solution: To 75ml ddH_20 add 6.7ml of ***1 M Tris HCl (pH 8)***, 5.3ml of ***0.5 M EDTA (pH 8)***, 520µl of ***5M NaCl*** and 8.7g of trisodium phosphate dodecahydrate. Stir mixture

until all solids dissolve. Adjust to pH 9 with 5M HCl. Bring to final 100ml volume with ddH_20.

Tissue Lysis Additive: To 60ml (2 volumes) ddH_2O add 30 ml (1 volume) of ***20% SDS***.

Proteinase K: Dissolve 100mg of Proteinase K (PK) in 3.5 ml of ddH_2O. Add 200µl of ***1M Tris HCl (pH 8)***, 50 µl of ***3% Calcium chloride*** and bring to 5ml with ddH_2O. Add to 5ml of glycerol and vortex to mix thoroughly.

Flocculant Solution: To 50ml (2 volumes) of ***5M Ammonium acetate*** add 25ml (1 volume) of ***180 mM Aluminium ammonium sulphate dodecahydrate***. Vortex briefly before adding 25ml (1 volume) of ***3% Calcium chloride***. Vortex briefly to mix.

Wash Solution: To 20ml (2 volumes) of ddH2O add 80ml (8 volumes) of 100% ethanol.

Elution Buffer**:** Add 1ml of ***1M Tris HCl (pH 8)*** and 200µl of ***0.5M EDTA (pH 8)*** to 75ml of ddH_2O. Bring to 100ml final volume with ddH_20.

DNA Extraction Bead Solution (for final 10 ml vol)**:** Mix 100µl of ***1M Tris HCl (pH 8)***, 20µl of ***0.5M EDTA (pH 8)*** and 3.2ml of ***5M NaCl***. Add 4ml of ***50% PEG 8000*** and invert to mix. Add 2.58ml of ddH_20. Invert to mix thoroughly.

Library Prep Bead Solution (for final 10 ml vol)**:** Mix 100 ul ***1 M Tris HCl (pH 8)***, 20 ul ***0.5 M EDTA (pH 8)*** and 3.2 ml ***5 M NaCl***. Add 4 ml ***50% PEG 8000*** and invert to mix.

Add 2.43 ml ddH_20. Invert to mix thoroughly. Add 50 ul of ***10% Tween 20*** then add 200ul prepared ***Bead suspension***. Vortex or invert to mix thoroughly.

Sera-Mag SpeedBeads preparation:

1. Vortex Sera-Mag SpeedBeads until completely resuspended
2. Transfer 100µl of Sera-Mag SpeedBeads to a 1.5 mL Eppendorf tube
3. Place on magnetic stand until supernatant is clear and beads are bound to the magnet
4. While on the stand carefully remove and discard the supernatant without disturbing beads
5. Add 1ml of ddH_2O. Vortex tube to resuspend beads
6. Place on magnetic stand until supernatant is clear and beads are bound to the magnet
7. While on the stand carefully remove and discard the supernatant without disturbing beads
8. Repeat steps 5 to 7 three more times
9. Add 100µl ***Elution Buffer***. Vortex tube to resuspend beads
10. Add resuspended bead mixture to ***Bead Solution*** as above. Invert and vortex to mix thoroughly

Protocol:

1. Create ***Lysis master mix***. For 1ml: 730µl of ***Lysis Solution***, 250µl of ***Tissue Lysis Additive*** and 20µl of ***PK***. Vortex to mix
2. Place the sample into a 200µl PCR tube. Add 100µl of ***Lysis master mix***. Invert to mix and centrifuge (bench top) briefly (1 to 2 seconds)
3. Incubate at 55°C (Hybard Shake 'n' Stack, Thermo Scientific) for 3 hours. Invert the tubes to mix occasionally throughout
4. Centrifuge (bench top) for 30 seconds. Transfer the supernatant to a 1.5ml Eppendorf tube
5. Add 50µl (or 0.5x volume of ***Lysis master mix***) of ***Flocculant Solution*** and vortex briefly. Then place on ice for 10 minutes
6. Centrifuge (VWR MicroStar12) at 10,000xg for 1 minute. Transfer the supernatant to a fresh 1.5ml Eppendorf tube
7. Add 1.5x volume (***Lysis master mix***) of ***Bead Solution***. Place on Hulamixer (Invitrogen HulaMixer, Thermo Fisher Scientific) at continual rotation for 5 minutes
8. Place on a magnetic stand until the supernatant is clear and beads are bound towards the magnet
9. Whilst on the stand, carefully remove and discard the supernatant without disturbing beads
10. Add 500µl of ***Wash Solution***. Incubate at room temperature for 30 seconds
11. Carefully remove and discard the supernatant without disturbing beads
12. Repeat steps 10 to 11 a further time

13. Centrifuge (bench top) briefly (1 to 2 seconds). Place back on the magnetic stand, ensuring beads are bound towards the magnet. Remove all the remaining ***Wash Solution*** with a 20µl pipette. Air dry the tube with cap open for 30 seconds
14. Add 100µl of ***Elution Buffer*** (55°C) and vortex briefly to resuspend beads. Ensure all beads are resuspended with no clumps. Centrifuge (bench top) briefly (1 to 2 seconds)
15. Place in a thermomixer (ThermoMixer C, Eppendorf) at 55°C for 5 minutes at 750rpm. Centrifuge (bench top) briefly (1 to 2 seconds)
16. Place on the magnetic stand until supernatant is clear and beads are bound towards the magnet
17. Carefully eluate into a fresh Eppendorf tube without disturbing beads

NOTE: Lysis and elution volumes were changed depending on sample size and type:
Lysis: small pollen baskets = 50 uL, medium to large baskets and all swab samples = 100 uL, very large baskets = 200 uL.
Elution: small baskets and all swab samples = 50 uL, medium to very large baskets = 100 uL.

DNA amplification

DNA extracts were amplified using a 2-step nested tagging protocol [modified from Kitson et al. (2015)]. The initial step 25µL PCR reaction contained 12.5µL MiFi^{TM} Mix

(Bioline, UK) 8.5µL molecular-grade water (Fisher Scientific), 1µL of 10µM forward primer [rbcL-Z1af, Hofreiter et al. (2000)]: 5'-ATGTCACCACCAACAGAGACTAAAGC-3') and reverse primer [rbcL-3CR, Macgregor et al. (2018)]: 5'-AGGGGACGACCATACTTGTTCA-3'), followed finally by 2µL of template DNA. This initial PCR amplified the DNA fragment and also added tags to uniquely identify each well within a plate. 12 variants of rbcl-Z1af and 8 variants of rbcl-3CR were combined to produce 96 unique combinations of forward and reverse primer. All PCRs were performed using a thermal cycler (Veriti, 96-well ThermalCycler, Applied Biosystems), with the first PCR using the following conditions: 95°C for 60s followed by 35 cycles of 95°C for 30s, 55°C for 45s, and 72°C for 90s ending with 72°C for 7 minutes. PCR products was then sealed with a drop of mineral oil.

For positive controls, 3 plant species (*Strelitzia reginae, Citrus maxima, Pachypodium lamerei*) were gathered from the greenhouses at Thwaite Botanical Gardens, Cottingham, UK. These plants were chosen as it was deemed highly unlikely that bees would come into contact with such species on farmland. 22 positive controls and 22 negative controls (molecular-grade water) were used.

PCR products were visualised on 1.5% agarose gel and samples were pooled to create a per plate library. Sample contribution to a library was chosen arbitrarily according to band strength, with weak bands contributing 15µl, average strength bands contributing 10µl, and strong bands and negative controls contributing 5µl. Positive controls were pooled at 1µl. Each plate library was then purified using double-size selection with SPRI library prep beads (bead solution 2%; see Working Solutions) to remove nonspecific products and primer dimers. First step of size selection used 0.4 x bead volume of product followed by adding a further 0.4 x.

A second PCR was then performed in order to add the plate-specific tags, providing a unique plate identification and allowing for differentiation between identically located samples on separate plates. The second 50µl PCR contained 25µl MiFi^{TM} Mix (Bioline, UK), 15µL of molecular-grade water (Fisher Scientific), 3µL of 10µM forward and reverse primer, and 4µL of each plate library. PCR conditions were as follows: 95°C for 3 minutes; 10 cycles of 98°C for 20 seconds and 72°C for 1 minute; a final step of 72°C for 5 minutes followed by 4°C for 10 minutes.

The DNA concentration of each pooled plate was then measured using a Qubit 3.0 Fluorometer (Invitrogen, Thermo Scientific), the results of which were used to inform the initial pooling of all plate products into a single library. This single library was then quantified using qPCR, the results of which were used inform the accurate pooling of a single final library from the individual plate libraries. An accurate base-pair length was then obtained for the final library using a tapestation (2200 Tapestation, Agilent Technologies) prior to being sequenced. Sequencing of amplified DNA was performed on an Illumina MiSeq using V3 chemistry loading at 12pM with 10% PhiX.

Bioinformatics & Data Processing

As it was deemed impractical to gather all locally-present flora at every site to the degree that would allow for the coverage of many bees', often large, foraging range, a custom reference database for the rbcL locus was constructed using a list of plants recorded in vice-county 61 (as in Macgregor et al., 2018). An initial BLAST search was used to identify plausible assignments that were not in the list (e.g. closely related garden varieties,

members of the same genus as the positive controls). These sequences were then appended, along with any sequences for plants found during the on-site floral survey that did not appear in the list. Sequences were then downloaded for this appended list. The subsequent database was then checked for any mis-labelled sequences using SATIVA (Kozlov et al., 2016) within the ReproPhylo environment (Szitenberg et al., 2015), using the mafft alignment algorithm (Katoh et al., 2002), resulting in a final number of 1089 plant species in the reference database.

Raw reads were initially demultiplexed according to the unique sample tag combinations using a custom Python script. Individual reads were then quality trimmed at both ends to a minimum phred score Q30 using Trimmomatic v0.32 and then clipped to remove PCR primers where upon all reads below 50bp were discarded. Read pairs were merged into single reads using FLASH v1.2.11 and for any unsuccessfully merged sequences, only the forward was kept. All reads were investigated for chimeric sequences using vsearch v1.1.0 and clustering was performed at 100% similarity (also in vsearch v1.1.0) with only clusters containing a minimum of 3 representative sequences retained. These clusters were then compared to the custom reference database describe above via BLAST and taxonomically assigned using a Last Common Ancestor approach [similar to that employed by the MEGAN software (Huson et al., 2016)] based on the top 10 BLAST matches. Initial matching via BLAST used at least 97% similarity across over 80% of the sequence length. Matches with a bit-score of a maximum 10% deviance from the top hit were retained and their lowest taxonomic level identified.

Once taxonomic assignment had been completed, any risk of contamination was accounted for by applying a 2-stage threshold for read counts based on the positive controls. In the initial stage, the average proportion of any biological sample OTU's reads occurring within the positive controls was applied across all biological samples as a cut-off

for that OTU i.e. each biological OTU occurring within a positive control had its own cut-off read proportion applied across all biological samples. Following this, for the remaining biological OTUs, a blanket proportional read cut-off was calculated by calculating the proportion of total reads across all samples that were assigned to the positive control. This proportion was then applied to each individual biological sample to determine the minimum read count threshold requirement for a positive OTU identification in that sample. As a product of this, some OTUs were removed entirely; these can be seen in Table S10.

5.6.2. Supplementary results

Host plant community similarity - species:

When visualised with NMDS ordination, the 4 clusters observed were not as clear as that seen at the genus level, however, do broadly correspond to farm and sample type (Fig. S1a). Despite this, for both permutation structures (by bee and by visit respectively), separation between sample type (PERMANOVA by bee, $R^2 = 0.033$, $p<0.001$; by visit, $R^2 = 0.033$, $p<0.001$) was still greater than farm type (PERMANOVA by bee, $R^2 = 0.014$, $p<0.001$; by visit, $R^2 = 0.014$, $p<0.001$). When permutations were restricted to within bee species within farm pairs, the plant species community similarity at farm sites was also explained by the two way interactions between farm type and visit month ($R^2 = 0.018$, $p<0.001$) and farm type and sample type ($R^2 = 0.0067$, $p = 0.003$). When restricted to within visit month within farm pairs, similarity was again explained by the two-way

interaction between farm type and sample type (R^2 = 0.0065, p<0.001), in addition to the interaction between farm type and bee species (R^2 = 0.05, p = 0.002).

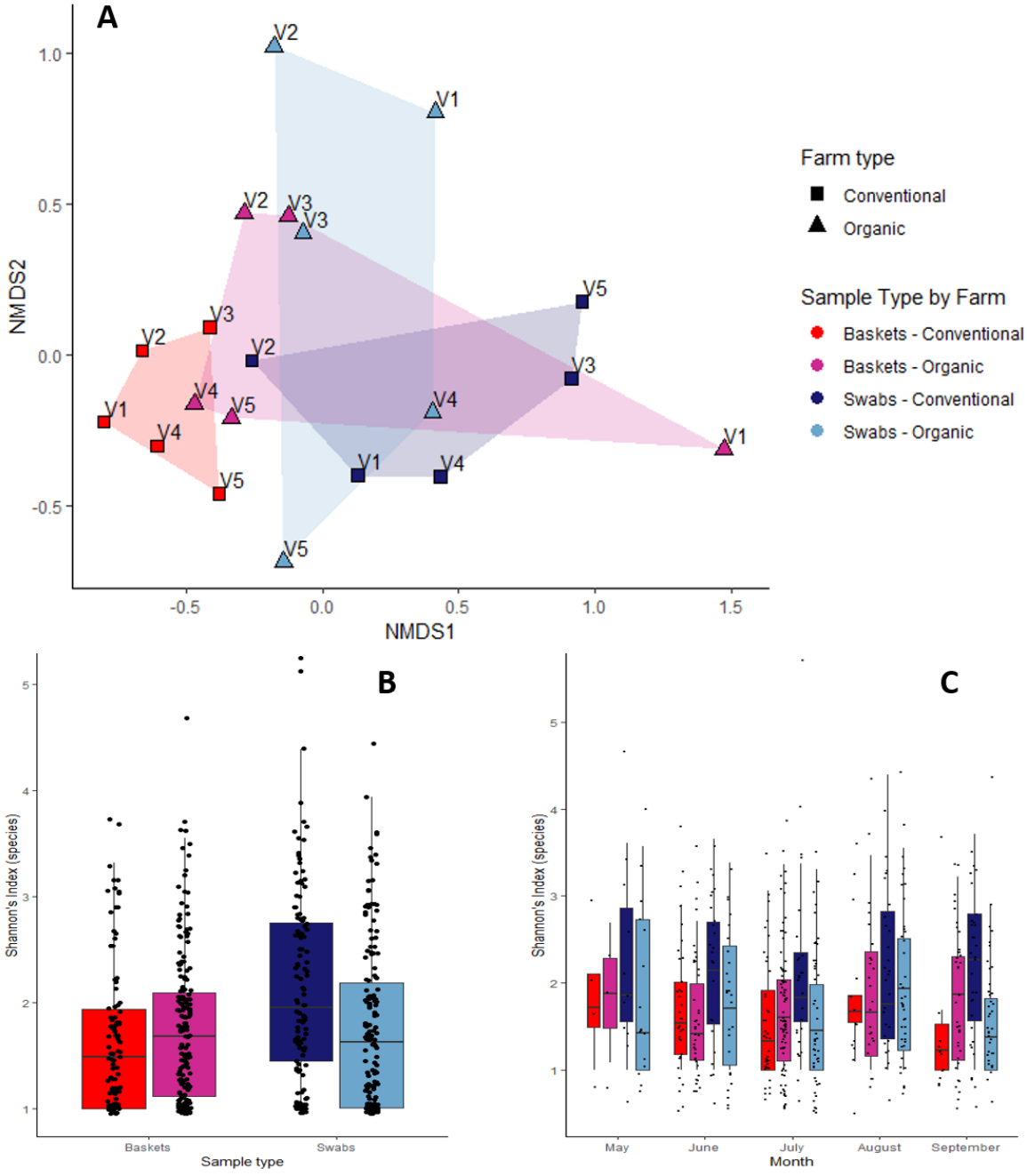


Fig. S1. Plant species community composition. Colour shades represent sample type; swabs (Blue) & baskets (Red). (A) NMDS plot for plant species found across farming practices for

both swab and baskets sample. (B) Boxplot of Shannon's Index by farm type & sample type pooled across the sampling season. (C) Boxplot of Shannon's Index by farm type and sample type split by sampling month. Points represent plant DNA from pollen taken from an individual bee sample.

Host plant community similarity - family:

When visualised with NMDS ordination, as with the plant genus level, the 4 clusters observed correspond to sample type and, to a lesser degree, farming type at the plant family level (Fig. S2a). For both permutation structures (by bee and by visit respectively), separation between sample type (PERMANOVA by bee, $R^2 = 0.11$, $p<0.001$; by visit, $R^2 = 0.11$, $p<0.001$) was greater than farm type (PERMANOVA by bee, $R^2 = 0.014$, $p<0.001$; by visit, $R^2 = 0.014$, $p<0.001$). At the plant family level, all two-way interactions were significant (PERMANOVA, farm type:sample type, $R^2 = 0.011$, $p<0.001$; PERMANOVA, farm type:visit month, $R^2 = 0.031$, $p<0.001$; PERMANOVA, sample type:visit month, $R^2 = 0.013$, $p = 0.015$) under the within bee species within farm pair permutation structure. Under the within visit month within farm pair permutation structure, however, only the two two-way interactions involving farm type were significant (PERMANOVA, farm type:sample type, $R^2 = 0.011$, $p<0.001$; PERMANOVA, farm type:bee species, $R^2 = 0.054$, $p<0.001$).

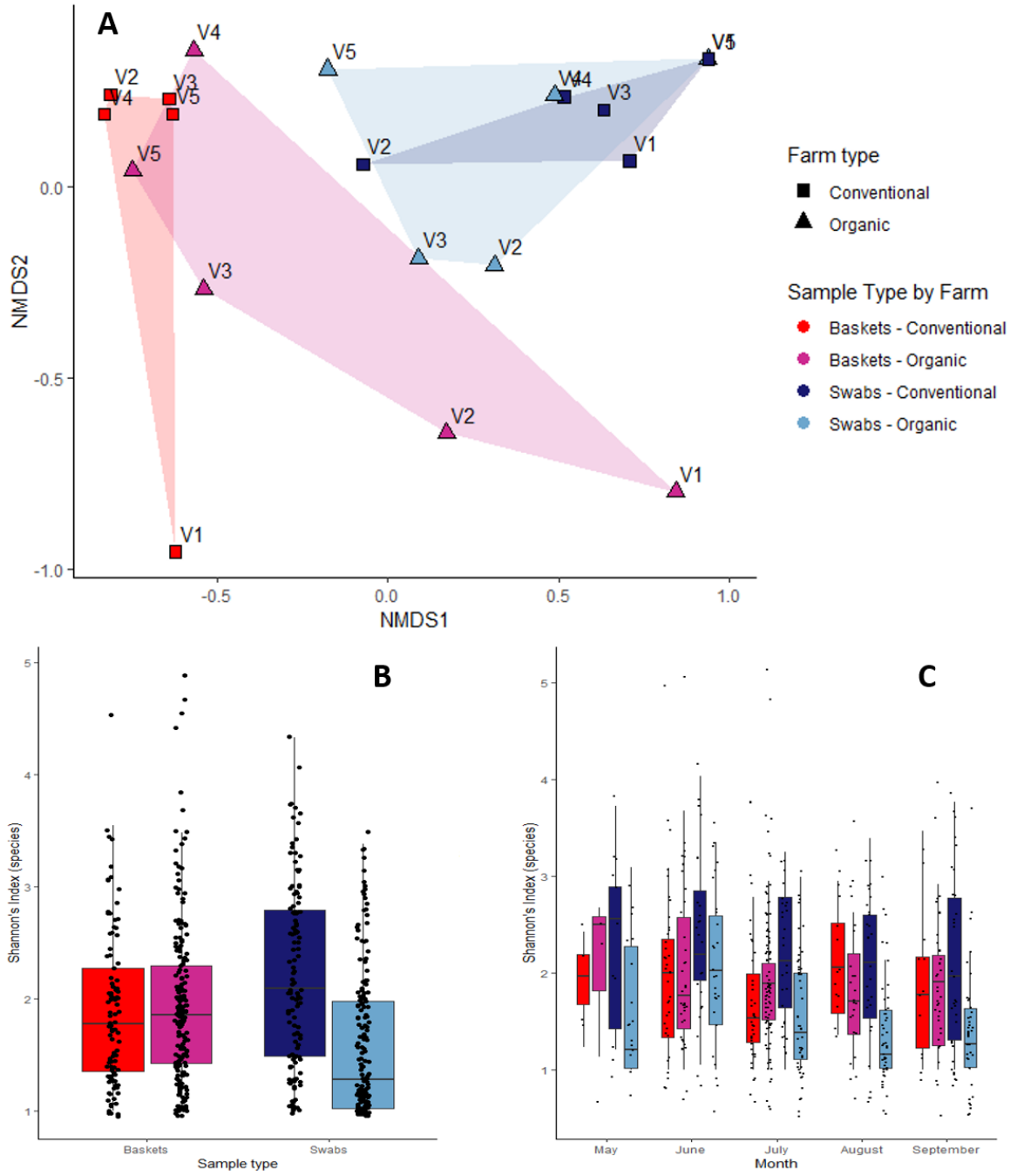


Fig. S2. Plant family community composition. Colour shades represent sample type; swabs (Blue) & baskets (Red). (A) NMDS plot for plant families found across farming practices for both swab and baskets sample. (B) Boxplot of Shannon's Index by farm type & sample type pooled across the sampling season. (C) Boxplot of Shannon's Index by farm type and sample type split by sampling month. Points represent plant DNA from pollen taken from an individual bee sample.

Host plant community indices: conventional swabs consistently show higher richness & diversity

Richness:

Swab samples how higher species richness on conventional farms as opposed to organic farms at all taxonomic levels analysed, but swabs only showed higher plant richness in comparison to baskets at conventional sites (Fig. S3). Looking at baskets alone however, there is little difference in richness when comparing organic and conventional sites (Fig. S3). At the plant species level richness was affected by the interaction of farming type with sample type (GLMM specifying a Poisson family and a log link function, $\chi^2_{1,10}$ = 7.95, p = 0.005). This interaction persisted when rolled to genus level (GLMM, $\chi^2_{1,10}$ = 20.97, p<0.001) and up to family level (GLMM, $\chi^2_{1,10}$ = 22.88, p<0.001). When split by visit month and farm type conventional swabs only show higher plant *species* richness during August and September (Fig. S3), however, show increased richness across all seasons when rolled to the genus or family level (Fig. S3).

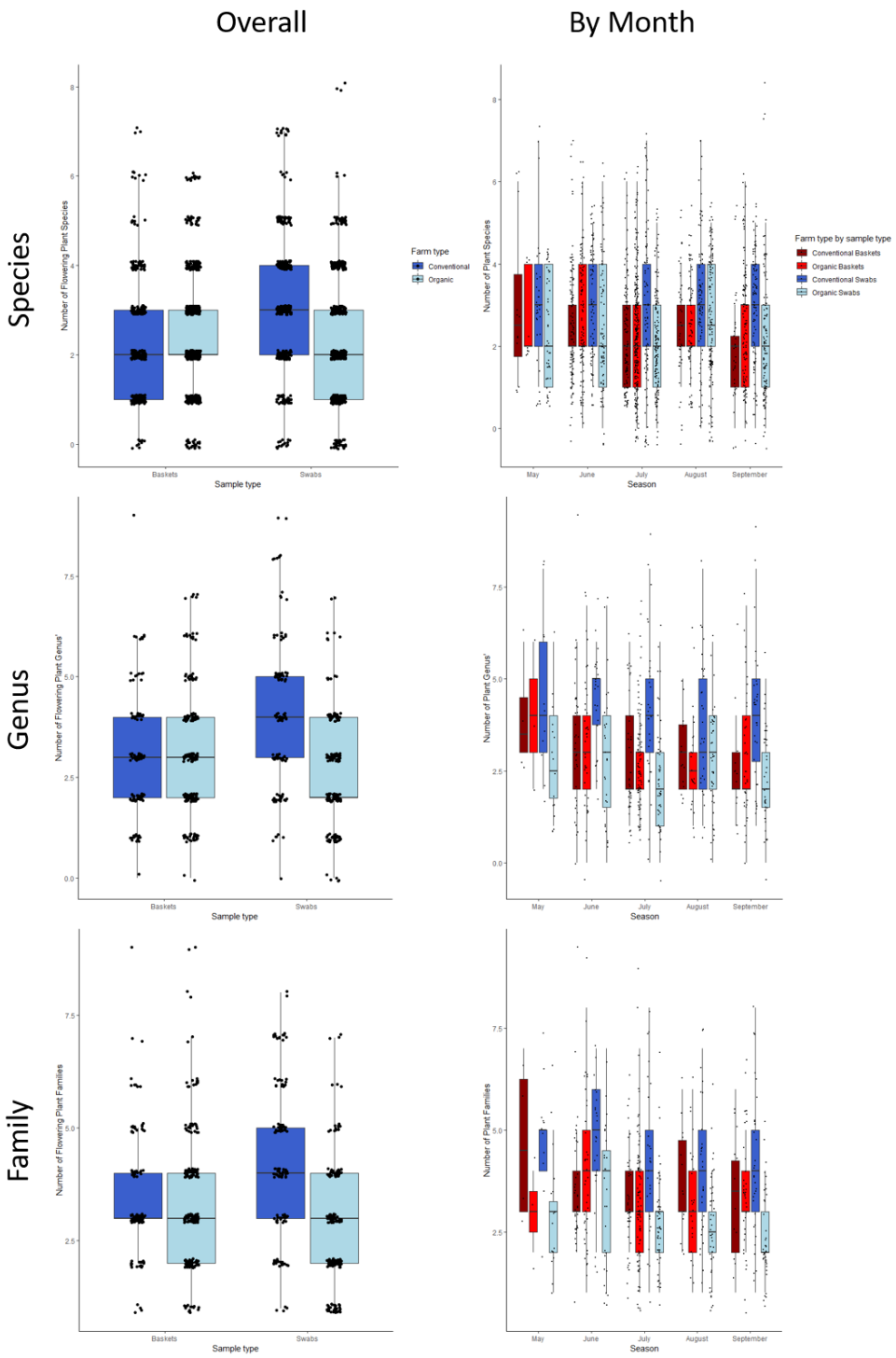


Fig. S3 - Boxplots of species richness by farm type & sample type. Columns represent level of pooling; across the entire sampling season (Overall) or by month. Rows represent taxonomic levels. Points represent plant DNA from pollen taken from an individual bee sample.

Simpson's Index:

Swabs tend to have higher index values compared to baskets on conventional farms but not on organic farms, and swabs (but not baskets) from conventional farms show higher index values than those from organic farms (Fig. S4). Additionally, conventional sites trended towards higher index values across months compared to organic farms (Fig. S4). Simpson's Index was affected by the interaction of farming type with sample type at the plant species level (GLMM, $\chi^2_{1,11} = 16.02$, $p<0.001$). However, at the genus level models revealed a significant 3-way interaction between farm type, sample type and season (GLMM, $\chi^2_{4,23} = 11.93$, $p = 0.018$), and at the family level both two-way interactions with farm type were significant (GLMM, farm type: sample type, $\chi^2_{1,15} = 16.85$, $p<0.001$; farm type:visit month, $\chi^2_{4,15} = 10.28$, $p = 0.036$).

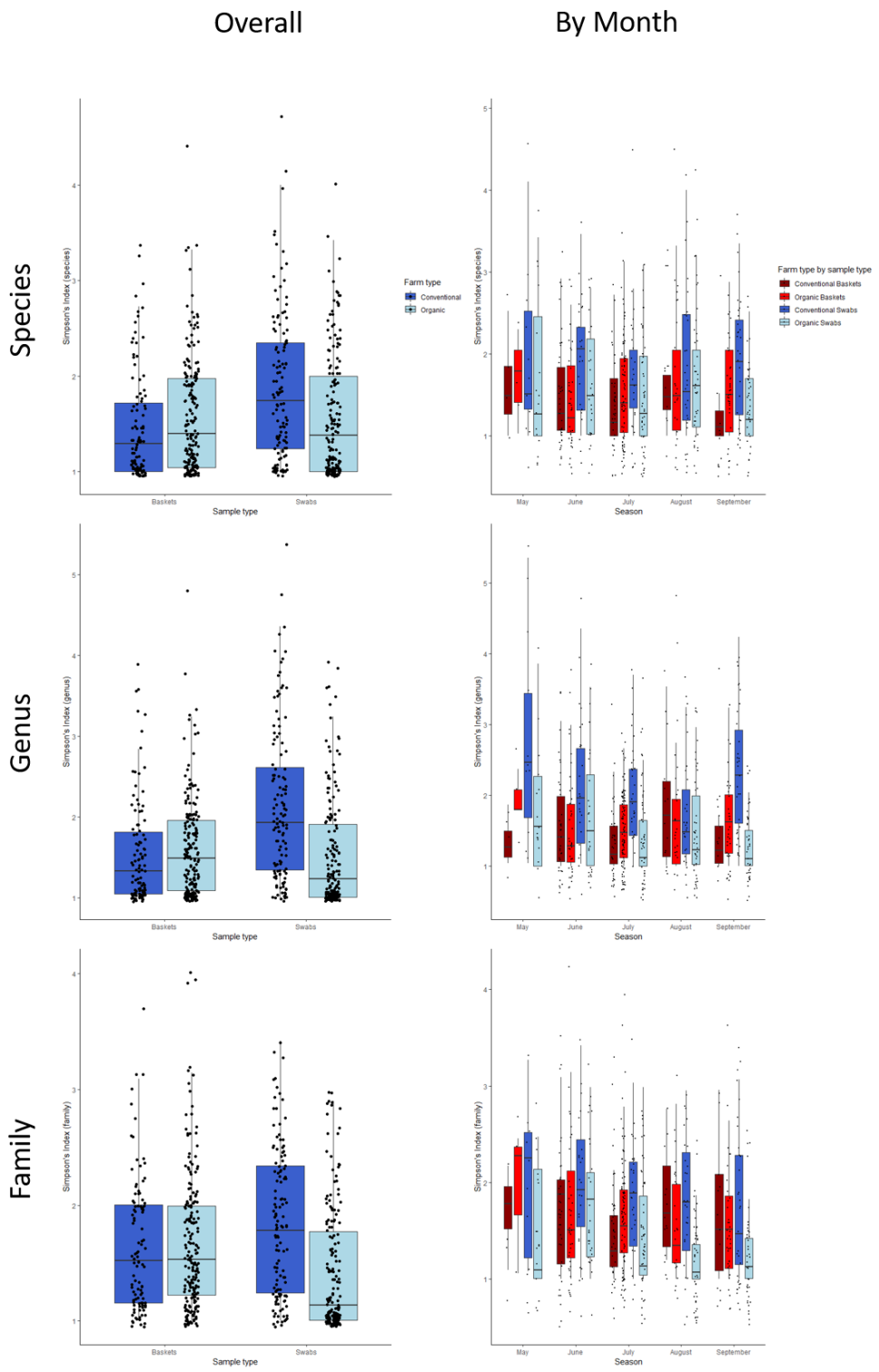


Fig. S4 - Boxplots of Simpson's Index by farm type & sample type. Columns represent level of pooling; across the entire sampling season (Overall) or by month. Rows represent taxonomic levels. Points represent plant DNA from pollen taken from an individual bee sample.

Shannon's Index (Species & Family):

Conventional swabs showed higher values than their equivalent basket samples, as well as when compared to swabs from their organic counterparts, at both the species and family level, with little variation seen between farm types in relation to basket samples (Fig. S1a, S2a). When split by visit month, swabs from conventional sites consistently show higher Shannon's index values than swabs from organic sites at both species (Fig. S1c) and family level (though more extremely at the family level; Fig. S2c). This is also true when compared to their equivalent baskets, though, again, this is more marked at the family level than at the species level (Fig. S1c, S2c). Shannon's Index was affected by the interaction of farming type with sample type at the plant species level (GLMM, $\chi^2_{1,11}$ = 16.02, $p<0.001$), with swabs on organic sites having lower values than those on conventional sites. This was true also at the family level (GLMM, $\chi^2_{1,11}$ = 37.75, $p<0.001$).

Bee-flower networks: description

The networks visualised at the plant family level support those findings at the plant genus level, with clear differences seen between swab and basket samples on both farm types (Fig, S5, 6). Also, as with the plant genus level, there was little difference between swabs from conventional and organic farms (Fig. S7) but noticeable difference between baskets samples (Fig. S8).

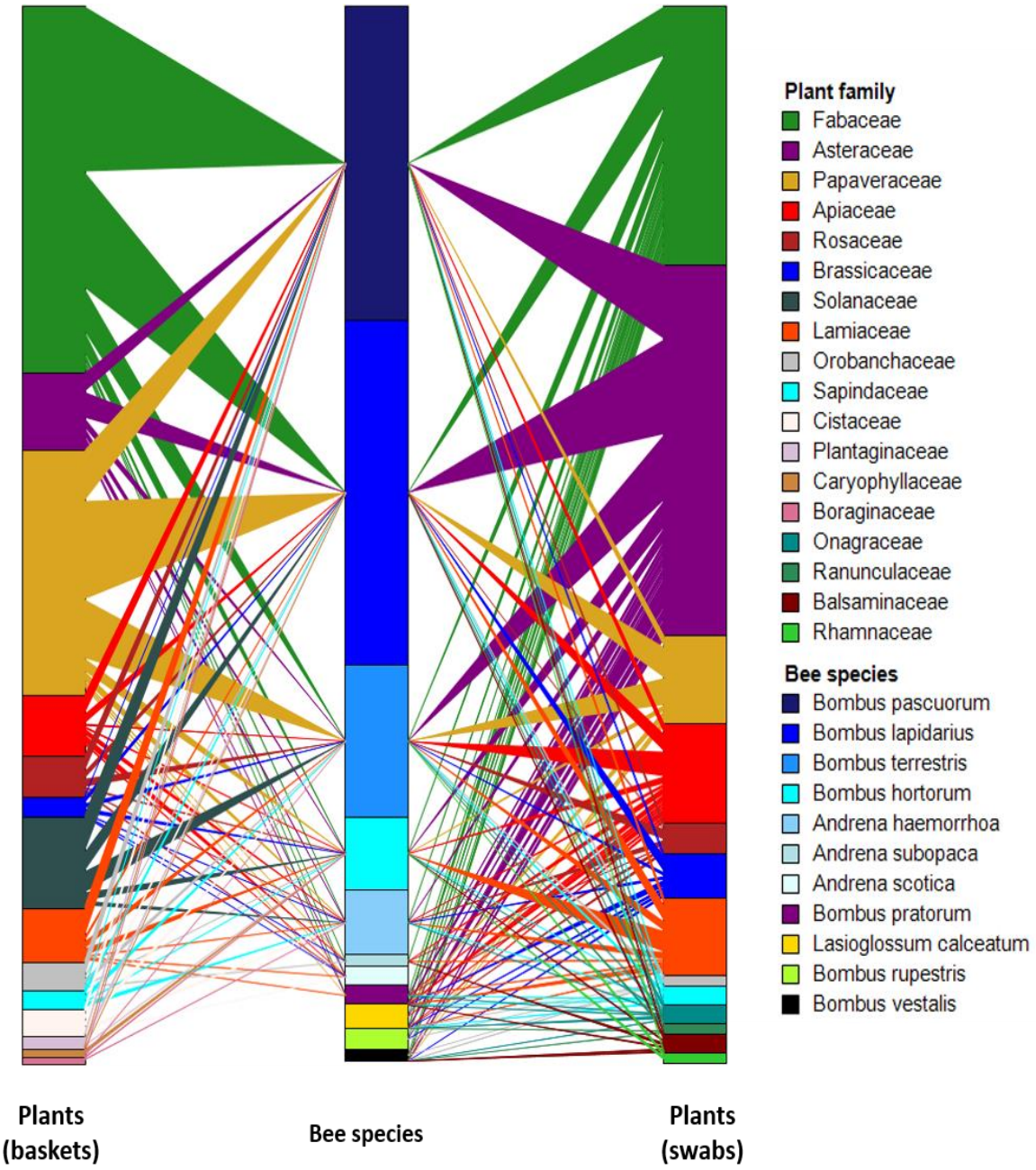


Fig. S5. Network constructed from pollen taken from bees on organic farms, split between swab and basket sample. Differing plant families are represented by differing colours and bees are coloured by species (see key). Plant families occur top to bottom as shown in the key. For bees bar depth denotes the species abundance. Bar depth for plants denotes the incidence of DNA occurrence on bees. Links represent the proportion of each plant family found on each bee species.

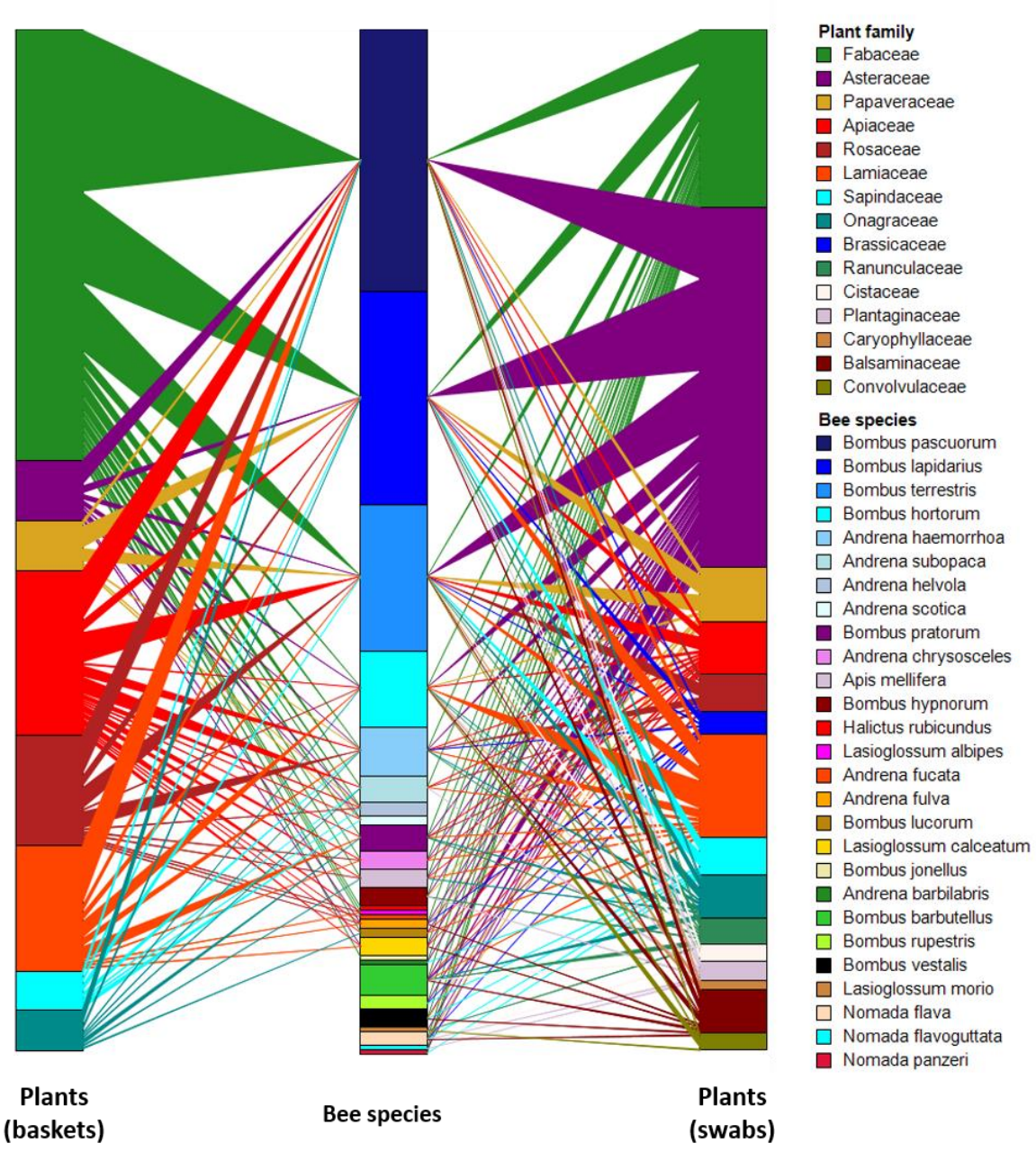


Fig. S6. Network constructed from pollen taken from bees on conventional farms, split between swab and basket sample. Differing plant families are represented by differing colours and bees are coloured by species (see key). Plant families occur top to bottom as shown in the key. For bees bar depth denotes the species abundance. Bar depth for plants denotes the incidence of DNA occurrence on bees. Links represent the proportion of each plant family found on each bee species.

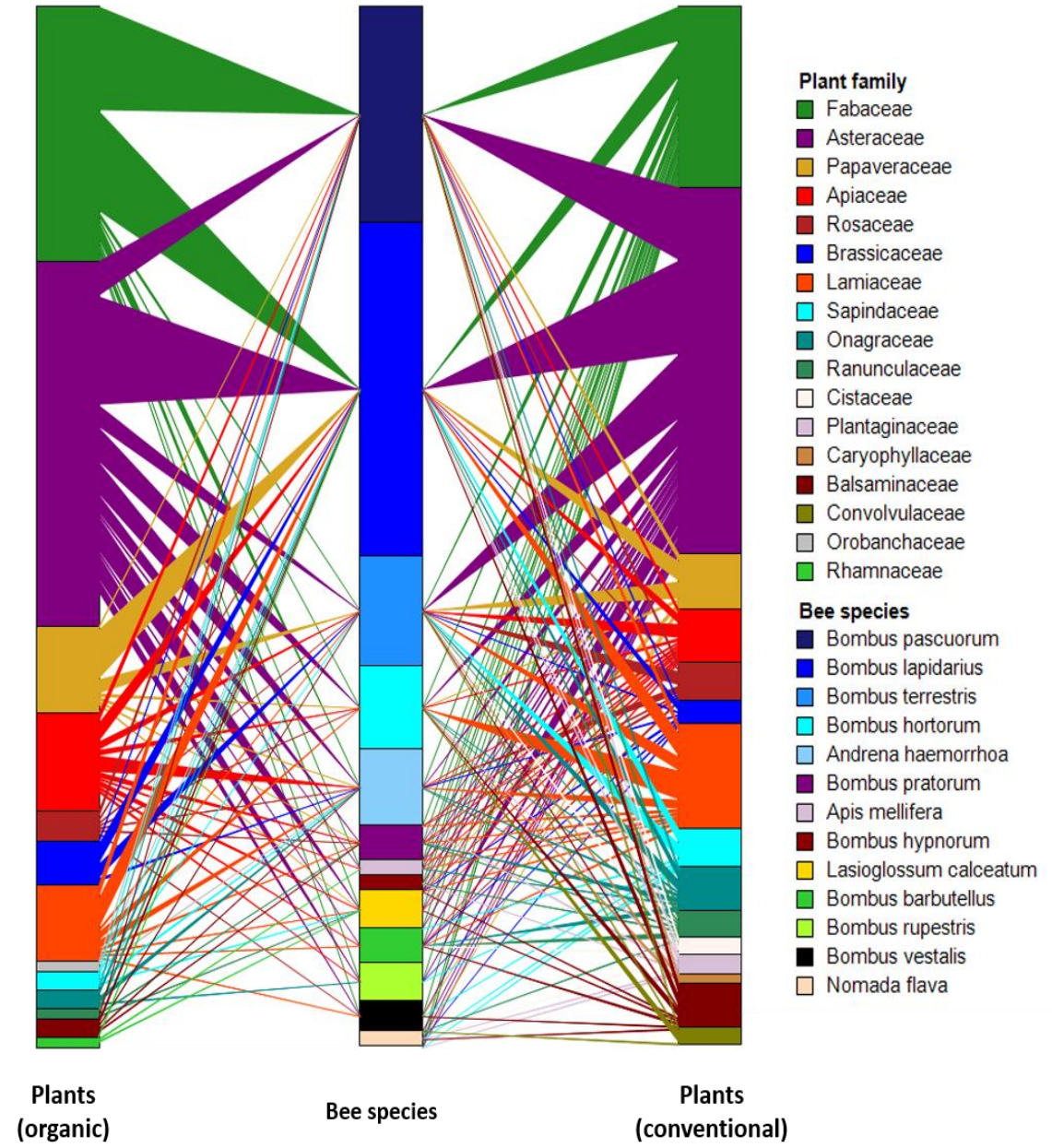


Fig. S7. Network constructed from pollen analysed via swabs from bees, split by farming practice. Differing plant families are represented by differing colours and bees are coloured by species (see key). Plant families occur top to bottom as shown in the key. For bees bar depth denotes the species abundance. Bar depth for plants denotes the incidence of DNA occurrence on bees. Links represent the proportion of each plant family found on each bee species.

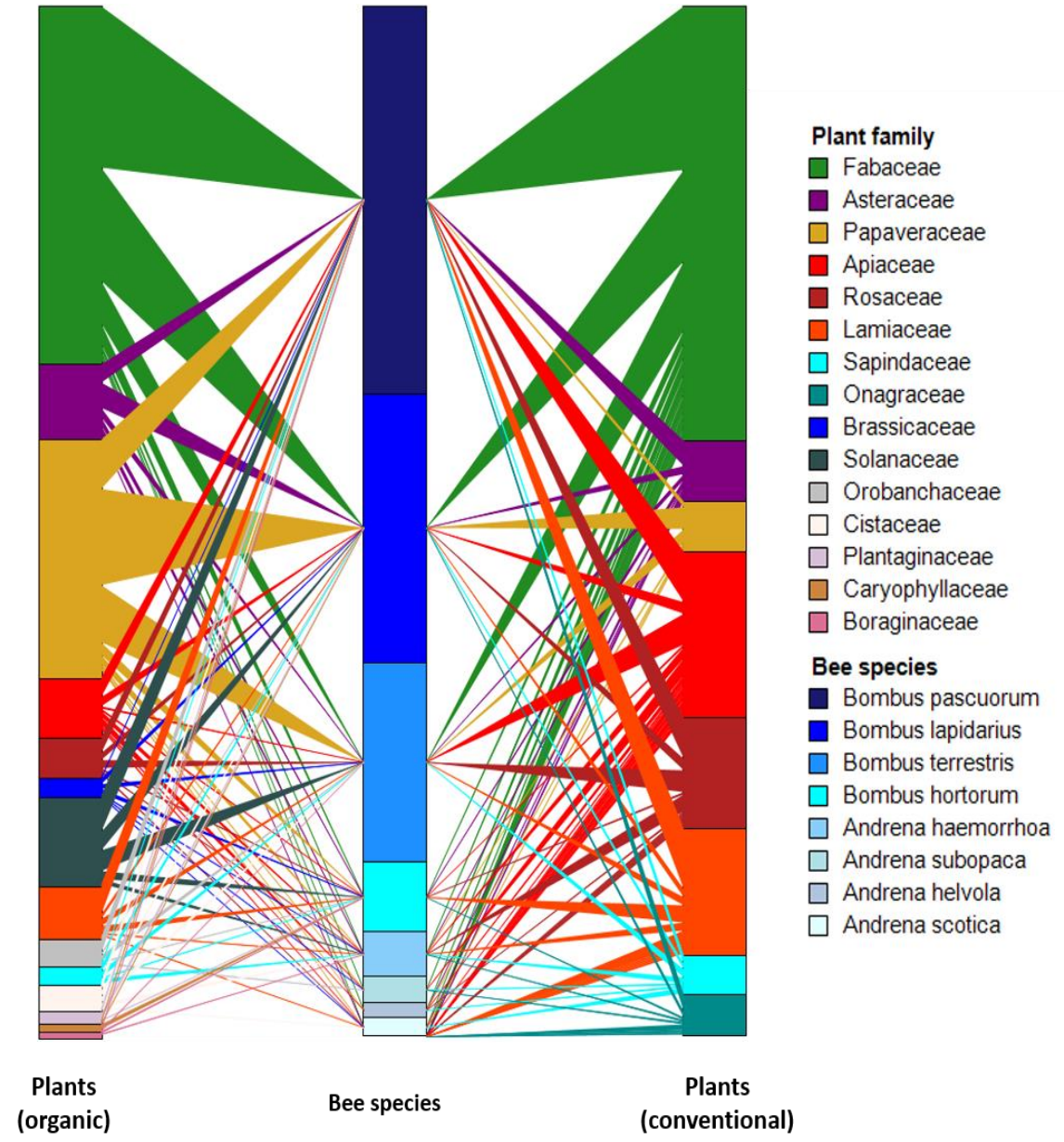


Fig. S8. Network constructed from pollen analysed from pollen baskets taken from bees, split by farming practice. Differing plant families are represented by differing colours and bees are coloured by species (see key). Plant families occur top to bottom as shown in the key. For bees bar depth denotes the species abundance. Bar depth for plants denotes the incidence of DNA occurrence on bees. Links represent the proportion of each plant family found on each bee species.

Bee-flower network metrics - overall

At the plant species level connectance appeared slightly higher for baskets when compared to swabs, a pattern that persisted when split across farm types (Fig. S9a,d). Linkage density appeared higher for swabs than baskets, however, when split by farm type this seemed to be driven by conventional sites, with organic baskets actually showing slightly higher linkage density than organic swabs (Fig. S9b,e). Generality was higher for swabs than for baskets, a trend that persisted when split between farming practice (Fig. S9c,f)

Sample type had no influence on any of the network metrics at the plant species level, with all metrics (bar nestedness which was not affected by any predictor variables across any taxonomic levels) being influenced by some or all of the other predictor variables. Linkage density was affected by plant species richness (LMM, χ^2=37.9, p<0.001), with linkage density increasing with the number of plant species. Network connectance was influenced by bee species richness only (LMM, χ^2=27.51, p<0.001), with connectance decreasing with increasing bee species richness. The same was true for generality (LMM, χ^2=26.46, p<0.001), however, generality increased with increasing bee richness.

At the plant genus level sample type did not influence linkage density or connectance. As at the plant species level, linkage density was influenced by plant richness (LMM, χ^2=34.13, p<0.001), with increased plant genus richness leading to increased linkage density. However, it was also influenced by bee species richness (LMM, χ^2=4.6, p=0.032), with linkage density decreasing with increasing bee species richness. Again, as with the plant species level, connectance was influenced by bee species richness only (LMM, χ^2=23.76, p<0.001), with connectance decreasing with increasing bee richness.

At the plant family level, as across other taxonomic levels, connectance was slightly higher for baskets, similarly so when split between farming practice (Fig. S10a,d). Swabs also showed slightly higher linkage density, however, when split across farming practice this was only true for conventional farms, with organic farms showing higher levels for baskets (Fig. S10b,e). Generality was also higher for swabs, a pattern maintained when split across farming type. (Fig. S10c,f).

At the plant family level sample type did not influence linkage density or generality. Similar to that found at the plant genus level, linkage density was influenced by both plant richness (LMM, χ^2=22.67, p<0.001) and bee richness (LMM, χ^2=3.97, p=0.046), with linkage density increasing with increasing plant family richness but then also increasing with increasing bee richness. As at the plant species level, and similar to that seen at the plant genus level, network generality was influenced by bee richness (LMM, χ^2=53.56, p<0.001), with generality increasing with increased bee richness.

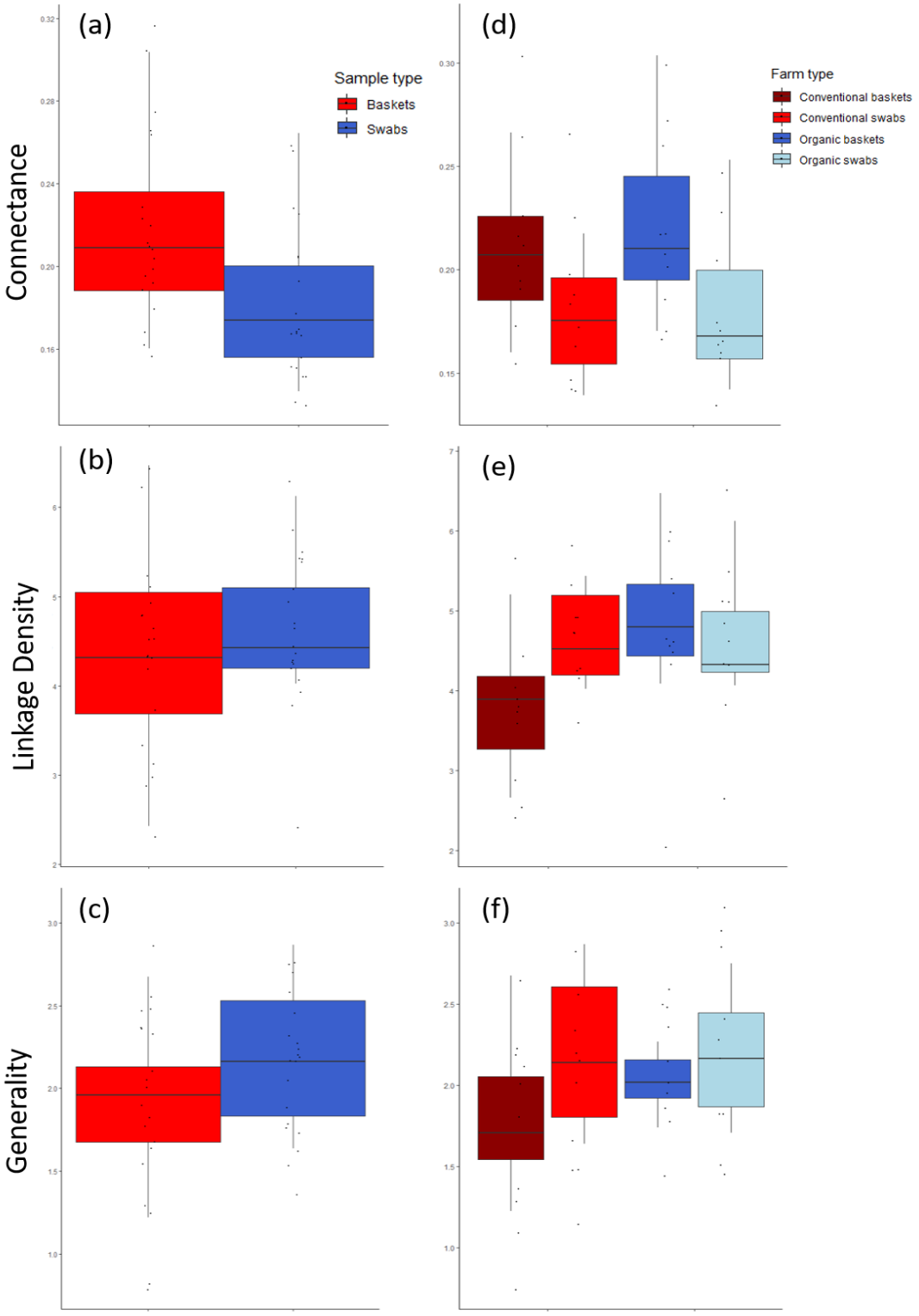


Fig. S9. Metric values, based on plant species, by sample type only (left) and by farm type and sample type (right). (a) and (d) network connectance, (b) and (e) network linkage density, (c) and (f) network generality

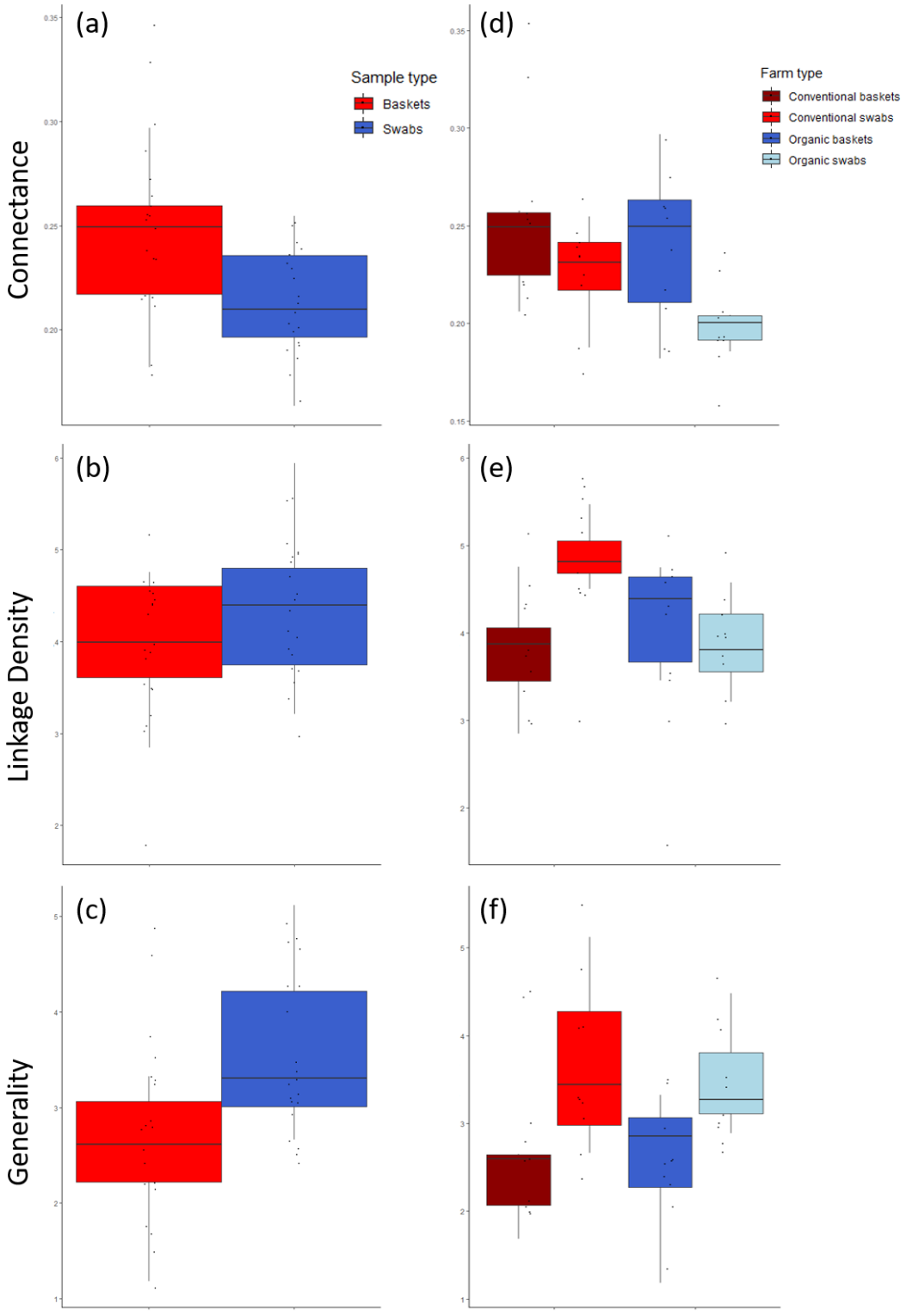


Fig. S9. Metric values, based on plant family, by sample type only (left) and by farm type and sample type (right). (a) and (d) network connectance, (b) and (e) network linkage density, (c) and (f) network generality

Bee-flower network metrics - by farm type

No effect of sample type was found on any of the network metrics on organic farms at the plant species level, nor on connectance or generality on conventional farms. Linkage density on organic farms was influenced by plant species richness only (LMM, χ^2=13.66, p<0.001), with linkage density increasing with increased plant richness. Connectance was influenced by bee species richness on both conventional (LMM, χ^2=16.48, p<0.001) and organic (LMM, χ^2=11.07, p<0.001) farms, with connectance decreasing with increased bee richness in both cases. Network generality also was influenced only by bee species richness in both systems (Conventional: LMM, χ^2=19.09, p<0.001; Organic: LMM, χ^2=7.63, p=0.006), with generality increasing with increased bee richness.

At the plant genus level no effect of sample type was found on linkage density on organic farms, nor on connectance and generality on conventional farms. As with the plant species level, linkage density at the plant genus level on organic farms was influenced by plant genus richness only (LMM, χ^2=16.77, p<0.001), with linkage density increasing with increasing plant genus richness. Again, as with at plant species level, connectance on conventional farms was influenced by bees species richness only (LMM, χ^2=16.97, p<0.001), with connectance decreasing with increasing bee richness. However, it is worth noting that there was a marginal effect of sample type (LMM, χ^2=3.15, p=0.07). Similarly with generality, only bee species richness was shown to have an effect (LMM, χ^2=36.66, p<0.001), with generality increasing with increasing bee richness.

At the plant family level we found no effect of sample type on linkage density on conventional farms or on generality in both farming systems. Linkage density on conventional farms was influenced by plant family richness only (LMM, χ^2=8.04, p=0.005), losing the interaction with sample type seen at the genus and species level, with

linkage density increasing with increasing plant richness. Generality was influenced by bee species richness in both farming systems (Conventional: LMM, χ^2=38.58, p<0.001; Organic: LMM, χ^2=22.23, p<0.001), with generality increasing with increasing bee richness in both cases. On conventional farms only, generality was also influenced by plant family richness (LMM, χ^2=5.06, p=0.024), with generality decreasing with increasing plant family richness.

Table S1. Number of basket and swab samples used in DNA analysis split by farm

Site	Farm type	No. basket samples	No. swab samples
MLS	Conventional	10	15
BES	Conventional	9	12
BEE	Conventional	4	11
LIT	Conventional	9	12
FRE	Conventional	12	14
BEC	Conventional	17	16
WAL	Conventional	8	9
HAS	Conventional	4	15
BXA	Conventional	15	9
THR	Conventional	17	12
DEN	Organic	12	17
CAR	Organic	7	18
BAR	Organic	17	16
COL	Organic	2	13
STR	Organic	31	18

BRO	Organic	21	20
YOR	Organic	38	23
STO	Organic	18	10
THO	Organic	25	18
LEA	Organic	24	22

Table S2. Number of basket samples used in DNA analysis split by farm and by visit month

	Conventional									
	BEC	**BEE**	**BES**	**BXA**	**FRE**	**HAS**	**LIT**	**MLS**	**THR**	**WAL**
May	0	0	0	0	0	0	0	1	2	1
Jun	7	2	2	6	8	2	2	0	7	1
Jul	7	1	6	7	4	0	1	5	2	5
Aug	1	1	0	1	0	1	5	4	0	1
Sep	2	0	1	1	0	1	1	0	6	0
	Organic									
	BAR	**BRO**	**CAR**	**COL**	**DEN**	**LEA**	**STO**	**STR**	**THO**	**YOR**
May	1	2	0	0	0	0	0	0	0	0
Jun	13	5	2	1	5	0	0	12	0	1
Jul	1	11	2	0	2	8	13	17	14	24
Aug	2	1	1	1	3	7	3	2	2	2
Sep	0	2	2	0	2	9	2	0	9	11

Table S3. Number of swab samples used in DNA analysis split by farm and by visit month

	Conventional									
	BEC	**BEE**	**BES**	**BXA**	**FRE**	**HAS**	**LIT**	**MLS**	**THR**	**WAL**
May	1	2	1	0	1	2	0	1	3	0
Jun	4	2	1	5	3	1	3	1	3	0
Jul	5	1	3	3	5	1	3	2	2	5
Aug	2	0	2	1	5	6	4	6	1	2
Sep	4	6	5	0	0	5	2	5	3	2
	Organic									
	BAR	**BRO**	**CAR**	**COL**	**DEN**	**LEA**	**STO**	**STR**	**THO**	**YOR**
May	2	3	0	4	2	1	1	1	1	1
Jun	4	4	2	3	3	3	0	6	1	1
Jul	3	5	5	0	5	7	4	7	6	7
Aug	3	5	6	3	4	4	3	2	6	8
Sep	4	3	5	3	3	7	2	2	4	6

Table S4. Contribution of each bee species used in DNA analysis to both swab samples and baskets samples

	Baskets	Swabs
Apis mellifera	2	4
Andrena minutula	1	0
Andrena barbilabris	0	1
Andrena chrysosceles	2	2
Andrena fucata	2	2
Andrena fulva	1	2
Andrena haemorrhoa	12	20
Andrena helvola	4	0
Andrena scotica	5	3
Andrena subopaca	8	3
Bombus barbutellus	0	9
Bombus campestris	0	1
Bombus hortorum	18	22
Bombus hypnorum	3	4
Bombus jonellus	1	0
Bombus lapidarius	74	87

Bombus lucorum	1	3
Bombus pascuorum	104	57
Bombus pratorum	3	9
Bombus rupestris	0	10
Bombus terrestris	54	29
Bombus vestalis	0	8
Coelioxys rufescens	0	1
Halictus rubicundus	2	0
Hylaeus communis	0	1
Lasioglossum albipes	1	3
Lasioglossum calceatum	2	10
Lasioglossum morio	0	1
Nomada flava	0	4
Nomada flavoguttata	0	2
Nomada panzeri	0	2

Table S5. List of unique plant taxa (species and genera) across farming practices and sample types

Organic	Conventional	Swabs	Baskets
Aegopodium podagraria	*Betonica officinalis*	*Bellis perennis*	*Aegopodium podagraria*
Bellis perennis	*Calendula officinalis*	*Cakile maritima*	*Betonica officinalis*
Cakile maritima	*Calluna vulgaris*	*Calendula officinalis*	*Calluna vulgaris*
Capsella bursa-pastoris	*Cardamine pratensis*	*Calystegia soldanella*	*Capsella bursa-pastoris*
Digitalis purpurea	*Centaurea cyanus*	*Cardamine pratensis*	*Crepis vesicaria*
Epilobium parviflorum	*Chamerion angustifolium*	*Centaurea cyanus*	*Daucus carota*
Galeopsis tetrahit	*Crepis vesicaria*	*Crepis paludosa*	*Digitalis purpurea*
Geranium lucidum	*Daucus carota*	*Dioscorea communis*	*Galeopsis tetrahit*
Hypericum perforatum	*Dioscorea communis*	*Epilobium parviflorum*	*Geranium dissectum*
Ilex aquifolium	*Fagus sylvatica*	*Fagus sylvatica*	*Geranium lucidum*
Leontodon hispidus	*Geranium dissectum*	*Hylotelephium telephium*	*Hypericum perforatum*
Ligustrum vulgare	*Hylotelephium telephium*	*Hypochaeris radicata*	*Ilex aquifolium*
Oenanthe lachenalii	*Hypochaeris radicata*	*Leontodon hispidus*	*Orchis mascula*
Orchis mascula	*Lysimachia vulgaris*	*Menyanthes trifoliata*	*Ranunculus sardous*
Phacelia tanacetifolia	*Ranunculus acris*	*Pyrola minor*	*Reseda lutea*

Pyrola minor	*Reseda lutea*	*Ranunculus acris*	*Rosa sherardii*
Ranunculus sardous	*Salix cinerea*	*Rhamnus cathartica*	*Sisymbrium orientale*
Rhamnus cathartica	*Sisymbrium orientale*	*Rubus armeniacus*	*Trifolium incarnatum*
Rosa canina	*Solanum lycopersicum*	*Salix cinerea*	*Trifolium striatum*
Rosa sherardii	*Trifolium micranthum*	*Sambucus nigra*	*Trifolium suffocatum*
Rubus armeniacus	*Viburnum opulus*	*Scutellaria galericulata*	*Viburnum opulus*
Sambucus nigra	*Betonica*	*Serratula tinctoria*	*Vicia villosa*
Scutellaria galericulata	*Calendula*	*Solanum lycopersicum*	*Aegopodium*
Serratula tinctoria	*Calluna*	*Trifolium medium*	*Betonica*
Tilia x europaea	*Cardamine*	*Vicia sepium*	*Calluna*
Trifolium incarnatum	*Chamerion*	*Bellis*	*Capsella*
Trifolium medium	*Daucus*	*Cakile*	*Daucus*
Trifolium striatum	*Dioscorea*	*Calendula*	*Digitalis*
Trifolium suffocatum	*Fagus*	*Calystegia*	*Galeopsis*
Vicia sativa	*Filipendula*	*Cardamine*	*Geranium*
Vicia sepium	*Hylotelephium*	*Dioscorea*	*Hypericum*
Aegopodium	*Hypochaeris*	*Epilobium*	*Ilex*
Bellis	*Lysimachia*	*Fagus*	*Orchis*

Cakile	*Prunus*	*Filipendula*	*Prunus*
Capsella	*Reseda*	*Hylotelephium*	*Reseda*
Digitalis	*Salix*	*Hypochaeris*	*Sisymbrium*
Epilobium	*Sisymbrium*	*Leontodon*	*Viburnum*
Galeopsis	*Viburnum*	*Menyanthes*	
Hypericum		*Pyrola*	
Ilex		*Rhamnus*	
Leontodon		*Rubus*	
Ligustrum		*Salix*	
Oenanthe		*Sambucus*	
Orchis		*Scutellaria*	
Phacelia		*Serratula*	
Pyrola		*Viola*	
Rhamnus			
Rosa			
Rubus			
Sambucus			
Scutellaria			
Serratula			

Tilia			
Viola			

Table S6. List of bee species across farming practices and sample types

Organic	Conventional	Swabs	Baskets
Bombus lapidarius	*Bombus lapidarius*	*Bombus lapidarius*	*Bombus lapidarius*
Andrena subopaca	*Andrena subopaca*	*Andrena subopaca*	*Andrena subopaca*
Bombus terrestris	*Bombus terrestris*	*Bombus terrestris*	*Bombus terrestris*
Andrena helvola	*Andrena helvola*	*Lasioglossum albipes*	*Lasioglossum albipes*
Lasioglossum albipes	*Lasioglossum albipes*	*Lasioglossum calceatum*	*Lasioglossum calceatum*
Lasioglossum calceatum	*Lasioglossum calceatum*	*Bombus pascuorum*	*Bombus pascuorum*
Bombus pascuorum	*Bombus pascuorum*	*Andrena scotica*	*Andrena scotica*
Andrena scotica	*Andrena scotica*	*Andrena fulva*	*Andrena fulva*
Andrena fulva	*Andrena fulva*	*Bombus hortorum*	*Bombus hortorum*
Bombus hortorum	*Bombus hortorum*	*Apis mellifera*	*Apis mellifera*
Apis mellifera	*Apis mellifera*	*Andrena fucata*	*Andrena fucata*
Andrena fucata	*Andrena fucata*	*Bombus pratorum*	*Bombus pratorum*

Bombus pratorum	*Bombus pratorum*	*Bombus lucorum*	*Bombus lucorum*
Bombus lucorum	*Bombus lucorum*	*Andrena chrysosceles*	*Andrena chrysosceles*
Halictus rubicundus	*Halictus rubicundus*	*Andrena haemorrhoa*	*Andrena haemorrhoa*
Andrena haemorrhoa	*Andrena haemorrhoa*	*Bombus hypnorum*	*Bombus hypnorum*
Nomada panzeri	*Nomada panzeri*	*Nomada panzeri*	*Andrena helvola*
Bombus vestalis	*Bombus vestalis*	*Bombus vestalis*	*Halictus rubicundus*
Bombus barbutellus	*Bombus barbutellus*	*Bombus barbutellus*	*Bombus jonellus*
Bombus rupestris	*Bombus rupestris*	*Lasioglossum morio*	*Andrena minutula*
Bombus hypnorum	*Bombus hypnorum*	*Andrena barbilabris*	
Nomada flavoguttata	*Nomada flavoguttata*	*Bombus rupestris*	
Nomada flava	*Nomada flava*	*Nomada flavoguttata*	
Andrena minutula	*Andrena chrysosceles*	*Nomada flava*	
Hylaeus communis	*Bombus jonellus*	*Hylaeus communis*	
Coelioxys rufescens	*Lasioglossum morio*	*Coelioxys rufescens*	
	Andrena barbilabris		

Table S7. Network metrics for each farm site by sample type (swab or basket) at the species level

Farm	Organic/ Conventional (O/C)	Farm Pair	Nestedness	Linkage Density	Connectance	Generality (Bees)	Basket/ Swab (S/B)
BEC	C	P6	0.2912022	4.459962	0.1393738	2.225678	S
LIT	C	P4	0.0314102	4.397242	0.1758897	2.7081	S
MLS	C	P1	0.4536376	5.257257	0.1752419	2.5032	S
THR	C	P10	0.1121648	5.439008	0.2175603	1.9366	S
BEE	C	P3	0.2363785	4.136481	0.1654592	1.7582	S
BES	C	P2	0.6218453	5.02384	0.2644126	1.7047	S
BXA	C	P9	0.4990676	5.334934	0.1975902	1.6379	S
HAS	C	P8	0.2542957	4.588398	0.1480128	2.8660	S
WAL	C	P7	0.3469382	4.022606	0.1915527	2.0494	S
FRE	C	P5	0.2485354	4.074592	0.1509108	2.6397	S
BAR	O	P3	0.273526	4.259693	0.1419898	2.1824	S
BRO	O	P6	0.499317	4.844004	0.1730002	1.8941	S
STO	O	P8	0.0786596	2.47688	0.154805	1.7071	S
STR	O	P5	0.6995512	5.821648	0.2531151	2.3820	S
CAR	O	P2	0.4492148	6.126312	0.1750375	2.7512	S

COL	O	P4	0.0545229	4.071077	0.1628431	1.8582	S
DEN	O	P1	0.3479822	5.048818	0.2195138	2.1422	S
LEA	O	P10	0.3830931	4.223929	0.1564418	1.7146	S
YOR	O	P7	0.3185889	4.292021	0.1589637	2.6042	S
THO	O	P9	0.5476215	4.370823	0.2081344	2.4690	S
BAR	O	P3	0.5083305	5.736777	0.2124732	2.0170	B
BRO	O	P6	0.4354455	5.137206	0.1712402	1.9202	B
STO	O	P8	0.1902148	5.02365	0.2184196	2.2675	B
STR	O	P5	0.4788251	5.402324	0.2077817	1.9228	B
CAR	O	P2	0.6566516	4.087141	0.2724761	1.7397	B
COL	O	P4	-0.12116	2.428571	0.3035714	1.2857	B
DEN	O	P1	0.3320749	4.476065	0.1946115	2.2135	B
LEA	O	P10	0.7491751	4.578399	0.2543555	2.0169	B
YOR	O	P7	0.2081869	4.429487	0.1703649	2.1170	B
THO	O	P9	0.4993378	6.471685	0.1961117	2.1676	B
BEC	C	P6	0.3517564	5.204601	0.1734867	2.5462	B
LIT	C	P4	0.4616716	4.144542	0.2302523	1.6638	B
MLS	C	P1	0.3884468	4.200849	0.2100424	1.5000	B
THR	C	P10	0.4512166	4.497087	0.204413	1.9943	B

BEE	C	P3	0.3117187	2.663008	0.2663008	1.7314	B
BES	C	P2	0.3701676	4.04527	0.2129089	2.0714	B
BXA	C	P9	0.3708961	3.202292	0.1601146	1.6794	B
HAS	C	P8	0.3739066	3.749306	0.3124422	1.2225	B
WAL	C	P7	NaN	2.846154	0.1897436	1.3077	B
FRE	C	P5	0.3590561	3.488474	0.1836039	2.6743	B

Table S8. Network metrics for each farm site by sample type (swab or basket) at the genus level

Farm	Organic/ Conventional (O/C)	Farm Pair	Nestedness	Linkage Density	Connectance	Generality (Bees)	Basket/ Swab (S/B)
BEC	C	P6	0.4833841	5.857163	0.1673475	2.734659	S
LIT	C	P4	0.1352625	5.256688	0.2021803	3.0249	S
MLS	C	P1	0.4573574	5.831048	0.1715014	2.5202	S
THR	C	P10	0.195603	6.604653	0.2277467	2.1358	S
BEE	C	P3	0.3843294	5.746257	0.2052235	2.2442	S
BES	C	P2	0.742121	5.911734	0.2364694	2.0728	S
BXA	C	P9	0.5444972	6.025038	0.2077599	2.1492	S

HAS	C	P8	0.15397	4.882526	0.1395007	2.8153	S
WAL	C	P7	0.2543309	4.555016	0.1822006	2.2701	S
FRE	C	P5	0.3533026	4.87772	0.1524288	3.3901	S
BAR	O	P3	0.2898852	4.119597	0.1373199	2.4430	S
BRO	O	P6	0.528125	4.51507	0.1672248	2.0101	S
STO	O	P8	-0.0453919	2.791774	0.1550985	1.7424	S
STR	O	P5	0.6596552	5.20214	0.2080856	2.3662	S
CAR	O	P2	0.3474261	5.26488	0.1548494	3.0803	S
COL	O	P4	0.0654958	3.797819	0.1519128	1.8746	S
DEN	O	P1	0.2512191	5.788763	0.1996125	1.9986	S
LEA	O	P10	0.4566148	3.957441	0.1364635	2.0416	S
YOR	O	P7	0.4211813	3.851406	0.148131	3.0500	S
THO	O	P9	0.3541775	3.682631	0.1673923	2.2900	S
BAR	O	P3	0.3712582	5.083014	0.1882598	2.1506	B
BRO	O	P6	0.4953173	4.680018	0.1613799	2.3734	B
STO	O	P8	0.3435054	4.351519	0.1891965	2.8202	B
STR	O	P5	0.6365546	4.003404	0.1601362	2.2244	B
CAR	O	P2	0.667702	3.705223	0.2470149	1.8203	B
COL	O	P4	-0.1296996	2.371654	0.2964567	1.2500	B

DEN	O	P1	0.5456777	4.946162	0.2060901	2.6526	B
LEA	O	P10	0.7499427	3.526804	0.1959336	2.0625	B
YOR	O	P7	0.1136677	3.263545	0.148343	2.7107	B
THO	O	P9	0.5992279	5.223932	0.1801356	2.6897	B
BEC	C	P6	0.4468701	4.865969	0.162199	3.2871	B
LIT	C	P4	0.5706594	5.229472	0.2490225	2.0350	B
MLS	C	P1	0.3671335	3.483306	0.1658717	1.5784	B
THR	C	P10	0.5307667	4.70385	0.188154	2.2675	B
BEE	C	P3	0.1809647	2.738994	0.2282495	1.7002	B
BES	C	P2	0.3434633	4.712936	0.2142244	2.2253	B
BXA	C	P9	0.6579595	3.37359	0.1606471	2.3082	B
HAS	C	P8	0.4862487	4.36239	0.3115993	1.1570	B
WAL	C	P7	0.188885	2.989211	0.1758359	1.5139	B
FRE	C	P5	0.3069495	3.993435	0.1736276	2.8995	B

Table S9. Network metrics for each farm site by sample type (swab or basket) at the family level

Farm	Organic/ Conventional (O/C)	Farm Pair	Nestedness	Linkage Density	Connectance	Generality (Bees)	Basket/ Swab (S/B)
BEC	C	P6	0.5664512	5.94263	0.2200974	3.575174	S
LIT	C	P4	0.2300469	4.747421	0.2157919	4.2025	S
MLS	C	P1	0.5705835	4.846554	0.2423277	4.2980	S
THR	C	P10	0.3523854	5.090606	0.2545303	2.8242	S
BEE	C	P3	0.5532206	4.93834	0.22447	2.9670	S
BES	C	P2	0.6082192	4.781274	0.2390637	2.6652	S
BXA	C	P9	0.4515382	4.65727	0.2451195	3.0212	S
HAS	C	P8	0.3537169	4.500523	0.1875218	4.6845	S
WAL	C	P7	0.2412515	3.414395	0.179705	3.3143	S
FRE	C	P5	0.585824	5.468542	0.2377627	5.1195	S
BAR	O	P3	0.3240833	3.7589	0.1634304	3.9409	S
BRO	O	P6	0.6739215	4.287355	0.2041597	3.2467	S
STO	O	P8	0.4199364	3.505126	0.233675	3.2980	S
STR	O	P5	0.7424538	4.229635	0.2349797	3.1449	S
CAR	O	P2	0.3587847	4.579226	0.1990968	4.4820	S

COL	O	P4	0.2400008	3.416339	0.2009611	3.3906	S
DEN	O	P1	0.3348309	3.852257	0.2027504	3.1054	S
LEA	O	P10	0.670753	3.709463	0.1854731	2.9763	S
YOR	O	P7	0.3811761	4.189509	0.1995004	4.2632	S
THO	O	P9	0.4089766	3.213738	0.1890434	2.8907	S
BAR	O	P3	0.415998	4.598293	0.2554607	2.7498	B
BRO	O	P6	0.5435719	4.637119	0.2107781	3.0584	B
STO	O	P8	0.4760199	4.644226	0.2580126	3.3264	B
STR	O	P5	0.7503014	4.180344	0.1900156	2.3566	B
CAR	O	P2	0.6137925	3.712693	0.2651924	2.2468	B
COL	O	P4	-0.3988106	1.973754	0.2819648	1.1818	B
DEN	O	P1	0.5709195	4.75077	0.2969231	3.3118	B
LEA	O	P10	0.7862821	3.657744	0.2438496	2.1419	B
YOR	O	P7	0.064238	3.45839	0.1820205	2.9624	B
THO	O	P9	0.683103	4.644785	0.2111266	3.0695	B
BEC	C	P6	0.5173442	4.754706	0.2264146	4.5908	B
LIT	C	P4	0.5466719	4.382134	0.2577726	2.5776	B
MLS	C	P1	0.4711976	3.36481	0.2243207	2.4080	B
THR	C	P10	0.5289856	4.064418	0.2540261	2.6166	B

BEE	C	P3	0.664993	2.991542	0.3323935	1.9575	B
BES	C	P2	0.4763355	3.930019	0.2456262	2.6135	B
BXA	C	P9	0.6948117	3.710494	0.2061386	2.6506	B
HAS	C	P8	0.7717184	3.818007	0.3470915	1.6843	B
WAL	C	P7	0.2059133	2.848126	0.2190866	1.7670	B
FRE	C	P5	0.2169912	4.052526	0.2532829	4.3561	B

Table S10. Names of plant OTUs removed as a result of quality control measures

Taxon	**Taxonomic level**
Achillea millefolium	species
Agrostis scabra	species
Alliaria petiolata	species
Anthemis cotula	species
Anthoxanthum odoratum	species
Apium graveolens	species
Artemisia vulgaris	species

Atropa belladonna	species
Bellardia viscosa	species
Berula erecta	species
Brachypodium pinnatum	species
Briza media	species
Calamagrostis epigeios	species
Calamagrostis neglecta	species
Cannabis sativa	species
Centaurea solstitialis	species
Ceratocapnos claviculata	species
Chelidonium majus	species
Conium maculatum	species
Cornus sanguinea	species
Cota tinctoria	species
Cruciata laevipes	species
Elymus repens	species
Erigeron canadensis	species
Eupatorium cannabinum	species
Festuca rubra	species

Filipendula ulmaria	species
Fraxinus excelsior	species
Galium aparine	species
Geranium robertianum	species
Glebionis segetum	species
Glyceria maxima	species
Gymnadenia conopsea	species
Hieracium umbellatum	species
Hordeum vulgare	species
Inula helenium	species
Iva xanthiifolia	species
Jasione montana	species
Knautia arvensis	species
Leonurus cardiaca	species
Linaria repens	species
Lithospermum officinale	species
Matricaria discoidea	species
Melilotus officinalis	species
Myosotis arvensis	species

Papaver argemone	species
Parapholis incurva	species
Pinus sylvestris	species
Plantago major	species
Plantago maritima	species
Poa pratensis	species
Potentilla reptans	species
Prunus dulcis	species
Prunus spinosa	species
Pyrus communis	species
Ranunculus aquatilis	species
Ranunculus flammula	species
Robinia pseudoacacia	species
Rosa rugosa	species
Salvia verbenaca	species
Scandix pecten-veneris	species
Scorzoneroides autumnalis	species
Senecio vulgaris	species
Silaum silaus	species

Silene dichotoma	species
Silene flos-cuculi	species
Sison segetum	species
Solanum nigrum	species
Solidago canadensis	species
Sonchus palustris	species
Spiraea alba	species
Stellaria media	species
Stellaria palustris	species
Symphyotrichum lanceolatum	species
Thymus pulegioides	species
Torilis japonica	species
Triglochin maritima	species
Tripleurospermum maritimum	species
Triticum aestivum	species
Tussilago farfara	species
Ulex europaeus	species
Urtica dioica	species
Valeriana dioica	species

Veronica chamaedrys	species
Veronica filiformis	species
Veronica serpyllifolia	species
Vicia lathyroides	species
Astragalus	genus
Barbarea	genus
Cerastium	genus
Euphorbia	genus
Hippocrepis	genus
Leucanthemum	genus
Lysimachia	genus
Myriophyllum	genus
Oxalis	genus
Primula	genus
Rubus	genus
Salix	genus
Torilis	genus
Urtica	genus
Apocynaceae	family

Campanulaceae	family
Cannabaceae	family
Cyperaceae	family
Orchidaceae	family
Rubiaceae	family
Saxifragaceae	family

6. Discussion

In this thesis, I aimed to investigate how larval bees deal with varying nutritional quality and how bees navigate their environment in relation to nutritional needs. Specifically, I wanted to contribute towards the integration of top-down and bottom-up approaches to nutrition in bees, with the intention of providing a more holistic view. I have shown that larval bees regulate to a carbohydrate target overall but that this shifts across development, with protein being more important to larvae during the early stages, highlighting the plastic nature of larval nutritional needs and the challenge this poses for foraging parents. I also provide evidence, for the first time, that bee larvae regulate their intake of lipids, drawing attention to the need to consider all macronutrients when investigating bee nutrition. I also show that the organic and conventional farmland plant communities that provide the nutritional resources required by bees are similar in diversity, but that organic farms show greater abundance of resources, suggesting that focussed management could allow for conventional farmlands to narrow the gap seen in floral abundance. Finally, I show that bees forage differentially for themselves and their larvae, utilising different floral resources when collecting pollen for their young than those they visit for themselves, emphasising the importance of considering both adult *and* larval needs when studying bee nutrition. These findings, in my mind, lead to two major messages: (1) that to better understand and support bees' nutritional needs we must combine nutritional knowledge gained through experimentation with the understanding of the plant communities with which they interact, and that (2) in order to improve our knowledge of the behavioural ecology of bees, in relation to adult- and larval-focussed foraging, we must integrate knowledge of the quality and availability of forage resources in the community, as

this likely shapes bees' nutritional decisions as much as their own needs. Below I will focus on a selection of specific ideas that I believe are key next steps in the research outlined in this thesis and that fall broadly under the major messages described above.

Networks have frequently been used to investigate bees' interactions with their floral hosts (Johanson et al., 2019; Kovács-Hostyánszki et al., 2019). The importance of these interactions are often based on characteristics such as interaction frequency, interaction strength or the number of partners a species interacts with (Burgos et al., 2007; Vázquez et al., 2012, 2005), which allow for predictions of the relative importance of particular species in network maintenance to be made (Grass et al., 2018; Lance et al., 2017). However, networks are more complex than this. For example, species traits, as well as the network characteristics outlined above, are now known to also be important in the structure and maintenance of communities (Albrecht et al., 2018; Valdovinos et al., 2018). How then, when we are considering bee nutrition, can we judge the true importance and structure of species interactions without also considering the quality of the nutritional resources available? I believe that a logical next step for this research would be to address this issue by the construction of a Nutritional Network. I have shown in this thesis that bees interact differently when visiting floral resources for adult- and larval-focussed nutrition, as well as identifying the groups of flowers with which they interact, but what we don't yet know is what the *quality* of those specific pollen resources are in relation to bees' specific needs [though a picture of how pollen quality varies across landscapes is beginning to emerge; see (Donkersley et al., 2014)]. By analysing the nutritional quality of pollens taken from those plants utilised by the bees, it could be possible to identify the most important interactions in a network by the nutritional contribution of each plant. This weighting of interaction importance by nutritional quality could highlight unappreciated links that may go unrecognised based on standard ways of assessing interaction importance. We have an

idea about what larval bees aim for nutritionally, both in terms of macronutrients and elemental components (Chapter 2; Filipiak, 2019), as well as how their needs differ between sexes (Filipiak, 2019), and we know that the abundances of resources differ based on which habitat bees inhabit (Chapter 3). These could provide an excellent reference for which to compare the Nutritional Network and highlight species of particular nutritional importance to bees.

Another, more applied, angle which I feel would be a natural progression from this research thesis is to apply its findings to practical interventions in bee conservation, for example, a nutritionally informed seed mix. Seed mixes and wildflower strips are popular ecological management interventions on farmland and have seen much attention in the literature (Blackmore and Goulson, 2014; Cresswell et al., 2019; Galea et al., 2016). However, a common fault with these types of interventions is that their composition is often dictated by visitation interactions rather than by nutritional information (Filipiak, 2018). Previous work has assessed the volumes of available nectar and pollen (Hicks et al., 2016) but a true nutritionally constructed mix is still lacking. In this thesis I provide insights into what flowers bees visit for both themselves and for their offspring, how the abundance of resources changes across the season and across farming systems, and the levels of nutrients larval bees aim to achieve during development. I also provide evidence of key '*hungry gaps*' on farmland (Nowakowski and Pywell, 2016), demonstrating the need for seed mixes that span across the season and highlighting the level of nutrition such a mix would need to offer. We know that pollen can vary in both macronutrients and elemental composition (Filipiak et al., 2017; Roulston and Cane, 2000a), and, for example, Filipiak (2019) suggests that (for *Osmia bicornis* at least) plants such as *Vicia faba* and *Trifolium repens* could provide dietary elemental balance for bees. These plants are both from the Fabaceae family, a group shown to be of particular interest to bees foraging for larvae in

this thesis, especially on conventional farmland. Combining these sorts of information with the nutritional analysis of other plant species commercially available, whilst also considering the morphological ability for bees to interact efficiently with specific flowers (Hrncir and Maia-Silva, 2013; Minckley and Roulston, 2006), would be an important step towards a universal and nutritionally optimal seed mixture for bees, as well as informing other forms of farmland management such as hedgerow planting.

When considering the behavioural ecology of bees I think there are some other promising avenues to pursue in light of this thesis' findings. I have provided evidence that larvae are able to regulate their nutrient intake (Chapter 2), and what plants adult bees interact with in order to feed their young (Chapter 5). However, to truly complete the picture of how bees' regulate nutrition we must also investigate whether the nutrition gathered by the adult matches that which the larvae aims for i.e. does the adult regulate the nutrition it gathers for its young to the same level that the larvae regulates its own nutritional intake? We know that larval and adult nutritional needs, as well as the needs of males and females, differ (Filipiak, 2019) so identifying whether adults are able to gather this resource specifically for their young will provide crucial knowledge as to whether bees can navigate unbalanced nutritional landscapes. This is of particular importance in understanding bees' foraging capabilities in the modern world, as not only does pollen nutritional composition differ among species in the environment (Filipiak et al., 2017; Roulston and Cane, 2000a) but the influence of human-induced climate change is also affecting the quality of nutritional resources *within* plant species (Ziska et al., 2016), adding another level of complexity to the nutritional challenge bees face. Results for whether adult bees are able to assess pollen quality remain mixed however (Corby-Harris et al., 2018; Muth et al., 2016; Nicholls and Hempel de Ibarra, 2016; Roulston and Cane, 2002; Ruedenauer et al., 2016, 2015), yet the truth of this ability will undoubtedly have

implications for how adults interact nutritionally with their environment. This uncertainty in bee's ability to detect the quality of pollen resources may leave them vulnerable to anthropogenic changes in floral availability and quality. Such a scenario could lead to bees being caught in a 'nutritional trap', i.e. where rapid changes in their nutritional environment outstrip their evolved capacity to detect those changes, impairing their fitness. Through network construction I have shown how bees interact differently with flowers based on whether they forage for themselves or for their young, as well as that resource abundance differs between farming practices and across seasons. By combining this information with the nutritional quality of the resources available, and with experimental work investigating adult bees' regulatory capacity, key landscapes where nutritional traps are more likely could be identified as well as whether such traps may only occur at certain times during the flight season. A natural extension would be to then investigate such patterns under simulated climate change conditions. As stated above, such changes can influence the quality of pollen within a plant species (Ziska et al., 2016), therefore how would this influence the nutrition available to bees? And would shifting climates also affect the nutritional rules employed by adults and larvae in and of themselves?

The final avenue for further exploration I would like to highlight is the extension of the nutritional studies of dependent larvae reported in this thesis to social model species. Social nutrition is an emerging field (Dussutour and Simpson, 2009; Lihoreau et al., 2018, 2015) but, as I mentioned in chapter two of this thesis, to date larval nutrition has been hard to study directly in any social insects. Addressing this problem could provide novel insights into social nutrition, as the regulatory patterns I found in the solitary bee *O. bicornis* differed profoundly from those shown in honeybee larvae (Helm et al., 2017). Using solitary mass-provisioning bees for nutritional manipulation studies is useful as parentally provided nutrition can be isolated and tracked, whereas in social species, such as the

honeybee and bumblebee, nutrition is often manipulated within the nest prior to feeding and larvae are fed progressively (Field, 2005). Stingless bees (Meliponini), however, are fully eusocial like honeybees yet many species mass-provision their offspring (Roubik, 2006). They are also a key commercial pollinator group (Amano et al., 2000; Chapman et al., 2017; Heard and Dollin, 2000) and may help in mitigating the loss of other managed species such as honeybees (Cortopassi-Laurino et al., 2006), especially where such bees are not native (Cunningham et al., 2002). Recent advances in culturing larvae *in vitro* (Dorigo et al., 2019) lend themselves excellently to the nutritional manipulations utilised in this thesis, allowing for the investigation of nutritional regulation in a truly social species. Additionally, I demonstrate here that bees forage for plants differently based upon adult- or larval-focussed foraging, as well as that resource availability differs throughout the year. Stingless bees also forage from plants in order to create honey and, using DNA metabarcoding techniques, creating networks from the same hive based on both honey and pollen foraging could provide new insights into how social species differentially forage based on which resources they require, and how this may change in relation to floral availability. Such analyses have been done separately in honeybees, for honey (de Vere et al., 2017; Hawkins et al., 2015) and for pollen (Danner et al., 2017; Smart et al., 2017), but no study as yet has investigated this simultaneously, or in stingless bees. Moreover, integrating results from broader sets of bee species in general will aid in understanding the range of nutrition required by different bee species.

This thesis highlights the importance and usefulness of a holistic approach to investigating bee nutrition. I have shown that (1) larval bees can regulate their nutritional intake, (2) the importance of specific macronutrients changes over the developmental period, (3) the different types of farmland within which bees forage support similar plant communities but show distinct resource scarcity during key bee flight periods, and (4) that

bees forage differentially for themselves and for their offspring, highlighting the differences between pollination and pollen feeding. Bees are in decline (Potts et al., 2010a; Rhodes, 2018) and nutritional deficit is a major cause (Goulson et al., 2015; Roulston and Goodell, 2011). Therefore, understanding that (1) bees' nutritional needs differ between adults and larvae, and (2) the way they interact with their environment differs depending on whether they forage for themselves or their young, must be appreciated if we are to understand the effects of nutrition on bee ecology, and if we are to ensure that human-altered environments are managed in such a way so as to support strong, healthy bee communities.

7. References

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